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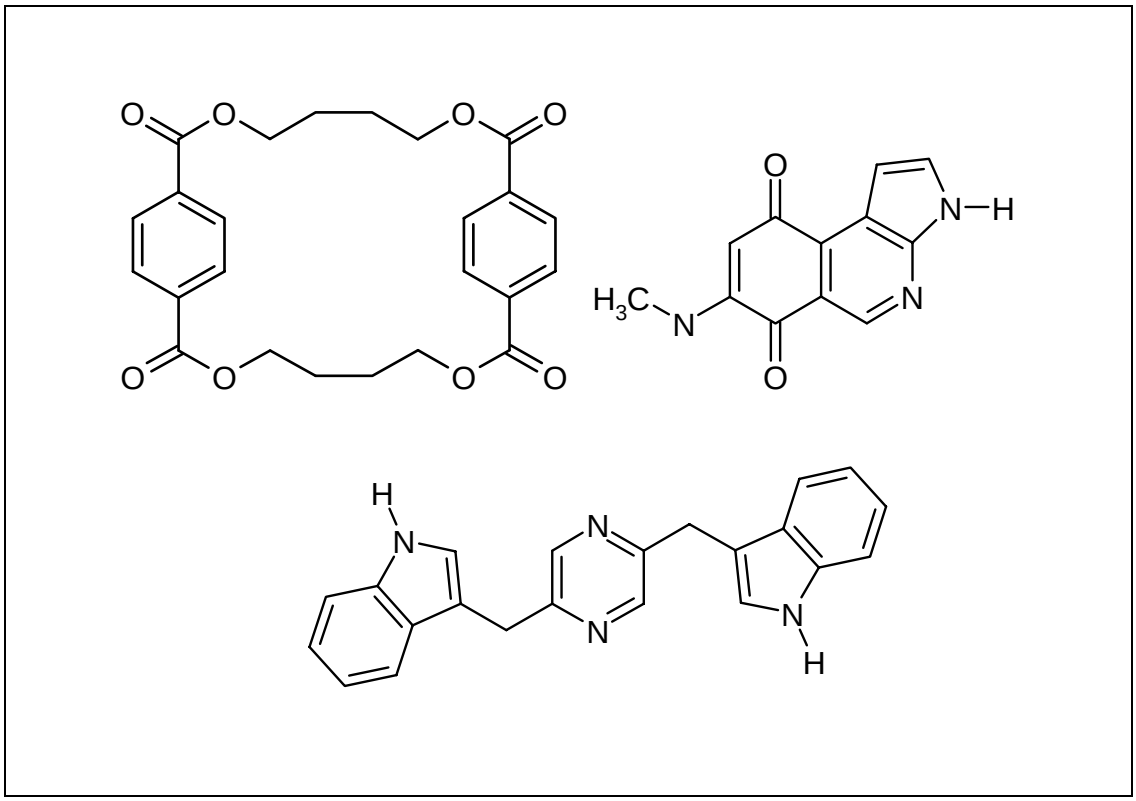
Bioactive Secondary Metabolites from Marine and Terrestrial Bacteria: Isoquinolinequinones, Bacterial Compounds with a Novel Pharmacophor



Dissertation

Bioactive Secondary Metabolites from Marine and Terrestrial Bacteria: Isoquinolinequinones, Bacterial Compounds with a Novel Pharmacophor

Dissertation

zur Erlangung des Doktorgrades

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der Georg-August-Universität zu Göttingen

vorgelegt von

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Herrn Prof. Dr. H. Laatsch danke ich für die Möglichkeit zur Durchführung dieser Arbeit sowie die ständige Bereitschaft, auftretende Probleme zu diskutieren.

Für meine Eltern,
meine Frau und meine Kinder

1. Introduction

Natural products, as the term implies, are chemical compounds derived from living organisms e.g. plants, animals and microorganisms. Natural product chemistry is related to the isolation, biosynthesis and structure elucidation of new products from macro or microorganisms, which may lead to new medical drugs. The pharmaceutical agents derived from natural sources are an important part of our therapeutic strategies since beginning due to their chemical diversity and various bioactivities against diseases. The low cost and availability of crude natural products have made them an important source of medicine especially in underdeveloped countries.

According to an estimation by the world Health Organization, 80% of the people on earth mainly depend on traditional medicines for their health care[1]. The use of natural products from specific plants as medicinal agents to treat illness presumably predates the earliest recorded history of humans. Records from as early as 2700 B.C. from China, to the Emperor Shennung, and the Eber's papyrus (written in about 1550 B.C.), indicate the usefulness of plants for treating diseases[2]. The scientific classification of plants started from Theophrastus (370-285 B.C.), while Dioscorides in his "De Materia Medica" (77 A.D.) reported the uses of over 600 plants as medicine. Later Ibn al-Baitar (1197-1248) listed over 1400 drugs and medicinal plants in his Corpus of Simples[3].

Microorganisms, and in particular the bacteria, have had a profound effect on the development of chemistry and upon medical science. Since the discovery of penicillin (**1**) in 1929, intensive studies of mainly soil derived bacteria and fungi have shown that the microorganisms are a rich source of structurally unique pharmaceutically important bioactive substances[4].

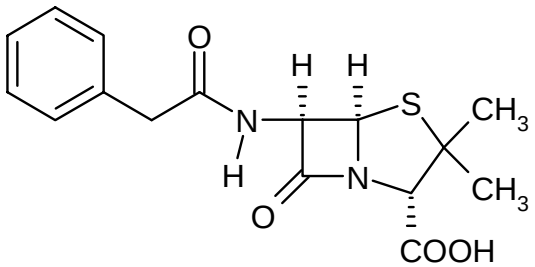
1.1 Developmental history of antibiotics

Antibiotics are defined[5,6] as low-molecular weight (MW < 2000 Dalton) secondary metabolites from natural sources including their chemically or biosynthetically produced derivatives, which show inhibition of the growth of higher organisms (e.g. tumour cells) or pathogens (e.g. bacteria, fungi, viruses) at low concentration, and subsequently can be used to cure the infectious diseases. Not all natural products serve as antibiotics, many of them function as signalling substances between micro-

organisms or have still unknown purposes. One of the most intensively studied bacterial intercellular signal substance is the A-factor (γ-butyrolactone) which stimulates the production of streptomycin by *Streptomyces griseus* and it is also responsible for the formation of mycelium and pigments[7].

It is customary to distinguish between primary and secondary metabolism. The former refers to widely distributed compounds, e.g. carboxylic acids of the Krebs cycle, α-amino acids, carbohydrates, fats, proteins and nucleic acids which are essential for the life processes. Secondary metabolites are in principle non-essential for life but they definitely contribute to the species' fitness of survival. They are often characteristic for the particular biological group[8].

A *Penicillium notatum* contamination showed a potent inhibition of the *Staphylococcus* sp. on agar plates, and this famous accident lead to the discovery of penicillin by Alexander Fleming[9]. Penicillin showed an inhibitory effect against *Staphylococcus* and *Streptococcus*, two Gram-positive bacteria, which exhibited a huge number of the human infectional diseases. The three researchers Fleming, Chain and Florey have got the Nobel Prize in medicine[10,11]in 1945 for the introduction of penicillin into medicine. Since then, many antibiotics have been isolated from various microorganisms like *Actinomycetes*, other bacteria, fungi etc. Antibiotics, such as vancomycin (**2**), daptomycin, cephalosporin, streptomycin, the antifungal amphotericin B, griseofulvin, the antiviral aciclovir, doxorubicin, and many others, play a pivotal role in therapeutics[12].



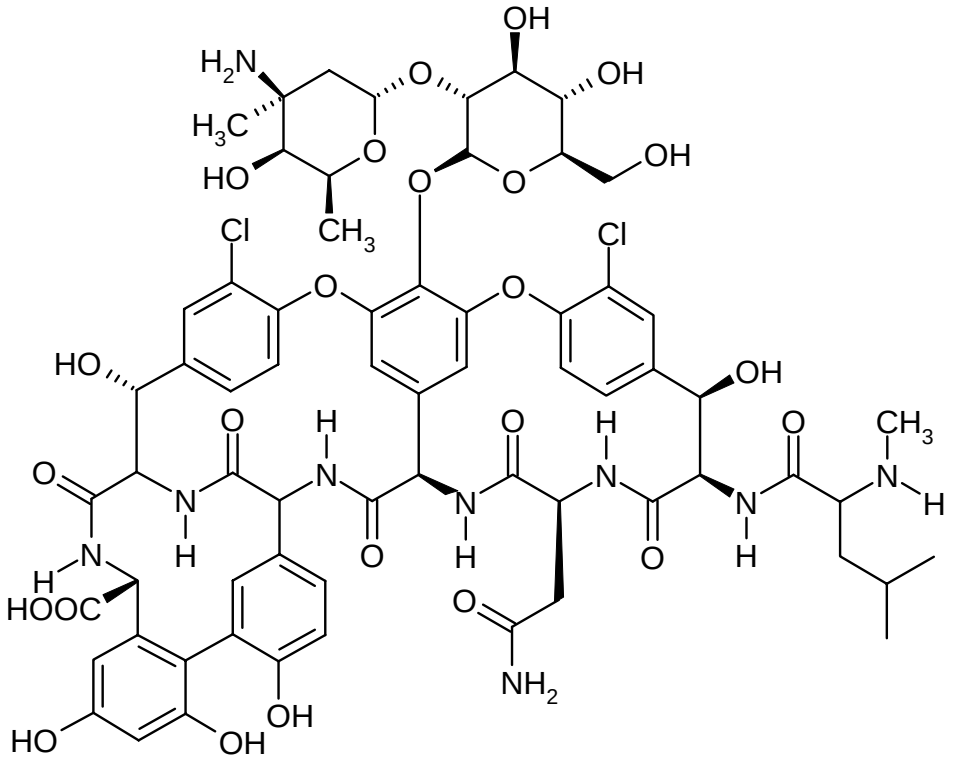
1

Over the past 60 years, about 28000 natural products have been isolated from microorganisms. More than 10000 of these compounds are biologically active and more than 8000 are antibiotic and antitumor agents[4,13-15]. Today over 100 microbial products continue to be used clinically as antibiotics, antitumor drugs and agrochemicals.

1.2 Searching for new Sources of Antibiotics

Antibiotics interfere with a metabolite or the synthetic pathway of another microorganism. They can block the DNA synthesis or compensate the transcription of selected enzymes or other cell function. In the beginning of the era of antibiotics, it seemed that all diseases could be defeated. However, with the prolonged use of the same antibiotics, it was shown no more effect and this is resonated to modification of the mutant of the infectious microorganism.

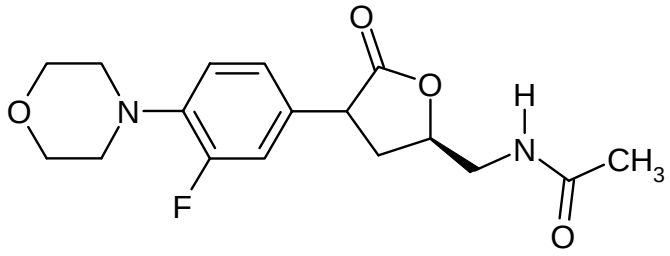
Infectional diseases and drug resistance phenomena cost ca. 17 million lives every year[16]. Certain *Staphylococcus aureus* strains presently show no response even against the aminoglycopeptide, vancomycin (**2**). The latter compound exhibits a high affinity to the cell wall building enzymes of bacteria, and is used as potent defence against multi-drug resistant (MDR) pathogenic bacteria. For this reason, there is worldwide researching to look for new medicine sources, as well as continuing international focus exploring the new antibiotics sources and especially pharmaceutically active microbial products, as well as synthetic approaches.



2

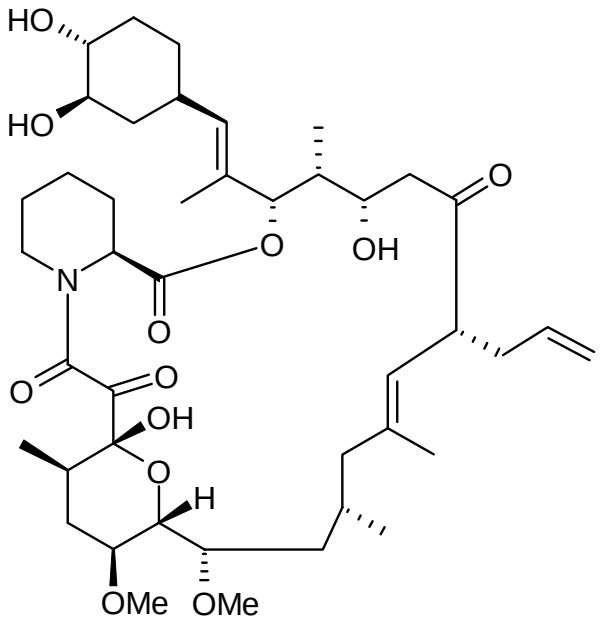
In accordance, the synthetic drug linezolid (**3**) was used to replace **2**. The mode of action of **3** is explained by its effect on the 30S-subunit bacterial ribosoms. It

blocks the formation of a functional capable initiator complex of the bacterial protein synthase[17].



3

Since 1983, the immunosuppressant cyclosporin A facilitates the transplantation of organs[18]. Four years later, the Japanese company Fujisawa isolated the novel immunosuppressant agent FK506 (**4**), which was discovered in a screening for inhibitors of interleukin-2 release on T-cells as the active principle of the culture broth of *Streptomyces tsukubaensis*[19]. Being 100-fold more active, **4** was expected to replace cyclosporin A.



4

Recently, chemists on a worldwide basis have paid attention towards the potential of marine microorganisms (e.g. bacteria, fungi, blue green algae, dinoflagellates etc.), as an alternative source for isolation of novel metabolites with interesting biological and pharmaceutical properties[20,21]. Most metabolites of bacterial origin come from one group, Gram-positive soil bacteria of the order *Actinomycetes*.

Various studies have shown that metabolites obtained from microorganisms are structurally more divers, and exhibit more interesting bioactivities compared to those of plant origin. Instead of monotonous alkaloids from plants, microorganisms produce many different chemical structure classes, such as polyketides (e.g. FK506[22] (**4**)), terpenes, polysaccharides (e.g. acarbose), polyethers, and nitrogen-containing compounds, such as indoles, peptides, pyrroles, glycopeptides (e.g. **2**).

Until now about 28000 secondary metabolites were isolated from microorganisms[23]. The number of natural products isolated from plants and animals is about 5 fold higher, however, they are limited mostly to peptides, terpenes, steroids and flavonoids[24].

The world oceans compose over 70% of the earth's surface and over 90% of the volume of its crust. Microbiologically, the oceans represent indeed a most diverse resource of life with huge dimensions and extreme variations in pressure, salinity, and temperature. These extreme conditions require unique adaptation strategies leading to new natural products, which differ from products known for terrestrial organisms. The carbonimide dichloride functionality (-N=CCl_2) and the sulphamate group (OSO_2-NHR) have been found only in marine natural products[25].

However, microbial diversity of oceans is still poorly understood. It is estimated that less than 5% of marine bacterial and fungal species are known. In parallel, studies on natural products of marine bacteria, *Actinomycetes*, or fungi are relatively rare. Okami[26] has reviewed the potential and problems of studying chemicals from marine microorganisms, and Fenical discussed the about 50 compounds known in 1993[4].

It was expected that marine bacteria might produce compounds, which differ basically from terrestrial products, as it is known from spongial metabolites. Many metabolites of marine microorganisms, especially those of *Streptomycetes* are, however, similar to or identical with those of terrestrial origin, and it is not easy to decide if a microorganism is truly marine. One of the principal difficulties appears to be therefore the definition of a marine microorganism. It was reported recently, that the marine microbial Picoplankton contains a high abundance of rare species, virtually none of which has ever been isolated and chemically investigated[27]. It can be expected that truly marine bacteria can be found in this or related sources. Fenical postulated that the newly described genus *Salinospora* is a rare *Actinomycete* restricted to the

sea[28], and further groups may exist as associated microorganisms in sponges, tunicates etc. As far as details are unknown, it is advisable for practical reasons to name marine bacterial isolates as 'bacteria of marine origin' and not as 'marine bacteria'.

The advantage of microorganisms as sources are: One bacterial cell is in principle enough for cultivation, and fermentations are independent of climate and seasons. A further advantage is the facile generation of new metabolites by feeding of suitable intermediates (precursor-directed biosynthesis). An example is the strain Tü3634 (*Streptomyces griseoviridis*) which produces various acyl and phenyl a-L-rhamnopyranosides in the presence of different supplements[29].

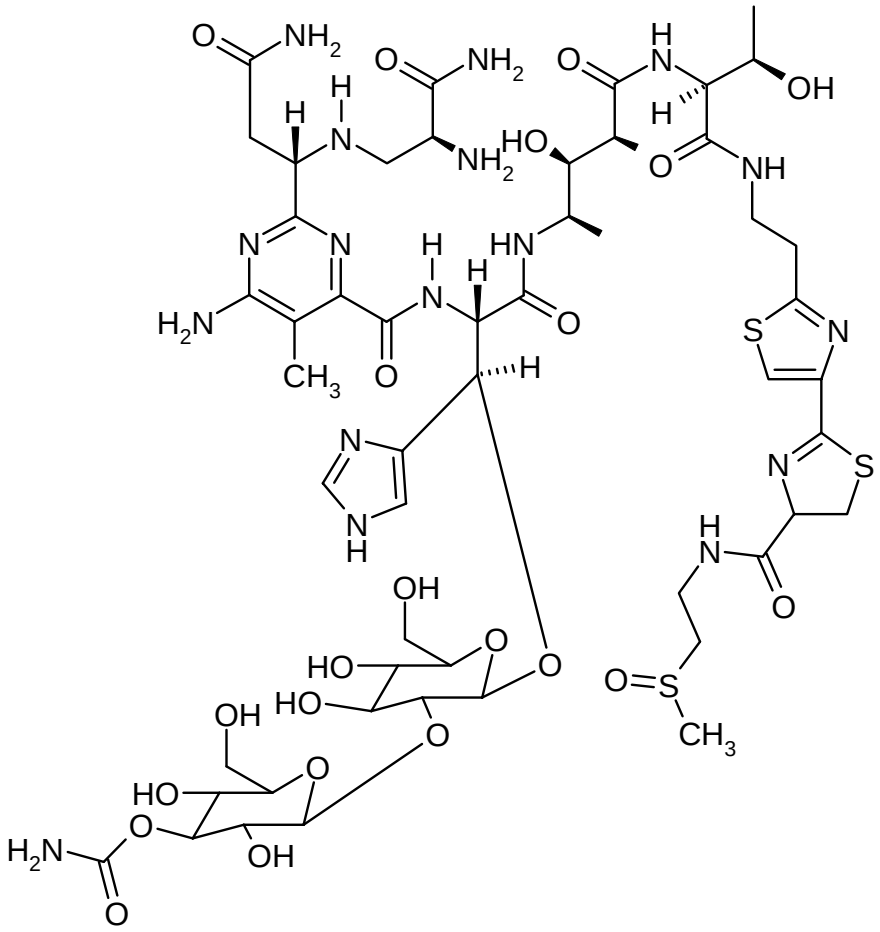
The increased frequency in clinically observed cases of antibiotic resistance and the appearance of vancomycin-resistant *Enterococci* (VRE) in 1988 can not be ignored. In addition many other widespread diseases such as MS, Alzheimer and cardiovascular diseases cannot be cured sufficiently until now and show an upwards tendency. New pathogens like HIV expand quickly and extend worldwide. Searching for new bioactive natural products with new skeletons, as leading structures in the development of medical drugs is becoming an urgent task for chemists, microbiologists, biochemists and pharmacists[30].

1.3 Approaches to get new natural products

The search for bioactive compounds in nature is a multi-step procedure, which begins with the selection of suitable sources. Biological, chemical or physical interactions of metabolites with test systems are then qualitatively or quantitatively evaluated[31].

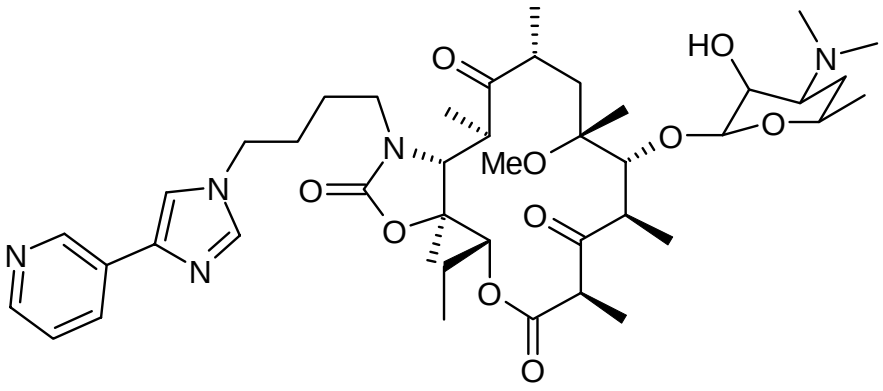
In recent years, genes of e.g. polyketide synthases were located and isolated. In accordance, genetically manipulated microorganisms can be used to generate new metabolites by realigning the synthetic capacities of different species. Novel natural products will be optimized based on their biological activities to yield effective chemotherapeutic and other bioactive agents[32]. Biotransformation techniques can be used to investigate new natural products. This method enables derivatization of known compounds by esterfication, reduction, oxidation, demethylation, or glycosylation utilizing the enzymes of living microorganisms. For example, glycopeptide antitumor metabolites from *Streptomyces verticillus*[33] were found to be good antibiotics or antineoplastic agents, e.g. bleomycin A1[34] (**5**). Bleomycins are currently

used for clinical treatment of Hodkin's lymphoma, carcinomas of the skin, head, and neck, and tumors of testis[34]. To achieve the same result by means of chemical transformations would be time consuming, requires many steps, needs chemicals and produces by-products.



5

Some antibiotics derived from natural resources need to be transformed structurally in order to optimise their pharmacological properties. Erythromycin is an antibacterial macrolide isolated in 1952[35]. Its *semi*-synthetic derivative telithromycin (**6**) which contains a 3-keto substituent in place of the cladinose sugar, is 10-40 times more active than the parent antibiotic[36].



6

The ketolides are a new class of antibiotics possessing excellent inhibitory abilities against Gram-positive or Gram-negative cocci like *Staphylococcus*, and *Enterococcus*, which are resistant against macrolides and other antibiotics[37]. Compound **6** is on the German market since 2001 as a new antibiotic.

1.4 Antibiotic screening

The discovery of penicillin and the actinomycins led to the new research field "antibiotic screening". Therefore, in order to discover new bioactive compounds, crude extracts are evaluated by chemical, biological and pharmaceutical screening approaches. The latter can focus on looking for bioactive substances and often provides the advantages of greater sensitivity and sample throughput for the industrial High-throughput-Screening (HTS)[38]. However, novel compounds, which may be active against other targets, are overlooked. To overcome this problem, Zähner and many other researchers started systematically a chemical screening of crude extracts in the 1980s[39]. The chromatographic characteristics of metabolites on thin layer chromatography (TLC) plates, as well as their chemical reactivity towards staining reagents under defined reaction conditions, allows visualizing of an almost complete fingerprint of the secondary metabolite pattern[38]. The use of this method has led to the isolation of nearly all metabolites of a given strain, and the various unknown compounds can then be biologically tested in pure state.

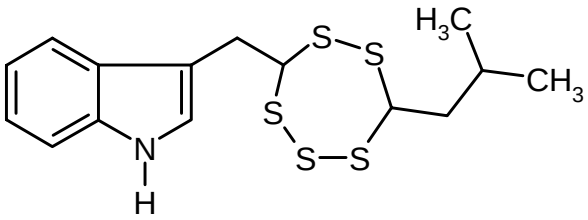
Due to the recent increase of the sensitivity of mass spectroscopy (MS), nuclear magnetic resonance (NMR) instruments, and the rapidly growing chemical databases (AntiBase, DNP, CA), allows the dereplication of known compounds and their structure determination. Presently the screening is conducted in combination with high-

performance liquid chromatography (HPLC), ultraviolet (UV), HPLC-DAAD, HPLC-CD, HPLC-MS, HPLC-NMR-MS or GC-MS systems[40].

In recent years, considerable attention has been paid to marine microorganisms as source of natural products. However, the marine bacteria show a low production rate under normal incubation conditions unlike the terrestrial bacteria. To increase the screening efficacy for secondary metabolites of marine bacteria, microbiologists developed a polymerase chain reaction (PCR)-based screening assay for genes e.g. Polyketide synthases, non-ribosomal polypeptide synthases (NRPSs), dNDP-glucose dehydratases and halogenases[41]. The problem of this approach is, however, that these genes occur very frequently, but may not be expressed (silent genes)

1.5 New Natural Metabolites from Marine Bacteria

The five sulphur atoms containing structure **7** was isolated from the marine thermophilic archaea *Thermococcus tadiuricus* and *Thermococcus acidaminovorans*, in addition to other 22 predominantly new cyclic polysulphides[42]. The strains were isolated from marine hydrothermal systems near Obock (Djibouti) and Vulcano (Italy), respectively.

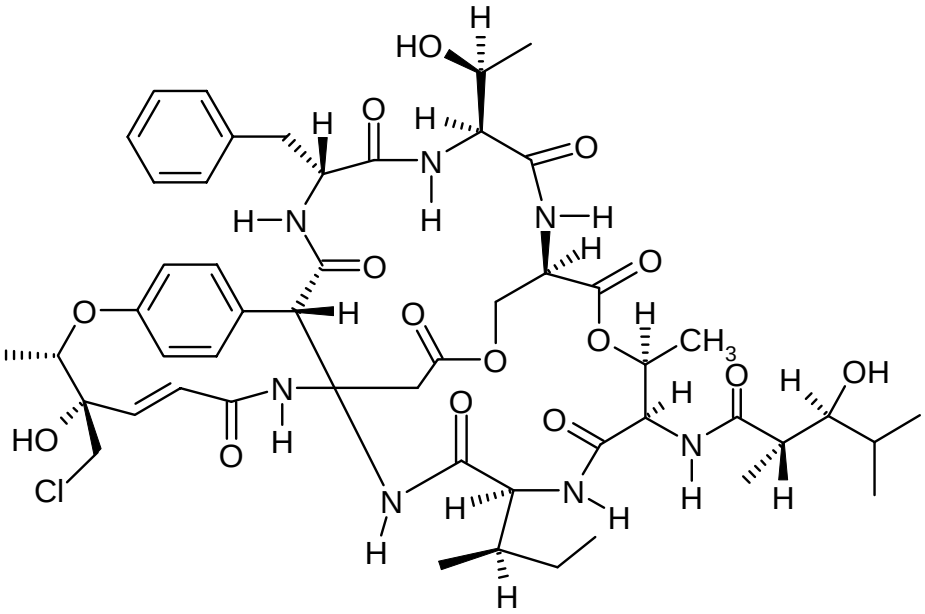


7

The chlorine-containing anti-inflammatory depsipeptide salinamide B (**8**) was isolated from a marine *Streptomycete*. The salinamide-producing actinomycete was isolated from the surface of the jellyfish *Cassiopeia xamachana* collected in the Florida Keys[43].

Pentabromopseudilin (**9**), an antibiotic and cytotoxic brominated marine natural product, was isolated from *Alteromonas luteoviolaceus.* 70 % of the compound's molecular weight consists of bromine[44-46]. Due to its highly toxic effects, it could not be used as medicine. The chloro analogue **10** was isolated in 1978 from the terrestrial *Actinoplanes* sp. ATCC 33002[47]. A recent study carried out on 136 marine

bacteria strains for searching a halogenated compounds confirmed that, it is very rare to get halogenated secondary metabolites from marine bacteria[48].



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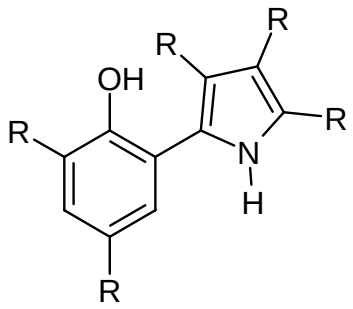
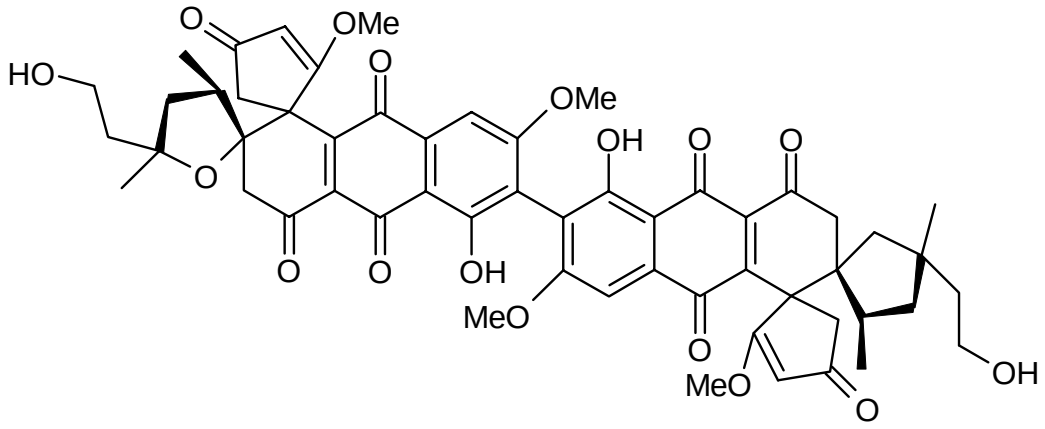


9: R = Br, **10**: R = Cl

Oceamycin[49] (**11**) and maremycine[50]A (**12**) and B were isolated in our research group from the marine *Streptomyces*. Oceamycin is the first reported anthraquinone dimer containing two five memberd Spiro rings.



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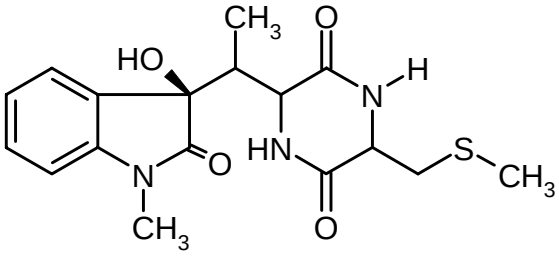
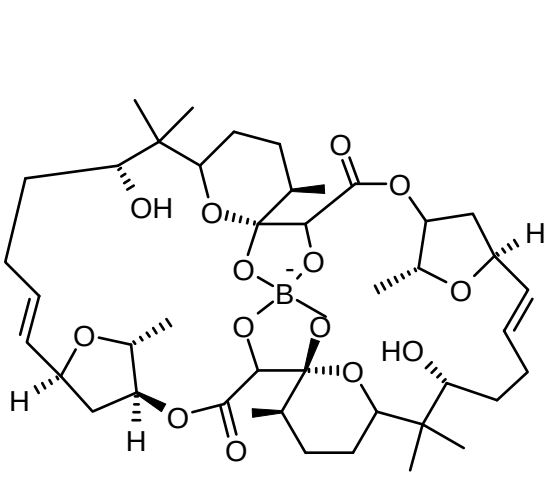


12

The boron containing aplasmomycins A-C (A **13**) were isolated from *Streptomyces griseus*[51,52]. Aplasmomycins are highly active against Gram-positive bacteria e.g. *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus anthracis* and *Corynebacterium smegmatis* with MIC-values 0.8-3.0 μg/ml in *vitro*. A subsequent marine boron component, borophycin (**14**), was discovered in the marine cyanobacterium *Nostoc linckia* and *N. spongiaforme*[53].



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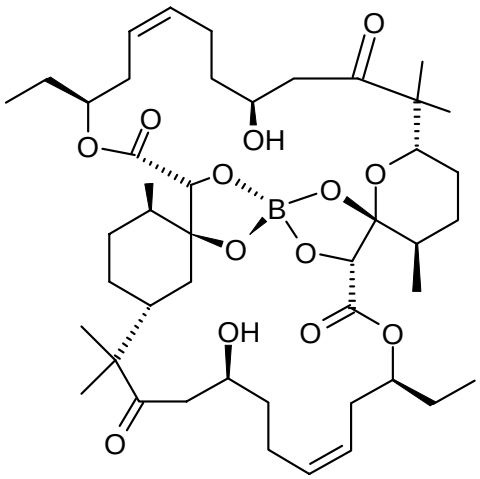
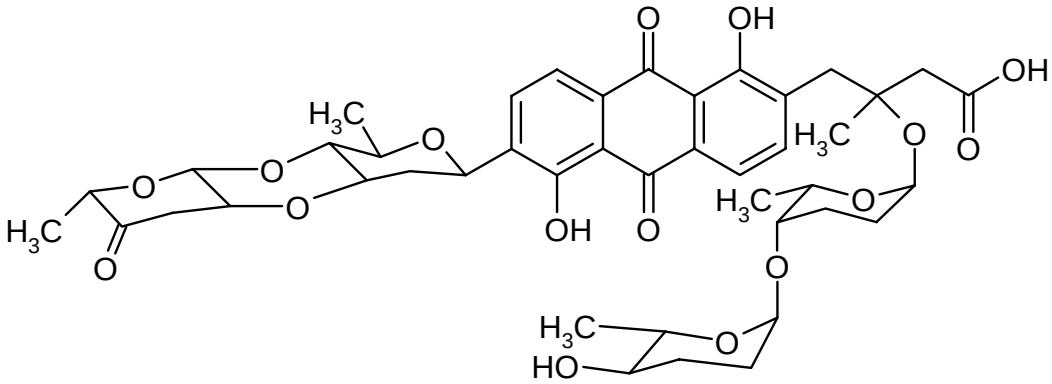


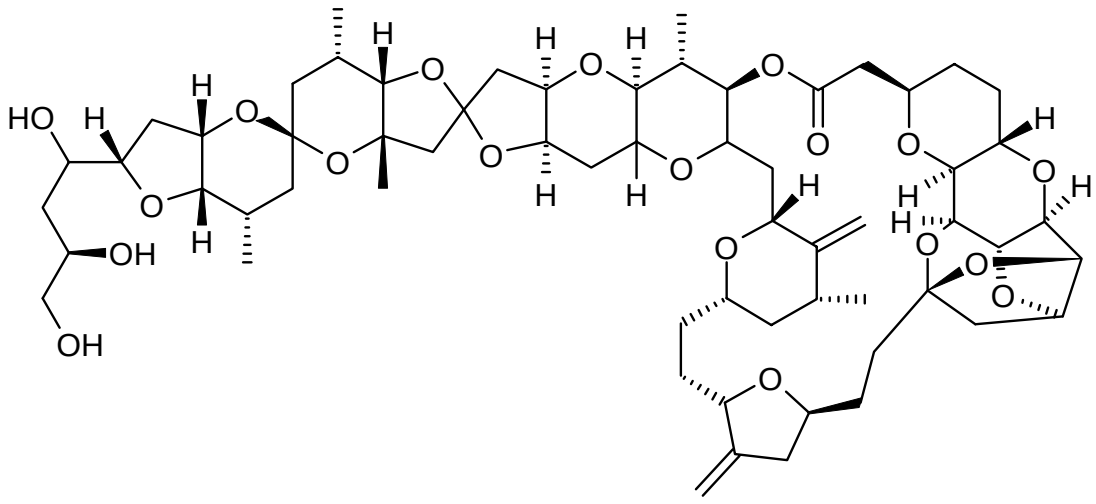
14

Hamilomycins[54] A, B (A, **15**) are C gylcosidic angular anthraquinone antibiotics which were isolated from a marine *Streptomyces* sp. The C gylcosidic moieties are relatively rare in nature. These antibiotic compounds possess antibacterial and antitumor activities as well.



15

Halichondrin B (**16**), a polyether macrolide, was first isolated from the sponge *Halichondria okadai* as the most potent member of a series of related antitumor compounds[55]. But because of its low concentration in the ranges of 10^{-5}-10^{-6} % in sponges, it is impossible to collect enough biomass. A total synthesis involves many steps with low yields[56]. Therefore, the mass cultivation of macroorganism, i.e. the sponge *Lissodendoryx sp*. has been first established for pharmaceutical purposes[57].

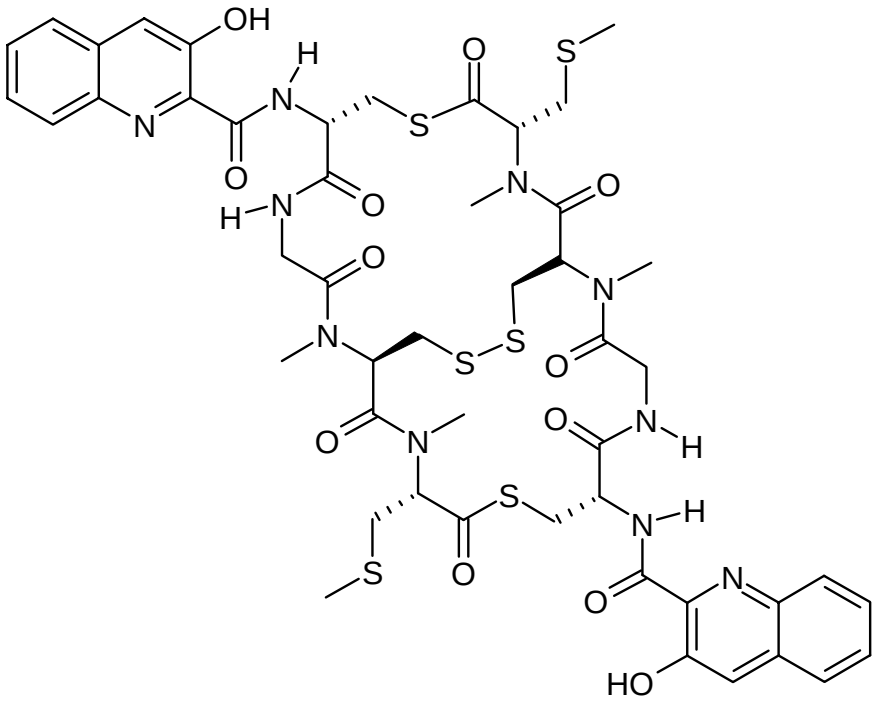


16

Increasing evidence suggests that many marine natural products are not produced by the source invertebrate, but rather attributed to symbiotic microorganisms

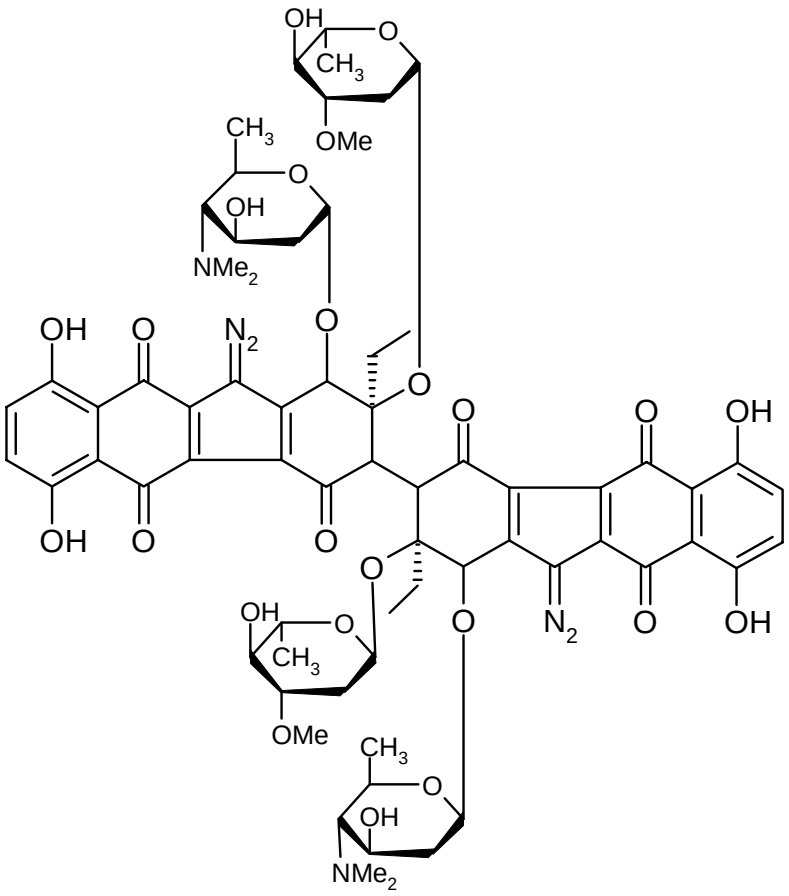
living within the tissues of invertebrates. The studies on their cultivation have a considerable meaning, because it is easier to get mass production from cultures of microorganisms than of macroorganisms.

Thiocoraline (**17**) is a new sulphur-containing depsipeptide with antitumor activity produced by the marine actinomycete *Micromonospora marina* from a marine soft coral, present in the Indian Ocean near the coast of Mozambique. It inhibits RNA synthesis and shows a potent antimicrobial activity against Gram-positive microorganisms and cytotoxic activities with an IC_{50} value on the ng level[58]. The high percentage of thiocoraline, enables its industrial production around 10 mg/l as compared to the other marine drug candidates. It is now in preclinical development at PharmaMar S. A. in Spain.



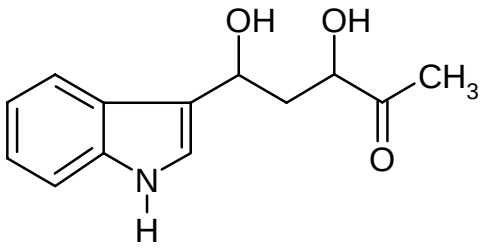
17

A previously unidentified strain with high activity against tumor cells was discovered during the isolation of *Actinomycetes* from Sea squirts. The bacterial source was defined as *Micromonospora lomaivitiensis* and its active components were named as lomaiviticin A (**18**) and B. These compounds also act as antibiotics and show activity against antibiotic-resistant strains of *Staphylococcus aureus* that cause infection and death in surgical treatments. Its MICs against tumor cells are lower than 10 pg/ml[59].



18

The indole **19** was isolated previously from the sponge *Dysidea etheria*[60] and has now been obtained from the Antarctic ice bacterium ARK 13-2-4371[61].



19

1.6 The Phylogenetic Tree of Bacteria

Bioactive compounds have been reported from marine bacteria belonging to the genus *Pseudoalteromonas*, *Cytophaga*, *Alteromonas*, *Micrococcus*, *Bacillus*, *Acinetobacter*, *Agrobacterium*, *Pseudomonas*[40] as well as *Streptomycetes*. Presently, there are two microbial phylogenetic hot spots known for the production of secondary metabolites:

- The *Streptomycetes*, a group of filamentous Gram-positive bacteria (Actinomycetes) that are the working horses of natural product isolation.
- The *Cyanobacteria*, the blue green algae, photosynthetic bacteria that are distributed globally and produce extremely potent metabolites.

1.7 North Sea Bacteria

The German North Sea is an unexplored marine habitat and a special ecological area due to the dynamic tidal water. Therefore, its microbiological and chemical features should differ from the other marine environments. Within the network Marine Biotechnology in Lower Saxony[62], an integrated approach between research groups in microbiology, natural product chemistry, biotechnology, and medicine was underway to discover the metabolic capabilities of North Sea bacteria for the production of bioactive compounds (Figure 1).

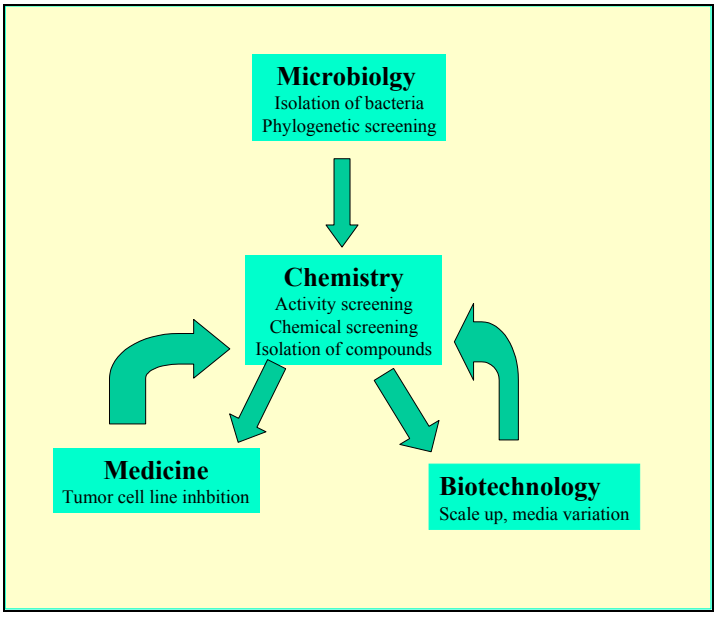
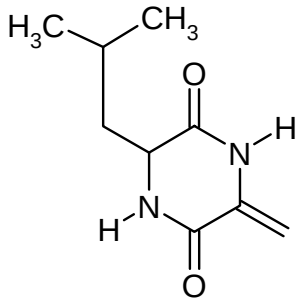


Figure 1: Integrated approach to explore the metabolic capabilities of North Sea bacteria for the production of bioactive compounds.

Screening of numerous crude extracts of North Sea bacteria using the agar diffusion method and a variety of test organisms has yielded inhibition zones of 15-25 mm diameter, while highly bioactive strains gave inhibition diameters of up to 50 mm. Tests with brine shrimps and human cell lines in screens for antitumor activity have given surprisingly positive results on the nanogram scale (Hel 3, Hel 38). They also exhibited high leishmaniacidal and antimalarial activities[63].

In the area of marine natural product, so far only a few publications have covered the small number of metabolites derived from North Sea bacteria. The North Sea strain Bio39 has been shown to produce the *α,ß*-unsaturated diketopiperazine (**20**). The same metabolite has been isolated from a *Penicillium* sp[64]. Compounds of type **21** and **22** show pronounced antitumor activity[65,23].



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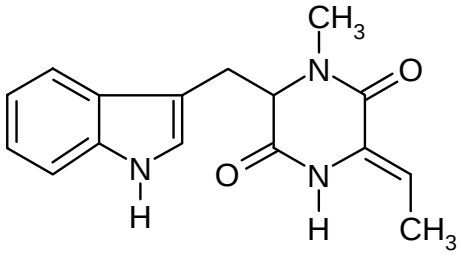

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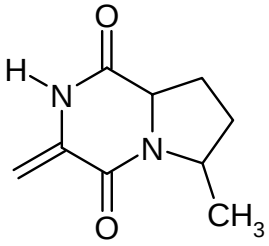
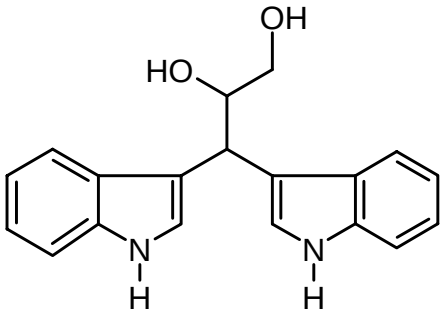


22

The extracts of strain Hel45 contained the diketopiperazines *cyclo*(phenyl-prolyl) and *cyclo*(tyrosyl-prolyl). However, they are dominated by large amounts of unsubstituted indole, the known dimer 3-(3,3'-diindolyl)-propane-1,2-diol (**23**)[66] and various new oligomeric indole derivatives[[67]]. The lipid phase of Hel45 delivered additionally *N*-(2-hydroxyethyl)-11-octadecen-amide, 17-methyl-16-octadecenoic acid[68] and indole-3-carboxylic acid thiomethyl ester (**24**).



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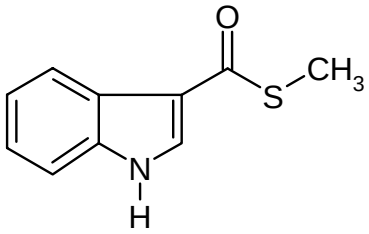
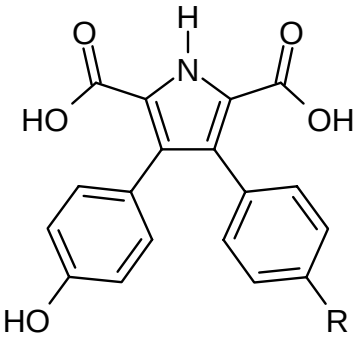


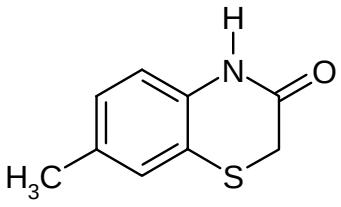
24

Zeeck and co-workers[69] have isolated 3-(4'-hydroxyphenyl)-4-phenylpyrrole-2, 5-dicarboxylic acid (**25a**), 3,4-di(4'-hydroxyphenyl)pyrrole-2,5-dicarboxylic acid (**25b**) and 7-hydroxy-2*H*-benzo[1,4]thiazin-3-one (**26**) from the culture broth of the North Sea strain RK377 fermented on MB medium with artificial sea water. From the same strain on SJ medium, two new imidazole and pyrimidin derivatives, namely glusun I (**27**) and glusun II (**28**), were isolated. Continuing these investigations, the same group has isolated tropodithietic acid (**29**), a novel carboxylic tropone skeleton

connected with a four-memberd disulphide ring system, from a North Sea strain T5. The compound exhibited antibacterial, antifungal and antitumor activities. The structure of tropodithietic acid (**29**) was elucidated by X-ray analysis and spectroscopic data.



25a: R = H, **25b**: R = OH



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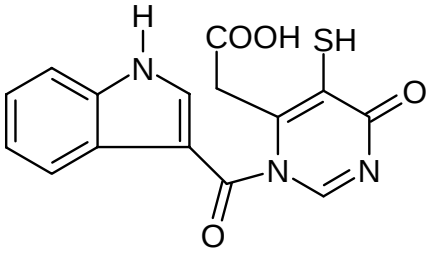

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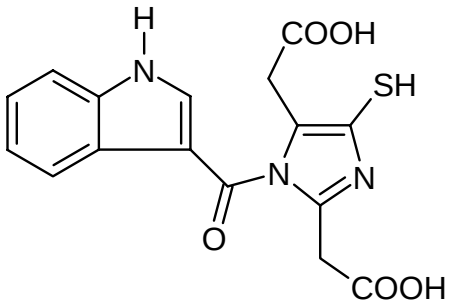

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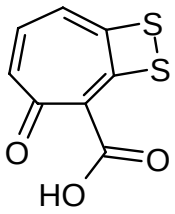
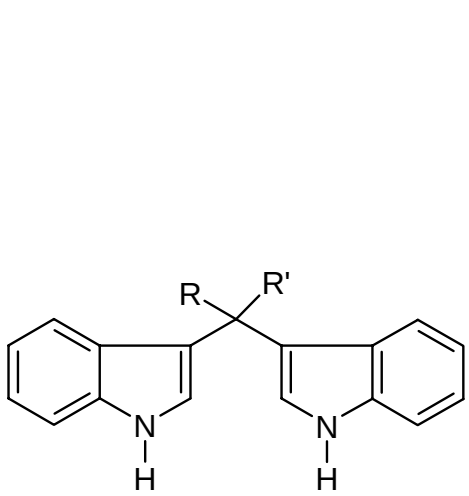
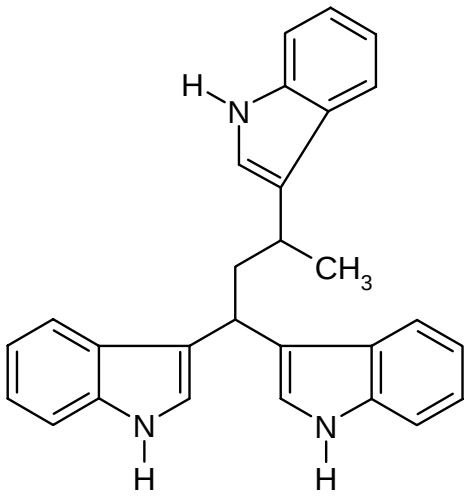


29

More than 1000 alkaloids with indole skeleton have been reported from microorganisms[70,23]. One third of theses compounds are peptides with mass beyond 600 Dalton where the indole is tryptoptophan-derived.



30: R = Ac, R' = Me
31: R = H, R' = H
32: R = 3-indolyl, R' = H



33

The strain Bio249 was isolated from a biofilm grown on a glass plate in the North Sea and taxonomically classified as closely related to *Vibrio parahaemolyticus*, and investigated recently by Veluri[67] in our research group. Two new indole alkaloids, namely 3,3-bis(3-indolyl)-butane-2-one (**30**) and 1,1,3-tris(3-indolyl)butane (**33**) have been isolated from strain Bio249. Additionally, the plant metabolite arundine[71] (**31**), 1,1,1-tris (3-indolyl)methane[72] (**32**) (previously known from synthesis) and several other metabolites have been found.

1.7.1 The marine *Roseobacter* Clade

Members of the *Roseobacter* clade are almost exclusively found in marine or hypersaline habitats and have an absolute requirement of sodium chloride for growth. Their physiological characteristics are divers. Two genera, *Erythrobacter* and *Roseobacter*, belong to the obligate aerobic phototrophic bacteria, which possess bacteriochlorophyll and are capable of aerobic photosynthesis. Representatives of the *Roseobacter* clade use thiosulfate as an electron donor, but are unable to grow autotrophically. By contrast, they are chemolitoheterophs, i.e., heterotrophic growth is enhanced by reduction of thiosulfate. Some strains can grow on diverse aromatic carbon sources (e.g. *Roseobacter*) including lignin. *Roseobacter* strains are also able to grow on dimethyl sulfopropionate (DMSP) that is produced by algal blooms at certain seasons in the Atlantic Ocean[40].

A striking feature of the *Roseobacter* clade is that many isolates are symbionts of marine organisms, e.g., algae, diatoms, dinoflagellates, or have been obtained from surfaces of marine macrophytes. The *Roseobacter* clade comprises an ecologically interesting phylogenetic group of marine microorganism that are distributed globally in coastal and open ocean marine bacterioplankton as judged from culture independent analyses. The *Roseobacter* clade should also be investigated for its ability to produce bioactive compounds, since these organisms are phylogenetically unique, marine, readily colonize surfaces and have co-evolved with marine invertebrates in close symbiotic relationships[40].

In the short research period of about 50 years on marine natural products, over 7000 compounds have been published[73,23]. Many of them possess unique functional groups or skeletons and potent biological activities. It is noteworthy that presently published new skeletons are almost exclusively found in marine organisms. Some of them got already into preclinical phase and may lead to pharmaceutical products[74]. Exploring marine organisms will be one of the essential focuses in the next years and should be more and more successful. It is worth to mention that most natural antibiotics came from bacteria and fungi so that the chance to get further drugs with this activity is high.

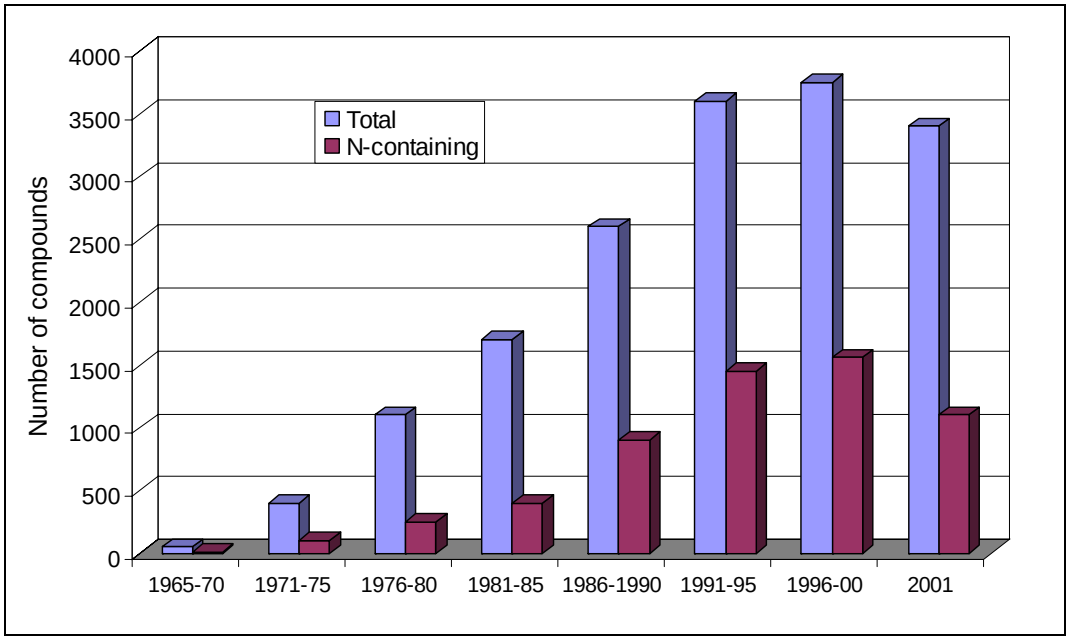


Figure 2: Statistics of the marine natural products (1965-2001)[75].

The most striking change in the direction of marine natural product chemistry since 1993 was reflected by a sudden increase in reports on new metabolites from

marine microorganisms. Although a total number of less than 600 compounds, is still small in comparison with more than ca. 28,000 structures from terrestrial microorganisms (bacteria and fungi). However, the most recent annual review[75] reported that during the period 1996-2001 there was a decrease in the number of new compounds published (Figure 2). This development suggests that we are observing a lessening in the rate of discovery of new as well as *N*-containing compounds reported earlier from the marine environment.

2 Aim of the present investigation

The main goal of the present investigation was the isolation, identification, and structural elucidation of new and preferably biologically active secondary metabolites from different bacterial species out of different environmental habitats. For this purpose, biological and chemical screening of interesting bacterial strains will be conducted chemically (TLC/HPLC-MS) and biologically, following the procedures below:

- After optimizing the cultivation conditions (nutrient type, pH, temperature and fermentation duration) of selected strains, the fermentation on a big scale should be performed to isolate the entire metabolic constituents.
- The microbial extracts will be subjected to various chromatographic methods (i.e. HPLC, Sephadex, silica gel column chromatography, PTLC, etc.) to obtain pure metabolites. The resulting components will be measured spectroscopically and identified through databases search (AntiBase, Dictionary of Natural Products, and Chemical Abstract).
- At the end, isolated new and pure metabolites will be evaluated through different bioassays (i.e. antimicrobial test, antinematodic and brine shrimp assay).

3 Classification of the investigated bacterial strains

There are a number of philosophies as to which organisms provide the most interesting bioactive metabolites. In the present work, we are focused on bacteria with a taxonomic diversity in order to provide the greatest possible chemical variety. For this purpose, the study was planned to be carried out on two marine bacterial classes living in different habitats in comparison to terrestrial *Streptomyces* bacteria.

- **Marine Bacteria other than *Streptomycetes***

North Sea bacteria were isolated from the free water column at the island of Helgoland from the German Wadden Sea by I. Wagner-Döbler (Braunschweig) and M. Meiners (Emden). The taxonomy of the *non-Streptomycetes* was unknown in advance and was determined only if the chemical results would justify it. The collected organisms were therefore described temporarily by colour, morphology, presence of mucus, odor etc. *In situ* and deckside photographs were important for the later taxonomic evaluation and were performed with a digital camera. The *non-Streptomycetes* were mainly cultivated on LB-medium with artificial sea water, and showed different coloured colonies (including white) on the agar plates, and most likely characterised by an increasing pH value through the fermentation process arriving at ≈ 8.5 at the end. Most of these types of strains produce indole alkaloids.

- **Marine *Streptomycetes***

The marine *Streptomycetes* were deposited in the Actinomycetes culture collection of the Alfred-Wegner Institute für Polar- und Meeresforschung in Bremerhaven and identified by E. Helmke. They were mainly cultivated on M_2^+ medium (= M_2 medium + sea water). The fermentation process showed mostly a decrease in the pH value (< 5) and increased at the last stages of the cultivation.

- **Terrestrial *Streptomycetes***

The terrestrial *Streptomycetes* were obtained from the strain collection of bioLeads, Heidelberg. They were also cultivated on M_2 medium, however, with tap water. They showed the same fermentation behaviour as in case of marine *Streptomycetes*.

3.1 Working up of selected strains

The general working up procedure of the investigated strains can be summarized in the following steps (Figure 3). The strains were evaluated first through chemical and biological screening. The interesting strains were then cultivated at large scale and after isolation of the metabolites, structure elucidations were performed.

3.1.1 Primary screening

Due to the highly different metabolic capabilities of the available bacterial strains, it was essential to select effective strains either for their biological activity, or for their production of new natural products. Hence well-grown 1-L shaker cultures were freeze-dried and the resulting residue was extracted with ethyl acetate (3 times), and evaporated under vacuum at 40 °C.

To evaluate the antibiotic activity of the extracts, they were subjected to agar diffusion tests using *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Bacillus subtilis, Staphylococcus aureus*, *Mucor miehei* (Tü284), *Candida albicans, Chlorella vulgaris*, *Chlorella sorokiniana, and Scenedesmus subspicatus* as test organisms. In parallel, the cytotoxic activity was evaluated against brine shrimps (*Artemia salina*) and nematodes (*Caenorhabditis elegans*). The extracts were also chemically screened by TLC, using UV and spray reagents, as well as by HPLC analysis to dereplicate the known compounds and to avoid the unwanted strains. Chemical and biological screenings complement each other very well: The sensitivity of biological methods is much higher than that of the chemical analyses and can detect even traces, whereas the chemical screening targets new structures even if they are not obviously bioactive. The strains that produce interesting metabolites are subjected to the scale-up cultivation.

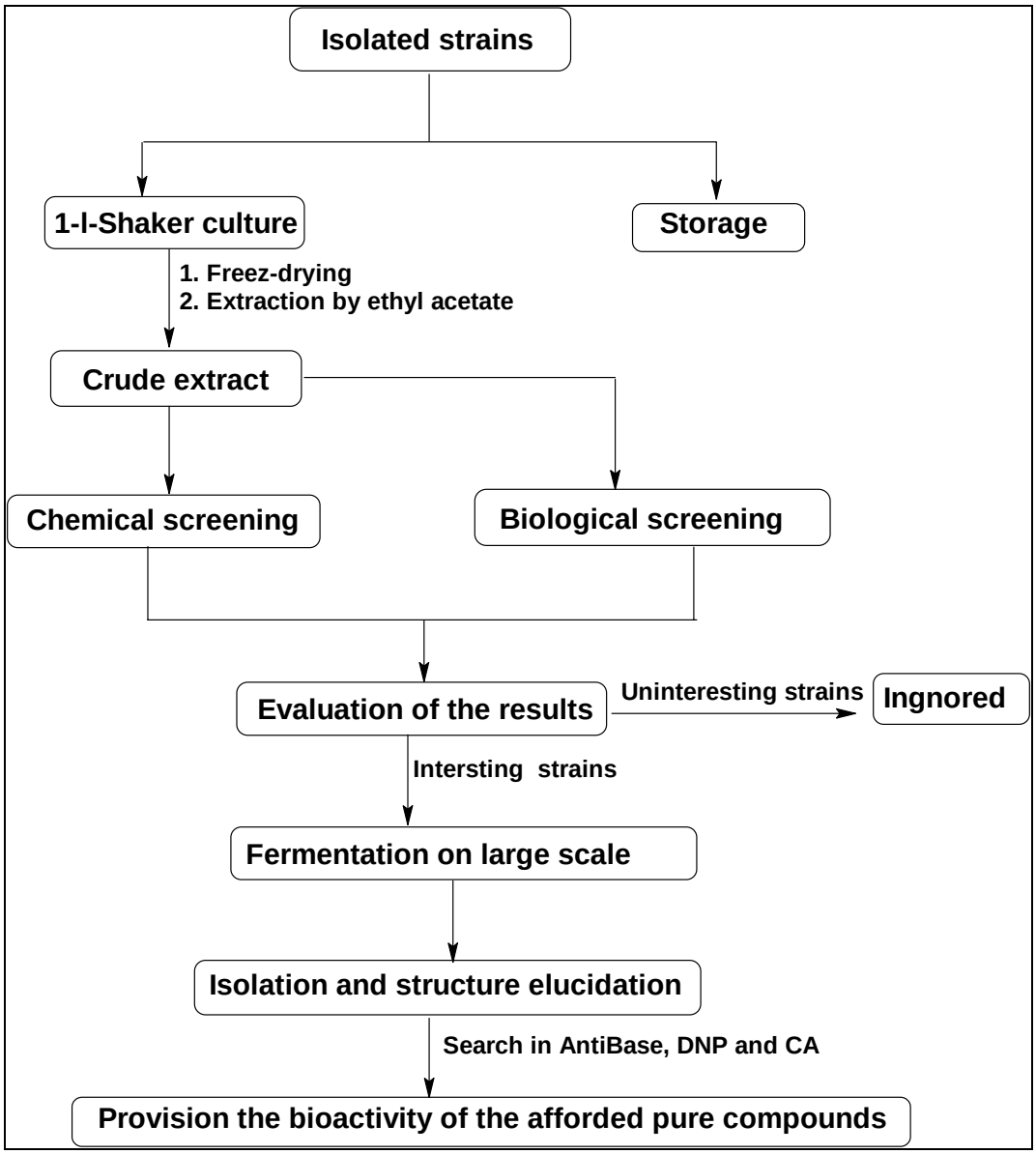


Figure 3: Working up of the selected strains in a general screening.

3.2 Large scale cultivation and extraction

As most strains are producing only metabolite concentrations of 0.1–1 mg/l, fermentation in at least 20~50 liters scale is necessary to get an adequate amount of product. This is mostly a two-step process: The initial agar culture of the producing organism will be transferred to a 2-liter liquid culture and then scale-up to provide up to 50 liters of culture broth. The fermentation may be carried out in shaking flasks or in a fermenter. It is worth to mention that, most of coloured compounds (e.g. quinones), are produced better in shaker culture than in a jar fermenter.

After harvesting, the culture broth is filtered through a filter press by adding celite. After filtration, the water phase and mycelial cake are extracted with ethyl acetate and the process continued until no further colour is extracted. Extraction can be considered complete when little or no additional residue is obtained after concentration. Storing extracts in ethyl acetate at room temperature can lead to degradation of the compounds and lower overall yields. Solutions should be therefore evaporated as soon as possible, and it is strongly recommended to store the residues at the coldest temperature possible to minimize degradation of compounds.

Adsorption on XAD resin is another efficient extraction method for obtaining the crude extracts. For this purpose, the culture filtrate is passed at a suitable flow rate through a glass column containing XAD resin (XAD-2). The compounds are eluted from XAD usually with methanol or a methanol/water gradient. Extraction with XAD is more advisable than the commonly used ethyl acetate extraction because of its cheapness. Also highly polar water-soluble compounds can be obtained if lipophilic interactions are possible, good recovery rates are obtained, and it is easy to recover and purify the resin for further use. In contrast to solvents, the resin is not harmful.

The isolation procedures depend mainly on the polarity of the compounds of interest (which can be determined by thin layer chromatography with eluents of varying polarity). There are two preliminary separation systems, which are commonly suitable for most metabolites:

- Flash chromatography of the extract on silica gel using a stepwise gradient of dichloromethane/methanol or ethyl acetate/cyclohexane. This system classifies the fractions depending on their polarity. Disadvantage is the contact with silica gel, as this may rearrange, oxidise, cleave or even destroy metabolites.
- Size-exclusion chromatography using Sephadex LH-20. The separation is based on the molecular weight. Sephadex does not have the former disadvantages and the recovery rate for the compounds is also higher.

The afforded fractions are monitored by TLC to decide the next isolation steps which may be by PTLC, silica gel column chromatography, Sephadex LH-20 or HPLC, etc.

3.3 Dereplication concept

It is obvious that despite of the existence of modern methods, the isolation and structural elucidation of natural compounds is a time-consuming and expensive process. The *dereplication* is an important step with the aim to distinguish between known compounds and unknowns, and consequently allowing to exclude the known compounds at an earlier stage.

The principle of this method is to compare data fragments of mixtures or pure metabolites with suitable literature data. This might be carried out by comparing the UV[76] or MS data and HPLC retention times with appropriate reference data collections. This method needs only negligible sample amounts and affords reliable results, if authentic samples had been available to measure the reference data. UV data and MS fragmentation patterns are also useful to identify unknown metabolites, if these show similar chromophores or fragmentation patterns as known analogues. Presently, ESI MS/MS spectra of more than 1000 of the most frequently isolated substances are included in our database of natural products. First results have shown that already known natural products can be identified easily even from crude extracts obtained from bacterial broths. Application of these methods is a very valuable tool to make the process of finding new biological and pharmacological active compounds more efficient.

As it will never be possible to collect a complete sample set and to measure all experimental data under identical conditions, reference values from the literature have to be used. If NMR data are selected, results from 1D measurements can be translated into substructures, which then will be used for a database search. In this case, normally sufficiently pure samples are required.

Databases with the NMR or UV data and a variety of other molecular descriptors can be searched using computers[77]. The most comprehensive data collection of natural compounds is the Dictionary of Natural Products (DNP)[24], which compiles metabolites from all natural sources, including plants. Our own data collection (AntiBase[23]) is, however, more appropriate for the dereplication of microbial products, as the identification depending on structural features and spectroscopic data is more comprehensive, faster and more reliable. In the case of new compounds, a database search is also helpful because novel skeletons are rare and usually related compounds

are already known which are easily revealed by a database search, thus identifying at least the compound class. Finally, the Chemical Abstracts, the most comprehensive bank of information worldwide, is used for a final confirmation that a given structure is new. Sub-structure searches with small fragments are not possible here for technical reasons.

The combination of liquid chromatography with detection methods such as NMR spectroscopy (HPLC NMR) and tandem mass spectrometry (HPLC-MS/MS) has recently led to new strategies by which biological matrices, e.g., crude plant extracts[78] or extracts from marine organisms[79], are screened to obtain as much information as possible about known constituents even with a minimum amount of material. As most compounds of interest are thermally labile, HPLC-ESI MS/MS would be the method of choice to identify known molecules from multi-component mixtures with high selectivity and sensitivity[80]. The *absolute* configuration of the pure components can be confirmed by application of circular dichroism (CD) spectroscopy[81]

4 Investigation of selected Bacterial Strains

4.1 *Cytophaga marinoflava* sp. AM13,1

The ethyl acetate extract of the North Sea bacterium *Cytophaga marinoflava* strain Am13,1 exhibited moderate antibacterial, antifungal and antimicroalgal activities against *Streptomyces viridochromogenes* (Tü57)*, Mucor miehei* (Tü284), *Candida albicans*, *Chlorella vulgaris*, and *Chlorella sorokiniana*.

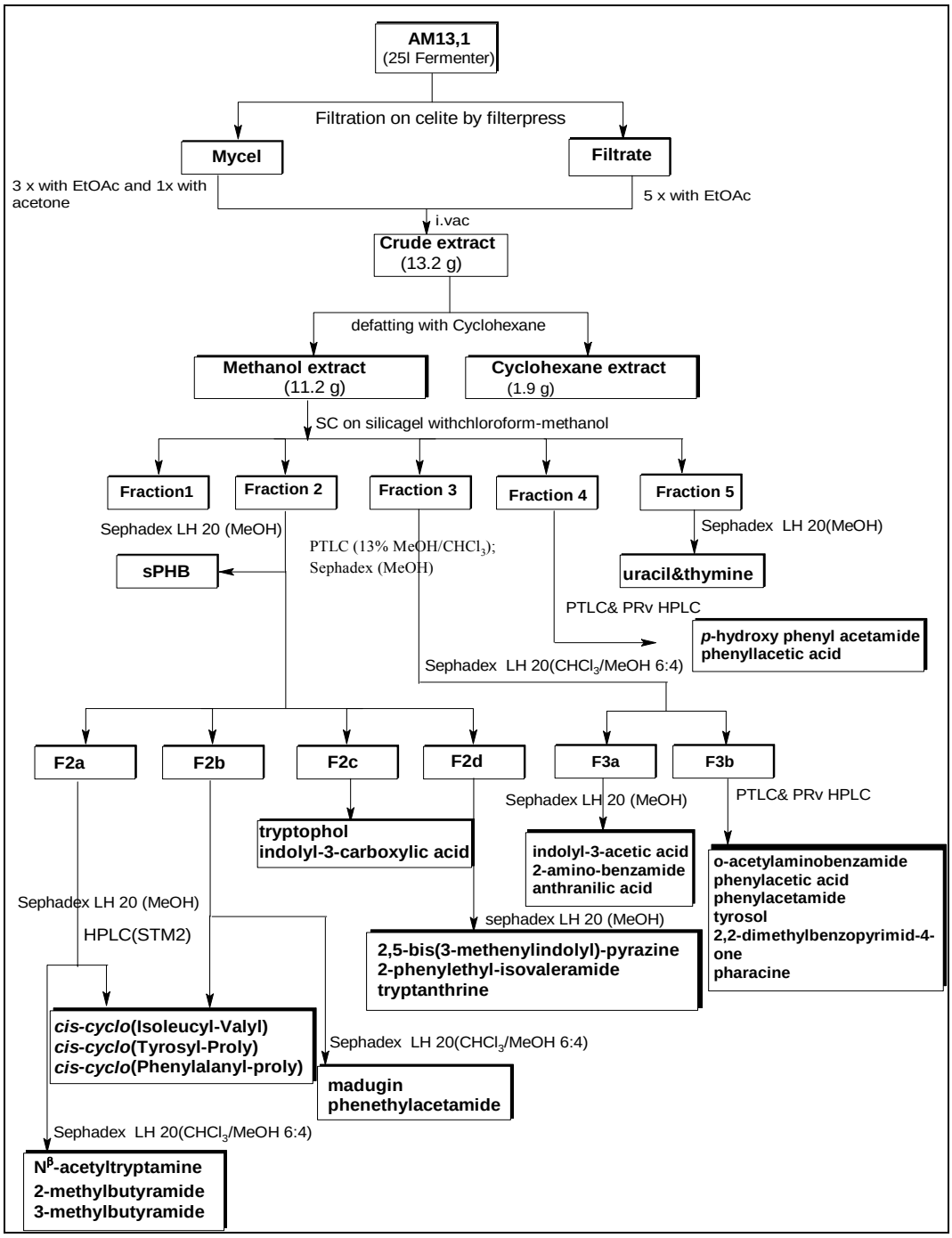


Figure 4: Work-up scheme of *Cytophaga marinoflava* strain Am13,1

In the TLC screening, the extract showed a low polar yellow band. In addition, numerous UV absorbing and fluorescent zones were observed, which were coloured orange to violet when sprayed with anisaldehyde/sulphuric acid, or pink to blue with Ehrlich's reagent.

Well grown agar plates of the strain AM13,1 were used to inoculate 3 liters of LB-medium and cultivated as shaker culture, and the resulting culture broth was used to inseed a 25-liter jar fermenter. The obtained fermenter broth was filtered over celite. The water phase and the biomass were extracted with ethyl acetate and the organic solution evaporated to dryness under vacuum giving a brown crude extract. The extract was defatted and the methanolic part was pre-separated by column-chromatography on flash silica gel into five fractions. Further purification on Sephadex LH 20, by PTLC and HPLC afforded 26 compounds (Figure 4), which are described as shown below.

4.1.1 N^{β}-Acetyltryptamine

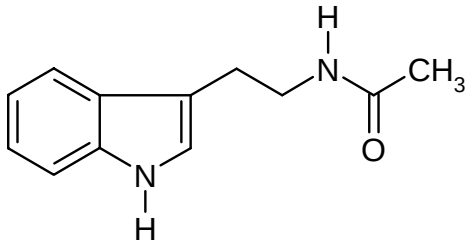
A colourless solid compound **34**, was isolated from sub-fraction F2a. It showed an UV absorbing band, which turned to orange/violet by anisaldehyde/sulphuric acid and pink by Ehrlich's reagent, as indication of an alkaloid derivative.

The ^{1}H NMR spectrum displayed a broad singlet at δ 8.30 of NH proton, five aromatic signals as well as three signals in the aliphatic region. In the aromatic region, it showed two doublets each with intensity of 1H at δ 7.60 and 7.38, two overlapped triplets at δ 7.26-7.08 forming an 1,2-disubstituted benzene ring, as well as a doublet at δ 7.03 of a proton with a *m*-coupling or next to an amino group. In the aliphatic region, a broad singlet at δ 5.70 of an NH moiety was observed, a quartet at δ 3.60 for methylene protons which showed a triplet after H/D exchange, confirming that it is adjacent to an amino group. Furthermore, a triplet at δ 2.98 of methylene protons indicated an ethandiyl group, while a singlet of a methyl group typical for an acetyl group was observed at δ 1.93.

The ^{13}C/APT NMR spectra delivered 12 carbon signals, from which nine were sp^2 carbons in the aromatic and carbonyl region, and three were in the aliphatic region. A carbonyl signal was displayed at δ 170.5 of an ester or amide. In addition, five methine sp^2carbon signals were observed. Three quaternary sp^2 carbons at δ 136.4, 127.2 and 112.3 confirmed the presence of two fused rings, an 1,2-

disubstituted benzene fused with a five-memberd ring containing a heteroatom (e.g. O, N, or S) with one substituent (δ 112.3). In the aliphatic region, three signals at δ 40.0, 25.2 and 23.1 for two methylene carbons and one methyl were exhibited.

The molecular weight of compound **34** was determined as 202 Dalton by CI MS. A search in AntiBase using the ^{1}H and ^{13}C NMR data as well as the molecular weight revealed N^{β}-acetyltryptamine (**34**), which was further confirmed by comparison with an authentic spectrum and the literature[82].



34

The N^{β}-aceyltryptamines are known as secondary metabolites from different species of plants[83] e.g. leaves of *Prosopis nigra* (*Liguminosae*)[24], as well as from bacteria e.g myxobacterium *Archangium gephyra* strain Ar T205, and are known for their antifungal properties[84]. The mass specrometric studies of N-acyl-tryptamines showed a Mclafferty rearrangement[83] (Figure 5).

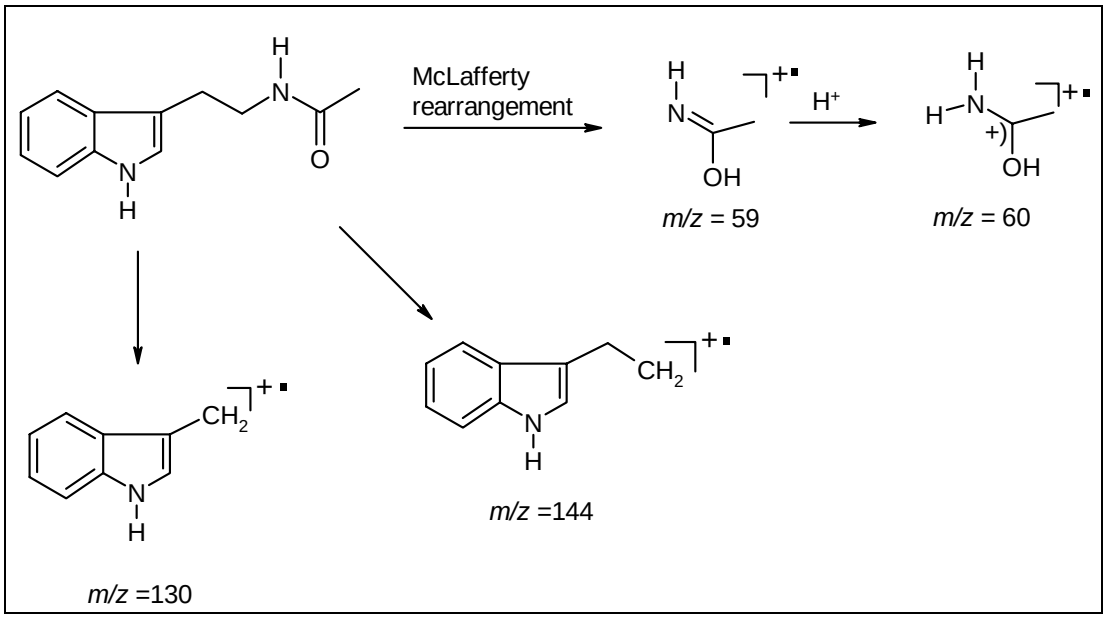
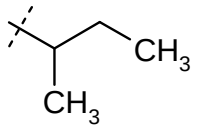


Figure 5: Mass fragmentation of N-acyl-tryptamines.

4.1.2 2-Methylbutyramide and 3-methylbutyramide

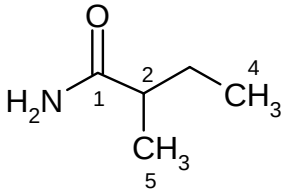
Compound **35a** was isolated by HPLC as colourless middle polar solid from sub-fraction F2a (minor product) and gave a faint pink colour with Ehrlich's reagent.

The ^{1}H NMR spectrum showed a broad singlet at δ 5.51 of two acidic protons (NH_2). In addition, one multiplet of 1H was observed at δ 2.19 as a methine proton, which could be attached to a sp^2 carbon. Furthermore, two multiplets each of 1H were exhibited at δ 1.68 and 1.48 of a methylene group flanked by two different resonance groups. Moreover, two methyl groups were exhibited, the first of which was observed as doublet at δ 1.17, while the second one was observed as triplet at δ 0.92. In accordance, the fragment **B** was suggested.



B

The molecular weight was determined by CI and EI mass spectra as 101 Dalton. The molecular ion *m/z* 101 showed a fragmention in EI mass spectrum to give a fragment at *m/z* 86 ($[M\text{-}Me]^{+\cdot}$, as well as a base peak at *m/z* 73. The resulting molecular formula $C_5H_{11}NO$ (101.08403) referred to the presence of an amino group and fixed the structure of **35a** as 2-methylbutyramide.

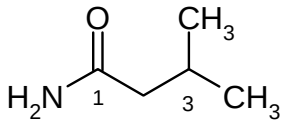


35a

A search in the AntiBase afforded no results. However, the Dictionary of Natural Products delivered **35a** in the (S)- and the (±)-form[85]. Compound **35a** is not known as a natural product, however, synthetically available.

In addition to the ^{1}H NMR spectrum of compound **35a**, three additional resonance signals were observed in the aliphatic region for compound **35b** at δ 2.07 (2 H), 1.65 (1H) and 0.98 (6 H), indicating a mixture. The first signal was assigned as a methylene group linked to sp^2 carbon, while the other signals, (1.65 and 0.98) are

characteristic for an isopropyl group. According to the mass of 101 Dalton, compound **35b** is an isomer of **35a**. In accordance, compound **35b** was established as 3-methylbutyramide, a positional isomer of compound **35a**.



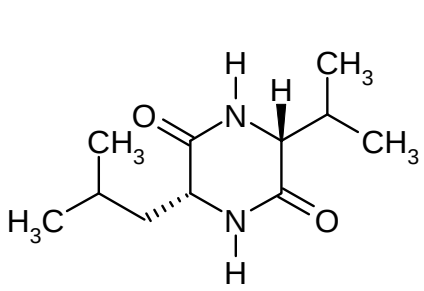
35b

4.1.3 *cis-Cyclo*(Isoleucyl-Valyl)

Compound **37** was isolated as a colourless solid from both sub-fractions F2a and F2b and showed an UV absorbing zone which turned to pink with Ehrlich's reagent or violet with anisaldehyde/sulphuric acid, respectively. The compound was further stained to blue by chlorine/*o*-anisidine indicating its peptidic character.

The ^{1}H NMR spectrum showed no protons in the aromatic or olefinic region, except one abroad singlet of an acidic proton (OH or NH) at δ 6.14-6.05. Additionally, two multiplets each of 1H at δ 4.11 and 3.60 were observed, which could be linked to a heteroatom (e.g. O or N). At δ 2.41 (1H), it showed the multiplet of a third methine proton attached to two methyl groups with resonances at δ 0.95 and 0.85 forming an isopropyl group. An additional multiplet at δ 2.18 of a fourth methine was found, as well as two multiplets of two CH_3 groups at δ 1.11 and 1.01. At δ1.55, it exhibited a multiplet of a methylene group. This established the presence of 4 CH, 1 CH_2, 4 CH_3 and two NH or OH groups in the compound **37**. The complex spectral signals could be attributed to the presence of chiral carbon atoms in the structure.

The CI mass spectrum confirmed the molecular weight of **37** as 212 Dalton. A search in AntiBase led to the isomers *cis-cyclo*(leucyl-valyl) (**36**) and *cis-cyclo*(isoleucyl-valyl) (**37**). However, comparison with an authentic ^{1}H NMR spectrum pointed to *cis-cyclo*(isoleucyl-valyl) (**37**).



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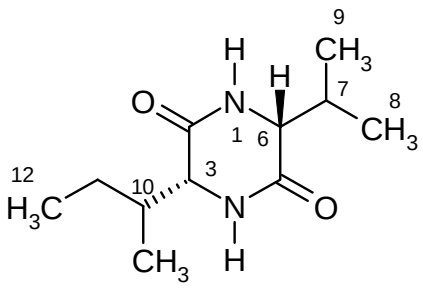


37

4.1.4 *cis-Cyclo*(Tyrosyl-Prolyl)

Similarly, compound **39** was isolated as colourless solid from sub-fractions F2a and F2b by Sephadex LH-20 and HPLC. It showed an UV absorbance and turned to violet by anisaldehyde/sulphuric acid. It showed also a colour staining to blue by chlorine/anisidine reaction, pointing to an additional peptide moiety.

The ^{1}H NMR spectrum showed an 1,4-disubstituted aromatic ring due to the presence of a AA',BB' signals at δ 7.15 and 6.72. It displayed a broad singlet at 6.08 of an acidic proton (NH), as well as two signals at δ 4.25 and 4.08 for two methines attached to electron withdrawing substituents. The spectrum revealed doublet signals at δ 3.45 and 2.79 for the ABX system of a methylene group, and a multiplet at δ 3.65-3.30 (9-CH_2) for a methylene group attached to a heteroatom. In addition, multiplets of two further methylene groups at δ 2.30 and 2.00-1.78 were exhibited.

The EI mass spectrum of the component showed a mol peak at *m/z* 260. The obtained molecular ion peak showed a further fragmentation to give a base peak at *m/z* 154, could be attributed to glycyl-prolyl moiety (Figure 6).

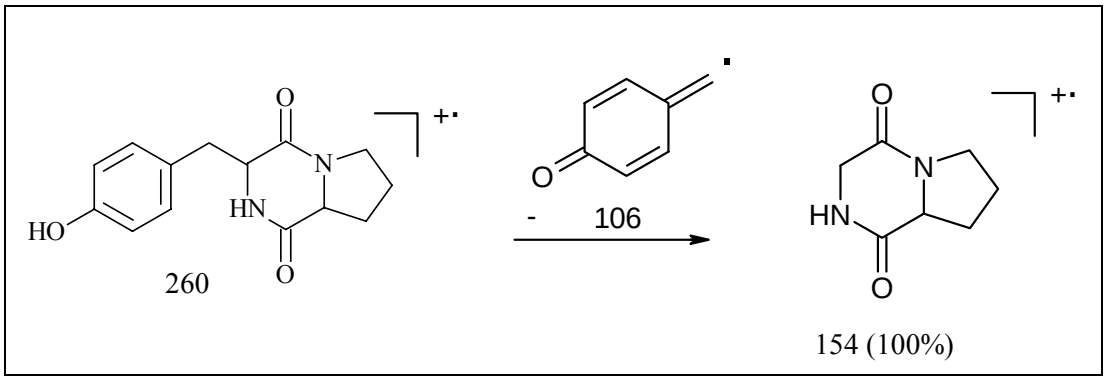
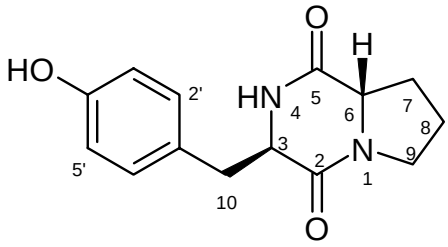


Figure 6: EI fragmentation pattern of *cis-cyclo*(tyrosyl-prolyl) (**39**).

A search in AntiBase led to the structural isomers *trans-cyclo*(tyrosyl-prolyl)[86] (**38**) and *cis-cyclo*(tyrosyl-prolyl) (**39**). Comparing the chemical shifts of H-3 and

CH_2-10 in both isomers **38**, and **39** with the discussed data, confirmed the compound as *cis*-form (**39**). The diketopiperazines are characterised by the presence of two chiral centres at positions 3 and 6 to afford four isomers. All isomers are known since a long time.



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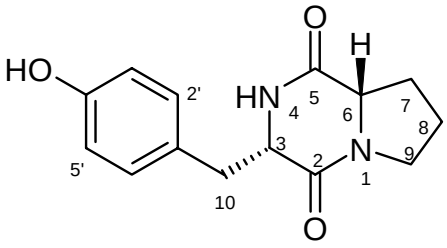


39

4.1.5 *cis-Cyclo*(Phenylalanyl-Prolyl)

Another Diketopiperazine derivative **40** was isolated as colourless solid from sub-fractions F2a and F2b by Sephadex LH-20 and purified by HPLC. It showed the same chemical colourations like **39** The EI mass spectrum displayed a mol peak at *m/z* 244 $[M]^{+\cdot}$.

The ^{1}H NMR spectrum showed a replacement of the two doublets of the 1,4-disubstituted aromatic ring in compound **39** by a multiplet of a phenyl group at δ 7.31, as well as a broad singlet of an acidic proton at δ 5.82. The same ^{1}H NMR pattern of the aliphatic part of component **39** was exhibited, especially of both H-3 and CH_2-10, indicating the same *cis*-configuration as for **39**.

The ^{13}C/APT NMR spectrum showed two quaternary carbonyl carbons at δ 169.3 (C-2) and 164.9 (C-5), as well as six sp^2 carbons of the phenyl group. In the aliphatic region, it exhibited two nitrogen-connected methine carbons at δ 60.0 (C-6), 56.1 (C-3), as well as four methylene carbons at δ 45.2 (C-9), 36.7 (C-10), 28.2 (C-7) and 22.2 (C-8).

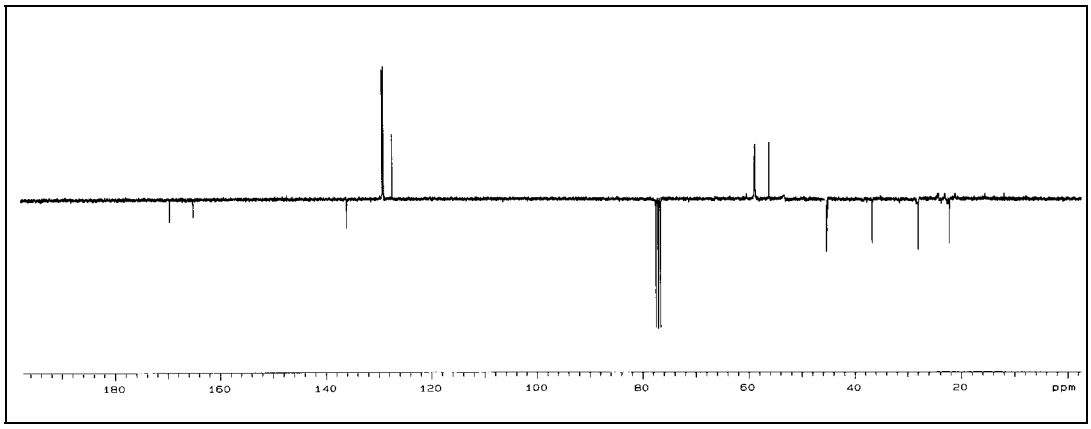
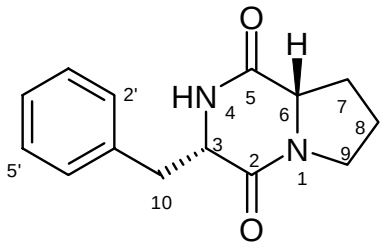


Figure 7: APT NMR spectrum ($CDCl_3$, 75 MHz) of *cis-cyclo*(phenylalanyl-prolyl) (**40**).

A search in AntiBase afforded again two isomers, namely *cis-cyclo*(phenylalanyl-prolyl) (**40**) and *trans-cyclo*(phenylalanyl-prolyl) (**41**). Comparing the ^{1}H NMR and carbon values of CH-3 and CH_2-10 in both isomers with literature data[82] pointed to *cis-cyclo*(phenylalanyl-prolyl) (**40**) (Table 1).



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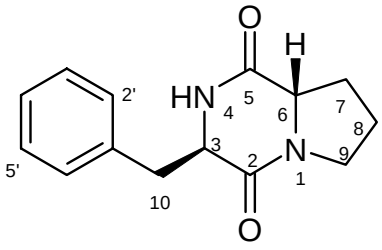


41

Table 1: ^{13}C NMR data of C-3 and C-10 of *cis-cyclo*(phenylalanyl-prolyl) (**40**) and *trans-cyclo*(phenylalanyl-prolyl) (**41**).

Position	**40** (exp)	**40** (lit)	**41** (lit)
3	56.1	56.2	58.9
10	36.7	36.8	40.5

4.1.6 Indolyl-3-ethylisovaleramide (Madugin)

A colourless solid **42** was isolated from sub-fraction F2b using Sephadex LH-20 and was further purified by HPLC. The component was identified as indole derivative due to its positive colour reaction with anisaldehyde/sulphuric acid (violet) and Ehrlich's reagent (pink changing to blue). Moreover, it was UV absorbing.

The ^{1}H NMR spectrum showed a broad singlet at δ 8.08 of 1H characteristic for NH proton of an indole moiety. Five aromatic proton signals between 7.62 and 7.0 were shown, four were characteristic for *ortho*-couplings in an 1,2-disubstituted

aromatic ring. The fifth one appeared as a singlet at δ 7.05 and is indicative for H-2 of an indole system. A broad singlet at δ 5.48 (3'-NH) of an acidic amido group was observed. The AB signal of a methylene group was displayed at δ 3.64, and a methylene triplet at δ 3.0 was typical for two vicinal methylene groups constructing an ethandiyl moiety. In addition, an isobutyl system was present due to the observation of three signals at δ 2.13 (m, 1 H, 6'-H), 2.00 (CH_2), and 0.90 ($2CH_3$). The side chain of the component could be attached to position 3 of the indole moiety, as the 3-H signal of indole (which is normally at δ ≈ 6.5) was missing.

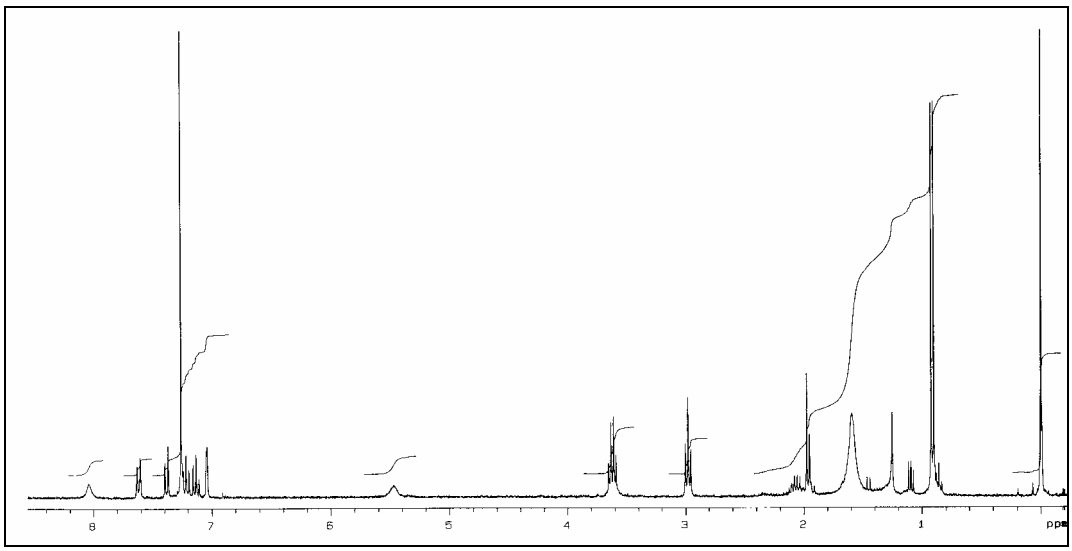


Figure 8: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of indolyl-3-ethylisovaleramid (**42**).

The ^{13}C NMR spectrum with CH-signals at δ 122.2, 122.0, 119.5, 118.7, and 111.2 (C-7) and quaternary carbons at δ 136.4, 127.3, and 113.1 confirmed an indole moiety. Furthermore, the spectrum afforded a quaternary carbon at δ 172.5 of a carbonyl group assigned as amide, ester or acid. In addition, three methylene carbon signals at δ 39.5, 25.4 and 46.2 linked to sp^2 carbon or likely to heteroatom were displayed. Finally, it revealed at δ 26.1 and 22.4 (2 C) two additional signals characteristic for an isopropyl group.

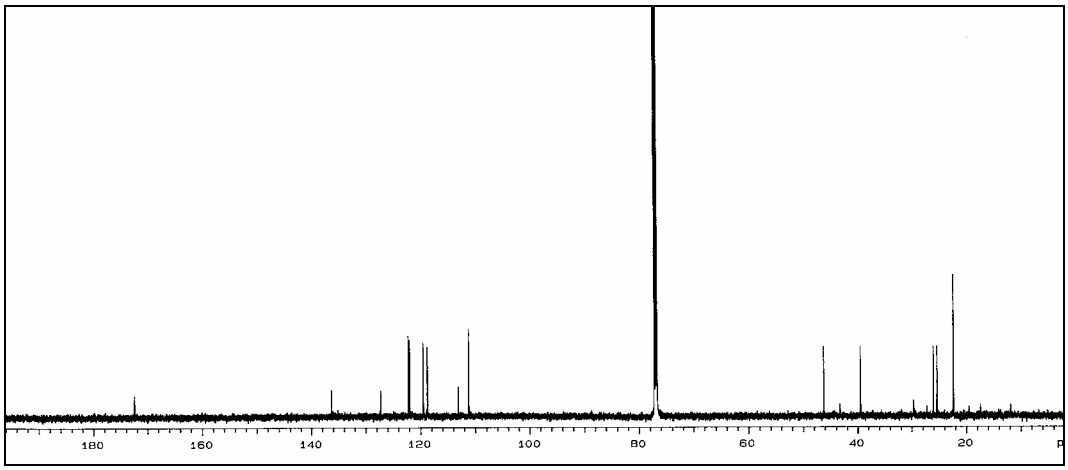
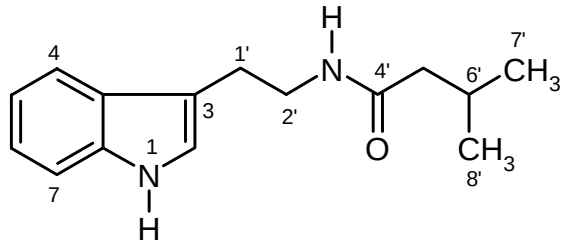


Figure 9: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of indolyl-3-ethylisovaleramid (**42**).

The CI and EI mass spectra led to a molecular weight of 244 Dalton. The EI mass spectrum showed a base peak at *m/z* 143, characteristic for the fragment ([M - (H_2N-C=O-CH_2-CH (CH_3)2)]$^{+\cdot}$), and an additional fragment at *m/z* 130, characteristic for the indolin-3-methylen ion[87]. The EIHR MS gave the corresponding molecular formula $C_{15}H_{20}N_2O$.

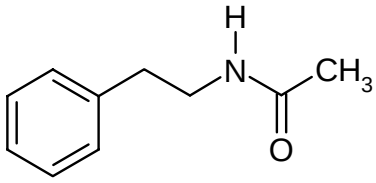
A search in AntiBase with the molecular weight and the substructures determined from the ^1H NMR and ^{13}C NMR data resulted in indolyl-3-ethylisovaleramide (**42**), a secondary plant metabolite[88], which had not been isolated from bacteria before[89].



42

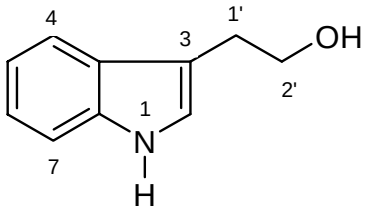
4.1.7 Trivial compounds

Working up of sub-fraction F2b exhibited an UV absorbing band, which turned to violet and pink by spraying with anisaldehyde/sulphuric and Ehrlich's reagent, respectively. After chromatography, compound **43** was obtained as colourless solid, which identified as N-(2-phenylethyl)–acetamide by its ^1H NMR and MS data, as well as by a search in AntiBase and comparison with the literature[61]



43

Two colourless solid compounds were isolated from sub-fraction F2c which exhibited an UV absorbing bands, turned to violet with anisaldehyde/sulphuric acid and pink with Ehrlich's reagent. On the basis of the NMR and MS data, the compounds were identified as tryptophol (**44**) and indolyl-3-carboxylic acid (**45**). The compounds were further confirmed by direct comparison with authentic spectra. Indolyl-3-carboxylic acid **(45)** was isolated frequently in our research group, and is also known as fungal metabolite from *Lasiodiplodia theobromae*[90].



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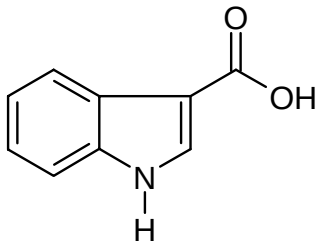
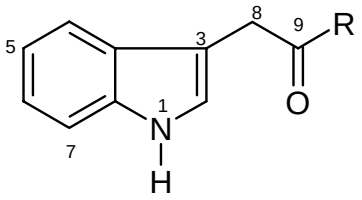


45

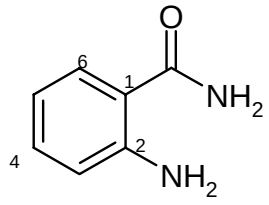
Working up of sub-fraction F3a on Sephadex LH-20 led to compound **46a** as a colourless solid. It showed an UV absorbing band, which turned to orange and pink with anisaldehyde/sulphuric acid and Ehrlich's reagent, respectively. By subjecting the compound to NMR and MS as well as searching in AntiBase, compound **46a** was identified as indolyl-3-acetic acid[48]. Compound **46a** was further confirmed by its methylation affording indolyl-3-acetic acid methyl ester (**46b**) as a colourless solid.

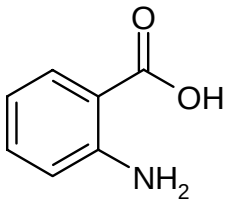
Indolyl-3-acetic acid (**46a**) is widespread in human urine, fungi, maize corn, and could be assigned as an auxin (phytohormon)[91]. It induces a prolongation of the important hormones responsible for the acceleration of growth of higher plants (plant growth regulator). It plays an important role in a number of plant activities, including fruit development, abscission, and root initiation[92]. On the other hand, it is used as fungicide for the protection of plants. Compound **46a** is biosynthesised in the plants starting with tryptophane, followed by indolyl-3-acetamide (**46c**) as an intermediate.

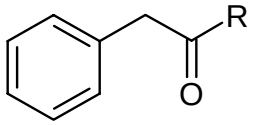


46a: R = OH, **46b**: R = OCH_3, **46c**: R = NH_2

Additionally, compounds **47** and **48** were obtained as pale yellow solids from sub-fraction F3a using PTLC and Sephadex LH-20. Based on NMR and MS data and a search in AntiBase, compounds **47** and **48** were elucidated as anthranilamide and anthranilic acid, respectively. The compounds **47** and **48** are characterised by their intensive UV blue fluorescence at 366 nm and their yellow colour reaction with anisaldehyde/sulphuric acid or Ehrlich's reagent. Anthranilic acid (**48**) is widely abundant in bacteria and fungi. It is a biosynthetic key intermediate for several alkaloids having the quinoline, acridine, quinazoline skeletons[93,94] and of the phenazines, which formally can be dissected into two molecules of anthranilic acid[95].





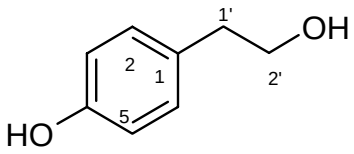


47 **48** **49a**: R = OH, **49b**: R = NH_2

Three colourless solid compounds were isolated from sub-fraction F3b, two of which showed blue UV fluorescence at 366 and 254 nm. These two compounds were identified as phenyl acetic acid (**49a**) and phenyl acetamide (**49b**), according to their ^{1}H NMR and MS data and a search in AntiBase as well as by comparison with authentic spectra of both. The third compound showed an UV absorbing band at 254 nm, gave no colour reaction with the spraying reagents and was identified as *p*-hydroxyphenyl-2-ethanol (tyrosol) (**50**) according to NMR and MS data.

Phenyl acetic acid is widespread in higher plants, fungi, e.g. *Aspergillus niger*[96], and bacteria. In the microbial antibiotic production, compound **49a** was added to the culture of *Penecillium* sp. to increase the production of penicillin G (parasticin)[97].

Tyrosol (**50**) was reported to be antibiotically weakly active against *Saccharomyces cerevisiae* (>100), *Nematospora corlyi* (> 100), is moderately phytotoxic and antifungal[23]. The compound is widespread in fungi e.g. *Candida* sp.[98], *Ceratocystis fimbriata*, *Cochliobolus lunata*, *G. fuikuroi*, *Pyricularia oryzae*[99] as well as several *Ceratocystis* sp. isolated from leaves of *Ligustrum ovalifolium* and the bark of *Fraxinus excelsior*, or *Gibberella fuikuroi* and *peanuts*[100].



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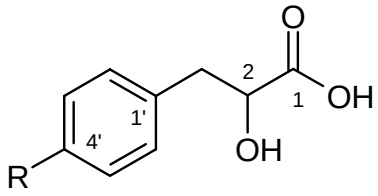
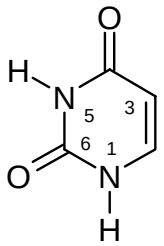


51a: R = H, **51b**: R = OH

Fraction IV was subjected to chromatography to give the known metabolite 2-phenyllactic acid (**51a**) as a colourless solid. It showed an UV absorbing band, and turned to pink with Ehrlich's reagent. Compound **51a** and its *p*-hydroxy analogue **51b** are naturally occurring in plants and fungi[101,102].

Fraction V was chromatographically processed to give two known metabolites, uracil (**52**) and thymine (**53**). They were elucidated depending on their characteristic chromatographic behaviour and their spectroscopic data. Compounds **52** and **53** are components of nucleic acid, and present also in free state in bacteria[24].



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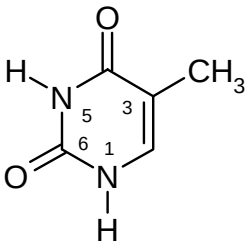


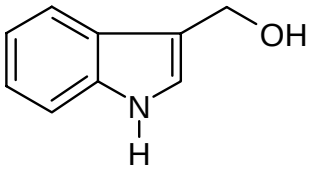
53

4.1.8 2,5-Bis(3-methenylindolyl)-pyrazine

A faint yellow band of compound **55** was observed in the sub-fraction F2d. This band showed an UV absorption at 254 and 366 nm and turned to pink with Ehrlich's reagent (and later to faint brown). The band was isolated and purified by PTLC followed by Sephadex LH-20 column to give **55** as a faint yellow solid.

In the UV spectrum, a peak at λ_{max} 300 nm was characteristic for an aromatic system. The IR spectrum exhibited several bands characteristic for olefinic or aromatic and for aliphatic CH groups, and a sharp peak at 1457 cm^{-1} was characteristic for methylene groups.

Compound **55** was slightly soluble in chloroform and less polar solvents. However, it showed a good solubility in dimethylsulfoxide and methanol. The ^1H NMR spectrum in dimethylsulfoxide showed a broad NH singlet of indole at δ 10.91, in addition to the signals of an 3-substituted indole. The spectrum showed also a singlet at δ 4.17 of a methylene group, which could be attached to a heteroatom or sp^2 carbon, in addition to position 3 of the indole skeleton. This pointed to 3-indole-3-carbinol (**54**) or 3,3'-diindolylmethane; arundine (**31**). Indole-3-carbinol is an autolytic break down product of the glucosinolate glucobrassicin present in *Brassica* plants such as cabbage, kale, and Brussels sprouts[103]. Compound **31** is known as an alkaloide from roots of *Arundo donax*, however, was isolated and investigated recently by Veluri[67] in our research group as new metabolite from the North Sea bacteria Bio249, closely related to *Vibrio parahaemolyticus*.



54

The molecular weight was determined by EI mass spectrum as 338 Dalton, which was further confirmed by CI mass spectrum giving *quasi*-molecular ions at *m/z* 356 ($[M + NH_4]^+$) and 339 ($[M + H]^+$). The EIHR MS data gave the corresponding formula $C_{22}H_{18}N_4$ referring to the existence of two indole moieties in addition to a heterocyclic ring with two equivalent methine atoms, four carbons and further two nitrogen atoms.

Therefore, compound **55** was measured again in $CDCl_3$. The ^1H NMR spectrum showed an additional surprising deep field singlet with intensity of 1H at δ 8.43 suggesting an electron-deficient heteroaromatic ring.

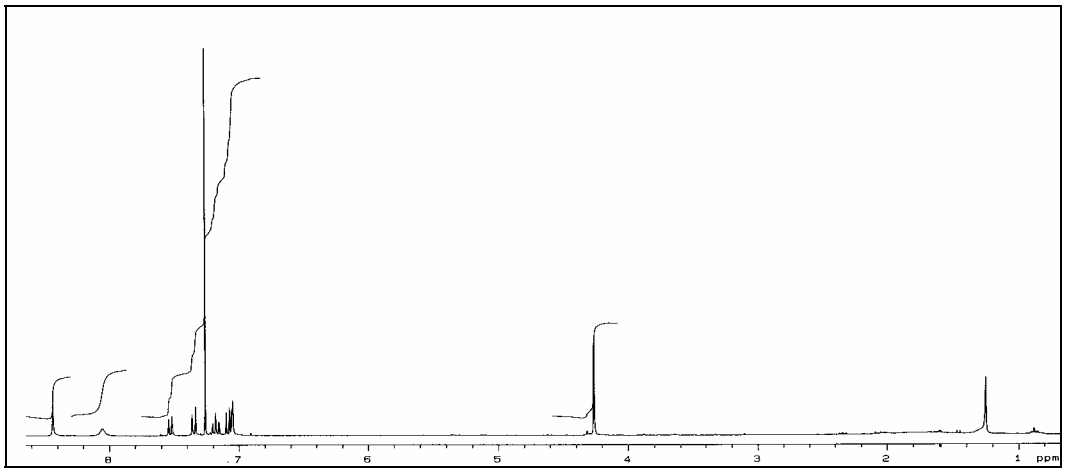


Figure 10: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 2,5-Bis(3-methenylindolyl)-pyrazine (**55**).

The ^{13}C/APT NMR spectrum of **55** confirmed the 3-substituted indole skeleton as 3-substituted moiety due to the presence of five methine aromatic sp^2 carbons at δ 123.4, 121.0, 118.4 (2 C), and 111.4. The three quaternary carbons of the indole moiety were shown at δ 136.2, 126.8 and 111.6. In the aliphatic region, a methylene carbon was observed at δ 30.8 suggesting its existence between two sp^2 carbons. Furthermore, it showed two deep magnetic field sp^2 carbon signals at δ 153.7 and δ 142.2, which both must be linked to a heteroatom. In accordance with the formula $C_{22}H_{18}N_4$, a 1,4-disubstituted pyrazine with substituents at C-2,5 or C-2,6 was suggested. Because of the symmetry of compound **55**, the two probable structures, 2,5-bis(3-methenylindolyl)-pyrazine (**55**) and 2,6-bis(3-methenylindolyl)-pyrazine (**56**) were suggested.

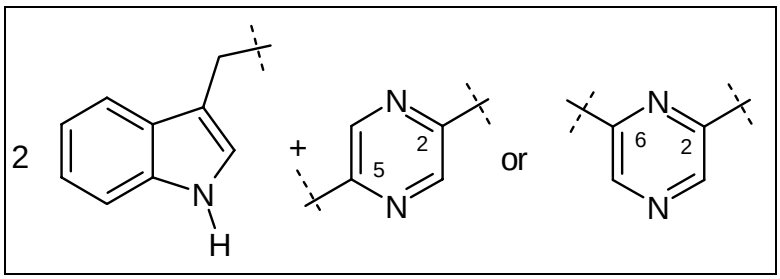


Figure 11: Fragments of 2,5- and 2,6-substituted of compounds **55** and **56**, respectively.

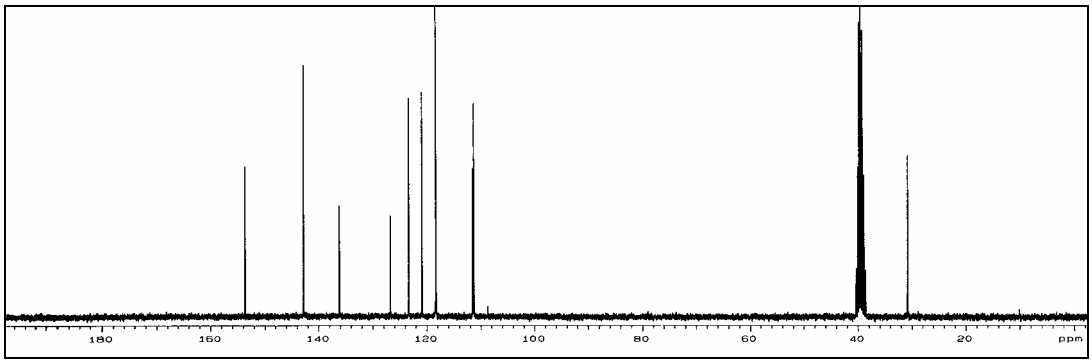


Figure 12: ^{13}C NMR spectrum ([D_6]DMSO, 75 MHz) of 2,5-bis(3-methenylindolyl)-pyrazine (**55**).

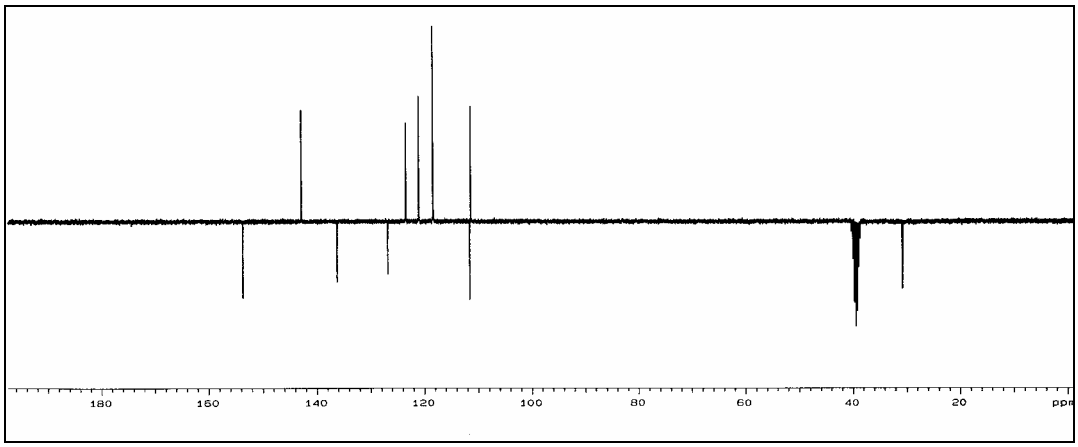
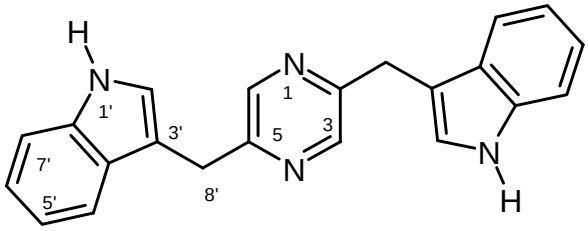


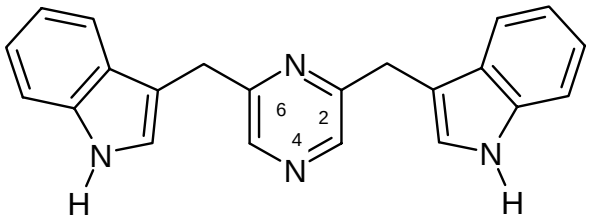
Figure 13: APT NMR spectrum ([D_6]DMSO, 75 MHz) of 2,5-bis(3-methylindolyl)-pyrazine (**55**).

A search in AntiBase and DNP resulted in no hits. This indicated that both isomers **55** and **56** are new natural products. According to computer calculations, the two methine carbons (3,5-) and their corresponding protons of compound **56** are expected at same position, δ 139.9 and 7.86, while the two quaternary pyrazine carbons, C-2,C-6 were predicted low field (δ 160.9). However, the measured ^{13}C and ^{1}H values, for compound **55** were found down field at δ 153.7 (C-2/5), 142.2 (C-3/6), and at δ 8.43 for the methine protons (H-3,6). These values are not matched with compound **56**. On the basis of these values, compound **56** could be excluded. In accordance, we concluded the structure of isomer **55** as 2,5-bis(3-methenylindolyl)-pyrazine.

Compound **55** was further confirmed by comparing the data with a similar analogues of pyrazine moiety e.g. 2,5-dihydroxy methyl pyrazine (**57**). The latter compound (**57)** was previously isolated from marine *Streptomyces* in our group[104].



55



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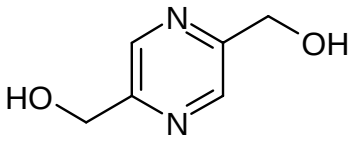


57

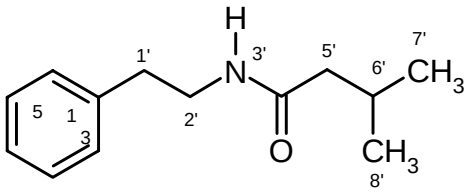
4.1.9 N-Phenylethyl-isovaleramide

Compound **58**, another colourless solid, was isolated from sub-fraction F2d. It showed an UV absorbing band and turned to pink with Ehrlich's reagent and violet with anisaldehyde/sulphuric acid.

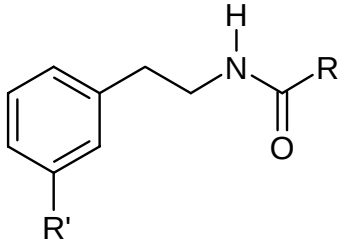
The ^{1}H NMR spectrum showed a deep field multiplet of an aromatic phenyl group between δ 7.34~7.18, and a broad singlet of an acidic proton at δ 6.46 could be of an aliphatic amide group. Two vicinal methylene groups, one of which gave a quartet at δ 3.69, which changed to a triplet after H/D exchange, pointed to a CH_2-CH_2-NH fragment. Additional three signals showed a splitting pattern as in madugin (**42**).

The molecular weight of compound **58** was determined as 205 Dalton by an EI mass spectrum. A search in AntiBase resulted in N-phenylethyl-isovaleramide (**58**). Compound **58** was isolated previously in our group[105] along with other related N-phenylethylamides e.g. **58, 59, 60**. On the basis of the FAME profiles, the producing strains were assigned to the *Nocardiopsis* group of organisms but could not be identified further. Sequencing of the 16S rDNA showed GW73a to be most similar but not identical to *Bacillus cereus* (98 % sequence identity), GW90a to an unknown *Bacillus* strain (96 % sequence identity) and GW102b to *Bacillus thuringiensis* (96 % sequence identity). Thus, these strains probably represent new species or subspecies within the genus *Bacillus*.

The compounds **58**, **59**, **60**, and **61** possess a potent antimicroalgal activity and are of interest as herbicides. Recently, it was reported that N-phenethylbutyramide (**61**) and N-phenthylisovaleramide (**58**) exhibited additionally significant cytotoxic effects against human cancer-cell line, *viz*. gastric adenocarcinoma, colon adenocarcinoma, and lung adenocarcinoma[106]. 3-Methyl-N-(2-phenylethyl)-butyramide (**62**) was also isolated from a novel *Bacillus* and protected by a patent[107].



58



59: R = CH_2CH_3, R' = H
60: R = CH $(CH_3)_2$, R' = H
61: R = $CH_2CH_2CH_2CH_3$, R' = H
62: R = $CH_2CH_2CH_2CH_3$, R' = CH_3

4.1.10 Tryptanthrine

Compound **63,** was obtained from sub-fraction F2d as an intensive yellow solid. It showed no colour change by treatment with either sodium hydroxide or sulphuric acid, and was therefore not a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum showed eight deep field aromatic protons, however, no aliphatic or olefinic protons. Two highly deep field doublets each of 1H at δ 8.62 and 8.42 pointed to a heteroaromatic ring and/or a *peri*-position to carbonyl groups. A doublet at δ 8.05, as well as three multiplets each with intensity of one proton between δ 7.95~7.75 were exhibited. Two triplets, each with 1H at δ 7.65 and 7.43 were observed. From the discussed proton spectral data and due to the similar coupling constant (≈8 Hz), the compound contained two different 1,2-disubstituted aromatic rings fused with others containing heteroatoms, carbonyl groups or both.

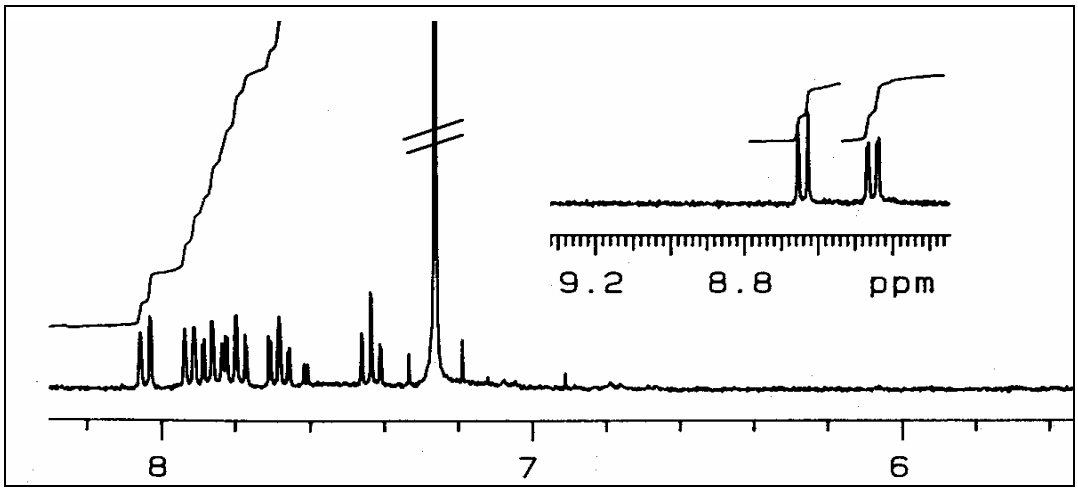
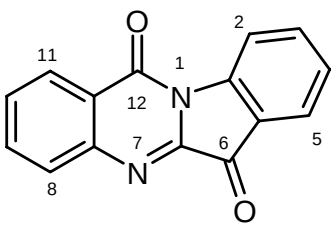


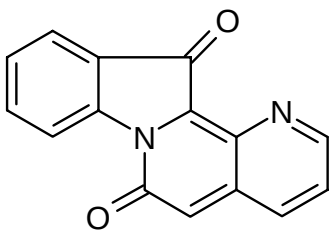
Figure 14: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of tryptanthrine (**63**).

The ^{13}C/APT NMR spectra showed two quaternary carbon signals at δ 182.6, and 158.1. The first signal could be due to a keto-carbonyl group attached to an aromatic ring, and the last one could be assigned as carbonyl of an amide in a conjugated system. Eight sp^2 methine carbons were displayed corresponding to the signals in the ^{1}H NMR spectrum. In addition, five quaternary sp^2 carbons were visible from which three were at deep field (δ 146.6, 146.3, and 144.3) pointing to their connection with heteroatoms, namely nitrogen or oxygen. The last two carbons were displayed at δ 123.7 and 121.9. In agreement, four aromatic rings were proposed, from which two were 1,2-disubstituted, while the other two are most likely heterocycles containing nitrogen and at least two carbonyl groups.

The molecular weight of compound **63** was determined as 248 Dalton using CI mass spectrum, due to the ions at *m/z* 266 ($[M + NH_4]^+$) and 249 ($[M + H]^+$). A search in AntiBase using the NMR spectra in combination with the MS led to assign compound **63** as tryptanthrine. The compound was further confirmed by comparison with authentic spectra as well as the literature[108].



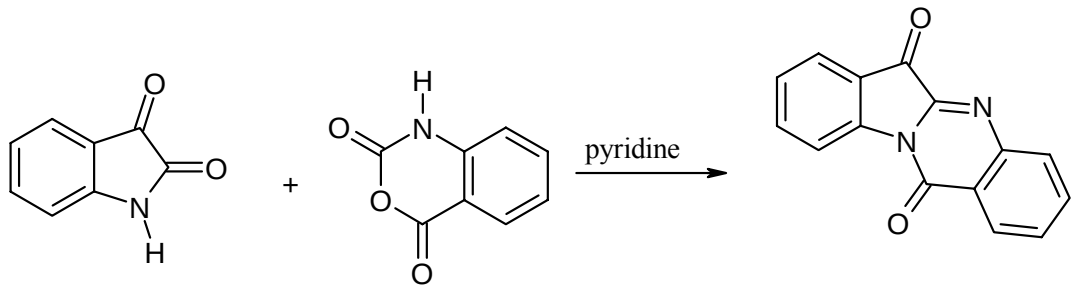
63



64

Tryptanthrine was isolated first in 1974 by Dutta *et al.*[109] and designated as couroupitine A (**64**), basing on spectroscopic evidence, as a novel type of alkaloids

from the fruits of *Couroupita guianensis*[110]. Recently the structure has been confirmed by x-ray crystallography as **63** [108].



65 **66** **63**

A compound with structure **63** has been isolated by Zähner *et al.*[111] from *Candida lipolytica* and described as antibiotic. Many trials have been done to synthesize **63** using isatine (**65**) and isatoic anyhdride (**66**)[112]. Tryptanthrine (**63**) is biosynthetically produced from isatine (**65**) and anthranilic acid (**48**). Most of antibacterial and antifungal activities of the crude extract could be attributed to tryptanthrine (**63**).

4.1.11 *o*-Acetylaminobenzamide

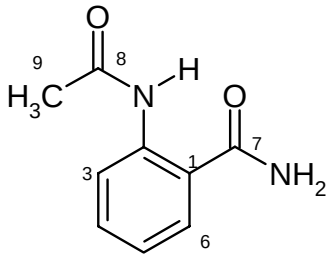
Compound **67** was found as a third middle polar band with blue UV fluorescence at 366 and 254 nm in sub-fraction F3b. However, the band showed a different colour reaction (grey-brown) with anisaldehyde/sulphuric acid. The constituent was isolated and purified as colourless solid using PTLC followed by HPLC.

The ^{1}H NMR spectrum showed three broad singlets at δ 11.72, 7.82, and 7.03 of three acidic protons and a 1,2-disubstituted aromatic ring due to the presence of two doublets at δ 8.68 and 7.86 and two triplets at δ 7.49 and 7.10. In addition, a singlet of a methyl group was at δ 2.14, which could be attached to an aromatic system or a carbonyl group.

The ^{13}C/APT NMR spectrum displayed two carbonyl groups at δ 170.6 and 168.9, which could be assigned to amides, esters or acids. Six aromatic sp^2 carbons were revealed, from which four were CH and two quaternary sp^2 carbons at δ 139.5 and 119.7. The deep field carbon atom (δ 139.5) must be attached to a heteroatom, and the up field one (119.7) should be adjacent to sp^2 or sp^3 carbon. Finally, in the aliphatic region the spectrum revealed a methyl carbon of an acetyl group at δ 24.8.

The molecular weight of compound **67** was confirmed as 178 ($[M]^{+\cdot}$) by EI mass spectrum. The molecular ion peak *m/z* 178 of **67** showed a further fragmentation to

give a peak at *m/z* 136 due to the loss of ketene. This confirmed the presence of an acetyl group. In addition, a base peak fragment was observed at *m/z* 119, confirming a structure similarity of **67** with **47** and **48**. EIHR MS of **67** gave the corresponding molecular formula $C_9H_{10}N_2O_2$. A search in AntiBase led to only one consistent structure, *o*-acetylaminobenzamide (**67**). The assignment was further confirmed by comparison with the literature[113] and authentic spectra.



67

4.1.12 2,2-Dimethyl-2,3-dihydro-1*H*-quinazolin-4-on

Compound **68** was found also as blue UV fluorescent zone in sub-fraction F3b and was not affected by anisaldehyde/sulphuric acid or Ehrlich's reagent. It was isolated as colourless solid by applying the fraction to PTLC and HPLC.

The ^{1}H NMR spectrum of compound **68** displayed the typical pattern of a 1,2-disubstituted aromatic ring. In addition, one up-field singlet at δ 1.48 with the intensity of six protons indicated two equivalent methyl groups linked to a quaternary sp^3 carbon atom.

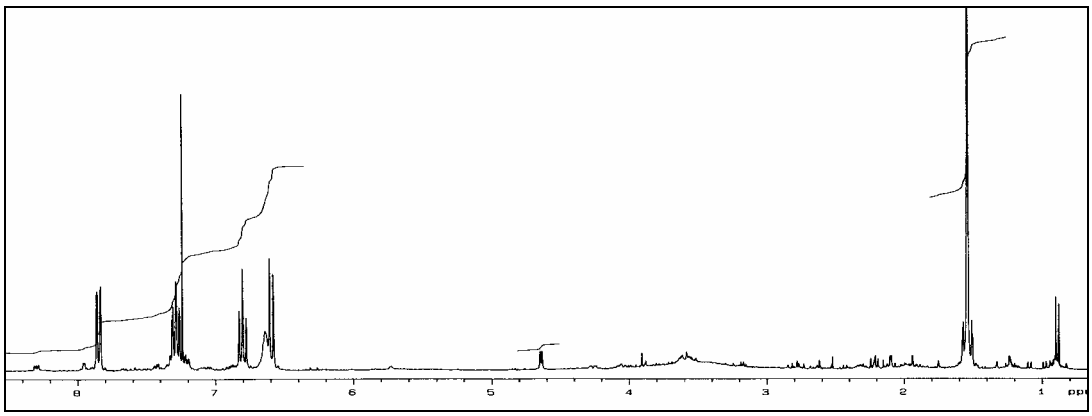
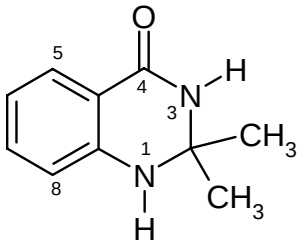


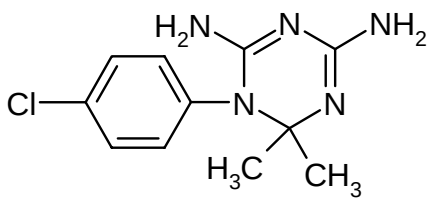
Figure 15: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 2,2-dimethyl-2,3-dihydro-(1*H*)-quinazolin-4-on (**68**).

The EI mass spectrum of **68** showed a molecular ion peak at *m/z* 176, followed by a base peak at *m/z* 161 ($[M - Me]^{+\cdot}$). HREI MS led to the corresponding molecular

formula $C_{10}H_{12}N_2O$. A search in AntiBase led to 2,2-dimethyl-2,3-dihydro-(1*H*)-quinazolin-4-on (**68**). The compound was further confirmed by comparing its data with authentic spectra. Compound **68** was isolated at the first time from bacteria by Speitling[114] in our group.



68



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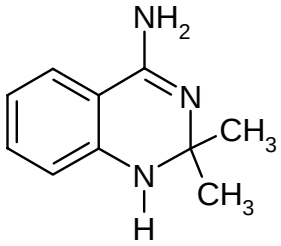
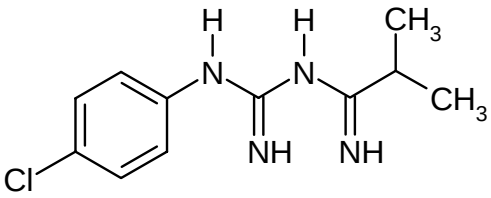


72



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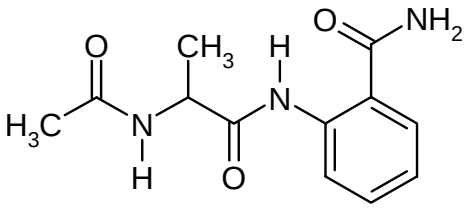


71

The first trial to synthesize the compound **68** was carried out by Carrington[115] in connection with the active dihydrotriazin[116] (**69**), a metabolite of the antimalarial drug paludrine[117] (**70**). Compound **69** was isolated from the urine of rabbits and of human volunteers. It is noteworthy that there have been only few reports of isolation of anthranilamide derivatives: the fungal metabolite 2-pyruvoylaminobenzamide (**71**), which was considered a precursor of quinazoline-type alkaloids[118,119]. The 1,2-dihydro-4-quinazolinamines e.g. **72** were reported recently as potent highly selective inhibitors of inducible nitric oxide synthase (NOS) and show anti-inflammatory activity *in vivo*[120].

4.1.13 Pharacine

The highly lipophilic compound **73** was found in sub-fraction F3b and obtained by PTLC, followed by Sephadex LH-20, and the resulting still impure compound was further purified by HPLC to get a colourless solid as a minor product. It showed UV absorption and no colour reaction with anisaldehyde/sulphuric acid or Ehrlich's reagent.

The IR spectrum showed no signals in the region beyond ν 3000 cm^{-1}, indicating the absence of acidic groups (OH, NH, or COOH). However, it showed two bands at ν 2962 and 2928, which are characteristics for C-H stretching vibrations of aliphatic compounds. It showed additionally a characteristic band for an ester carbonyl at ν 1721 cm^{-1}, in addition to a band at ν 1635 cm^{-1}. Additional several bands were observed between ν 1280 and 1019 cm^{-1} due to the existence of C-O bonds.

The ^{1}H NMR spectrum showed only three signals each of 1 H. A singlet at δ 7.86 can be interpreted as the *β*-proton of a double bond conjugated with a carbonyl group or as an aromatic proton in conjugation with an electron-withdrawing substituent. Two broad singlets at δ 4.41 results from a group connected to oxygen, and that at δ 2.02 could be due to a group attached either to a carbonyl group or to sp^2 carbon. From the spectral data of the ^{1}H NMR spectrum, it was expected that the compound could be very simple and small.

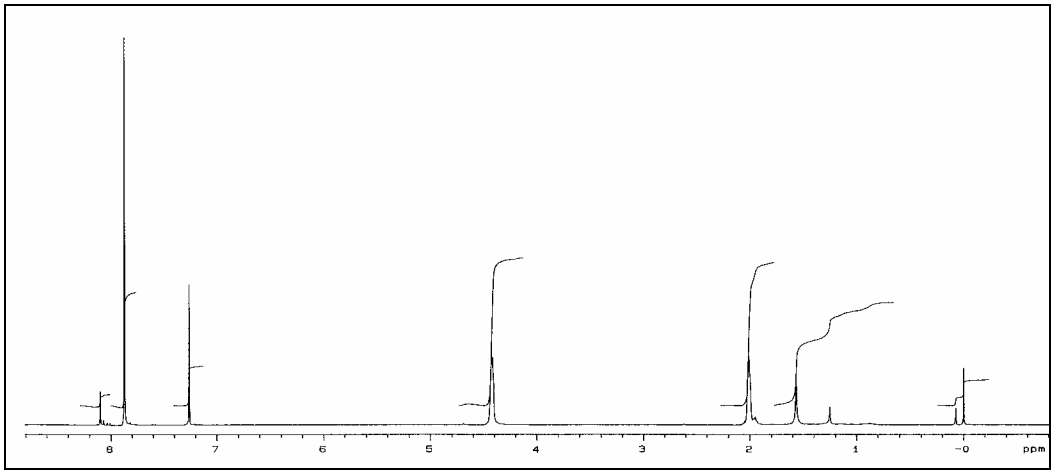


Figure 16: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of pharacine (**73**).

The ^{13}C/APT NMR spectrum displayed a quaternary carbon signal at δ 165.5, which could be assigned as a CO of carboxylic acid, ester, or amide. Two additional sp^2 carbon signals were observed, one of which was a quaternary carbon at δ 139.9, and the other one was a methine at δ 129.4. In the aliphatic region, two carbon signals for methylene carbons revealed at δ 64.6 and 25.8, from which the first one was assigned as oxygenated methylene carbon (OCH_2), while the other one could be flanked by two sp^2 and sp^3 carbons. The resonance of the sp^2 carbons indicated that the molecule contains more likely a symmetrically substituted aromatic ring than a conjugated olefinic double bond.

The CI mass spectrum led to a molecular mass of 440 Dalton because of the existence of two peaks at 898 ($[2M + NH_4]^+$) and 458 ($[M + NH_4]^+$), and it was further confirmed by EI mass spectrum. The high resolution of the molecular ion delivered the formula $C_{24}H_{24}O_8$ suggesting that the structure should be highly symmetrical.

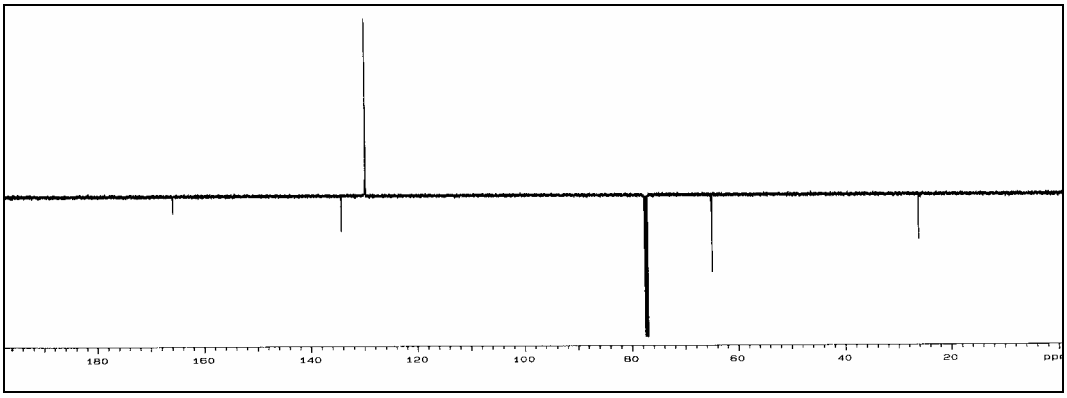


Figure 17: APT NMR spectrum ($CDCl_3$, 150 MHz) of pharacine (**73**).

The H,H COSY spectrum showed a correlation between the two methylene groups, confirming their direct attachment.

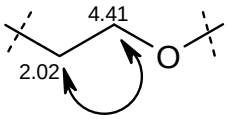


Figure 18: H,H COSY fragment of pharacine (**73**).

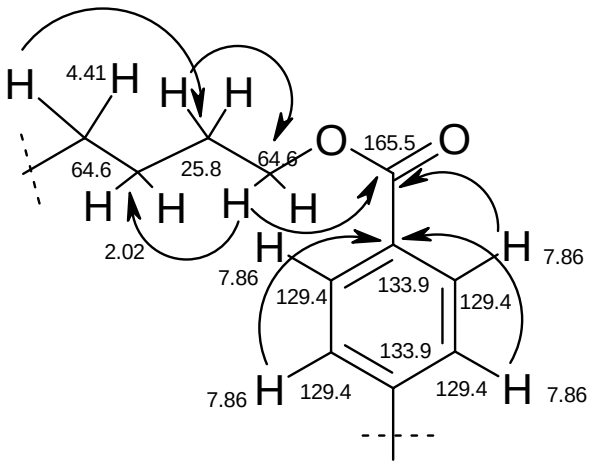


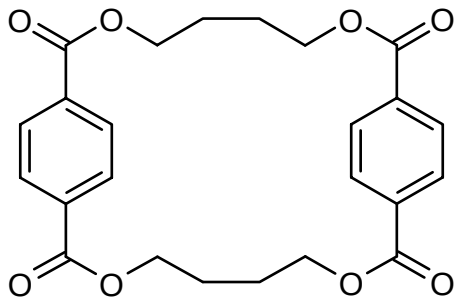
Figure 19: Partial structure of pharacine (**73**) delivered from HMBC correlations.

The continual interpretation using HMBC spectrum led to the correlation between the aromatic proton (δ 7.86) with both the quaternary carbon (133.9, ^2J) and 165.5 (^3J), and in the same, it showed a coupling between the methylene proton at δ

4.41 with the carbonyl carbon at δ 165.5 (^3J). This led to the partial structure as in Figure 19.

Here, the more plausible three-bond correlation have been drawn, however, two-bond correlations cannot be excluded completely due to the showed identical shifts.

From the molecular formula, it could be suggested that the molecule consists of four equivalent parts resulting in the 1,4-aromatic cyclic structure **73** that was named pharacine. The compound can not be a 1,2- or 1,3-aromatic system as in **74** and **75**, because of the existence of only one aromatic proton singlet in the spectrum. In case of a 1,2-disubstituted benzene moiety (**74**), two aromatic singlets should be observed. Also, in case of a 1,3-disubstituted aromatic one (**75**), three aromatic protons should be exhibited. Therefore, both positional isomers **74** and **75** were excluded.



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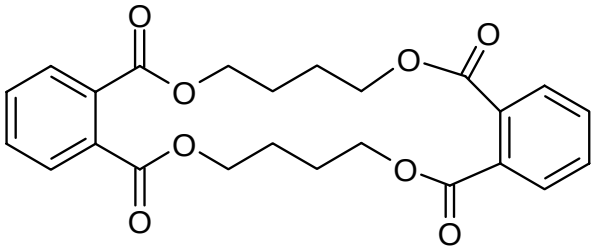

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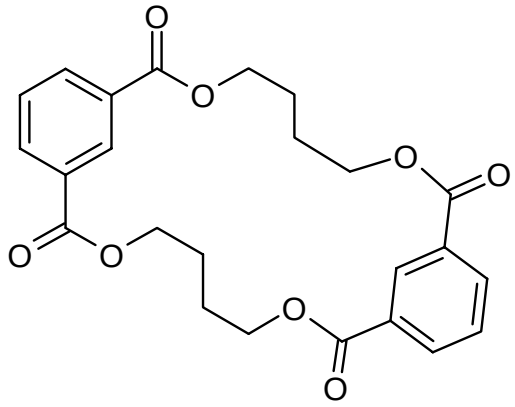

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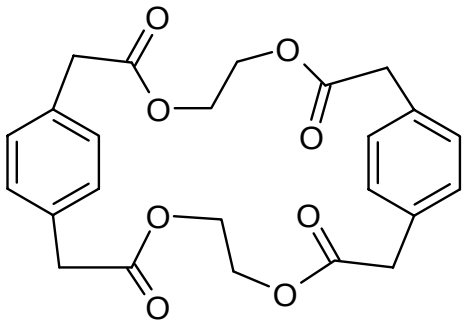
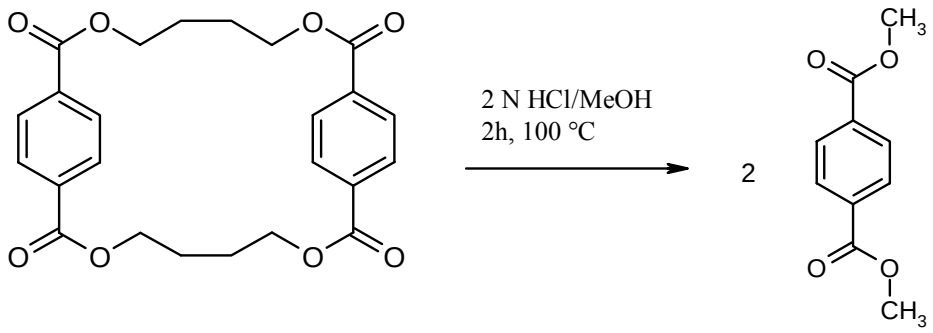


76

The structure of pharacine (**73**) was further confirmed by hydrolysis in methanol / 2 N HCl followed by methylation with diazomethane, which delivered terephthalic acid dimethyl ester (**77**) with a molecular ion at *m/z* 194. This excluded the alternative of 1,4-phenylenediacetic acid as an aromatic constituent in addition to ethylene glycol (as aliphatic part) as in compound **76**. In addition, the molecular ion of 1,4-phenylenediaceticacid acid would be displayed at *m/z* 222 and the chemical shift of

the methylene groups should be completely different (δ 3.62). The broadened singlets (narrow multiplets after expansion) for the butanediol bridges may not correspond to the expectation; however, the data were identical with published values[121] and very similar to simulated ^{13}C and ^{1}H NMR spectra[122].



73 **77**

Terephthalic acid and several of its esters have been isolated from plants[24]. Pharacine (**73**) is the first cyclic terephthalic acid ester from a natural source[89]. However, it has already been described as a cyclization product from terephthalolyl chloride and butanediol[123] or as a trace component of poly-(1,4-butylene glycol terephthalate)[124]. In addition, the mass spectra were nearly identical with those in the literature[125]. As terephthalic esters are often used as additives in plastics, pharacine (**73**) could be present in the materials used in the laboratory and extracted as an artefact during workup. However, it was possible to reproduce the isolation from *Cytophaga* sp. AM13,1 strictly avoiding all contact with plastic materials, yet it could be not isolated from other bacterial strains. To definitely rule out an artificial origin of **73**, biosynthetic studies should be done, which are, however, presently not possible due to the very low yield. Pharacine (**73**) showed no antibiotic or phytotoxic activity.

4.1.14 *p* –Hydroxyphenyl acetamide

Compound **78** was isolated from fraction IV as a colourless solid by PTLC and HPLC. By TLC screening, it showed an UV absorbing band, which turned to faint pink by Ehrlich's reagent after heating.

The IR spectrum showed a broad band between ν 3500 and 2500 cm^{-1} along with a sharp band at 3400 (characteristic for non-chelated OH groups). One band at ν 1660 cm^{-1} might be due to CO attached to an electron-donating group (e.g. NH_2).

Furthermore, two bands were observed at ν 1616 and 1500 are possibly characteristic for sp^2 carbon moieties.

The ^{1}H NMR spectrum displayed three broad singlets at δ 9.10, 7.15 and 6.70 characteristic for phenolic hydroxyl and /or amino groups, two doublets each with intensity of 2H at δ 7.05, and 6.65 of a 1,4-disubstituted aromatic system, and a singlet of a methylene group at δ 3.27, which could be a linkage between two sp^2 groups.

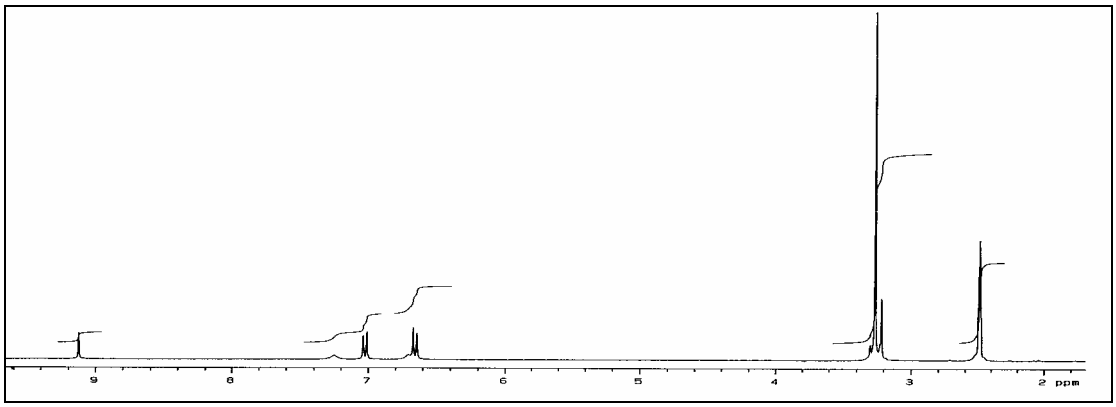
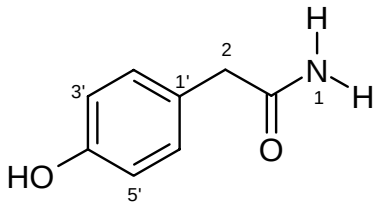


Figure 20: ^{1}H NMR spectrum ([D_6]DMSO, 300 MHz) of *p*-hydroxyphenyl acetamide (**78**).

The ^{13}C/APT NMR showed a quaternary carbon signal at δ 172.7 due to an acid, ester, or amide, two quaternary carbons at δ 155.7 and 126.5, from which the first one should be linked to oxygen. Two methine carbon signals were shown at δ 129.8 and 114.8, are corresponding to a 1,4-disubstituted aromatic ring. In the aliphatic region, one methylene carbon was observed at δ 40.1.

The molecular weight of compound **78** was determined as 151 Dalton using EI MS. Furthermore, a base peak was obtained at *m/z* 107, as a result from the expulsion of $CONH_2$. EIHR of **78** led to the molecular formula $C_8H_9NO_2$ and established the structure as *p*-hydroxyphenyl acetamide.

A search in AntiBase resulted no hits, as indication of a new natural product from microorganisms. However, **78** is synthetically known[126]. In contrast, the acid analogues **79a** is widespread in plants e.g. sweet clover (*Melilotus officinalis*) and other biological sources[24]. Also the nitrile analogue **80** was obtained from *Brassica alba* as a decomposition product of 4-hydroxybenzyl glucosinolate[24]. The methyl ester **79b** is also naturally known from *Fusarium oxysporium* and shown to be a phytotoxin[24].



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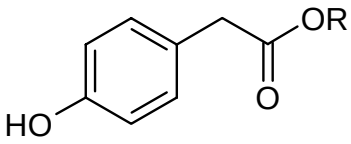
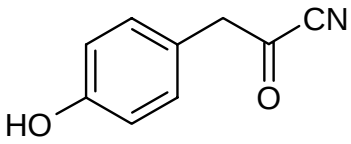


79a: R = H, **79b**: R = CH_3



80

4.2 Marine *Streptomyces* sp. B1848

The ethyl acetate extract of the marine *Streptomyces* sp. B 1848 drew our attention due to the presence of two dark red spots and one orange band during TLC. Their negative colouration with dilute sodium hydroxide solution excluded *peri*-hydroxyquinones. The strain showed a high activity against *Mucor miehei* (Tü284) and *Candida albicans*, a moderate activity against *Escherichia coli* and the alga *Chlorella vulgaris*.

Well-grown agar cultures of B 1848 served to inoculate 12 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2^+ medium. The flasks were incubated at 28 ºC while shaking with 110 rpm on a linear shaker for 3 days. The seed culture was inoculated to 25-liter jar fermenter, and the fermentation was carried for additional 3 days at 28 °C. The pH was automatically maintained (pH 6.5± 1.25). The culture broth was filtered and extracted with ethyl acetate. The brown crude extract was defatted with cyclohexane, and the methanolic extract was subjected to silica gel flash column chromatography, eluting with gradient of dichloromethane-methanol.

Working up and purification of fractions II~V delivered the metabolites N^{β}-acetyltryptamine (**34**), N(2-phenethyl)acetamide (**43**), 1-acetyl-*β*-carboline (**81**), anthranilic acid (**48**), 2'-deoxyadenonsine (**87**), tyrosol (**50**), indolyl-3-acetic acid (**46a**), phenyl acetamide (**49b**), 2'-deoxythymidine (**95**), 2'-deoxyuridine (**96**), indolyl-3-carboxylic acid (**45**), N-acetyltyramine (**142**), *p*-hydroxybenzoic acid (**105a**) and 4-methylamino-7,8-dimethyl-isoquinoline-3,6-dione **(97)**, in addition to the two new compounds, 6-hydroxy isatine (**93**) and 7-methylamino-3*H*-pyrrolo[2,3-c]isoquinoline-6,9-dione (**102**) (Figure 21).

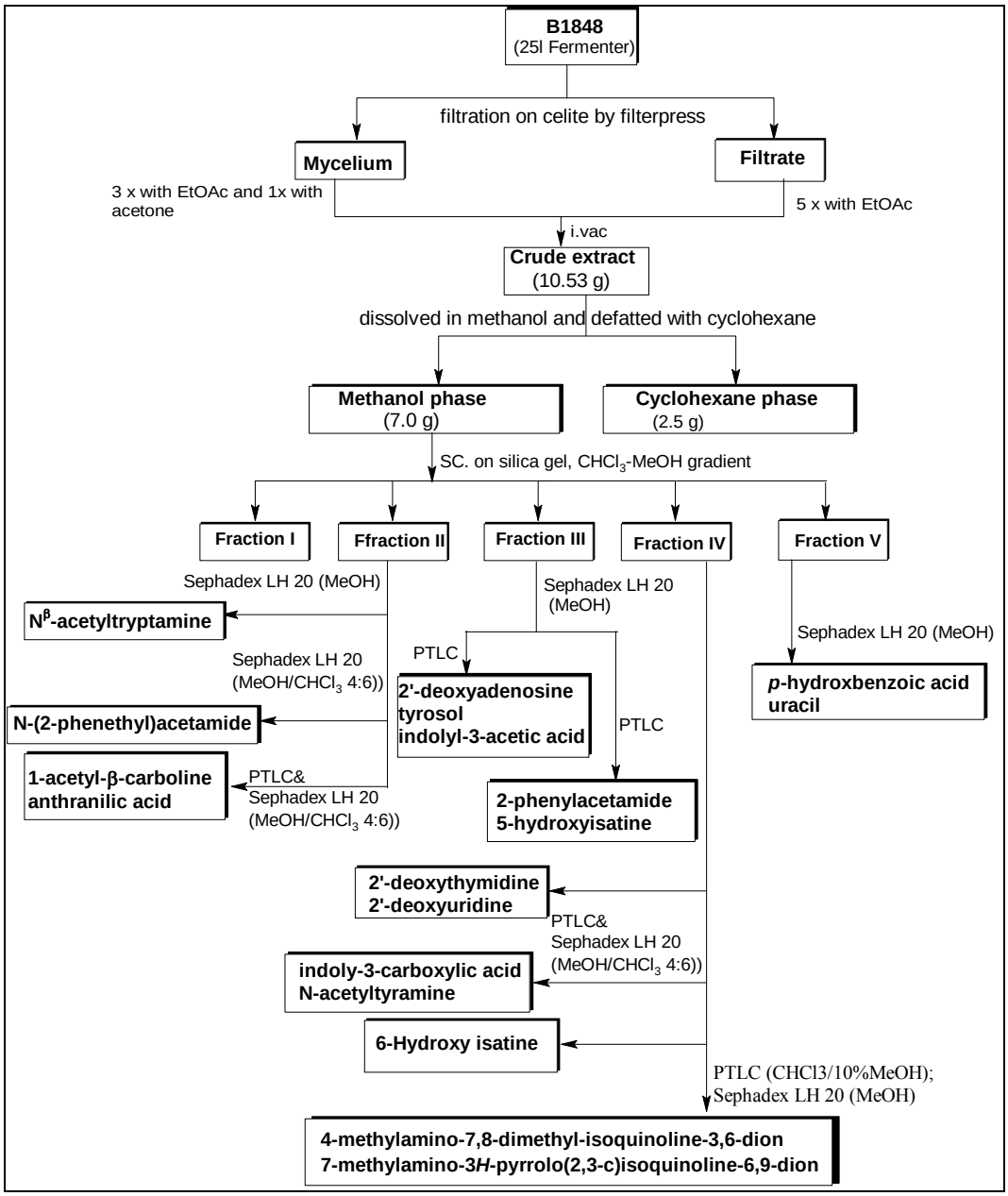


Figure 21: Work-up procedure of marine *Streptomyces* sp. isolate B 1848

4.2.1 1-Acetyl-β-carboline

Compound **81** was isolated from the fast moving phase of fraction II as faint yellow solid (blue UV fluorescence at 366 nm), which was stained to faint yellow by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed a strong solvent dependency. In $CDCl_3$, it showed a broad singlet at δ 10.30 of 1 acidic H, two *o*-coupled doublets each of 1H at δ 8.54 and 8.16 which, may belong to a heteroaromatic ring due to their small coupling constant (^{3}J = 5.1 Hz). An additional 1,2-disubstituted aromatic ring was observed due to the presence of four multiplet protons at δ 8.15 (5-H), 7.62 (7,8-H) and 7.31 (6-H). In the aliphatic region, it showed a

singlet of an aromatic bound methyl group at δ 2.96, which could be present in *peri*-position to a carbonyl group, or may be present as NCH_3. The ^1H NMR spectrum of compound **81** was better resolved in acetone (Table 2).

Table 2: ^1H NMR comparison of **81** in $CDCl_3$ and [D_6]acetone, (*J* in Hz)

Position	$CDCl_3$	[D_6]acetone
1	-	-
3	8.54 (d, ^3J = 5.1)	8.52 (d, *J* = 5.1)
4	8.16 (d, ^3J = 5.1)	8.36 (d, *J* = 5.1)
5	8.15 (m)	8.28 (dd, *J* = 8.1,1.3)
6	7.31 (m)	7.33 (td, *J* = 8.1, 1.3)
7	7.62 (m)	7.61 (td, *J* = 8.1, 1.3)
8	7.62 (m)	7.84 (dd, 8.1, 1.3)
9	10.30 (s br.)	11.25 (s br.)
11	2.90 (s)	2.75 (s)

The ^{13}C/APT NMR spectrum revealed an acetyl carbonyl carbon at δ 202.5, five quaternary sp^2 carbons at δ 142.7, 137.2 and 135.6, 132.1 and 121.4, of which the first three carbons could be attached to nitrogen or oxygen. In addition, six aromatic methine carbons at δ 138.6, 129.8, 122.5, 121.2, 119.8 and 113.5 were observed. Furthermore, one sp^3 carbon of a methyl group appeared at δ 25.7.

CI and EI mass spectra determined the molecular weight of **81** as 210 Dalton. The obtained molecular ion *m/z* 210 of **81** in EI mass spectrum showed an expulsion of CO to give a peak at *m/z* 182. In addition, it showed a base peak at *m/z* 168 ([M - $(COCH_2)$]$^{+\cdot}$, which is most likely attributed to *β*-carboline moiety (**83a**), on the basis of comparison with a closely related moiety was previously investigated by our research group. A search in AntiBase led to elucidate the compound as 1-acetyl-*β*-carboline (**81**). Compound **81** was isolated at the first time from bacteria in our group[48].

According to ^2J and ^3J of hetero coupling (H→C) correlations (HMBC), the sequence of carbon values in **81** were confirmed (Figure 22). It is worth to mention that the carbon values of compound **81** were previously reported with wrong assignment [127], perhaps due to the missing 2D measurements. Although, the compound reported in literature was measured in a different solvent (dimethylsulfoxide), it showed no deviation in the shift of most carbons except for the wrongly assigned signals (Table 3).

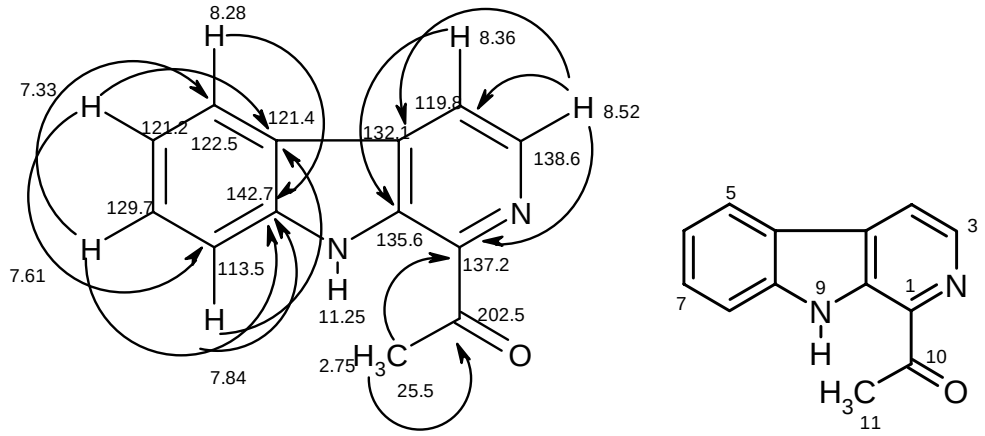


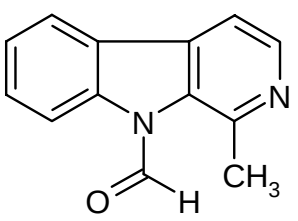
Figure 22: HMBC correlations of 1-acetyl-β-carboline (**81**)

Table 3: Comparison of ^{13}C NMR of 1-acetyl-β-carboline (**81**) using 2 D with those reported in the literature[127]

Pos.	Exp. ([D_6]acetone)	Lit. ([D_6]DMSO)
C-1	137.2	141.8
C-3	138.6	137.3
C-4	119.8	119.3
C-4a	132.1	130.9
C-5a	121.4	119.8
C-5	122.5	121.7
C-6	121.2	120.1
C-7	129.7	128.8
C-8	113.5	113.0
C-8a	142.7	135.9
C-1a	135.6	133.9
CO	202.5	201.3
CH_3	25.5	25.8

Compound **81** was isolated from several plant species. It is identical with the lycii Alkaloid I which was previously wrongly assigned as N-9-formylharman (**82**). Recently compound **82** was confirmed by Bracher *et al.*[127] to be 1-acetyl-β-carboline (**81**). Compound **81** was isolated also from the bark of *Ailanthus malabarica* (*Simaroubaceae*), as well as the sponge *Tedania ignis*[24,128]. Harman (**83b**) is the simplest 1-alkyl-β-carboline-alkaloid, occurs in plants[129], fungi, microorganisms and marine animals[130,131]. Harman (**83b**) displayed a high pharmacological effect e.g. inhibition of the mononaminooxidase and the cAMP-phosphodiasterase[132] as well as antimicrobial activity[133]. 1-Ethyl-β-carboline (**84**), a simple β-carboline derivative, exhibited a pronounced antimicrobial activity. It is used as an antagonist of catecholamines[133,134]. The observed antifungal activity of the marine *Streptomyces* sp. B 1848

could be attributed to the existence of some *β*-carboline analogues in low concentrations, which could not be isolated.



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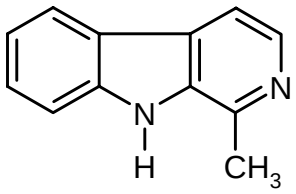
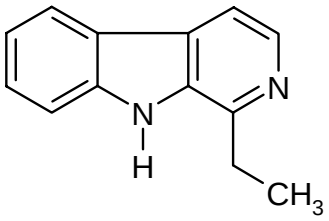


83a: R = H, **83b**: R = CH_3



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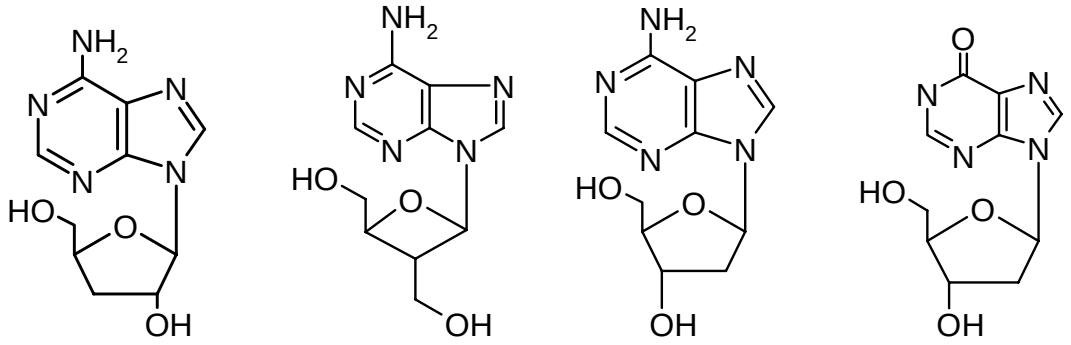
4.2.2 2'-Deoxyadenosine

Compound **87** from fraction III was isolated as colourless solid exhibiting an UV absorbing band, which turned to green-blue with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of **87** exhibited two singlets each of 1H at δ 8.31 and 8.12, and a broad singlet of 2 acidic protons at δ 7.23 (NH_2). In the aliphatic and sugar regions, it showed one doublet of 1H at 6.34, two broad singlets (δ 5.25 and 5.15) of 2 OH groups, two oxygenated methines at δ 4.40 (dd) and 3.87 (ddd), and one ABX signal at δ 3.64~3.51 of an oxygenated methylene group. Furthermore, two methylene multiplets each with 1H at δ 2.68 and 2.24 of a cyclic sugar were observed.

The ^{13}C NMR spectrum of compound **87** showed three sp^2 quaternary carbon signals at δ 156.1, 148.9 and 119.3, and two sp^2 methines (δ 152.4 and 139.5). In the sugar moiety, five sp^3 carbons signals were observed, from which three were oxygenated methines at δ 88.0, 84.0 and 71.0, and two at δ 61.9 and 40.3 were methylene carbons, of which the first one was oxygenated.

The molecular weight of compound **87** was established as 251 Dalton by EI mass spectrum. A search in AntiBase led to four possible structures, namely cordycepin (**85**), oxetanocin, (**86**) 2'-deoxyadenosine (**87**) and 2'-deoxyinosine (**88**).



85 **86** **87** **88**

However, comparison of our spectral data with the literature of the above compounds, as well as with authentic spectra, led to identify the compound as 2'-deoxyadenosin (**87**)[48,135]. Cordycepin (**85**) is one of the first adenine-nucleoside analogues, which was isolated from cultures of the fungus *Cordyceps militaris*[136]. Cordycepin (**85**) is a strong inhibitor of RNA synthesis showing cytostatic activity. In contrast, 2'-deoxyadenosin (**87**) is an essential base of DNA.

4.2.3 6-Hydroxy isatine

Compound **93** formed an orange middle polar band in both fractions III and IV. It exhibited no colouration with NaOH and sulphuric acid confirming the absence of *peri*-hydroxyquinone moieties. It was isolated by PTLC and Sephadex LH-20 as an orange solid. The ^{1}H NMR spectrum showed three different spectral resonances in the aromatic region each with intensity of 1 H, two ortho-coupled protons at δ 7.42 (d) and 6.37 (dd), and a doublet of a m-coupled proton at δ 6.26 (^4J = 2.4 Hz) as of an 1,2,4-trisubstituted aromatic ring.

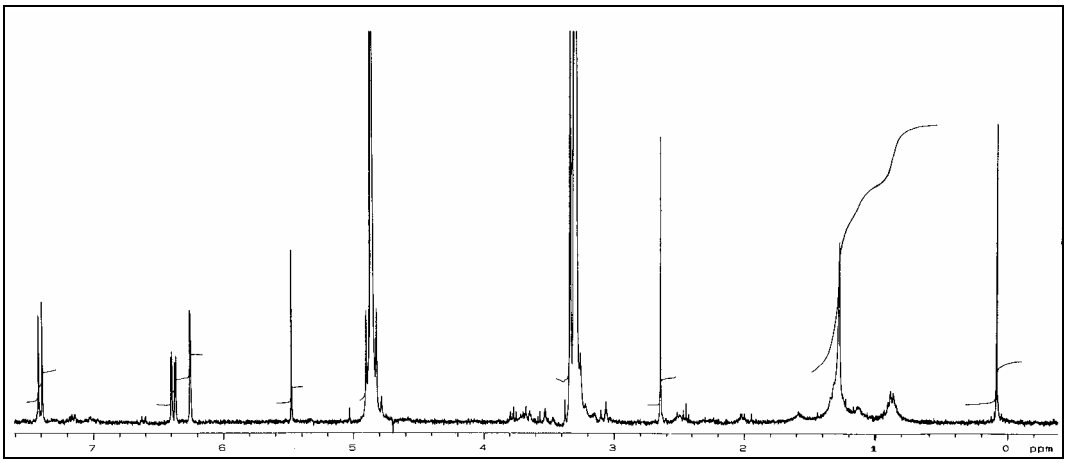


Figure 23: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 6-hydroxy isatine (**93**).

It is worth to mention that, the ^{1}H NMR signals showed a deviation in the chemical shift depending on the impurities by other compounds. The crude product exhibited shift values, which were Δδ 0.2-0.4 higher than those after complete purification. This could be due to the interaction with other compounds forming a complex. The molecular weight of compound **93** was found to be 163 Dalton in ESI MS. HREI MS of **93** gave the molecular formula $C_8H_5NO_3$.

The ^{13}C NMR spectrum exhibited three sp^2 down field carbon signals at δ 178.0, 166.8, and 155.9, where the first one could be the carbonyl of an ester, flanked by two sp^2 carbon systems. The second one (166.8) could be an oxygenated sp^2 carbon, carbonyl of acid or amid, while the third one could be of an oxygenated sp^2 carbon or an imide group. The spectrum showed three additional sp^2 methine carbons at δ 129.7, 116.8, and 102.8.

According to the above spectral data as well as the molecular formula, the formula could be constructed by two rings, one as a six memberd aromatic ring with three protons, two in *o*- and the third in a *m*-position, and a second five memberd ring containing two carbonyls in lactone or lactam form. This allowed suggesting five possible structures: **89**, **90**, **91**, **92** and **93**.

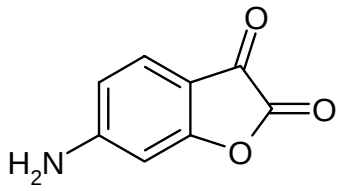
Compounds **89**, **90** and **91** were plausible, but should be colourless and so do not match. In addition, the *peri* proton in **89** and **90** should be displayed at higher chemical shift (≈ 7.4) due to the adjacent carbonyl group. Compounds **89** and **90** are not known from nature, even not as constituents of natural compounds[23], while **91** is known as a constituent of several active compounds e.g. Staurosporin (**94**).

Compounds **92** and **93** are isatine derivatives, which are characterised by their orange-red colour, and one of them could match with our chemical data. The hydroxyl group could be either at 5-position as in **92** or at 6-position as in **93**. ^{1}H and ^{13}C NMR predictions with ACD pointed to isomer **93** as the more plausible one (Table 4).

Table 4: ^{1}H NMR predicted values of both 5- and 6-hydroxy isatine compounds (**92**, **93**) in comparison with the experimental data.

H no.	Exp. Val. of **93**	**93**	**92**
4	7.42 (d, *J* = 8.1)	7.69 (d, *J* = 8.1)	7.07 (d, *J* = 2)
5	6.37 (dd, *J* = 8.1, 1.5)	6.56 (dd, *J* = 8.1, 1.5)	-
6	-	-	7.31 (dd, *J* = 8.2, 2)
7	6.26 (d, *J* = 1.5)	7.11 (d, *J* = 1.5)	7.77 (d, *J* = 8.3)

The UV spectrum of compound **93** was measured in comparison with isatine (**65**): compound **93** showed maxima at λ_{max} 263, 280 (sh), 348.9 (br) and 420 nm (br). The observed UV values of compound **93** are very similar to isatine [λ_{max} 241, 296.6 and 419 (br)]. The differences could be attributed to the bathochromic effect of the hydroxyl group (n-electrons) in **93**.



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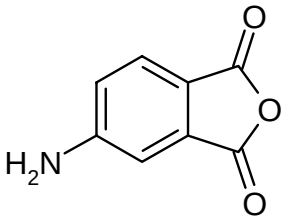

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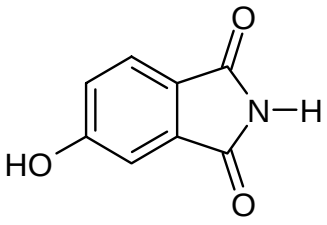

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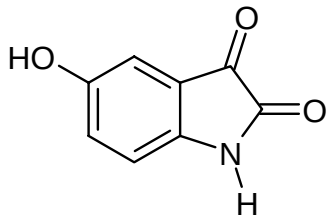

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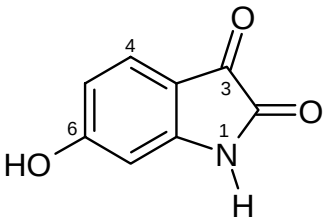

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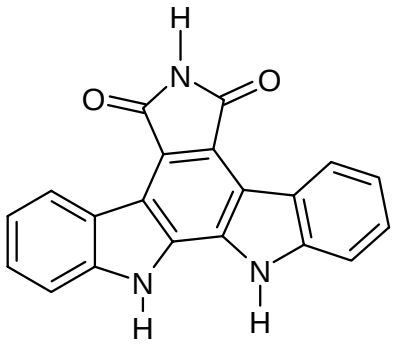


94

A search in AntiBase for both isomers **92** and **93** resulted without any hit. This referred to that **93** is a new natural product. However, by search in CA, both compounds were found as synthetic products, but no spectral data have been reported. Compound **93** was obtained after demethylation of the corresponding methoxy derivative by pyridinium hydrobromide perbromide ($Py^+ HBr_3^-$)[137].

4.2.4 2'-Deoxythymidine, and 2'-Deoxyuridine

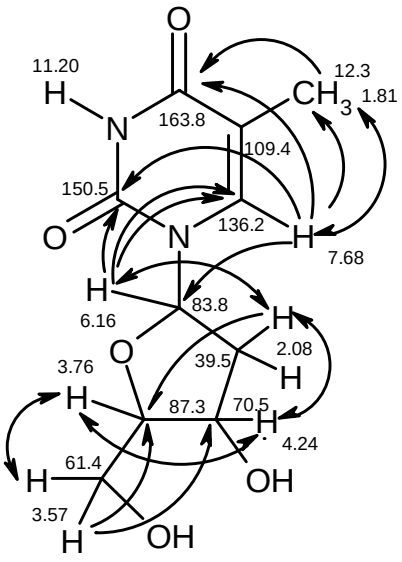
Compound **95** was obtained from fraction IV as colourless solid. It exhibited an UV absorbance, which turned to blue-green by anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum showed three signals characteristic for the thymine moiety, a broad 1H singlet at δ 11.20 (NH), an aromatic 1H singlet at δ 7.68 (6-H), and an up-field singlet of a methyl group at δ 1.78, probably linked to an sp^2 carbon in an α,β-unsaturated system. Furthermore, the spectrum exhibited a triplet at δ 6.16 (1 H), signals of two oxygenated methine protons at δ 4.24 (td) and 3.76 (dd), two multi-

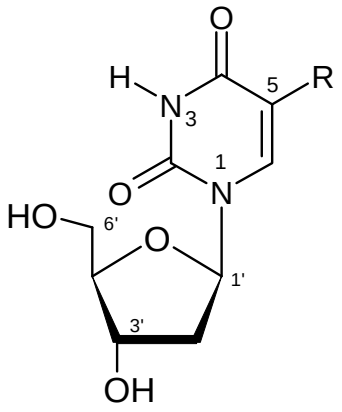
plets of two methylene groups at δ 3.60 and 2.08, of which the first one is oxygenated.

The ^{13}C NMR spectrum exhibited 10 carbons, five of which are similar as in thymine. In addition, it contains five sp^3 carbon atoms, three oxygenated methines and two methylene carbons.

The molecular weight was determined by EI mass spectrum as 242 Dalton. Further more, two characteristic peaks were observed at *m/z* 126 and 117, of which the first of due to a thymine moiety, and the second of deoxypentose, respectively. A search in AntiBase led to 2'-deoxythmidine (**95**), which was confirmed by the literature data[138].



95



95: R = CH_3, **96**: R = H

Figure 24: H,H COSY and HMBC correlations of 2'-deoxythymidine (**95**).

In addition to 2'-deoxy-thymidine (**95**), 2'-deoxyuridine (**96**) was isolated from fraction IV as colourless solid, which turned to blue by anisaldehyde/sulphuric acid. Compound **96** was identified by comparing its spectral data with the literature[48].

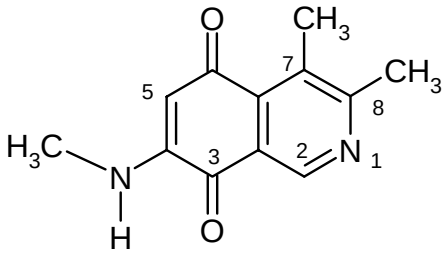
4.2.5 4-Methylamino-7,8-dimethyl-isoquinoline-3,6-dione

From fraction IV, the dark red middle polar compound **97** was isolated using PTLC and Sephadex LH-20. It showed no colour reaction with sodium hydroxide indicating the absence of *peri*-hydroxyquinones. The ^1H NMR spectrum showed three 1H singlets at δ 9.02, 5.82 and 5.72. The broad singlet at δ 5.82 is an exchangeable NH proton of an amide. In addition, a methyl doublet was detected at δ 2.93 (*J* =

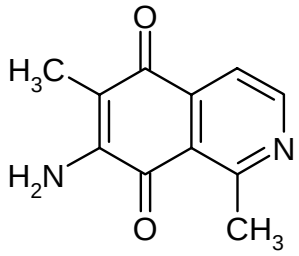
4.6 Hz), indicating a CH_3NH- fragment. Two CH_3 singlets at δ 2.76 and 2.70 for aromatic bound methyl groups were detected. The molecular weight of compound **97** was determined as 216 Dalton by EI and CI mass spectra. HREI MS led to the molecular formula $C_{12}H_{12}N_2O_2$.

The ^{13}C and APT NMR spectra indicated two aromatic methines at δ 145.1 and 103.3, and three aliphatic carbon signals were found at δ 29.0, 24.6 and 16.0, corresponding of three methyl groups. Furthermore signals of seven quaternary carbons, of which two at δ 185.0 and 181.9 representing carbonyl groups, were observed. A search in AntiBase led to 4-methylamino-7,8-dimethyl-isoquinoline-3,6-dione (**97**), which was further confirmed by direct comparison with the literature. The compound was isolated previously in our group by M. Speitling[114]. The unusual quinone exhibited four peaks at λ_{max} 234, 273, 338 and 443 in the UV spectrum, which were identical with the previous data[114].

Cribrostatin 1[139] (**98**) is a similar structural analogue. It was isolated recently together with some related compounds from the marine sponge *Cribrochalina* sp. and is characterised by its cytotoxicity against lymphocyte P388 leukaemia cells. The biosynthetic origin of these compounds is still unknown. Additionally, four isoquinoline quinones as well as two isoquinoline iminoquinones were obtained recently from the marine Bryozoan *Caulibugula intermis*. They are designated as novel cytotoxic compounds, and assigned as caulibugulones A-F (A, **99**; F, **100**)



97



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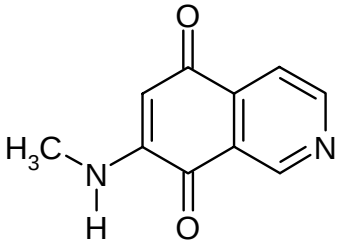
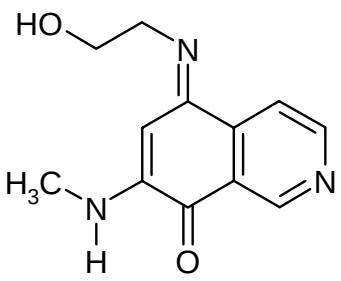


99



100

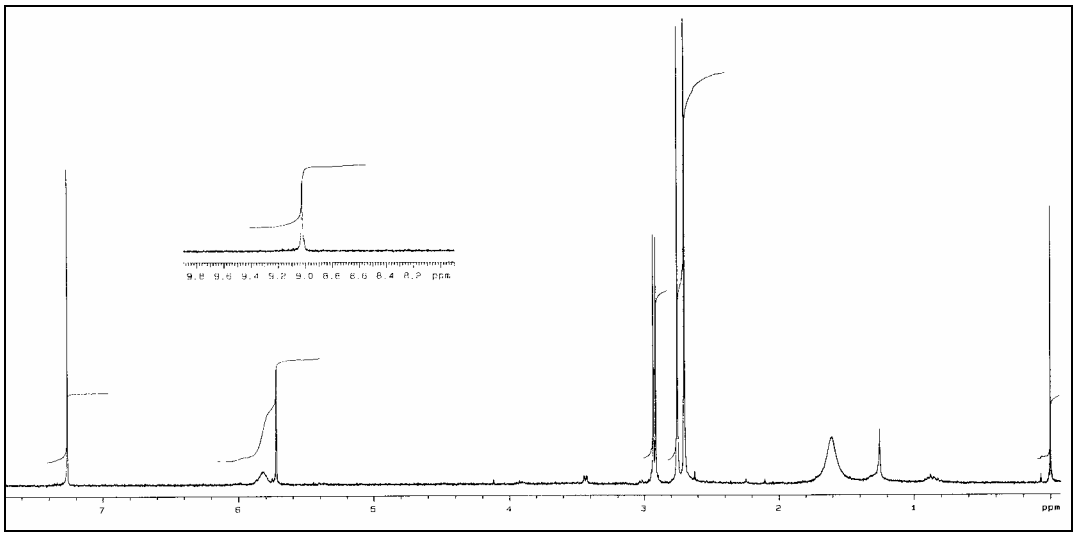


Figure 25: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 4-methylamino-7,8-dimethyl-isoquinoline-3,6-dione (**97**).

4.2.6 7-Methylamino-3*H*-pyrrolo[2,3-c]isoquinoline-6,9-dione

Compound **102** was found in fraction IV as a second dark red middle polar component, which showed high similarity with compound **97**, pointing to a related structure analogue. This was confirmed by the UV spectrum, which showed similar maxima as compound **97 (**Figure 26).

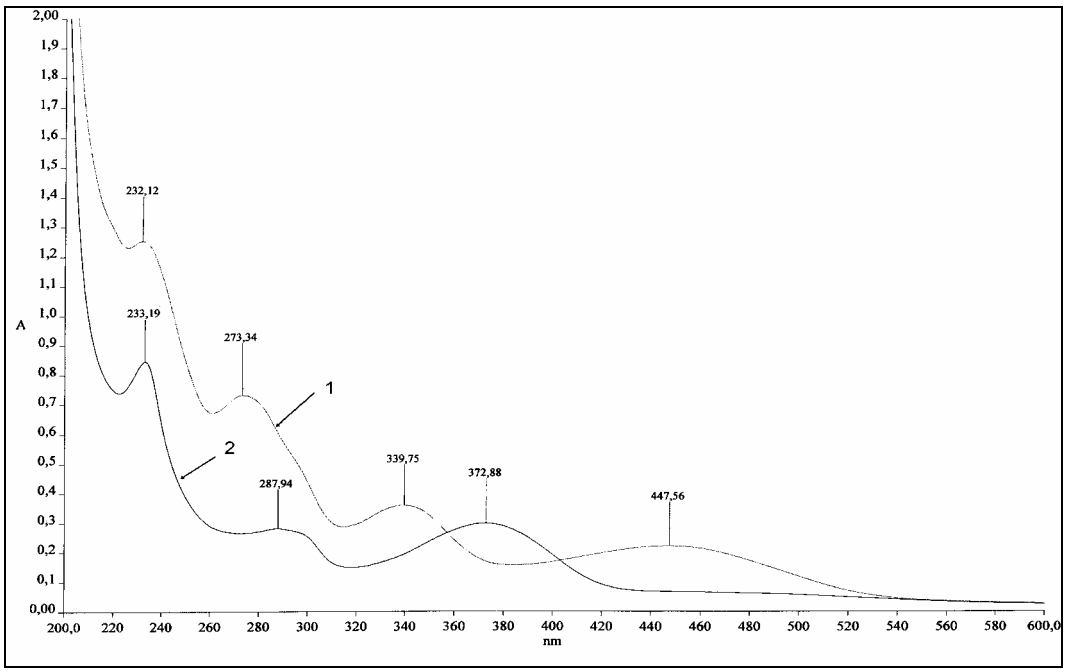


Figure 26: UV Spectra (methanol) of compounds **97** (1) and **102** (2)

The ^{1}H NMR spectrum revealed a broad H/D exchangeable singlet at δ 11.88 and two singlets at δ 8.98 and 5.61 confirming the protons at positions 2 and 5 in **97**.

It showed also a doublet of an $NHCH_3$ group at δ 2.83 (J = 5.0 Hz). The signal of the NH proton at 7-position appeared in DMSO as a broad quartet at δ 7.82 with higher shift than that in **97**, measured in $CDCl_3$. It displayed furthermore two 1H doublets at δ 7.92 and 6.69 with small coupling constants (< 4.0 Hz) characteristic for a heterocyclic five membered heterocycle. This also excluded the possibility of a benzene or pyridine derivative, which exhibits normally higher coupling constants (>5 Hz). The molecular weight (*m/z* 227 by EI and CI mass spectra) of **102** was odd referring to an odd number of nitrogens. HREI MS revealed the molecular formula $C_{12}H_9N_3O_2$.

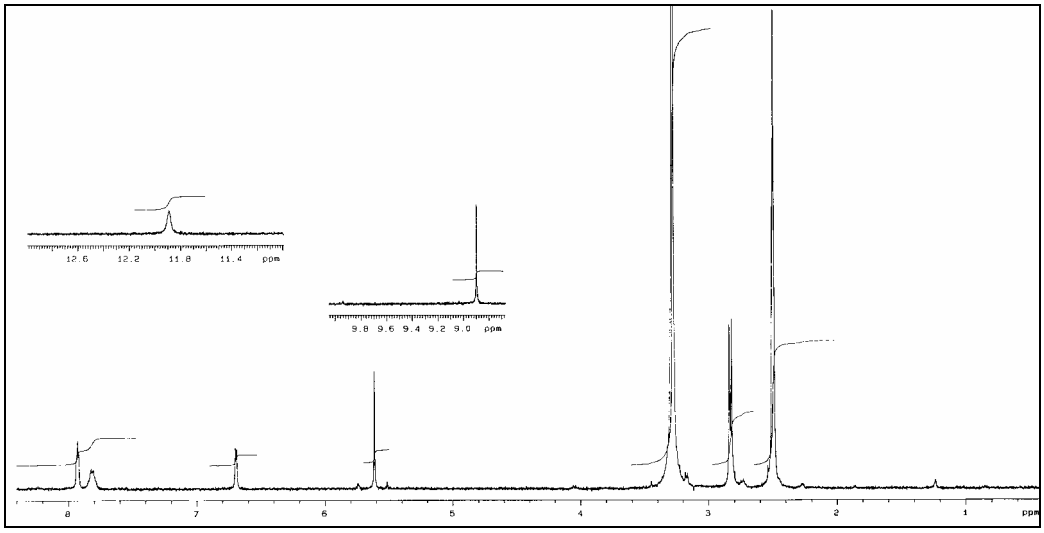


Figure 27: ^1H NMR spectrum ([D_6]DMSO, 300 MHz) of 7-methylamino-3*H*-pyrrolo[2,3-c]isoquinoline-6,9-dione (**102**)

The ^{13}C and APT NMR spectra indicated four aromatic methines, two of which were displayed at δ 140.7 and 99.0, corresponding to the two methine carbons C-2, C-5 in **97**. The other two aromatic carbons at δ 137.6 and 102.7 are belonging to the two pyrrole methine carbons. Additionally, one aliphatic carbon signal was found at δ 28.9, fixing the $NHCH_3$ fragment. Furthermore, signals of seven quaternary sp^2 carbons were detected, of which two at δ 182.8 and 181.2 are representing the carbonyl groups in positions 6 and 3, similar as in **97**

The spectral data and molecular formula allowed three structural alternatives, 7-methylamino-1*H*-pyrrolo[3,2-c]isoquinoline-6,9-dione (**101**), 7-methylamino-3*H*-pyrrolo[2,3-c]isoquinoline-6,9-dione (**102**) and 7-methylamino-2*H*-pyrrolo[3,2-c]isoquinoline-6,9-dione (**103**).

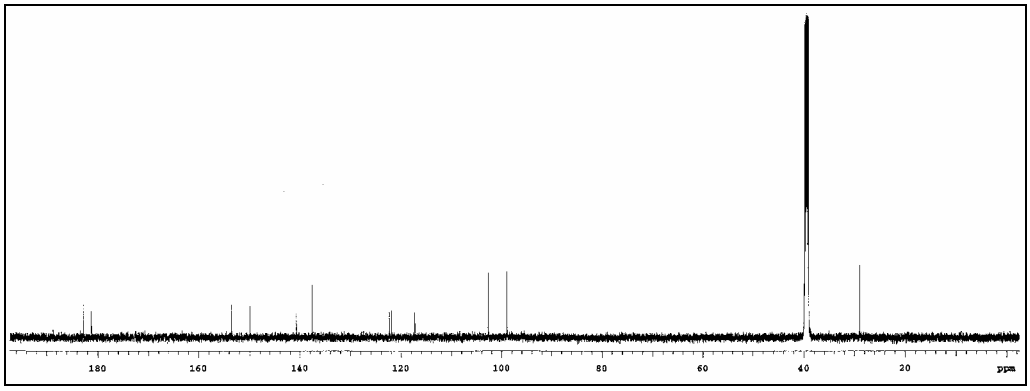
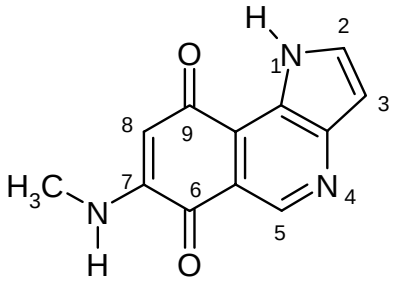
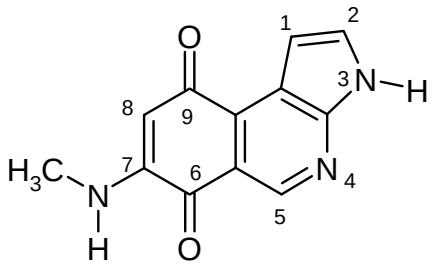


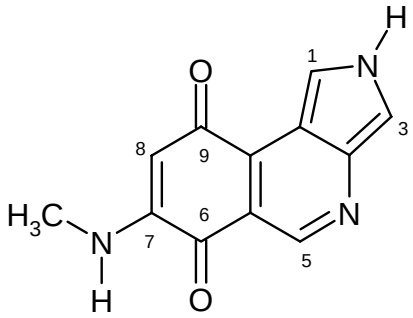
Figure 28: ^{13}C NMR spectrum ([D_6]DMSO, 150 MHz) of 7-methylamino-3*H*-pyrrolo[2,3-c]isoquinoline-6,9-dione (**102**)



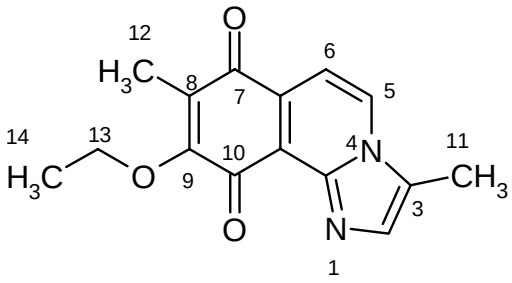
101



102



103



104

The observed similarity between the signal patterns of pyrrole protons and those of the 2,3-protons in indole (**106**) confirmed their direct *o*-arrangement. Furthermore, this conclusion was established by the H,H COSY spectrum, which showed a ^3J correlation between the two protons (δ 7.92, 6.60). The proton at δ 7.92 showed a ^3J coupling to the pyrrol NH proton (11.88), and the proton at δ 6.60 exhibited a long range coupling (^4J) with the same NH proton (Figure 29). Additionally, the ^1J correlations (HMQC) confirmed the attachment of the two protons (7.92 and 6.60) to the corresponding carbons at δ 137.6 and 102, respectively. This agrees with the assump-

tion that the methine carbon at δ 137.6 is linked with the NH of the pyrrol ring, while the other one (δ 102.7) is attached to an sp^2 carbon atom. This excluded compound **103**.

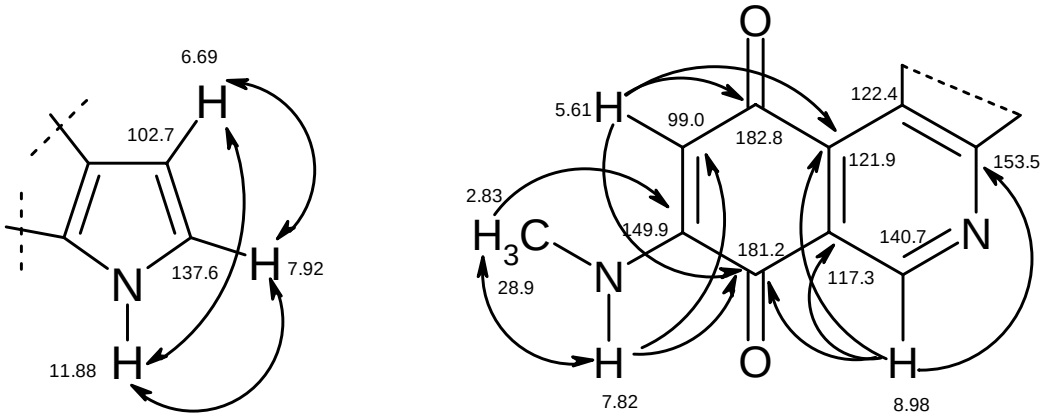


Figure 29: Partial structures of 7-methylamino-3*H*-pyrrolo[2,3-c]isoquinoline-6,9-dione (**102**) delivered from H,H COSY and HMBC correlations

Based on the long-range correlations (HMBC), both structures **101** and **102** are possible. The observed long range coupling (^3J) between the pyrrol proton (δ 7.92) and the quaternary carbon in *o*-position of the pyridine ring (δ 153.6) exhibited the same ^3J coupling in both cases of compounds **101** and **102**. However, the low δ value of the *m*-carbon in the pyridine ring (δ 122.4) directed its linkage to a carbon atom as in compound **102** rather than to nitrogen (**101)**. This assumption was confirmed by comparison with similar fused pyrrolo-pyridine skeletons[140] (**A** and **B**), establishing the compound to be **102** but not **101**. Furthermore, the residual main isoquinolone part of compound **102** was confirmed with the aide of 2 D correlations, presenting the same skeleton as in compound **97 (**Figure 29**)**.

The structurally related cribrostatin 6[141] (**104**) was recently obtained from the marine sponge *Cribrochalina* sp. It exhibited anticancer activity and was highly bio-active against bacteria and fungi.

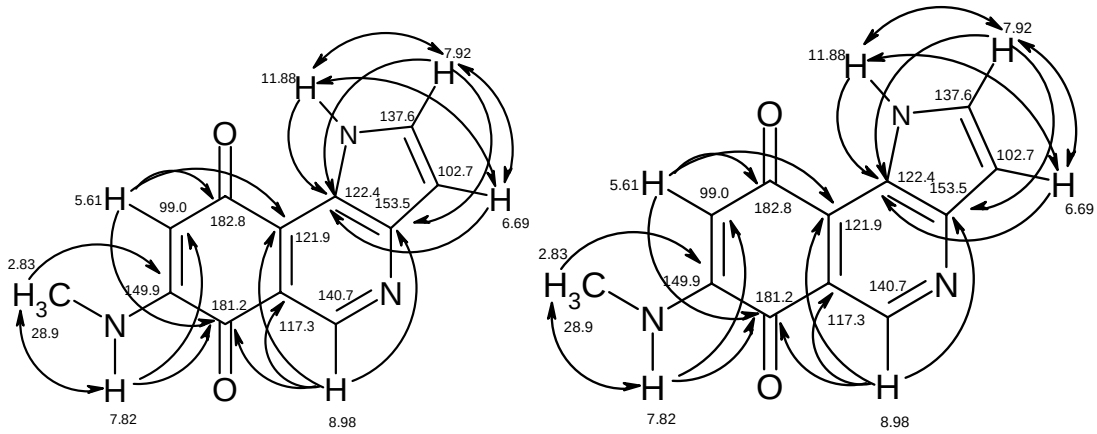
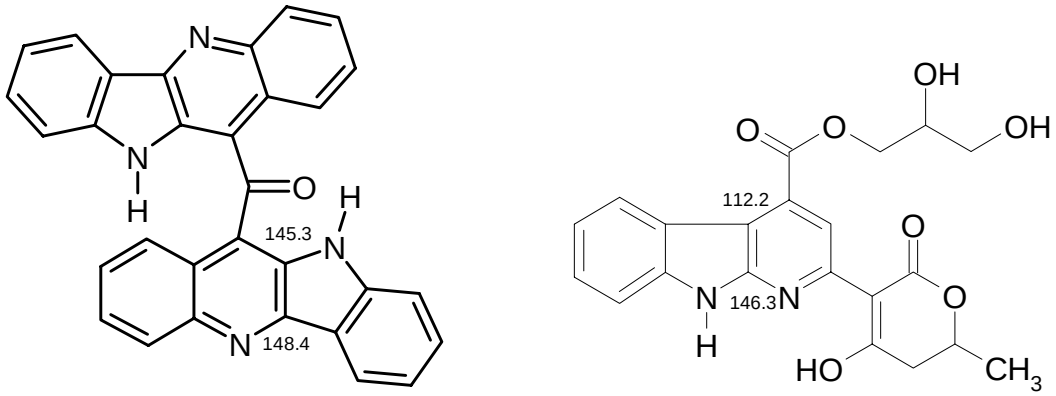


Figure 30: H,H COSY and HMBC correlations of compounds **101** and **102**



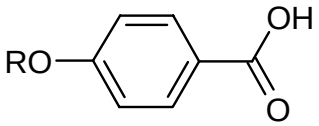
A **B**

Compound **102** has not been described so far, pointing to a novel natural product from bacteria. It is worth to mention that the compound **102** was isolated in a very little amount from the strain using M_2^+ medium (two 25-liter shaker cultures). However, after optimisation, it was found that the C medium + 50 % sea water (meat extract, 1 g; glucose, 10 g, peptone, 2 g; yeast extract, 1 g) is much better for cultivation of the strain. A 50-liter fermentation delivered now sufficient material for all spectroscopic and biological measurements. Additionally, three further new isoquinoline quinones were isolated, which are still under investigation.

4.2.7 *p*-Hydroxy benzoic acid

Working up of fraction V led to a colourless solid substance **105a**. On the basis of chromatographic and spectral data, compound **105a** was identified as *p*-hydroxy benzoic acid, which further confirmed by methylation giving *p*-methoxybenzoic acid methyl ester (**105b**) as a colourless solid. *p*-Hydroxy benzoic acid (**105a**) is wide-

spread in many plants, as well as in microorganisms free or in form of derivatives. It was first isolated from fruits of *Catalpa bignonioides*[24].



105a: R = H, **105b**: R = CH_3

4.3 *Alteromonas distincta* sp. Hel69

Chemical screening of the North Sea bacterium *Alteromonas distincta* isolate Hel 69 afforded several UV absorbing bands which gave mostly orange-violet colours by spraying with anisaldehyde/sulphuric acid, turned to pink by Ehrlich's reagent and blue by the chlorine/anisidine reaction, referring to indole derivatives as well as simple dipeptides. The crude extract possessed moderate activity against *Streptomyces viridochromogenes* (Tü57) and *Escherichia coli*, weak against *Bacillus subtilis* and *Staphylococcus aureus* in the agar diffusion test.

Fermentation of Hel69 was carried out in LB medium+50% Sea water using a 20-liter fermenter for 3 days at 28 °C. The ethyl acetate extract was defatted with cyclohexane. The cyclohexane extract was treated with acetonitrile to afford an insoluble white solid substance, which was identified as polyhydroxybutyric acid; sPHB (**317**, see page 254). Both methanol and acetonitrile extracts were combined and separated on Sephadex LH-20 to afford five fractions. Working up of these fractions resulted in indole (**106**), brevianamide F (**107**) and other known metabolites: N^{β}-acetyltryptamine (**34**), *cis-cyclo*(tyrosyl-prolyl) (**39**), *cis-cyclo*(phenylalanyl-prolyl) (**40**) and uracil (Figure 31).

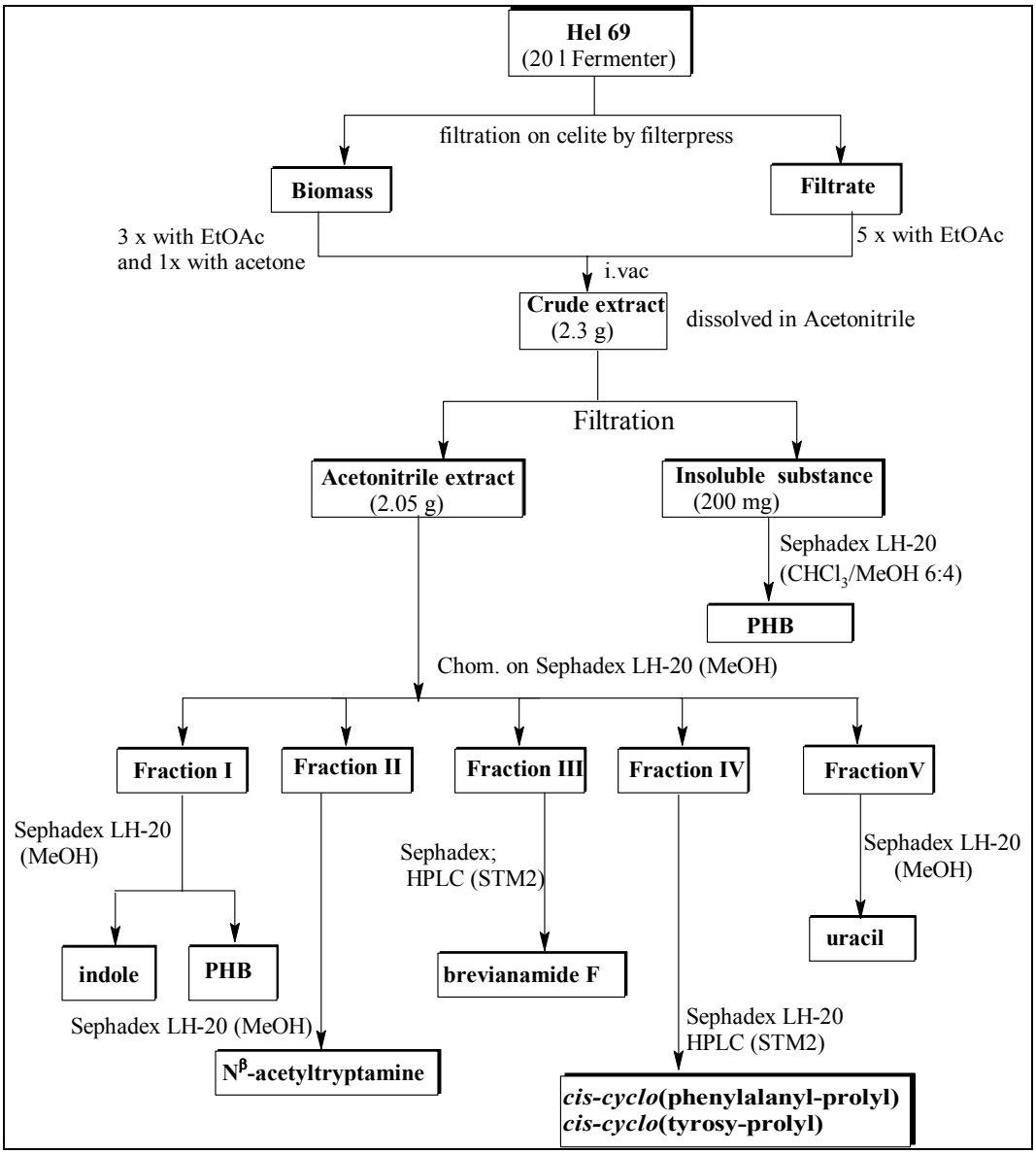
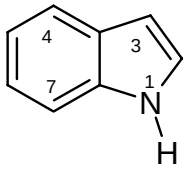


Figure 31: Work-up procedure of *Alteromonas distincta* isolate Hel 69

4.3.1 Indole

Indole (**106**) was easily identified by the high R_f value, the characteristic colour reactions with anisaldehyde/sulphuric acid (orange) and Ehrlich's reagent (pink), the ^{1}H NMR and mass spectra (*m/z* 117 Dalton). Indole is known for long as a constituent of oils e.g. Jasminum and Citrus species (*Oleaceae*, *Rutaceae*), as well as a product of bacterial decomposition of proteins, it is ubiquitous in faeces, is a constituent of coal tar, and used as an ingredient in Perfumery and flavouring[24]. Indole was found to have a wide antifungal spectrum and to inhibit few species of bacteria[142].



106

4.3.2 Brevianamide F

Compound **107** was isolated from fraction III as a colourless solid. It exhibited an UV absorbing band at 254 nm, gave a dark red colouration with anisaldehyde/sulphuric acid, and became pink with Ehrlich's reagent and blue with chlorine/*o*-anisidine as indication of a peptide.

The ^{1}H NMR spectrum of **107** showed a broad 1H singlet at δ 8.30 characteristic for an NH of an indole moiety, five aromatic protons at δ 7.59, 7.40, 7.24, 7.14 (m, 2 H) confirming the indole skeleton substituted at 3-position. In the aliphatic region, another broad 1H singlet of an acidic proton at δ 5.84 due to NH of an amide, two oxygenated or aminated methines at δ 4.39 (dd) and 4.08 (t), three different resonances each of 1 H, 2 H, and 1 H, respectively at δ 3.76 (dd), 3.64 (m) and 2.97 (dd) were seen. In addition, three multiplets of 4H between δ 2.36 and 1.90 were representative for two methylene groups.

The ^{1}H NMR pattern of the compound pointed to a 3-substituted indole moiety and a tyrosyl residue. This was supported by the molecular weight of 283 Dalton (EI mass spectrum) and by a fragment at *m/z* 154 due to a glycinyl-proly part, and a base peak at *m/z* 130 assigned to the indolyl-3-methylene ion[143].

A search in AntiBase revealed L,L-*cyclo*(tryptophanyl-prolyl); brevianamide F (**107**), which was further confirmed by comparison with the literature[144,61].

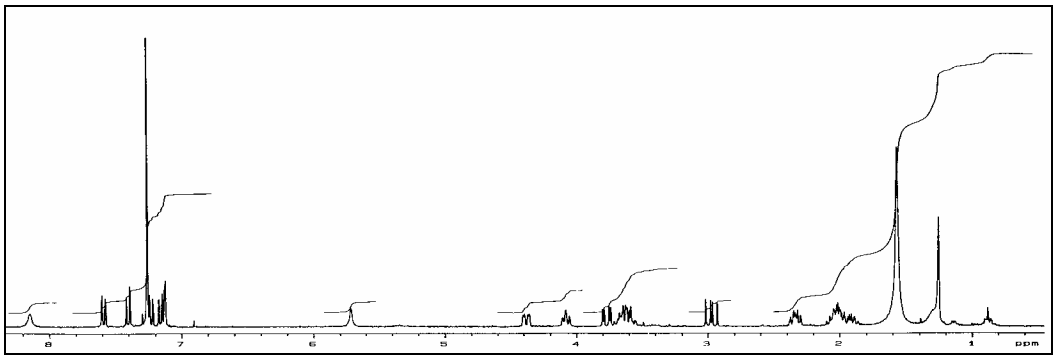
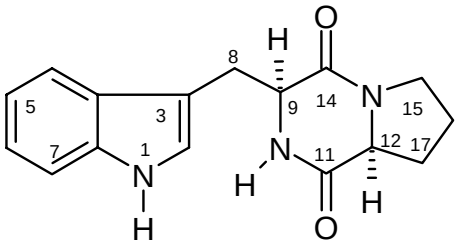


Figure 32: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of brevianamide F (**107**)

Compound **107** was isolated previously as a fungal metabolite of *Penicillium brevicompactum* by Birch and his co-workers[145] as a tryptophane-proline derivative. Compound **107** was also isolated from the marine sponge *Hyrtios altum.* The compound was isolated at the first time from a marine bacterium by Schröder[61] in our group. Compound **107** played a vital role as an intermediate in the biosynthesis of brevianamide A (**109**). In the presence of mevalonic acid, it is first converted to (1,1-dimethylallyl)tryptophyl-L-prolin (**108**) and then later into brevianamide A (**109**).



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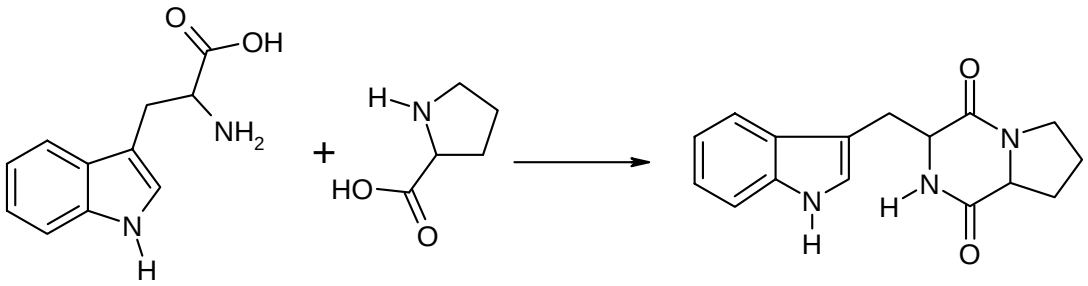

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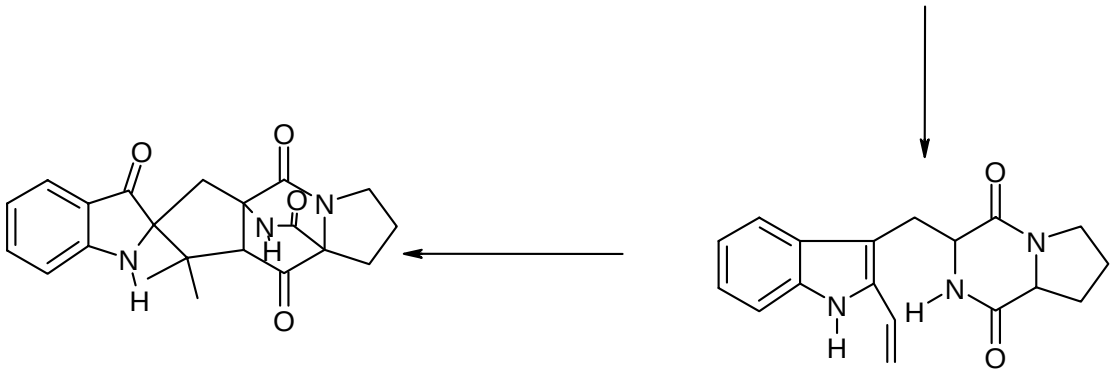


109 **108**

4.4 Terrestrial *Streptomyces* sp. GW3/1538

The terrestrial *Streptomyces* sp. GW3/1538 showed by TLC different UV absorbing zones at 254 nm which were coloured mostly brown to violet by spray re-

agents (anisaldehyde/sulphuric acid; Ehrlich's reagent). The biological screening proved a moderate activity of the crude extract against *Staphylococcus aureus*, *Escherichia coli,* and weak against *Streptomyces viridochromogenes* (Tü57), *Bacillus subtilis*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*.

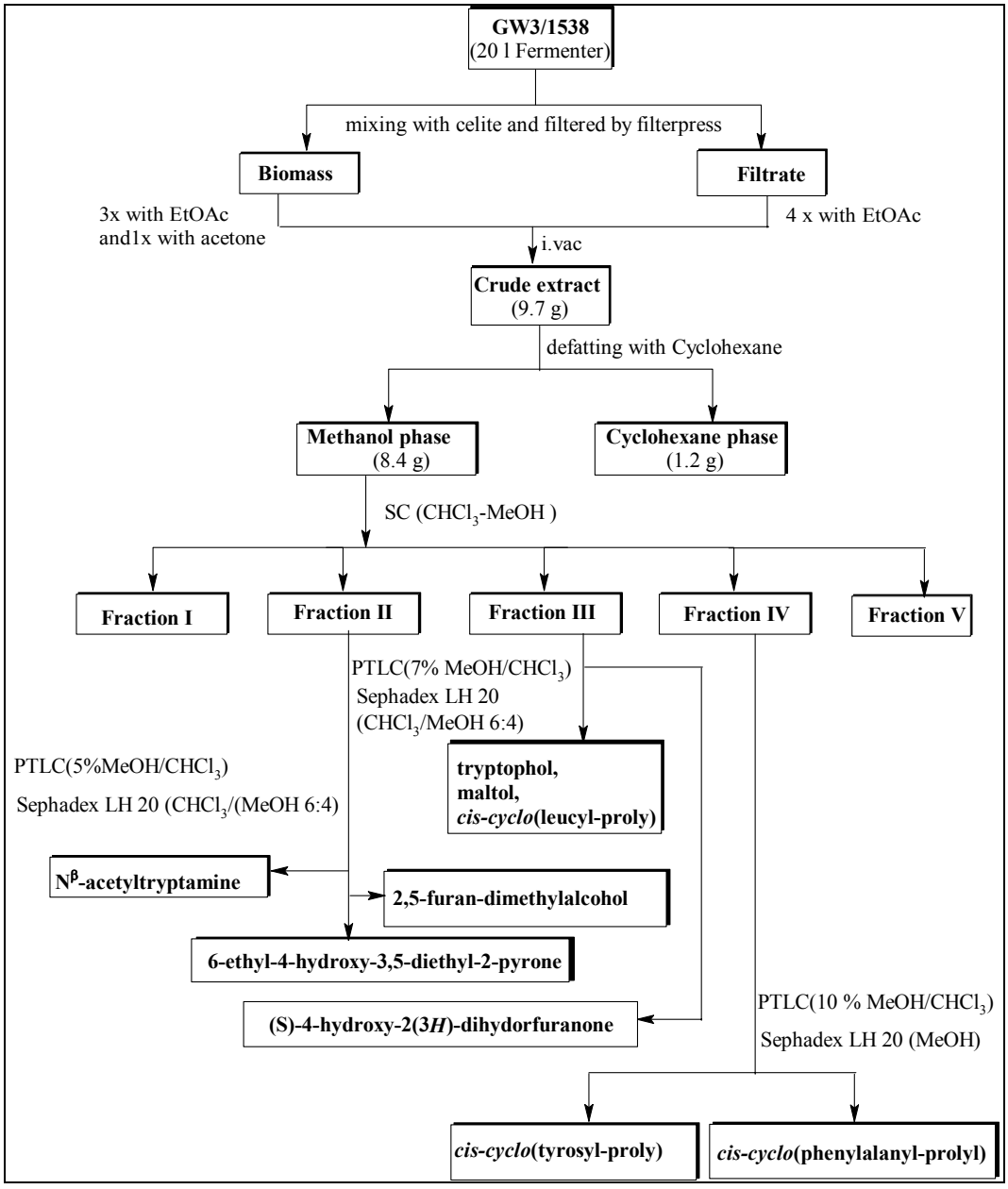


Figure 33: Work-up procedure of terrestrial *Streptomyces* isolate GW3/1538.

Fermentation of GW3/1538 was carried out in M_2 medium in a 20-liter fermenter for 3 days at 28 °C. The ethyl acetate extract was subjected to flash column chromatography on silica gel with a chloroform-methanol gradient to afford five fractions. Further purification of the fractions using PTLC and Sephadex LH-20 resulted in the isolation of N^{β}-acetyltryptamine (**34**), 6-ethyl-4-hydroxy-3,5-dimethyl-

2-pyrone (**113**), 2,5-furandimethanol (**114**), tryptophol (**44**), 3-hydroxy-2-methyl-γ-pyrone (**116**), *cis-cyclo*(leucyl-prolyl)(**119**), *cis-cyclo*(tyrosyl-proly) (**39**), *cis-cyclo*(phenylalanyl-proly) (**40**) and (S)-4-hydroxy-2(3*H*)-dihydrofuranone (**122**) (Figure 33).

4.4.1 6-Ethyl-4-hydroxy-3,5-dimethyl-2-pyrone

Compound **113** was found in fraction II as an UV absorbing band at 254 nm which showed no colour reaction with our spray reagents. It was isolated as a colourless solid.

The ^{1}H NMR spectrum displayed only three up-field spectral resonances in the aliphatic region. A 2H quartet at δ 2.55 (^{3}J = 7.4 Hz) of a methylene group attached to a sp^2 carbon atom and a methyl group at δ 1.20 (t, ^{3}J = 7.4 Hz) formed an ethyl group, two methyl groups each with 3H with singlets at δ 2.00 and 1.98 could be attached to olefinic, aromatic or carbonyl carbons.

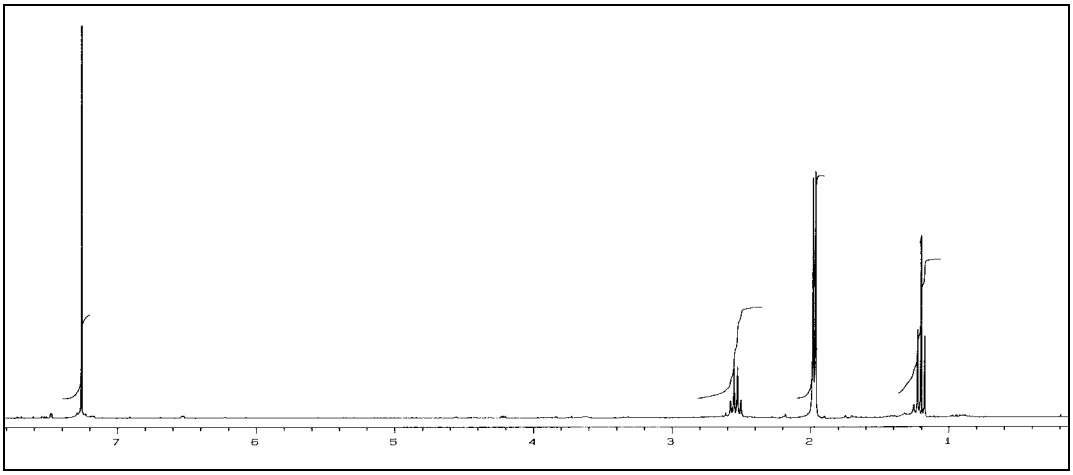


Figure 34: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 6-ethyl-4-hydroxy-3,5-dimethyl-pyron (**113**).

The ^{13}C/APT NMR spectra showed two quaternary carbons at δ 166.0 and 164.6, of which the first one could be assigned as a carbonyl group of ester, amide or carboxylic acid, while the other one (δ 164.6) is possibly of an oxy-sp^2 β-carbon conjugated with a carbonyl group, or as carbonyl group of acid or amide. Additionally, one quaternary phenolic carbon was observed at δ 160.1. Furthermore, two quaternary sp^2 carbons were displayed at δ 105.8 and 98.1. In the aliphatic region, it showed one methylene carbon at δ 24.2 adjacent to sp^2 carbon. Additionally, the spectrum exhibited three carbon signals at δ 11.7, 9.5 and 8.4, are corresponding to

three methyl carbons, at which the first two are possibly linked to olefinic carbons. The third one (δ 8.4) is corresponding to a methyl carbon at sp^3 carbon.

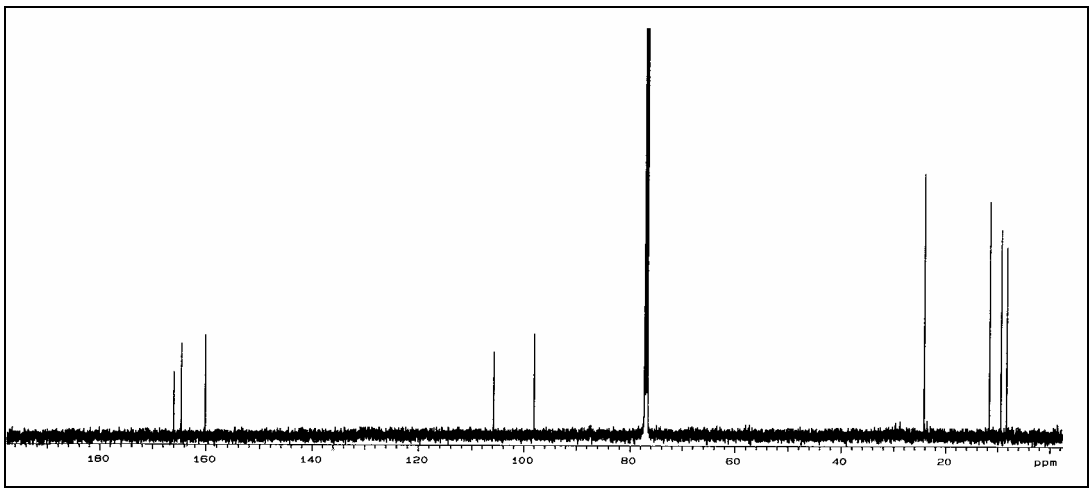
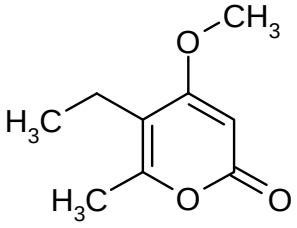


Figure 35: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of 6-ethyl-4-hydroxy-3,5-dimethyl-pyrone (**113**).

The molecular weight of the compound was established as 168 Dalton by CI MS. Based on the Rule of 13[146], this directed to the corresponding molecular formula $C_9H_{12}O_3$. A search in AntiBase led to four possible structures: 5-Ethyl-4-methoxy-6-methyl-2H-pyran-2-one; macommelin (**110**), nectriapyrone-B (**111**), 2,4-dimethylocta-(2E,4E)-dienoic acid amide (**112**) and 6-ethyl-4-hydroxy-3,5-dimethyl-pyron (**113**).

However, compounds **110**, **111**, **112** were excluded as they contain sp^2 CH groups which do not match with the spectra. Therefore the sole consistent structure is **113**, which was further confirmed by direct comparison with the literature[147]. Compound **113** was isolated first from *Emericella heterothalica* as a fungal metabolite but was not known from bacteria. Compound **113** was also synthetically obtained by condensation of three molecules of propionyl chloride[148].



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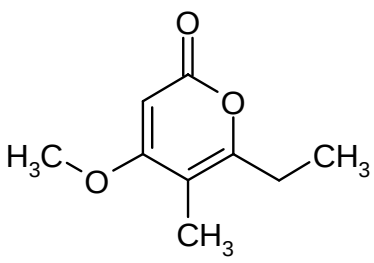

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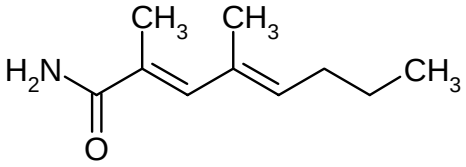

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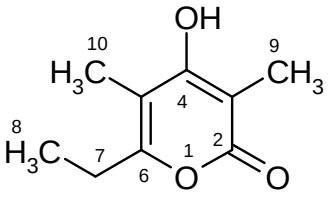


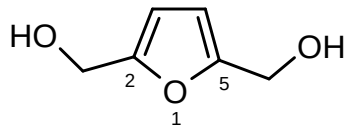
113

4.4.2 2,5-Furandimethanol

Compound **114** was isolated as colourless semi-solid from fraction II. It exhibited a weak band at 254 nm and gave an intensive brown colour with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum revealed three singlets between δ 6.20~4.40, the first of which at δ 6.18 (1H) could be due to an olefinic double bond or a hetero five-memberd heterocycle, a 1H triplet at δ 5.09 (^{3}J = 6.1 Hz) (OH) which disappeared in the presence of TFA, and a 2H doublet at δ 4.39 (d, ^{3}J = 6.1 Hz) of an oxygenated methylene group.

The ^{13}C NMR spectrum showed only three carbon signals. That at δ 154.0 was a quaternary sp^2 atom attached to a hetero atom (O; N), it showed an sp^2 methine carbon at δ 108.4, and finally sp^3 oxygenated methylene carbon at δ 56.8.

The EI mass spectrum revealed a molecular weight of 128 Dalton for **114**. The molecular ion lost a hydroxyl group to give a fragment at *m/z* 111; the spectrum showed a base peak at *m/z* 97 due to a loss of hydroxy methylene group (CH_2-OH). A search in AntiBase resulted in 2,5-furandimethanol (**114**) and (Z)-2-octen-1-ol (**115**).



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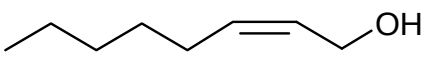


115

The olefin **115** exhibits several multiplets, both in the olefinic or aliphatic region of the ^{1}H NMR spectrum, and was easily excluded. Compound **114** was further confirmed by comparison with an authentic spectrum and the literature[114]. 2,5-Furandimethanole (**114)** has a moderate antimicrobial activity especially against fungi, *Nematospora coryli* and the yeast *Saccharomyces cerevisiae*[23].

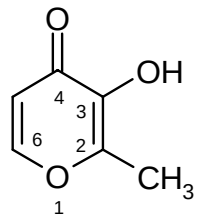
4.4.3 3-Hydroxy-2-methyl-γ-pyrone; Maltol

Working up of fraction III led to compound **116** as colourless solid and was UV absorbing at 254 nm. The ^{1}H NMR spectrum revealed two 1H doublets at δ 7.71 and 6.43 of an α,β-unsaturated lactone, in addition to the singlet of a methyl group, which must be positioned at an sp^2 carbon due to the shift of δ 2.38. The molecular weight of **116** was determined by EI mass spectrum as 126 Dalton. The spectrum showed an expulsion of 29 due to the loss of a CHO group and gave a peak at *m/z* 97.

A search in AntiBase resulted in two possible γ-pyrone isomers, 3-hydroxy-2-methyl-(4*H*)-4-one (**116**) and 2-hydroxy-3-methyl-(4*H*)-4-one (**117**). Comparison of the NMR data with an authentic spectrum and comparison with the literature[149-151] led to confirm the compound as 3-hydroxy-2-methyl-(4*H*)-4-on **(**maltol, **116**) (Table 5).

Table 5: ^{1}H NMR comparison of the maltol **116** and its isomer **117** with the literature [*J* in Hz]:

Position	**116** exp. ($CDCl_3$)	**116** ($CDCl_3$)Lit[149].	**116** (C_5D_5N) Lit[150].	**117** ([D_6]DMSO)lit.[151]
2	2.38 (s)	2.38 (s)	2.28 (s)	-
3	-	-	-	2.25 (s)
5	6.43 (d, *J* = 6)	6.43 (d, *J* = 6)	6.46 (d, *J* = 6)	6.32 (d, *J* = 5.8)
6	7.71 (d, *J* = 6)	7.71 (d, *J* = 6)	7.74 /d, *J* = 6)	8.01 (d, *J* = 5.8)



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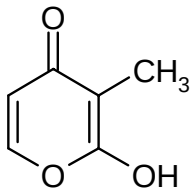
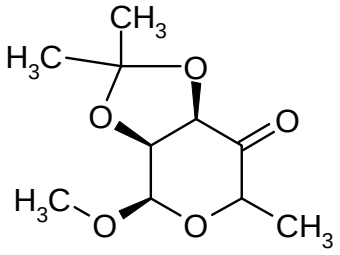


117



118

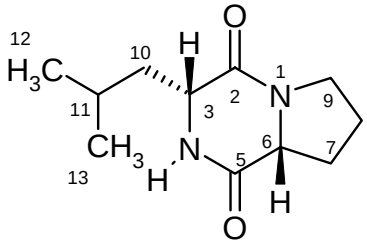
Maltol (**116**) is known as plant metabolite e.g. from fern leaves and larch bark *(Larix deciduas*), and occurs in food materials[152]. Compound **116** is of great value as flavouring agent in food industry as well as pharmaceutical formulations[24]. Therefore, several approaches to synthesize **116** have been published, one of which was carried out by Chawla *et al.*[153] during the hydrolysis of 2,3-O-isopropylidene-6-deoxy-α-L-lyxo-hexopyran-4-ulose (**118**).

4.4.4 *cis-Cyclo*(Leucyl-Prolyl)

During the isolation of maltol (**116**), an additional compound **119** was isolated as a colourless solid from fraction III. It showed a violet colour reaction with anisaldehyde/sulphuric acid and turned blue with chlorine/*o*-anisidine, pointing to a peptide. The ^{1}H NMR spectrum showed a broad 1H singlet of an amide at δ 5.98, two methine protons at δ 4.13 (t), and 4.02 (dd). Furthermore, a 2H multiplet between 3.64-3.53 (9-H_2), a 1H multiplet at δ 2.45-2.28 (10-H_a), and a multiplet between δ 2.24-1.64 of 5H were observed. In addition, a multiplet of 1H at δ 1.61-1.45 (7-H_b), and two doublets each of 3H as of two equivalent methyl groups were found at δ 1.00 and 0.96, delivering an isopropyl system.

The ^{13}C/APT NMR spectra displayed two quaternary carbons of two CO groups at δ 170.3 and 166.1. In the aliphatic region, two methine carbon signals at δ 59.0 and 53.4 linked to a hetero atom were visible. Four methylene carbons at δ 45.5, 38.6, 28.1, and 22.7, as well as a third methine carbon at δ 24.7 were observed. It showed finally two methyl carbons at δ 23.3 and 21.2 of the previously mentioned isopropyl group.

The molecular weight of compound **119** was determined as 210 Dalton by CI and EI mass spectra. The EI spectrum showed a base peak at *m/z* 154 due to the loss of an isobutyl group. The fragment at *m/z* 154 was observed during the EI fragmentation of the diketopiperazine analogues containing proline, and assigned as prolylglycinyl ion. A search in AntiBase resulted in two possible structural isomers; *cis-cyclo*(leucyl-prolyl) (**119**) and *trans-cyclo*(leucyl-prolyl) (**120**). Comparison of the spectral data with the literature pointed to *cis-cyclo*(leucyl-prolyl)[154,82] (**119**). Compound **119** was isolated from cultures of *Norcardia restrica*[155] and reported as one of several phytotoxines produced by *Guignardia laricina*, and *Ceratocystis* sp.



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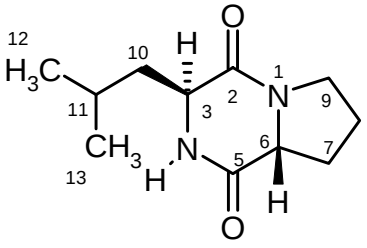


120

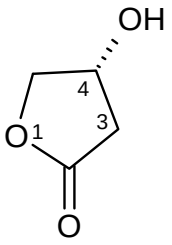
4.4.5 (*S*)-Dihydro-4-hydroxy-2(3H)-furanone

The oily colourless compound **122** was found in fraction III. The molecular weight was determined as 102 Dalton by CI mass spectrum. The ^{1}H NMR spectrum showed in the aliphatic region three different spectral resonances: one 1H multiplet at δ 4.72 (3-H), the ABX signals of a methylene group at δ 4.41 and 4.27 which could be due to an oxygenated methylene group in a ring system. An additional AB signal at δ 2.78 and 2.52, was due to an another methylene group. The spectral pattern seemed to indicate a cyclic five-memberd lactone ring. The ^{13}C NMR spectra of **122** exhibited four carbon signals, from which a quaternary carbon at δ 175.5 is attributed to a carbonyl group of amide or ester moiety. Three sp^3 carbon signals were detected at δ 76.7 (CH), 67.6 (CH_2) and 37.8 (CH_2), from which the first two could be of oxygenated methine and methylene groups, while the residual one could indicate a methylene carbon linked with sp^2 carbon.

A search in AntiBase led to no hits, pointing to a new natural product. However, a search in the Dictionary of Natural Products (DNP) resulted in (*R*)-4-hydroxy-2(3*H*)-dihydrofuranone (**121**) which was obtained as an acid hydrolysis product of anhydrooscillatoxin A[156]. Additionally, (*S*)-(-)-3,4-dihydroxybutanoic acid-γ-lactone (**122**) was obtained as synthetic product starting with (*S*)-malic acid[156]. The last compound (**122**) was recently obtained as secondary metabolite from the marine blue-green alga *Lyngbya majuscula* from the Hawaiian Islands[157]. Based on the comparison, the ^{1}H NMR spectral data of both isomers **121** and **122** are identical. However, throughout the optical rotation, they were distinguished. Compound **122** exhibited a negative rotation ($[\alpha]^{20}_{D}{}^{EtOH}$ -19.6°). Comparison of the reported optical rotation of **121** and **122** with the measured value of compound **122** directed to (*S*)-4-hydroxy-2(3*H*)-dihydrofuranone (**122**) (Table 6). This indicated that C-4 is above the C(3)-C(2)-O(1)-C(5) plane of the lactone ring in compound **122**. Both optical isomers **121** and **122** were found as parent structures of ca. 200 natural compounds produced by microorganisms[23].

Table 6: CD spectral data of the reported isomers **121** and **122** compared with the experimental value of **122**

Compound	$[\alpha]_D$	Solvent	*c* (mg/ml)
121[156]	+ 44°	EtOH	0.36
122[157,156]	-80; -72°	$CHCl_3$; EtOH	0.50
122 Exp.	-19.6°	EtOH	0.665



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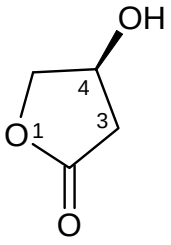


122

4.5 Strain Bio134

The ethyl acetate extract of the North Sea bacterium isolate Bio134 drew our attention due to the presence of several UV absorbing zones, from which a middle polar zone turned to blue with anisaldehyde/sulphuric acid. The strain exhibited weak biological activity against *Mucor miehei* (Tü284), *Candida albicans*, and the alga *Chlorella sorokiniana*.

Well-grown agar cultures of Bio134 were used to inoculate twelve of 1 liter-Erlenmeyer flasks each containing 250 ml of LB medium+50% Sea water. The flasks were incubated at 28 ºC on a shaker for 3 days. The culture broth was used to inseed a 25-liter fermenter which was kept for additional 3 days at 28 °C. After filtration, the water phase and biomass were extracted with ethyl acetate separately. The biomass extract showed no interesting compounds during TLC and was not further investigated therefore.

The extract from the filtrate was defatted with cyclohexane and subjected to flash silica gel column chromatography to give four fractions. Working up of the fractions resulted in the isolation of 13-methyltetradecanoic acid (**123**), N^{β}-acetyltryptamine (**34**), *cis-cyclo*(phenylalanyl-prolyl) (**40)**, anthranilic acid (**48)**, pyrrol-2-carboxylic acid (**125)**, phenyl acetic acid (**49a**), *cis-cyclo*(tyrosyl-prolyl) (**39)**, uracil (**52)**, thymine (**53**), and adenine (**175**), in addition to the new 3'-acetoxy-2'-deoxy-thymidine (**124**) (Figure 36).

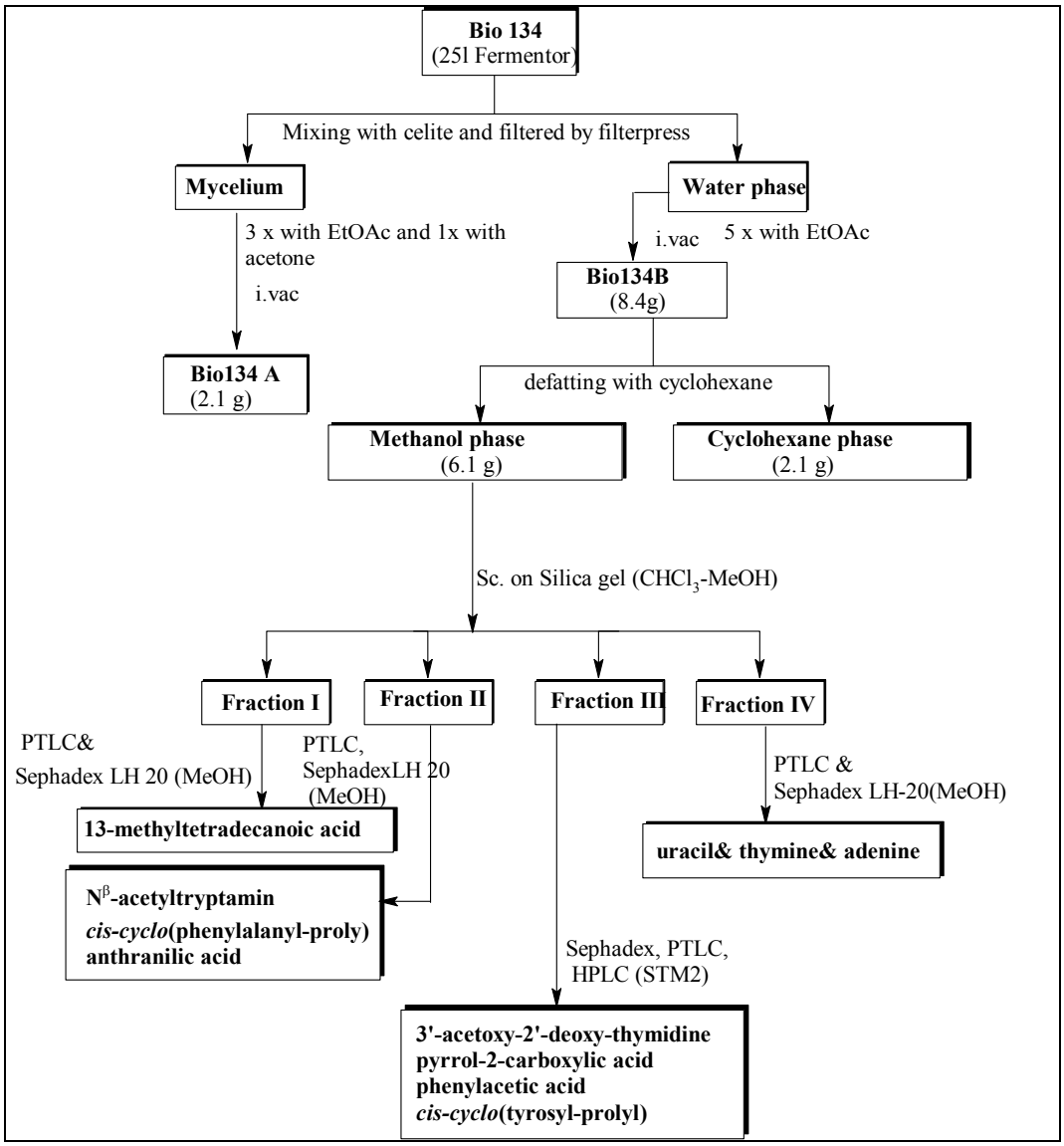


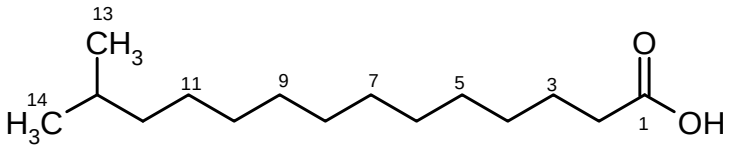
Figure 36: Work-up procedure of the marine bacteria isolate Bio134

4.5.1 13-Methyltetradecanoic acid

The fast moving compound **123** was isolated from fraction I by PTLC and Sephadex LH-20 as a colourless solid. The compound exhibited a violet colour reaction with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum showed a broad singlet of an acidic group at δ 10.38. In addition, several multiplets were found in the aliphatic region, one 2H triplet at δ 2.25 was of a methylene adjacent to sp^2 carbon. Furthermore, a 1H multiplet at δ 1.55-1.43, 6H doublets of two equivalent methyl groups at δ 0.83 (^3J = 7.7 Hz) were characteristic for an isopropyl group. The spectrum showed additionally, multiplets between δ 1.58~1.10 with an intensity of 20 H, according to 10 methylene groups, as indication of a long acyl chain.

The molecular weight of compound **123** was established as 242 Dalton by the EI spectrum, at which the molecular ion (*m/z* 242) afforded several consecutive fragments each with a difference of 14 Dalton corresponding to CH_2 groups, as it is characteristic for lipids. A search in AntiBase led to 13-methyltetradecanoic acid (**123**). Compound **123** exhibits an activity against *Fusarium* sp[23]. It is widespread in nature and especially in bacteria [24].



123

4.5.2 3'-Acetoxy- 2'-deoxy-thymidine

Compound **124** was a colourless solid obtained from fraction III by PTLC and Sephadex LH-20. It was observed as a middle polar UV absorbing band (254 nm), which gave a blue colour with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum showed a broad singlet at δ 8.45 of an acidic proton, at δ 7.45 the 1H singlet of a methine proton, two singlets each with intensity of two methyl groups at δ 2.10 and 1.90 were seen, which all must be attached to sp^2 carbon moieties. Furthermore, a triplet of 1H was observed in the sugar region at δ 6.22 (^3J = 7.2 Hz), two multiplets each of 1H at δ 5.33 and 4.08 could be assigned to two oxygenated methine protons. In addition, two 2H multiplets of two methylene groups were observed at δ 3.90 and 2.30, from which the first one could be assigned to an oxygenated methylene group.

The ^{13}C/APT NMR spectra showed, in addition to one sp^2 CH carbon signal at δ 136.3, four quaternary carbon signals at δ 170.7, 163.6 and 150.4 and 111.4. The first two of the previous four carbons could be of carbonyl groups as in an ester, acid, or amide, while the third one could be an sp^2 carbon linked to a hetero atom.

In the aliphatic region, three sp^3 oxygenated methine carbon signals at δ 86.8, 85.0 and 74.7 were exhibited. Two methylene carbon signals were observed, one at δ 62.6 of an oxygenated methylene, and the other at δ 37.2. The spectra exhibited furthermore two methyl carbon signals at δ 12.6 and 11.7.

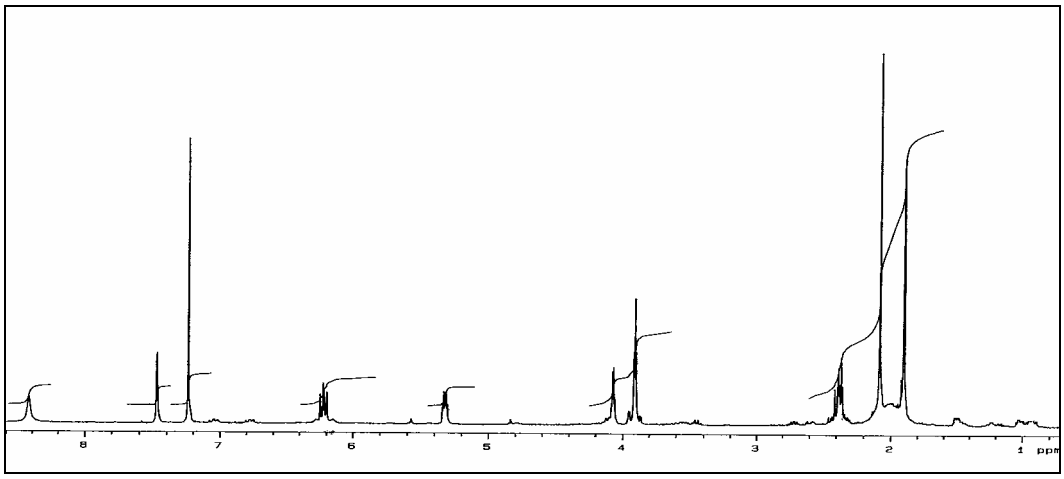
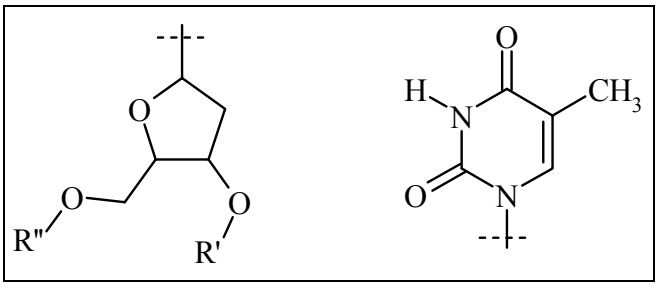


Figure 37: ^{1}H NMR ($CDCl_3$, 300 MHz) of 3'-acetoxy-2'-deoxy-thymidine (**124**)

The molecular weight of **124** was determined as 284 Dalton by CI and EI spectra. EI mass spectrum showed furthermore two fragments at *m/z* 159 and 126. The first fragment of which is could be assigned as furanosyl acetate group **(A**), and the other one as thymine (**B**) (Figure 38). HREI MS of compound **124** established its molecular formula as $C_{12}H_{16}N_2O_6$.



A: R' or R" = $COCH_3$ **B**

Figure 38: Partial fragments **A** and **B** of 3'-acetoxy- 2'-deoxy-thymidine (**124**).

Applying the above data of compound **124** to AntiBase resulted in no hits, pointing to a new natural product. So, the above partial structures **A** and **B** were further confirmed by the 2D correlations.

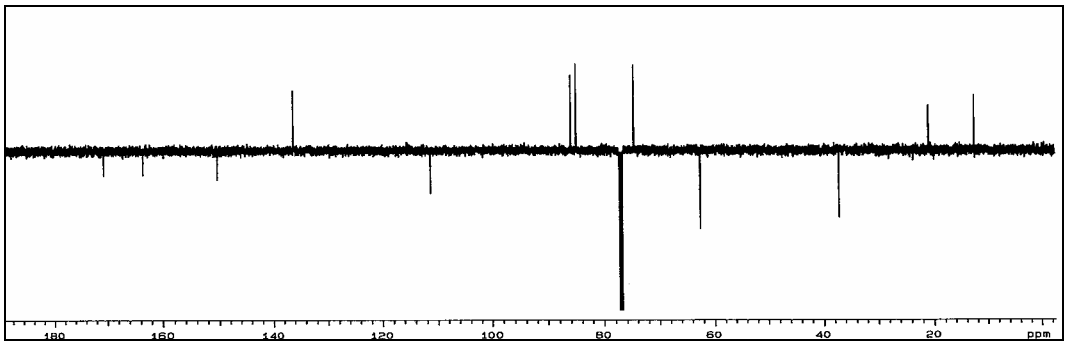


Figure 39: APT NMR spectrum ($CDCl_3$, 300 MHz) of 3'-acetoxy-2'-deoxy-thymidine (**124**).

The H,H COSY spectrum (Figure 41) showed a long range coupling (^4J) between the thymine proton (δ 7.45) and the methyl group at δ 1.90. In the sugar moiety, the triplet proton (δ 6.22) showed a coupling (^3J) with the methylene protons at δ 2.33, and the latter showed a coupling with the proton at δ 5.30. The proton at δ 4.08 displayed a coupling (^3J) with the methylene group at δ 3.90 (Figure 40).

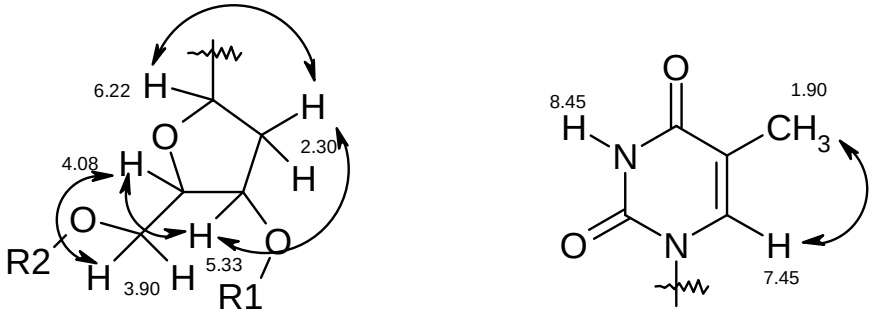


Figure 40: H,H COSY correlations of the fragments **A** and **B** of 3'-acetoxy-2'-deoxy-thymidine (**124**).

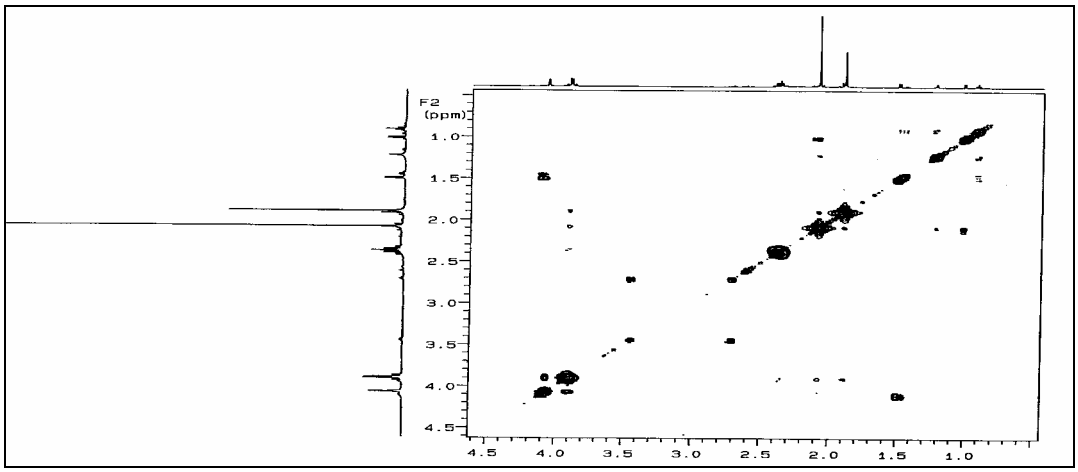


Figure 41: H,H COSY spectrum ($CDCl_3$, 500 MHz) of 3'-acetoxy-2'-deoxy-thymidine (**124**).

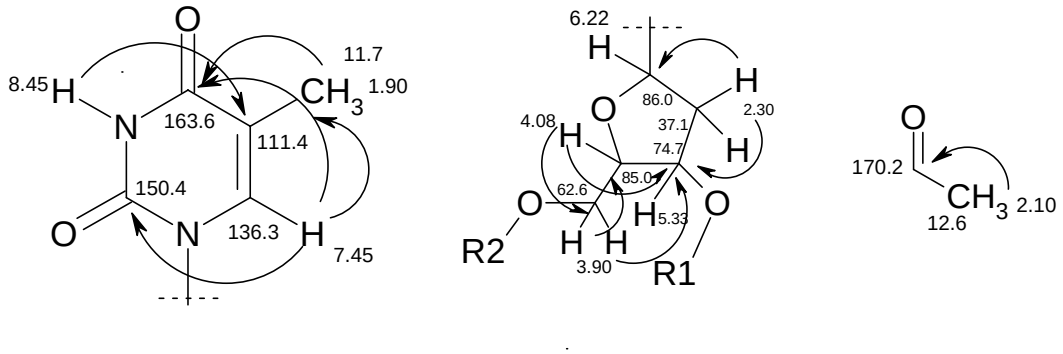


Figure **42**: HMBC correlations of the constituents of 3'-acetoxy-2'-deoxy-thymidine (**124**).

In the HMBC spectrum, the proton at δ 7.45 (C-2) showed a coupling (^3J) with the carbon at δ 86.0 (C-1') of the sugar moiety, and *vice versa*, confirming the attachment of the sugar part to thymine at N-1 position and not at N-3 position. The attachment of the acetyl group in the sugar moiety was confirmed at position-3' due to the presence of a long range correlation (^4J) between the methyl protons at δ 2.10 and the carbon at δ 74.7 (C-3') (Figure 44), confirming compound **124** as 3'-acetoxy-2'-deoxy-thymidine, a new natural product.

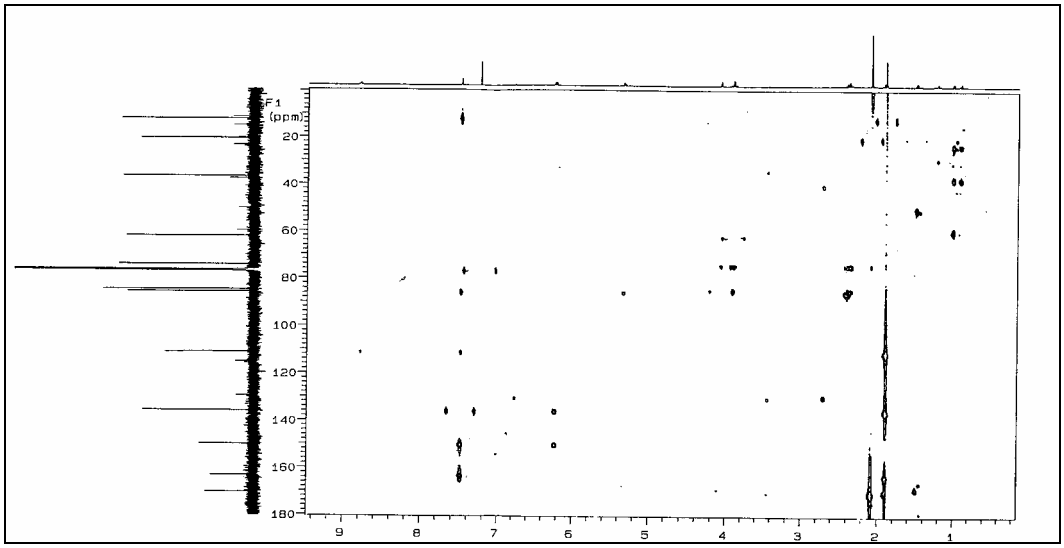


Figure **43**: HMBC spectrum ($CDCl_3$, 150 MHz) of 3'-acetoxy-2'-deoxy-thymidine (**124**).

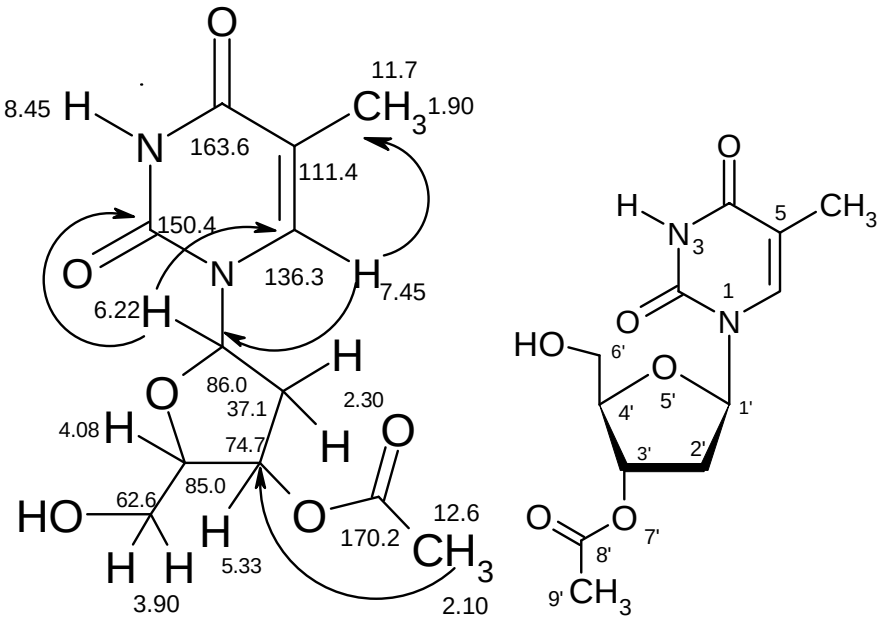
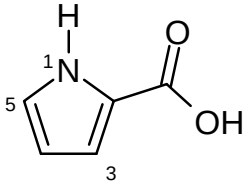


Figure **44**: Selected HMBC correlations of 3'-acetoxy- 2'-deoxy-thymidine (**124**).

4.5.3 Pyrrole-2-carboxylic acid

By further working up of fraction III, a colourless solid of compound **125** was obtained. It showed an UV absorbing band at 254 nm and turned to orange-brown with anisaldehyde/sulphuric acid. On the basis of NMR and mass spectra (*m/z* 111 Dalton), a search in AntiBase pointed to pyrrole-2-carboxylic acid (**125**) or pyrrole-3-carboxylic acid (**126**), respectively. By direct comparison the data with authentic spectra of both isomers, pyrrole-2-carboxylic acid (**125**) was established. Pyrrole-2-carboxylic acid (**125**) is naturally known from *Streptomyces*, and it was isolated first from *Streptomycetes griseoflavus* in 1983 by G. Höfle *et al*.[158].



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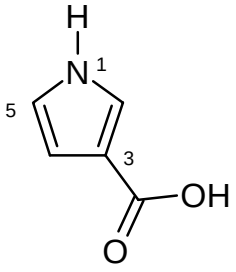


126

4.6 Marine *Streptomyces* sp. B8876

The marine *Streptomyces* sp. isolate B8876 was found to exhibit a moderate biological activity against *Escherichia coli* and *Bacillus subtilis*. Analytical TLC of the

ethyl acetate extract showed a reddish-pink band that gave a yellow colouration with NaOH. Additionally, other UV absorbing bands at 254 nm changed to violet by spraying with anisaldehyde/sulphuric acid.

The producing organism was cultivated in 12 of 1 liter-Erlenmeyer flasks each containing 250 ml of M_2^+ medium (50% artificial sea water) at 28 °C for 3 days. This small culture was used to inoculate a 20-liter jar fermenter with M_2^+ medium under the same conditions. After filtration, extraction and evaporation, the crude extract was subjected to silica gel column chromatography using a gradient of chloroform/methanol as eluting solvent. Fraction II was purified to give undecylprodigiosin (**128**), while working up of the fractions III-IV resulted in the isolation of the known metabolites N^{β}-acetyltryptamine (**34**), indolyl-3-carboxylic acid (**45**), 2,5-furandimethanol (**114**) and thymine (**53**) (Figure 45).

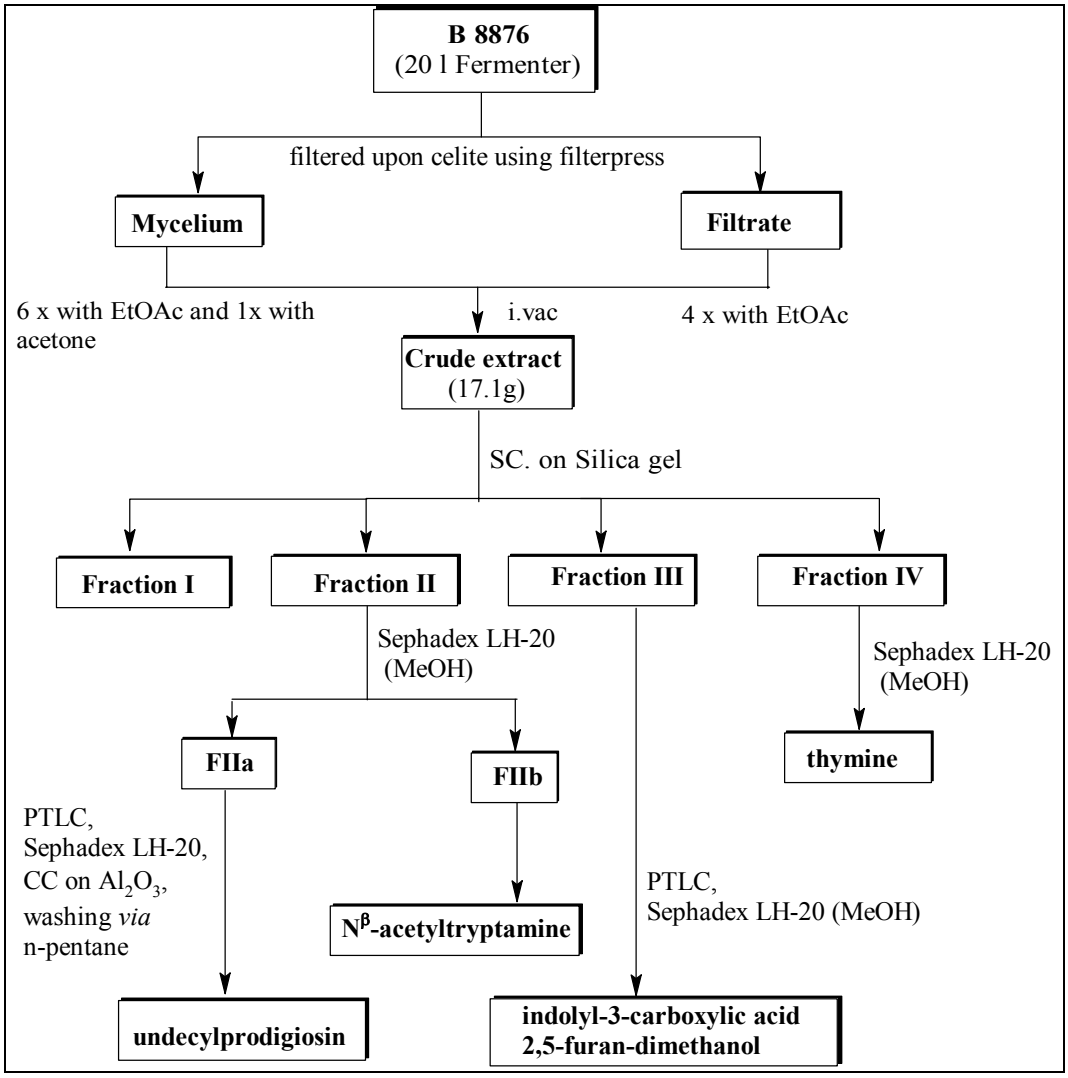


Figure 45: Work-up procedure of the marine *Streptomyces* isolate B8876.

4.6.1 Undecylprodigiosin

TLC of fraction II exhibited a pink band of a non polar compound, which was chromatographed on Sephadex LH-20. The resulting sub-fraction IIa was further purified by PTLC, followed by basic alumina column chromatography to give **128** as a dark violet solid. It showed a colour change to yellow by treatment with NaOH as an indication of prodigiosins.

The ^{1}H NMR spectrum displayed three 1H singlets of acidic protons at δ 12.93, 12.75 and 12.70. In the aromatic and olefinic region, it exhibited seven different resonance groups each of 1 H, from which two were singlets at δ 6.08 and 7.00, and the other five were exhibited as ddd signals at δ 7.25, 6.94, 6.85, 6.35 and 6.20. Their small coupling constant (<4) is characteristic for five memberd heterocyle (e.g. pyrroles). The aliphatic region exhibited a 3H singlet at δ 4.04 (OCH_3), and its low field shift is due to an adjacent sp^2 atom. In addition, a 2H triplet at δ 2.95 was observed, several multiplets of 18 protons (8 CH_2) between δ 1.78 ~1.30, and a triplet of a terminal methyl group were observed at δ 0.88, to give a chain of $C_{11}H_{23}$.

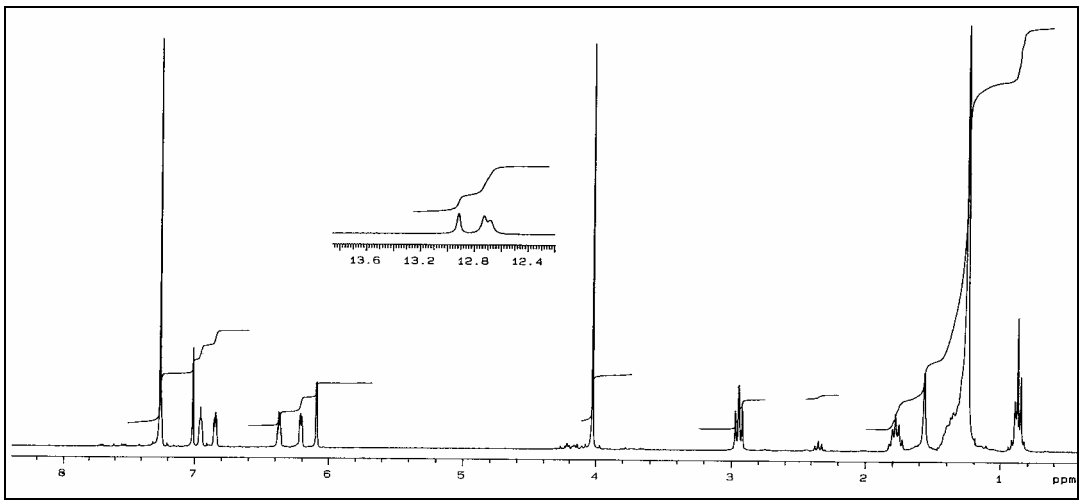
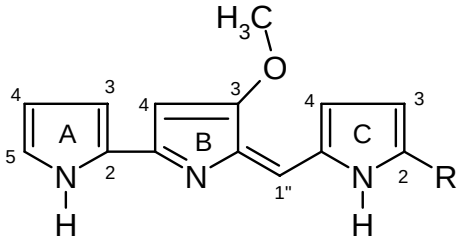


Figure 46: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of undecylprodigiosin (**128**).

The molecular weight of compound **128** was determined as 393 by EI mass spectrum. The spectrum showed a base peak at *m/z* 252 characteristic for the prodigiosin parent fragment (**127,** R = CH_2). There is a significant peak at *m/z* 238 corresponding to the loss of $C_{11}H_{23}$. This confirmed the presence of the undecyl-chain attached to the prodigiosin chromophor **127** [159,160]. The observed loss of $C_{11}H_{23}$ in the mass spectrum is thus indicative of an undecyl side chain. Compound **128** was established as undecylprodigiosin which was further confirmed by comparison of the

data with the literature[161]. Undecylprodigiosin is one of 16 natural prodigiosins which were isolated from microorganisms[23].

Prodigiosin **128** was isolated and identified first by Wasserman *et al.* as well as Harashima from various *Actinomycetes*[161-163], and by Gerber from *Actinomadura* (*Nocardia*) *pelletieri*[164], and frequently in our group. It has antimalarial activity[24] and exhibits a weak activity against Gram-positive bacteria as well as fungi[164]. Prodigiosin itself (methylpentyl-prodiginine) has considerable antibiotic and antifungal activity, but high toxicity precludes its use as a therapeutic agent[165]. The biosynthetic origin of prodigiosins was proposed by Gerber[166] (Figure 47).



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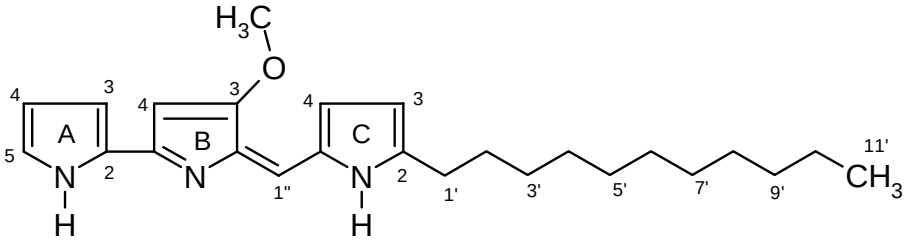

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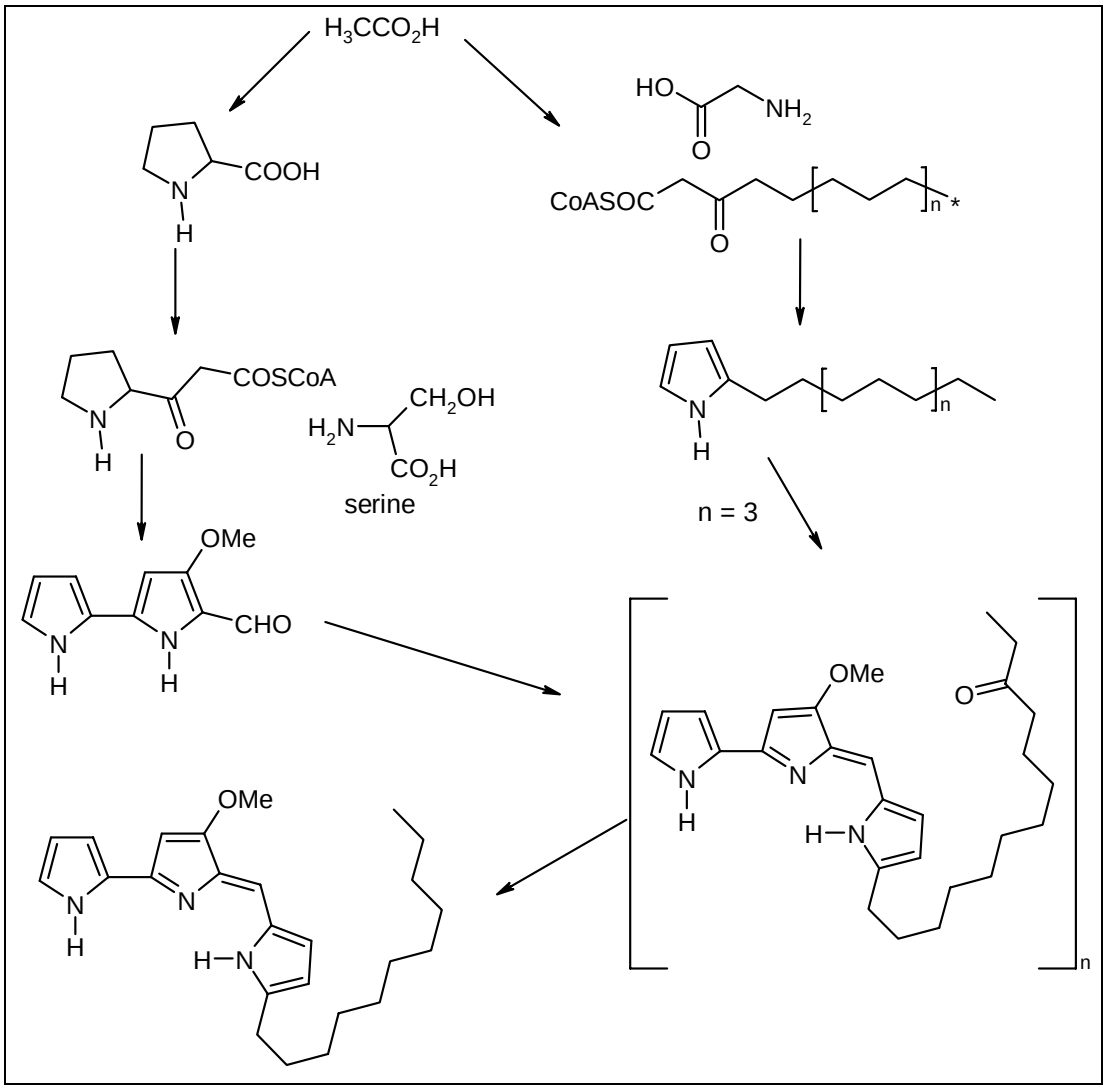


128

Figure 47: A possible biogenetic route to the actinomycete prodiginines.

4.7 Marine *Streptomyces* sp. B7936

The crude extract of the marine *Streptomyces* sp. B7936 exhibited high activity against *Escherichia coli*, *Bacillus subtilis*, *Mucor miehei* (Tü284), *Streptomyces viridochromogenes* (Tü57), moderate activity against *Candida albicans*, *Staphylococcus aureus*, the algae *Chlorella vulgaris*, and *Chlorella sorokiniana*, and weak activity against *Scenedesmus subspicatus*. TLC analysis of the extract showed three different coloured zones between orange to yellow, among which a greenish UV fluorescent (366 nm) polar band turned to blue with anisaldehyde/sulphuric acid. In addition, the yellow zone turned to brown with sulphuric acid but did not change with dilute sodium hydroxide solution.

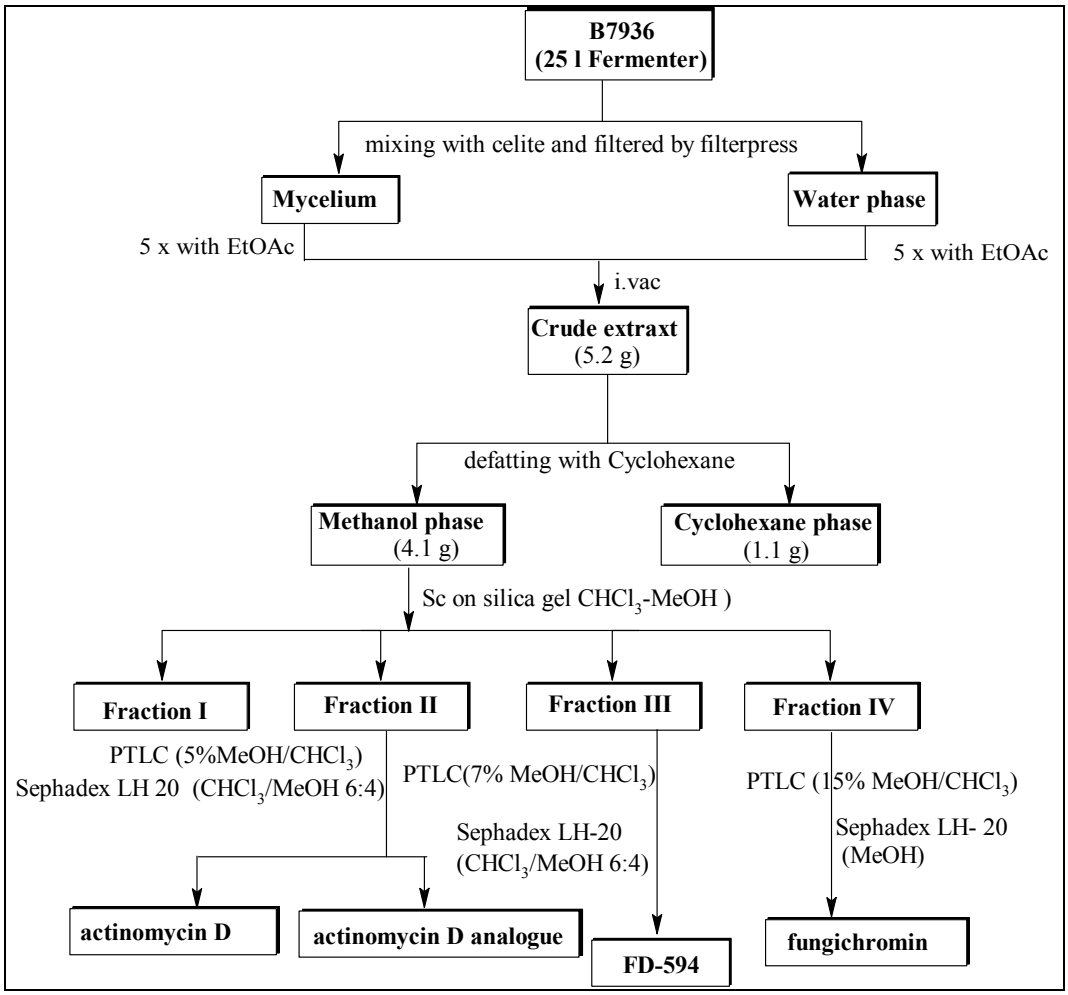


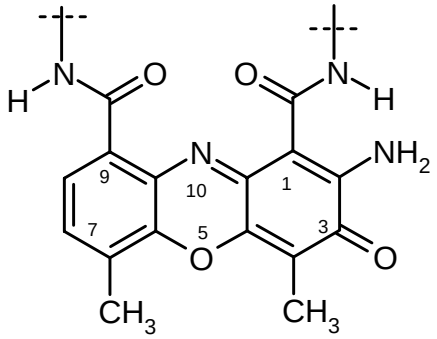
Figure 48: Working-up procedure of *Streptomyces* sp. B7936

Fermentation of the strain was carried out primarily in shaker flasks on a small scale on M_2^+ medium (50% artificial Sea water) for three days at 28 °C. The seed culture was used to inoculate a 25-liter jar fermenter. After 3 days, the culture was extracted with ethyl acetate. The reddish-brown crude extract was defatted, and applied to flash silica gel column chromatography, eluting by chloroform-methanol-gradient to give four fractions. Purification of the fractions II-IV led to the isolation of actinomycin D (**130**), actinomycin D analogue (**131**), FD-594 (**135**) and fungichromin (**138a**).

4.7.1 Actinomycin D

Compound **130** was obtained as major product from a less polar reddish orange zone in fraction II, which showed no colour change with NaOH but changed to red with anisaldehyde/sulphuric acid. It formed a red solid after purification on Sephadex and several times by PTLC, which gave an additional minor product **131**.

The ^{1}H NMR spectrum of **130** showed broad doublets at δ 8.13, 7.97 and 7.80 of three amide protons, two *ortho* coupled protons at δ 7.65 and 7.37 of a 1,2,3,4-tetrasubstituted aromatic ring, and two 3H singlets at δ 2.57 and 2.23 of methyl groups in *peri*-position of an aromatic system. This is characteristic for the phenoxazinone chromophore (**129**) in actinomycins.



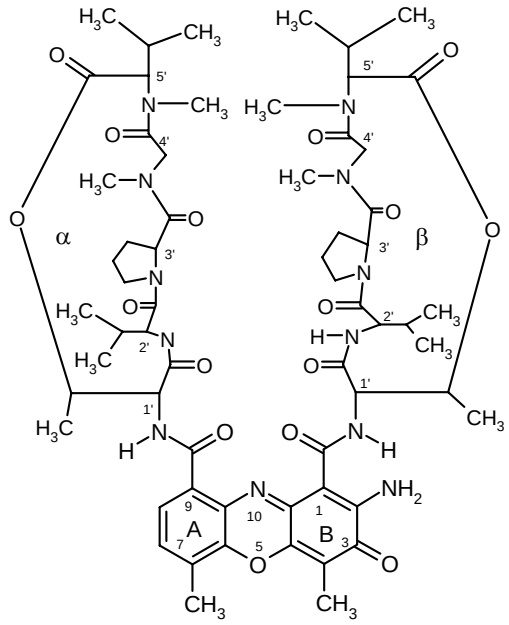
129

In addition, the spectrum showed overlapping NMR signals between δ 7.20 – 0.75 as it is indicative for a complex peptide. One broad 1H doublet at δ 7.20 (^{3}J = 6.4 Hz), 8 hydrogen signals of oxygenated or α-amino acid protons were observed at δ 6.03 (d), 5.96 (d), 5.25-5.15 (m, 2 H), 4.78 (d), 4.73 (d), 4.61 (dd) and 4.49 (dd). Unresolved signals of methylene and methine groups with an intensity of further 8H were observed between δ 4.03-3.31. Additionally, four singlets with intensity of 12H of four methyl groups were found between δ 2.93-2.88 and may be due to N-methyl groups, multiplets of several methylene groups of rings with intensity of 6H appear between δ 2.65-2.57, multiplets between δ 2.38-1.76 of 8 H, and 1.28 (m) of 6H as of two methyl groups. In addition, multiplets between δ 1.17-1.07 with intensity of two methyl groups and δ 0.99-0.85 for 12H as for 4 methyl groups, and finally a doublet of two methyl groups at δ 0.75 (^{3}J = 4.9 Hz) were exhibited.

The ^{13}C/APT NMR spectra exhibited 11 signals between δ 100-150 and one at δ 180, which are attributed to the twelve ring-carbon atoms of the phenoxazinone system. Twelve sp^2 carbonyls were observed around 170 and are characteristic to those of amide and lactone-carbonyl groups of a peptide system. The α-carbon atoms of the amino acids are shown in the region (δ 50-73). In addition, two signals of oxygenated methine carbons are at δ 74-76.

The ESI mass spectra determined the molecular weight of **130** as 1255 Dalton in (+)-ESI and *m/z* (-)-ESI modes.

A search in AntiBase resulted in actinomycin D (**130**). Compound **130** was further confirmed by comparing the spectra with an authentic one as well as the literature[167,61].



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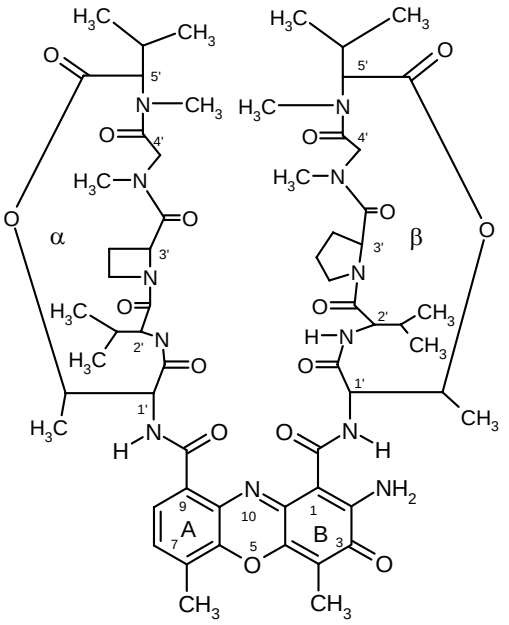


131

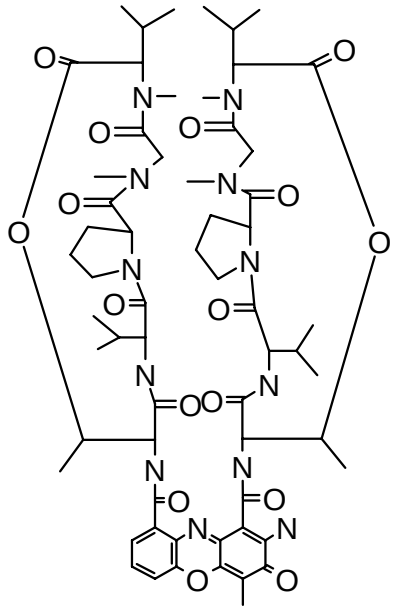
The orange-red actinomycins are a family of chromopeptide antitumor antibiotics isolated from various *Streptomyces* strains, of which more than 70 native and many synthetic variants are known[168-170]. They are known since 1940 when Waksman[171] and Woodruff isolated actinomycin A, as first ever crystalline antibiotic. Actinomycin complexes termed A, B, C, D, I, X, Z, as well as other actinomycin analogues have been reviewed[172]. The natural actinomycins all share the same phenoxazinone chromophore, varying only in the amino acid content of their two depsipentapeptide moieties. Actinomycins C_3 and D have found clinical application as anticancer drugs, particularly in the therapy of Wilm's tumors[173], and soft tissue sarcoma[174] of children, and are still of interest in molecular biology[175]. Recently, actinomycin D has been proposed as a therapeutic agent for AIDS, because it is a potent inhibitor of HIV-1 minus-strand transfer[176].

4.7.2 Actinomycin D analogue

Compound **131** was isolated along with compound **130** as a red solid phenoxazinone derivative, where the ^{1}H NMR spectrum showed a high similarity to that of

130. The molecular weight of compound **131** was determined as 1240 Dalton by ESI mass spectra and is by 14 au (CH_2) lower than that of **130**.

A search in AntiBase gave three possible structures: Azetomycin I (**131**), aurantin III[177] (**132**) and actinomycin D_0[178] (**133**). The first two actinomycins were previously isolated from *Streptomyces antibioticus* and *Streptomyces aurantiacus*, respectively, while the third one was found in *Actinomyces olivobrunneus*. However, azetomycin I (**131**) required a medium containing L-azetidine-2-carboxylic acid (**134**) or its derivatives, and was excluded therefore. Aurantin III (**132**) showed a replacement of the methyl group at 6-position in phenoxazinone chromophore (**129**) by a hydrogen, compared with actinomycin D, while the N-methyl group of valin (*β*-ring) was replaced by H in actinomycin D_0. Therefore, aurantin III[177] (**132**) or actinomycin D_0 (**133**) is supposed. Due to the small amount, no further attempts were made to differentiate between both structures.



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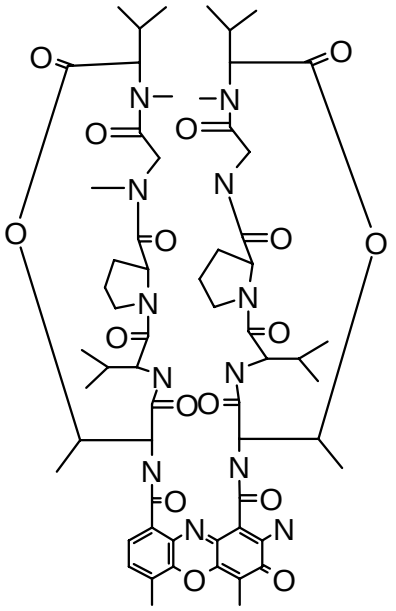

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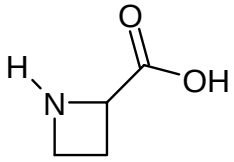
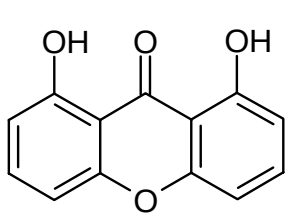


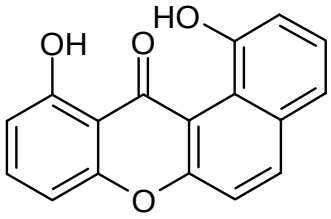
134

4.7.3 FD-594

In fraction III, a middle polar yellow zone of compound **135** was found which turned to brown by treatment with conc. sulphuric acid. It exhibited additionally a yellowish-green colour with Ehrlich's reagent, however, no colour change with sodium hydroxide or anisaldehyde/sulphuric acid was observed. PTLC and Sephadex LH-20 delivered a yellow solid. This pointed to compound **135** possibly as *peri*-hydroxy xanthone moiety, present in linear or angular form (**A**, **B**).



A



B

The ^{1}H NMR spectrum showed three *peri*-hydroxyl protons at δ 12.37, 11.98 and 11.88. In the aromatic region, it displayed two doublets each of 1H at δ 7.56 (d, ^{3}J = 9.1 Hz), and 6.96 (d, ^{3}J = 9.1 Hz) of a 1,2,3,4-tetrasubstituted benzene ring. An additional aromatic singlet of 1H was observed at δ 7.09 (H-5). In the sugar and aliphatic region, it showed three anomeric protons at δ 5.14 (dd, J = 9.7, 2.0 Hz), 4.51 (m) and 4.50 (dd, J = 9.8, 1.8 Hz), pointing to three sugar moieties in compound **135**. Between δ 4.8-3.0, it exhibited 12 oxygenated methine protons, two singlets of methoxy protons at δ 3.41 and 3.40, as well as two singlets of 2 OH at δ 4.54 and 4.49 which disappeared in the presence of TFA. Furthermore, it showed multiplets and twofold doublet signals between δ 2.60-1.60 of 10 protons, which could be assigned as 5 methylene groups. In addition, two other multiplets of 2 CH_2 of an open chain between δ 1.91- 1.45 are exhibited. Three 3H doublets of methyl groups at δ 1.36, 1.34, and 1.30 were pointing again to the existence of three sugar units in **135**. Finally, a terminal methyl triplet at δ 1.00 was observed.

The ^{13}C/APT NMR spectra displayed 44~45 carbon signals which were classified into several categories i.e. two α,β-unsaturated carbonyl carbons at δ 186.2 and 170.3, fifteen sp^2 quaternary carbons, three sp^2 methine carbons, fifteen oxygenated sp^3 methine carbons of which three were assigned as anomeric carbons, two methoxy carbons, five sp^3 methylene carbons and finally four methyl carbon signals.

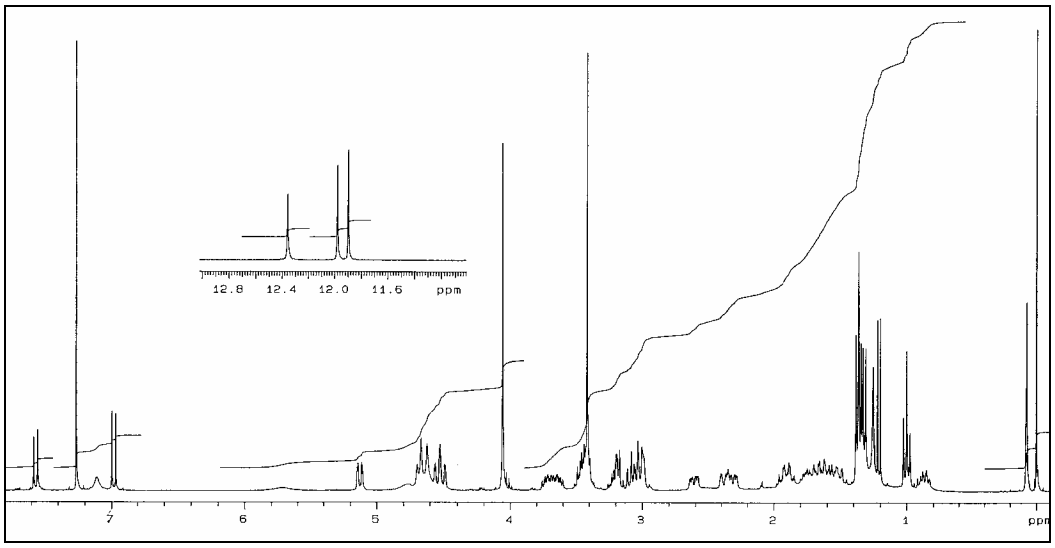


Figure 49: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of FD-594 (**135**).

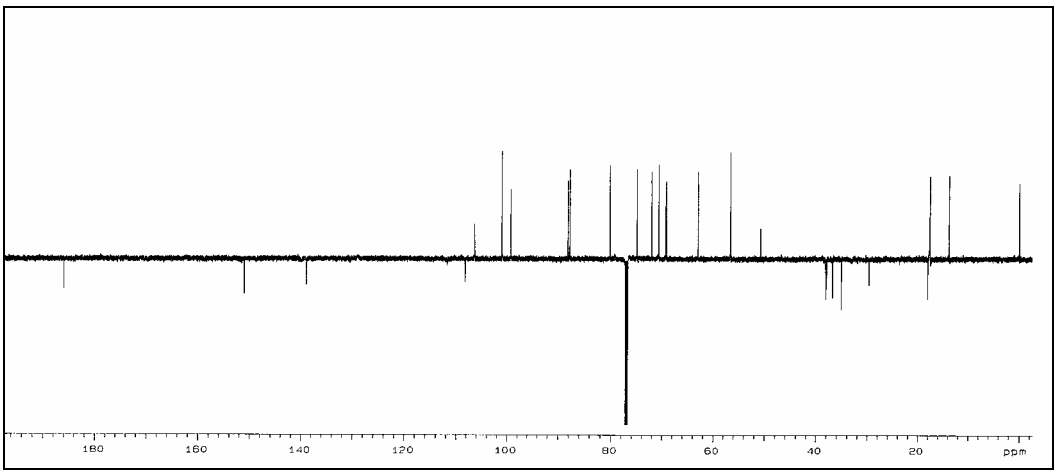
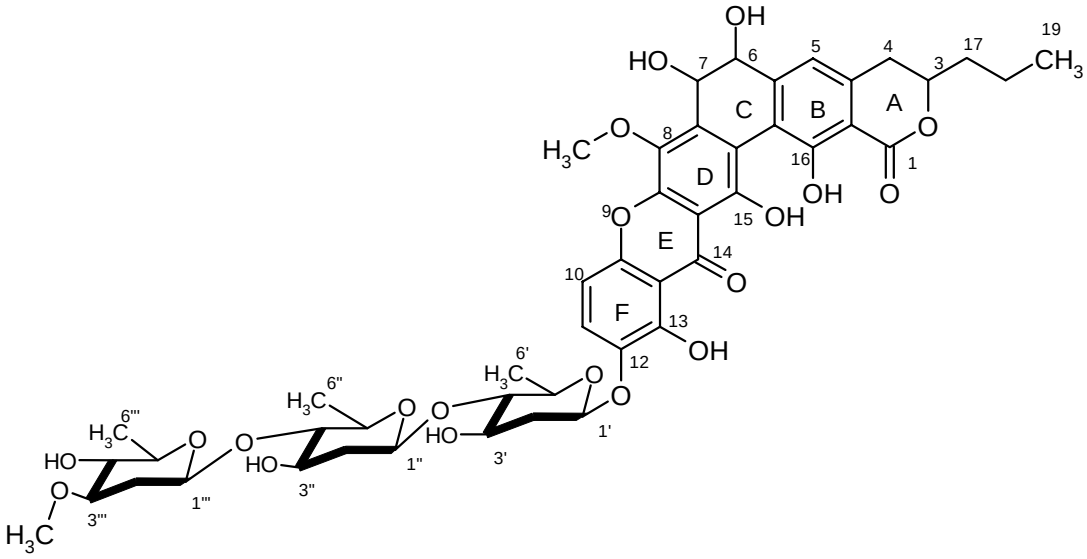


Figure 50: APT NMR spectrum ($CDCl_3$, 150 MHz) of FD-594 (**135**)

The ESI mass spectra confirmed the molecular weight of **135** as 940 Dalton due to the existence of two *quasi*-molecular ions at *m/z* 963 ($[M + Na]^+$) and 941 ($[M + H]^+$) in the (+)-mode, and 939 ($[M-H]^-$ in (-)-ESI mode.

Applying the above spectral data and the molecular weight of **135** to AntiBase resulted in FD-594 **(135**). The compound was confirmed by direct comparison of the data with the literature[179,180]. Due to the high interest of the compound **135** and its high antimicrobial activity, this drew our attention to confirm it furthermore by 2 D correlations (see the experimental part). Detailed analysis of the spin-coupling data and H,H COSY spectra as well as HMBC experiments revealed that the trisaccharide moiety of the compound (**135**) consisted of one 2,6-dideoxy-3-O-methyl-*β*-arabino-hexopyranoside (oleandrose) unit and two 2,6-dideoxy-*β*-arabino-hexopyranosides (olivose) units. The positions of the glycoside linkages between these hexo-pyranose

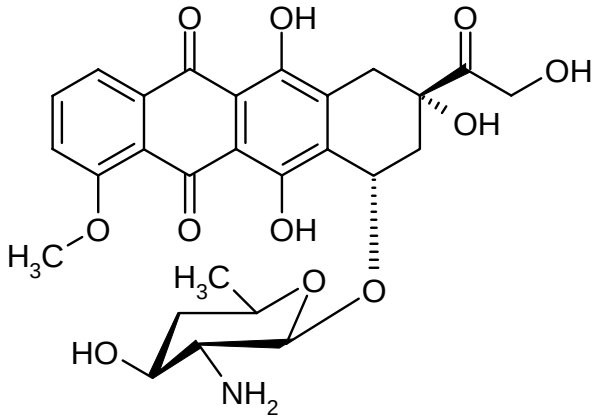
were established by HMBC spectra. The HMBC correlations of 1'''-H (4.51) with the C-4'' (87.9), and 1''-H (4.50) with C-4' (88.7) clearly indicated the connectivities of the pyranose units.



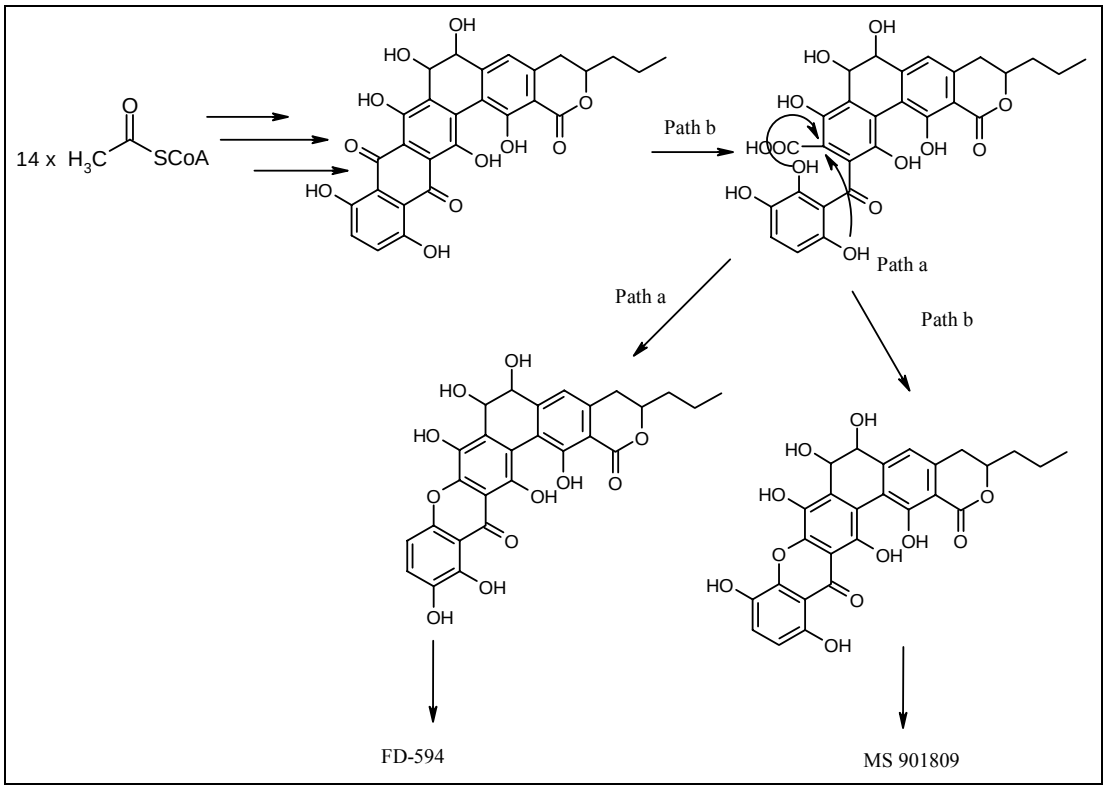
135

The antitumor antibiotic FD-594 (**135**) is produced by *Streptomyces* sp. TA-0256[179,180]. Compound **135** was reported to be closely related to BE-13973X and MS 901809, isolated from other *Streptomyces* sp.[181,182].

FD-594 (**135**) shows high activity against tumor cell lines, comparative to that of adriamycin[183] (**136**), as well as antibacterial activity against some Gram-positive bacteria.



136



135 **137**

Figure 51: Plausible biosynthetic pathway of FD-594 (**135**).

The biosynthetic pathway[180] of **135** appears to proceed *via* a polyacetate equivalent. In an early stage, the benzo[α]naphthacene quinone chromophore may first be derived from 14 acetate units. Thereafter, the Baeyer-Villiger type oxidation occurs at a quinone carbonyl group, followed by additional oxidation and decarboxylation to yield an open intermediate. Recyclization *via* path A leads to **135**, an alternative cyclization *via* path B may afford the structurally related MS 901809 (**137**).

4.7.4 Fungichromin (14-Hydroxyfilipin III)

Fraction IV showed a highly polar yellowish-green UV fluorescent band (366 nm), which turned to blue by anisaldehyde/sulphuric acid. Compound **138a** was isolated as a yellow solid by applying the fraction to PTLC and Sephadex LH-20.

The UV spectrum of **138a** showed three sharp absorption peaks (λ_{max} 300-400 nm) which are characteristic for a polyene system without conjugation with a carbonyl group. Comparison of the UV pattern of **138a** with AntiBase data pointed to five conjugated double bonds of a pentaene system.

The ^{1}H NMR spectrum displayed no signals in the aromatic region. However, it showed 9H of five olefinic double bonds between δ 6.5-5.9, the first of which was

that of a terminal one. In addition, between δ 5.29-4.36 many singlets with an intensity of 10H appeared, of which 9 disappeared by addition of TFA, to assign them as OH signals, and the residual one, a triplet at δ 4.67, is assigned as an oxygenated methine group. In addition, several multiplets with an intensity of 10H between δ 4.03-3.15 were observed which could be assigned to oxygenated methine protons. Furthermore, a twofold doublet of 1H at δ 2.50 (^3J = 8.1, 7.1 Hz) of a methine proton attached to sp^2 carbon (e.g. CO), a singlet of a methyl group at δ 1.73 (perhaps at an olefinic sp^2 carbon), multiplets of 18H at δ 1.54-1.23 of 9 methylene protons, a doublet of a methyl at δ 1.22 (^3J = 7.1 Hz), and a terminal methyl triplet at δ 0.90 (^3J = 7.1 Hz) were shown.

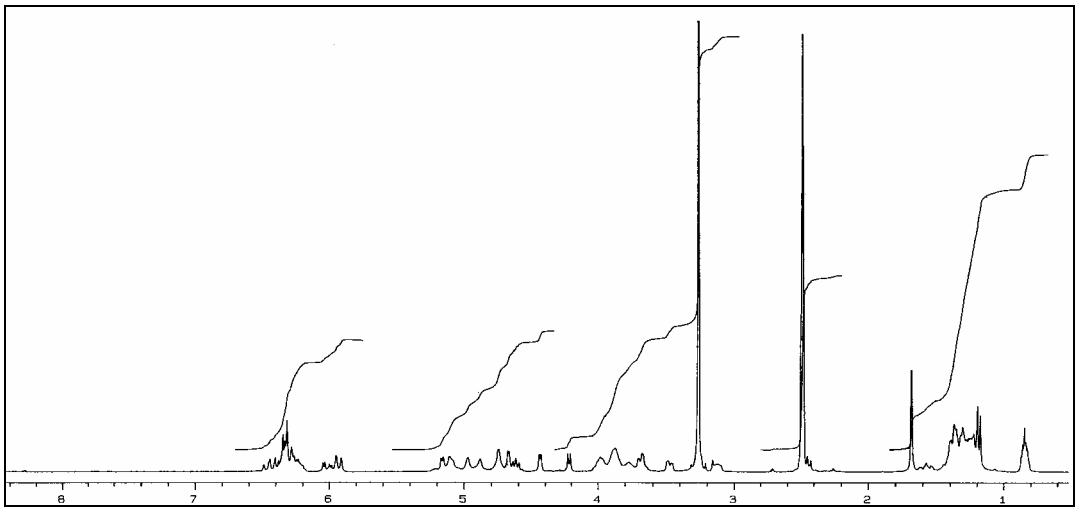


Figure 52: ^{1}H NMR spectrum ([D_6]DMSO, 300 MHz) of fungichromin (**138a**).

The ^{13}C/APT NMR spectra showed a CO signal at δ 171.0, in addition to another quaternary sp^2 carbon at δ 138.7, and 9 sp^2 olefinic methine carbons pointing to the presence of 5 conjugated double bonds, one of which being of the CH=C_q bond type. The spectra revealed also 11 oxygenated sp^3 methine carbons as well as one CH at δ 58.6 which could be assigned to a methine carbon attached to CO or to a hetero atom. In addition, 9 sp^3 carbon signals were displayed, which assigned as methylene groups, three methyl carbon signals at δ 17.7, 13.8 and 11.6, of which the first could be attached to sp^2 carbon of an olefinic double bond.

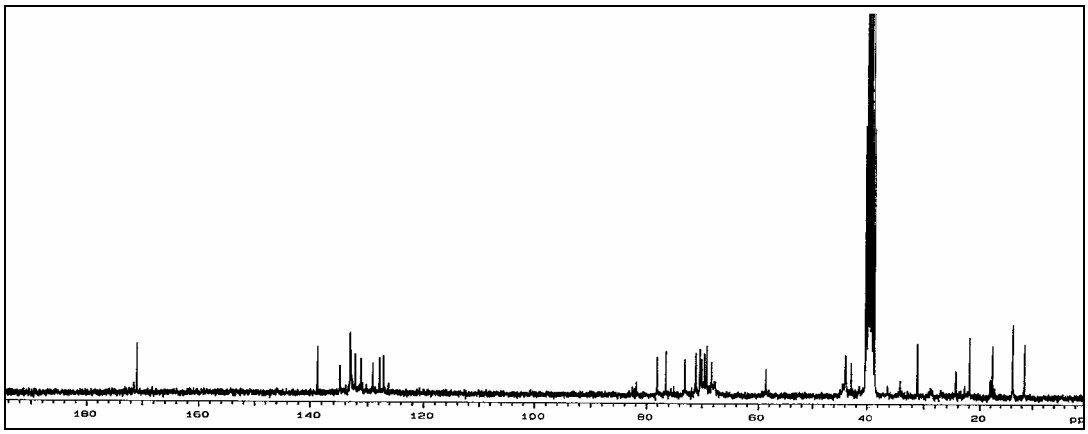
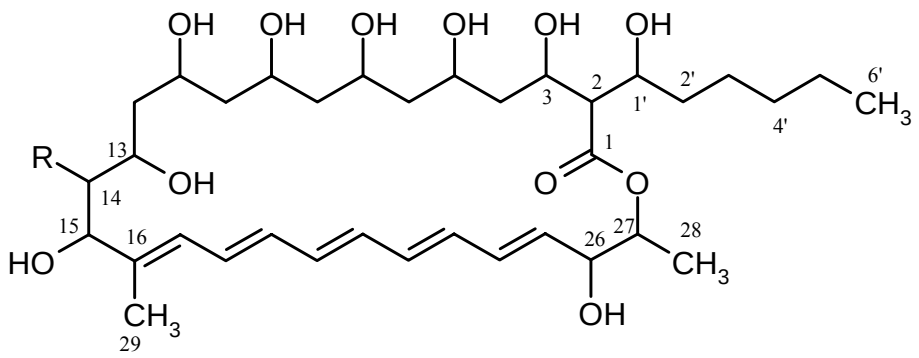


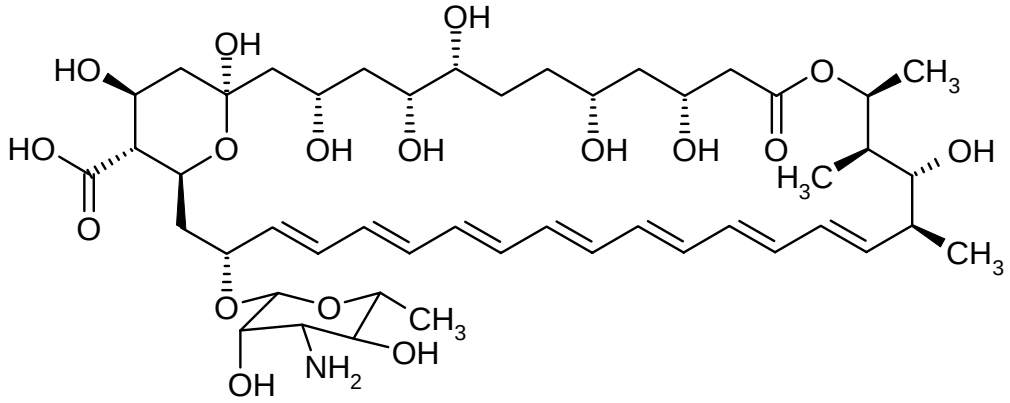
Figure 53: ^{13}C NMR spectrum ([D_6]DMSO, 125 MHz) of fungichromin (**138a**).

The molecular weight of the **138a** was established as 670 Dalton by ESI mass spectra. A search in AntiBase resulted in fungichromin (**138a**) which was further confirmed by direct comparison with the literature[184,48].

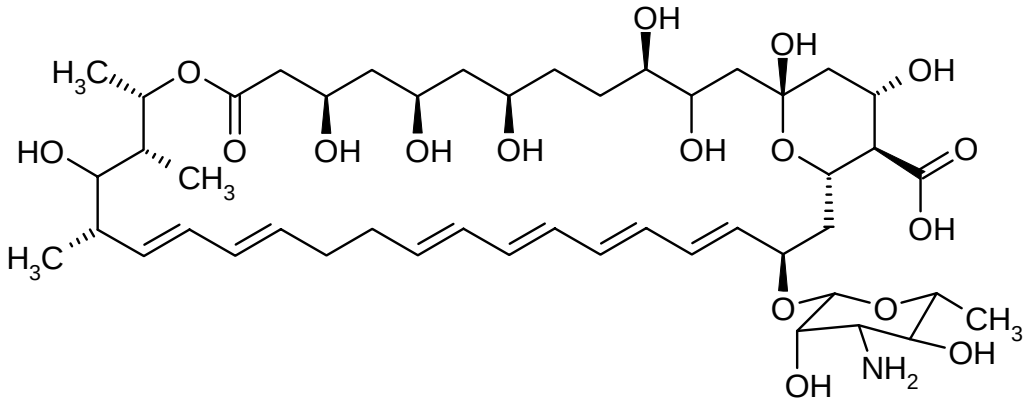
Fungichromin (**138a**), isolated first from a *Streptomyces* sp.[185,186], and the related analogue filipin **138b** belong to the class of macrocyclic polyene antibiotics, a group of over 200 compounds, produced mainly by *Streptomyces* sp., that possess antifungal and antiprotozoal activity[184]. Despite of their toxicity and the development of other classes of antifungal antibiotics, especially the polyene amphotericin B (**139**) and nystatin (**140**) remain the gold standard for treatment of many fungal infections in humans. The antihypercholesterolemic, antitumor, and antiviral activities of such steroid-binding polyenes also attracted considerable interest[184]. Biosynthetically, the macrocyclic ring of the polyenes derived from acetate and propionate[,184]. The compounds FD-594 (**135**) and fungichromin (**138a**) were subjected to cyctotoxcity measurements against nematode, and showed a weak activity (13%, 1mg/l) by **135**, and high activity (95 %, 1 mg/l) by **138a**.



138a: R = OH, **138b**: R = H



139



140

4.8 Terrestrial *Streptomyces* sp. GW2/577

Terrestrial *Streptomyces* strains are a prolific source of many diverse metabolic compounds that might represent useful leads in the development of new pharmaceutical agents. During the systematic search for new secondary metabolites from bacteria, the ethyl acetate extract of a terrestrial *Streptomyces* sp. isolate GW 2/577 was found to produce four new compounds designated as cPHB (**318**), *p*-hydroxyphenethyl propionamide (**144**), 3-hydroxy-N-phenethyl-butyramide (**145**) and 5-methyl-1*H*-quinazoline-2,4-dione **(148**), in addition to some other known compounds (Figure 54). Moreover, the crude extract possessed high activity against *Escherichia coli*, and moderate activity against *Bacillus subtilis*.

The strain was fermented in M_2 medium as 27-liter fermenter for 3 days at 28 °C. The obtained crude extract was applied to defatting and the resulting methanol extract was chromatographed on Sephadex LH-20 and eluted with methanol to give four fractions. Purification of the resulting fractions led to the described compounds.

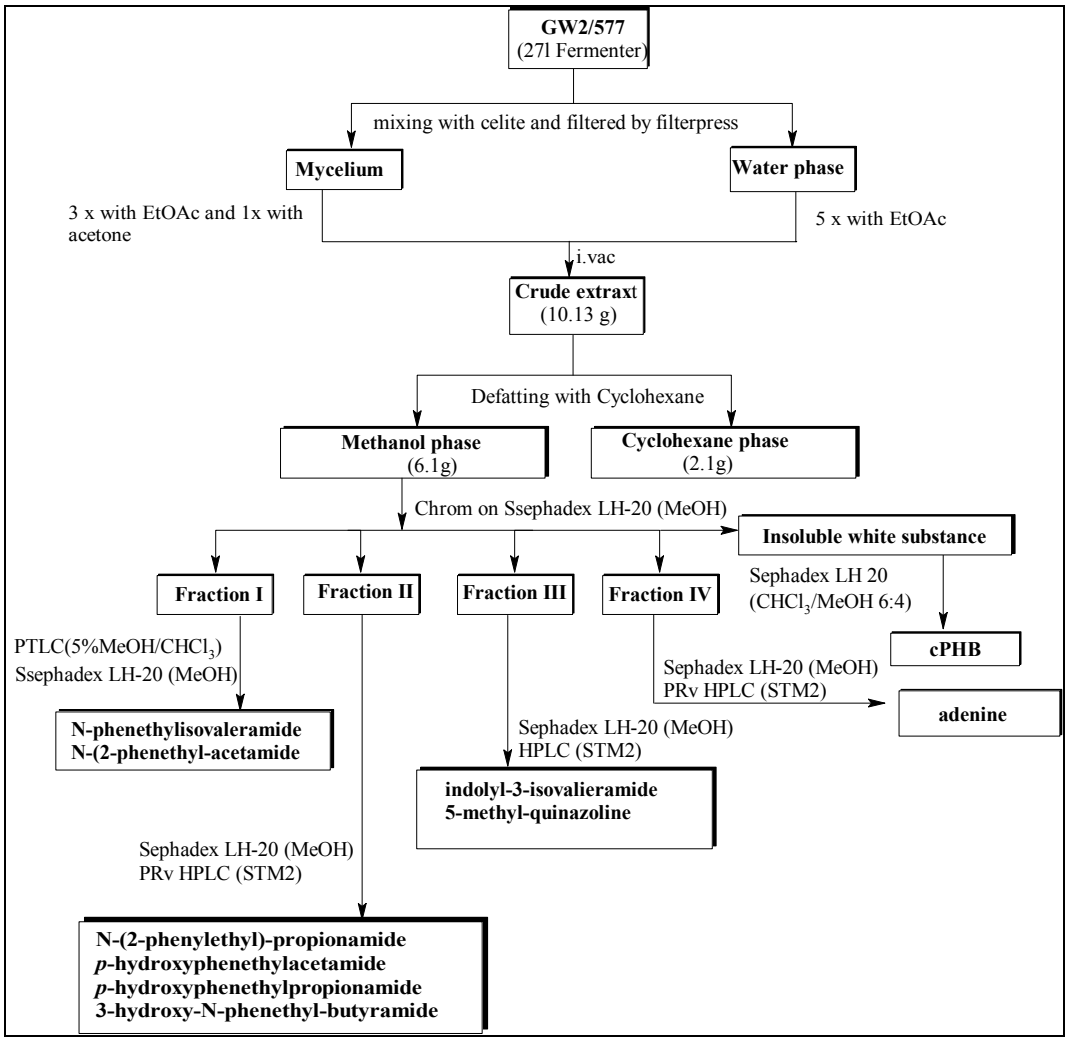


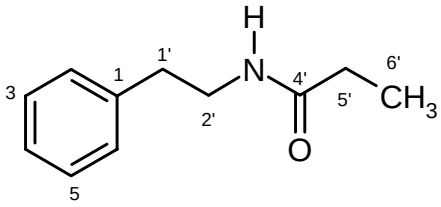
Figure 54: working-up procedure of strain GW2/577.

4.8.1 N-(2-Phenylethyl)-propionamide

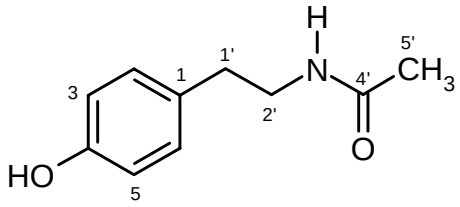
Compound **141** was isolated as colourless solid from fraction II by applying the fraction to Sephadex LH-20 and HPLC, was UV absorbing, and turned to violet and pink when sprayed with anisaldehyde/sulphuric acid and Ehrlich's reagent, respectively.

The ^{1}H NMR spectrum displayed a multiplet of a phenyl group at δ 7.24, in addition to a broad singlet of 1H at δ 5.5, was indicative for an amide (NH). Furthermore, an ethandiyl group was observed due to the existence of two aliphatic signals each of 2H at δ 3.40 (q) which changed to triplet after H/D exchanging, and the other one was a triplet at δ 2.77. An additional ethyl group was observed due to the presence of a quartet methylene group at δ 2.13, and the adjacent methyl group was found as triplet at δ 1.04.

The molecular weight of compound **141** was established as 177 Dalton by EI MS. The molecular ion exhibited a base fragment at *m/z* 104, which is attributed to an expulsion of acetamide group $[M - (HN\text{-}COCH_3)]^{+\cdot}$. A search in AntiBase resulted in N-(2-phenylethyl)-propionamide (**141**), which was further confirmed by its direct comparison with the literature. This type of compounds possesses a moderate antimicroalgal activity and can be used as herbicides[187].



141



142

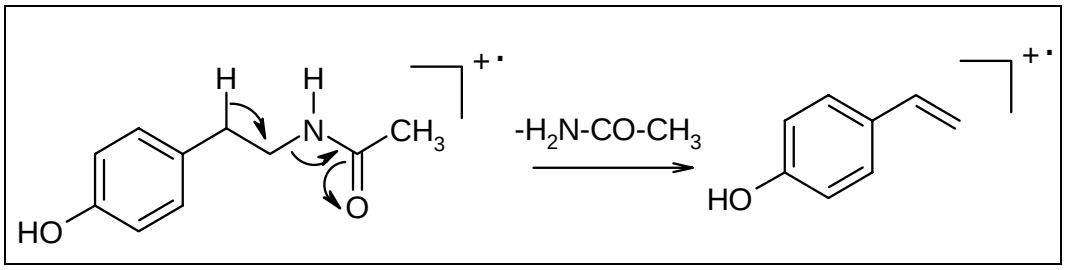
4.8.2 *p*-Hydroxyphenethyl acetamide; N-Acetyl-tyramine

Along with **141**, compound **142** was found in fraction II as an UV absorbing zone and stained similarly to violet with anisaldehyde/sulphuric acid. By the same isolation method as for **141**, compound **142** was isolated as colourless solid.

The ^{1}H NMR spectrum showed two acidic protons as broad singlets at δ 8.73, and 7.51, which could be due to phenolic hydroxyls or amide groups. An 1,4-disubstituted aromatic system was established due to the existence of two doublets each of 2H at δ 7.02, and 6.73. In the aliphatic region, an ethanediyl group attached to NH amide was observed due to the presence of two signals at δ 3.37 (q) and 2.67 (t) as two vicinal methylene groups like compound **141**. A 3H singlet of a methyl group was observed at δ 1.88, which could be assigned as an acetyl group.

The EI mass spectrum determined the molecular weight of compound **142** as 179 Dalton. The mass ion exhibited an expulsion of acetamide group to give a base peak at *m/z* 120. The last fragment is could be attributed to 4-hydroxystyrene (**143**) (Figure 55).

A search in AntiBase resulted in *p*-hydroxyphenethyl acetamide (**142**) [61]. Compound **142** and its derivatives are used as antitumor agents[188]. Compound **142** was isolated recently as a secondary metabolite from the marine fungus *Fusarium* sp.[189], *S. griseus*[190], as well as from plants[191]. It was found also as metabolite from pathogenic fungi, *Mycobacteria*, *Enterobacteria* and from *Bombyx mori* at the chrysalis stage[24].



143

Figure 55: A suggested EI MS fragmentation pattern of *p*-hydroxyphenethylacetamide (**142**).

4.8.3 *p*-Hydroxyphenethyl propionamide

In addition to **141** and **142**, compound **144** was found in the same fraction II as a colourless solid, UV absorbing substance, which was stained to violet by anisaldehyde/sulphuric acid.

The IR spectrum of **144** showed a broad band at ν 3309 cm^{-1} of OH and /or NH, a strong band at ν 1651 cm^{-1} due to a carbonyl group of an amide system, and multiple bands between ν 1555-1454 cm^{-1}. The ^{1}H NMR spectrum of **144** showed the same pattern as compound **142** referring to the presence of the tyramine chromophore, except that the acetyl group in compound **142** was replaced by an ethyl group found as quartet of methylene at δ 2.17 and a triplet of methyl group at δ 1.11.

The ^{13}C/APT NMR spectra of compound **144** exhibited 9 carbon signals, among them three quaternary sp^2 carbon signals were at δ 174.4, 155.0 and 130.0. Two methine signals of double intensity at δ 129.7 and 115.6 indicated an 1,4-disubstituted aromatic system. The first two quaternary carbon signals (174.4, 155.0) are characteristic for carbonyl of amide groups and an oxygenated carbon in the aromatic ring, respectively. In the aliphatic region, four sp^3 carbon signals were observed, three methylene groups at δ 40.8, 34.66, 29.7, and one methyl signal at δ 9.9. The compound **144** is assumed to be constructed from two main parts, an 1,4-disubstituted aromatic ring and an N-ethyl propionamide moiety (Figure 57)

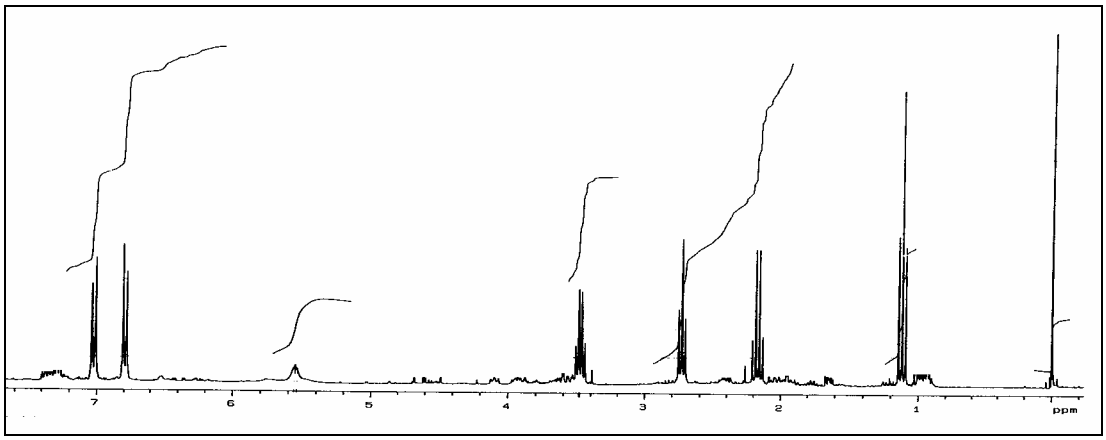


Figure 56: ^{1}H NMR ($CDCl_3$, 300 MHz) of *p*-hydroxyphenethyl propionamide (**144**)

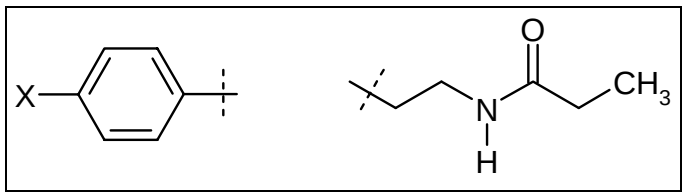


Figure 57: partial structures of *p*-hydroxyphenethyl propionamide (**144**)

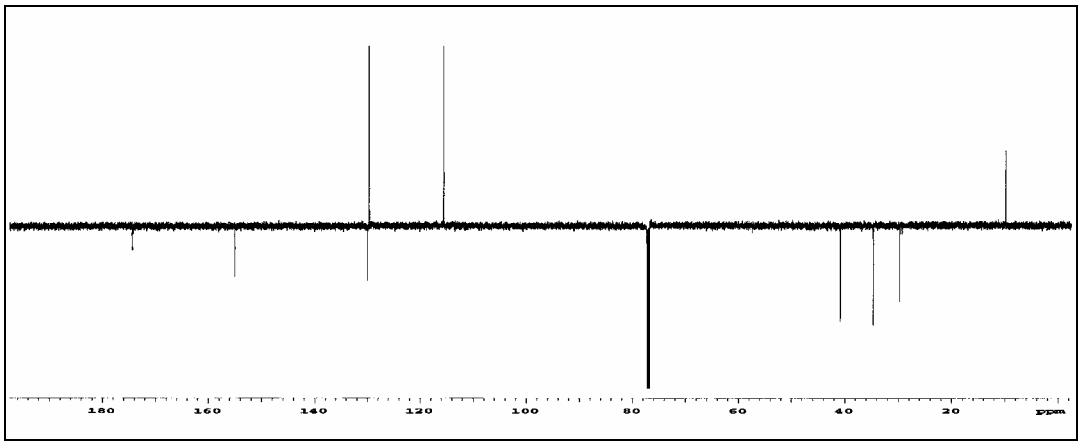


Figure 58: APT NMR spectrum ($CDCl_3$, 125 MHz) of *p*-hydroxyphenethyl propionamide (**144**)

The molecular weight of compound **144** was found by EI mass spectrum as 193 Dalton. The molecular ion showed the expulsion of an H_2N-CO-Et fragment to give a base peak at *m/z* 120 fragment **143** (Figure59). EIHR MS of **144** led to the molecular formula $C_{11}H_{15}NO_2$. In accordance, compound **144** was established as *p*-hydroxyphenethyl propionamide. A search in AntiBase and DNP resulted in no hits, as indication that *p*-hydroxyphenethyl propionamide (**144**) is a new natural product.

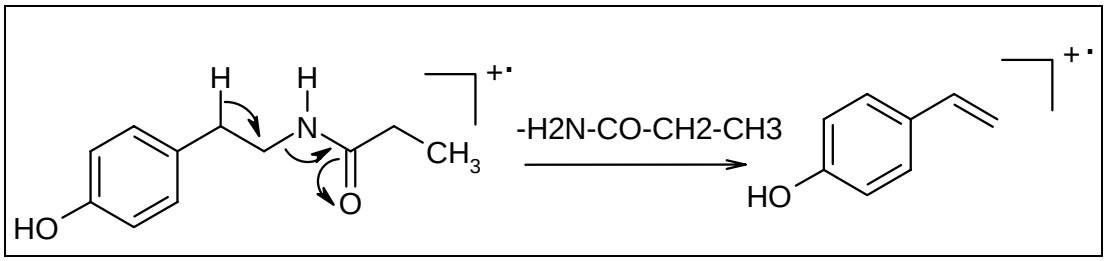
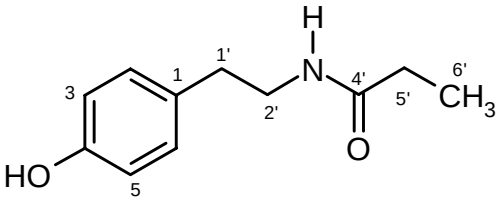


Figure59: EI MS fragmentation of *p*-hydroxyphenethyl propionamide (**144**) to give fragment **143**.



144

4.8.4 3-Hydroxy-N-phenethyl-butyramide

Compound **145** was obtained as slightly less polar colourless solid, UV absorbing substance by applying fraction II to Sephadex LH-20 and HPLC. It exhibited a pink colour with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of compound **145** showed a multiplet with intensity of 5H as phenyl group at δ 7.24, in addition to a broad singlet of an acidic proton at δ 5.55 (NH). Furthermore, a multiplet of 1H was observed at δ 4.03, which could be assigned as oxymethine. Besides, two signals of an ethanediyl group were detected at δ 3.42 (m) and 2.79 (t), of which the first one was transformed into a triplet after H/D exchange. Moreover, ABX system of methylene group was observed at δ 2.24 and 2.19, flanked by two different resonance groups. Finally, a doublet methyl group was displayed at δ 1.09.

The ^{13}C/APT NMR spectra of compound **145** showed a carbon signal (δ 172.3) of carbonyl group of acid, amide or ester system. In the aromatic region, four carbon signals were detected between δ 138.8-126.4, one of which at δ 138.8 was quaternary, while the other signals were assigned as 5 sp^2 methines, as it is indicative for a monosubstituted phenyl group. Three sp^3 methylene carbon signals were afforded at δ 43.9, 40.4 and 35.5, one oxymethine carbon displayed at 64.8, and finally one car-

bon signal of a methyl group was observed at δ 22.8. This pointed to the presence of a *β*-phenethyl amine and 2-hydroxybutyryl moiety in **145**.

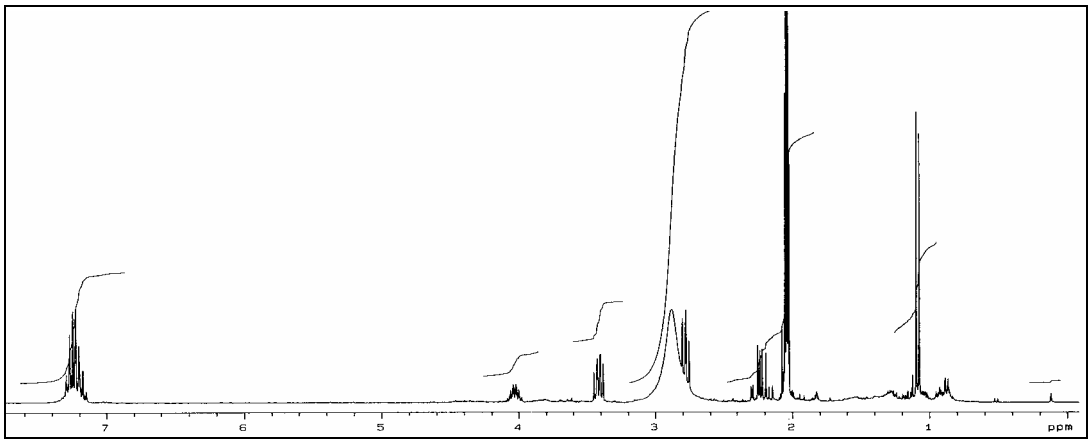


Figure 60: ^{1}H NMR spectrum ([D_6]acetone, 300 MHz) of 3-hydroxy-n-phenethyl-butyramide (**145**)

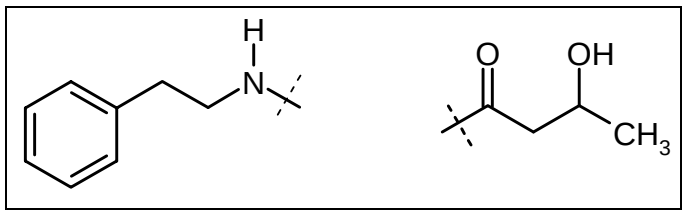


Figure 61: Proposed structural constituents of 3-hydroxy-N-phenethyl-butyramide (**145**)

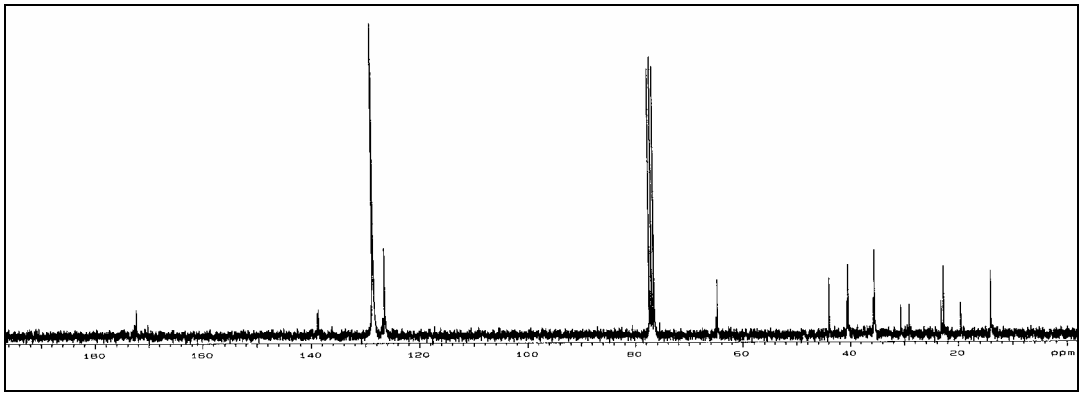


Figure 62: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 3-hydroxy-N-phenethyl-butyramide (**145**)

The molecular weight of **145** was determined by EI mass spectrum as 207 Dalton. Two further fragment peaks observed at *m/z* 192 and 189 due to the expulsion of a methyl group and water molecule from the molecular ion, respectively. Besides, a base peak fragment was detected at *m/z* 104, due to the expulsion of 3-hydroxybutyramide giving styrene (Figure 63).

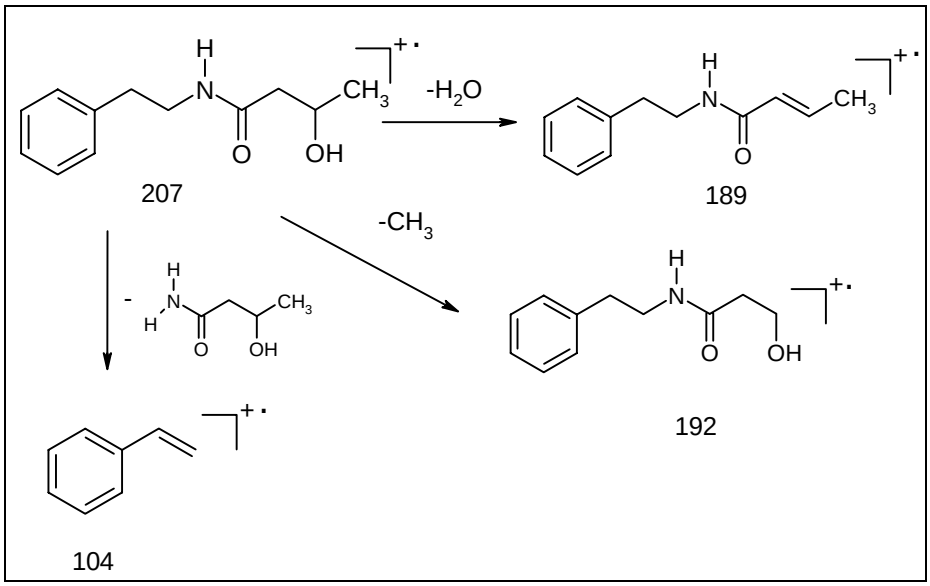
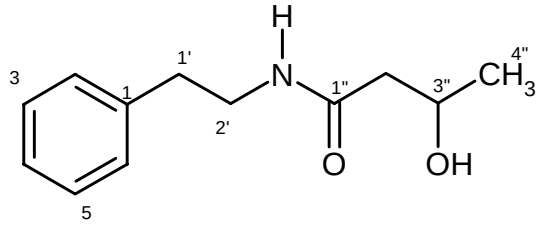
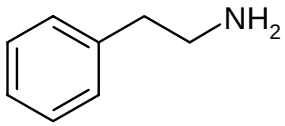


Figure 63: EI MS fragmentation pattern of 3-hydroxy-N-phenethyl-butyramide (**145**)

The HREI MS of **145** led to the molecular formula $C_{12}H_{17}NO_2$. According to the spectral data as well as the molecular formula, compound **145** was confirmed as 3-hydroxy-N-phenethyl-butyramide (**145**), which has not been found in nature so far. Compound **145** could be formed by aminolysis of polyhydroxybutyric acid (**317**) with *β*-phenethylamine (**146**).



145



146

4.8.5 Crotonic acid *β*-phenylethyl amide

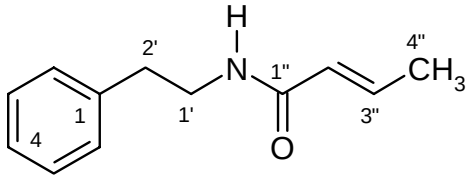
A further confirmation of structure **145** was attempted by synthesis from *β*-phenethylamine (**146**) and sPHB (**317**). At room temperature without solvent, no reaction occurred. Under reflux, however, the less polar compound **147** was formed.

The spectrum of compound **147** showed similarly as for **145**, a phenyl multiplet at δ 7.24, and the two vicinal methylene groups of the *β*-phenethylamine constituent. Two 1H signals were additionally observed in the olefinic region as doublets of

quartets at δ 6.76 and 5.96. In the aliphatic region, it showed an additional dd signal of a methyl group at δ 1.78 attached to an olefinic carbon.

In addition to the phenethylamine signals, the ^{13}C NMR spectrum of compound **147** exhibited two methine carbons at δ 139.9 and 124.9 corresponding to an olefinic double bond. The EI mass spectrum fixed the molecular weight as 189 Dalton.

Hence, compound **147** is obviously crotonic acid *β*-phenylethyl amide and was formed by dehydration of **145**. It was not known so far.



147

The amide **145** was finally obtained by deprotonation of *β*-phenyl ethyl amine (**146**) with *n*-butyl-lithium and successive reaction with a sPHB solution (**317**). The resulting product was purified by PTLC and Sephadex LH-20 to give colourless solid of **145**, which was identical with the natural product.

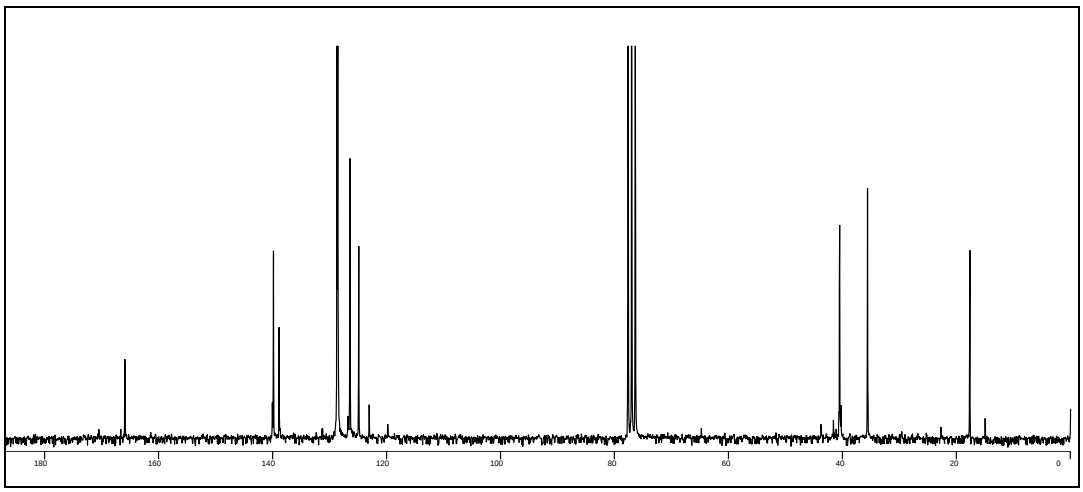
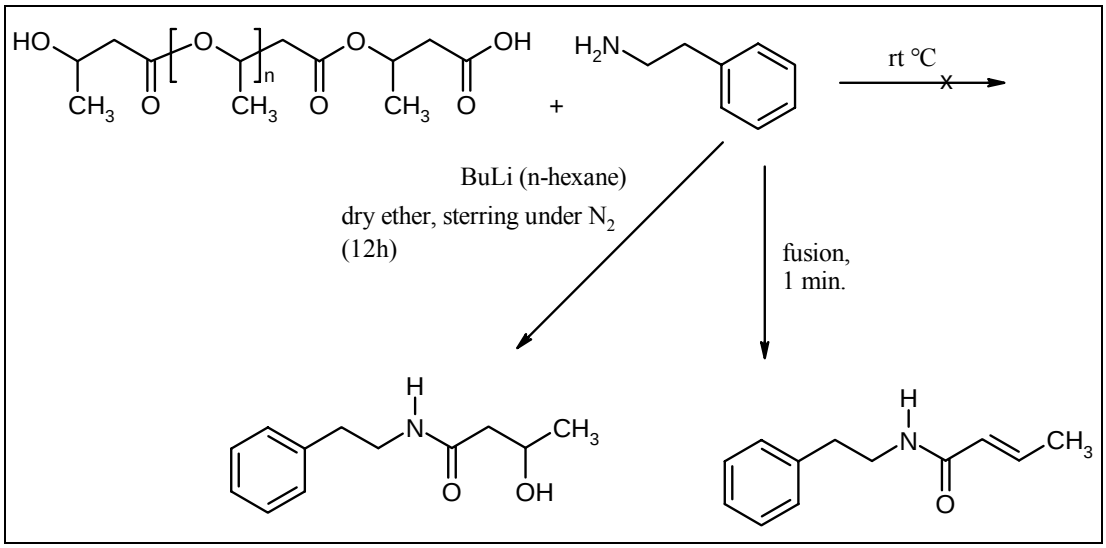


Figure 64: ^{13}C NMR spectrum ($CDCl_3$, 50 MHz) of crotonic acid-*β*-phenylethyl amide (**147**).



145 **147**

4.8.6 5-Methyl-1*H*-quinazoline-2,4-dione

Compound **148** was isolated as an UV absorbing zone from fraction III as a colourless minor metabolite. It showed no colour by treatment with anisaldehyde/sulphuric acid or Ehrlich's reagent.

The ^{1}H NMR spectrum of **148** showed broad singlet of 1H at δ 8.35 of phenolic or NH amide type. In addition, three aromatic signals were detected, two as dd at δ 7.57 and 7.19, while the third gave a triplet at δ 7.30 to create 1,2,3-trisubstituted aromatic system. In the aliphatic region, it showed a methyl singlet at δ 2.48, which could indicate an N-methyl, olefinic or aromatic methyl in *peri*-position to a carbonyl group, or an acetyl group.

The ^{13}C NMR spectrum delivered 3 sp^2 methine carbon signals at δ 126.7, 117.2 and 116.1. In addition, two quaternary carbon signals were found at δ 161.5 and 152.1, which are assigned as two carbonyl groups as in the uracil system. It showed also three additional quaternary carbons at δ 139.7, 137.7 and 121.5. Furthermore, one sp^3 carbon signal of methyl group was displayed at δ 21.1.

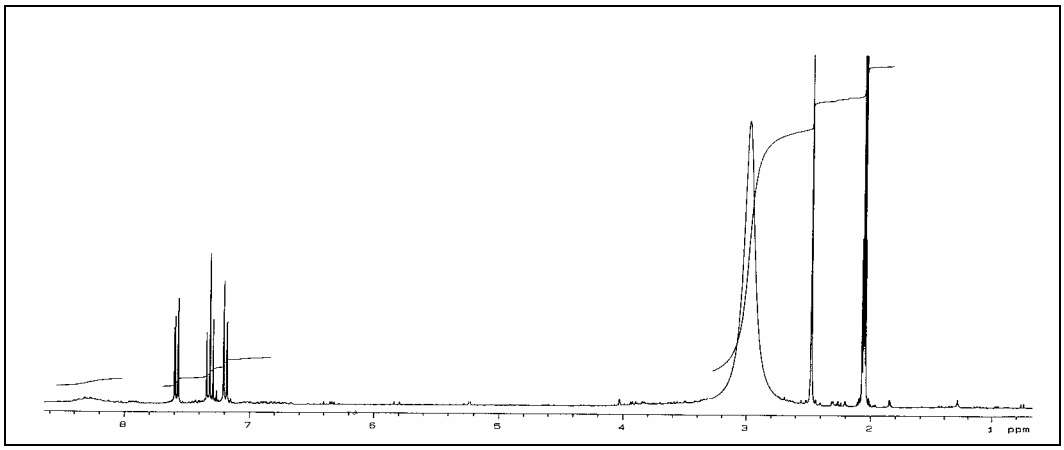
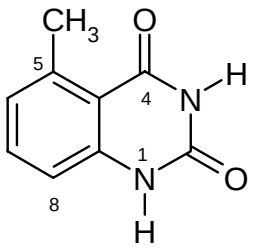


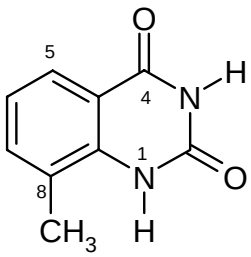
Figure 65: ^{1}H NMR spectrum ([D_6]acetone, 300 MHz) of 5-methyl-1*H*-quinazoline-2,4-dione (**148**)

The molecular weight of **148** was determined as 176 Dalton by CI and EI mass spectra. The EI mass spectrum revealed a further expulsion of a methyl group giving a fragment at *m/z* 162 $[M - CH_3]^{+\cdot}$). An additional peak was observed at *m/z* 134 due to a loss of an isocyanate group ($[M – (O=C=N)]^{+\cdot}$). EIHR MS of the molecular ion gave the corresponding molecular formula $C_9H_8N_2O_2$.

According to the spectral data, 5-methyl-1*H*-quinazoline-2,4-dione **(148**) and 8-methyl-1*H*-quinazoline-2,4-dione **(149**) were two possible structures. As the isolated amount of **148** was insufficient for further measurements, the structure was confirmed by synthesis as shown below.



148



149

4.8.7 8-Methyl-1*H*-quinazoline-2,4-dione

To illuminate the nature of the above compound either as **148** or **149**, the isomer **149** was synthesized by fusion[192] of 3-methylanthranilic acid with an excess of urea (Figure 66).

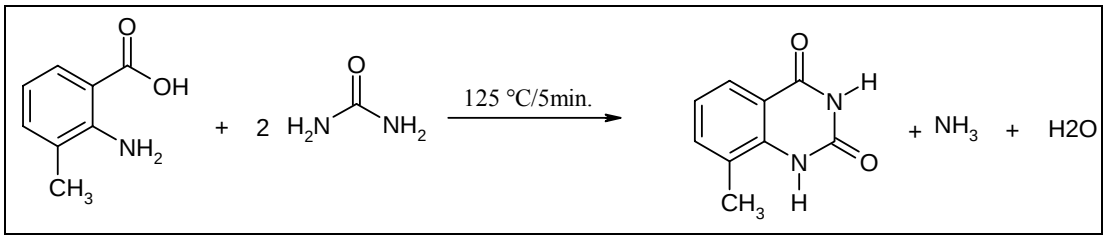


Figure 66: Synthesis of 8-methyl-1*H*-quinazoline-2,4-dione (**149**)

Comparison the spectral data of compound **149** (δ_H, δ_C and EI MS) and those of the natural one (**148**) showed, that they are different in both δ_H and δ_C values (Table 7). Also, the EI fragmentation patterns of their molecular ions were found to be different. The H and C sequences of compound **149** were established by 2 D spectra (Figure 67). This indicated that compound **148** was 5-methyl-1*H*-quinazoline-2,4-dione (**148**) but not as 8-methyl-1*H*-quinazoline-2,4-dione **(149)**.

Table 7: ^{1}H NMR comparison of the natural **148** and the its synthetic isomer **149**

Posit	**148**	**149**	**148**	**149**
	δ_H ([D$_6$] acetone)	δ_H ([D$_6$] acetone)	δ_C ([D$_6$] acetone)	δ_C ([D$_6$] DMSO)
1-NH	8.35	9.25	-	-
2	-	-	152.1	150.4
3-NH	-	10.10	-	-
4	-	-	161.5	162.9
4a	-	-	121.5	124.0
5	-	7.88 (dd)	137.7	124.7
6	7.19 (dd)	7.12 (t)	117.2	122.0
7	7.30 (t)	7.54 (dd)	126.7	135.9
8	7.57 (dd)	-	116.1	114.5
8a	-	-	139.7	139.1
5-CH$_3$	2.48 (s)	-	21.5	-
8-CH$_3$	-	2.47 (s)	-	17.0
8	7.57 (dd)	-	116.1	114.5

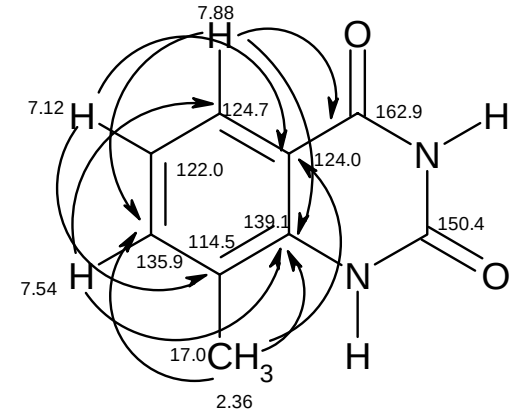


Figure 67: HMBC connectivities of 8-methyl-1*H*-quinazoline-2,4-dione (**149**)

4.8.8 1,8-Dimethyl-7*H*-quinazolino[3,2-a]quinazoline-5,12-dione

During the synthesis of **149**, compound **151** was obtained as a yellow solid minor product. The ^{1}H NMR spectrum of **151** exhibited 6 aromatic methine protons, out of them two triplets at δ 7.38 and 7.14, four dd at δ 8.32, 7.96, 7.70 and 7.40 to construct two 1,2,3-trisubstituted aromatic rings. In the aliphatic region, it revealed two singlets of two methyl groups at δ 2.63 and 2.38 linked to sp^2 aromatic systems. In addition, a broad 1H singlet at δ 5.5 could be assigned as amide group (NH).

The ^{13}C/APT NMR spectra delivered 15 sp^2 carbon signals from which 9 were due to quaternary carbon atoms. The signal at δ 159.5 corresponds an amide carbonyl group as in uracil and compounds **148** and **149**. The other two carbon signals at δ 146.5 and 146.0 could be due to carbonyl groups in an imide or guanidine skeleton. In addition, 6 sp^2 aromatic methines, and at δ 16.7 and 16.5 two methyl signals were observed.

The EI mass spectrum afforded a molecular weight of 291 Dalton, and HREI MS corresponded to the molecular formula $C_{17}H_{13}N_3O_2$. This indicated that obviously **149** had reacted with a second molecule of 3-methyl-anthranilic acid.

Three different isomers **150-152** can be expected, and all of them could exist as various prototrop-isomers (Figure 68).

Protonation of the azomethine nitrogen in the linear isomer **152** (Figure 69) should result in a symmetrical ion. As addition of TFA did not enhance the symmetry of the ^{1}H/^{13}C NMR spectrum, **152** was excluded[193].

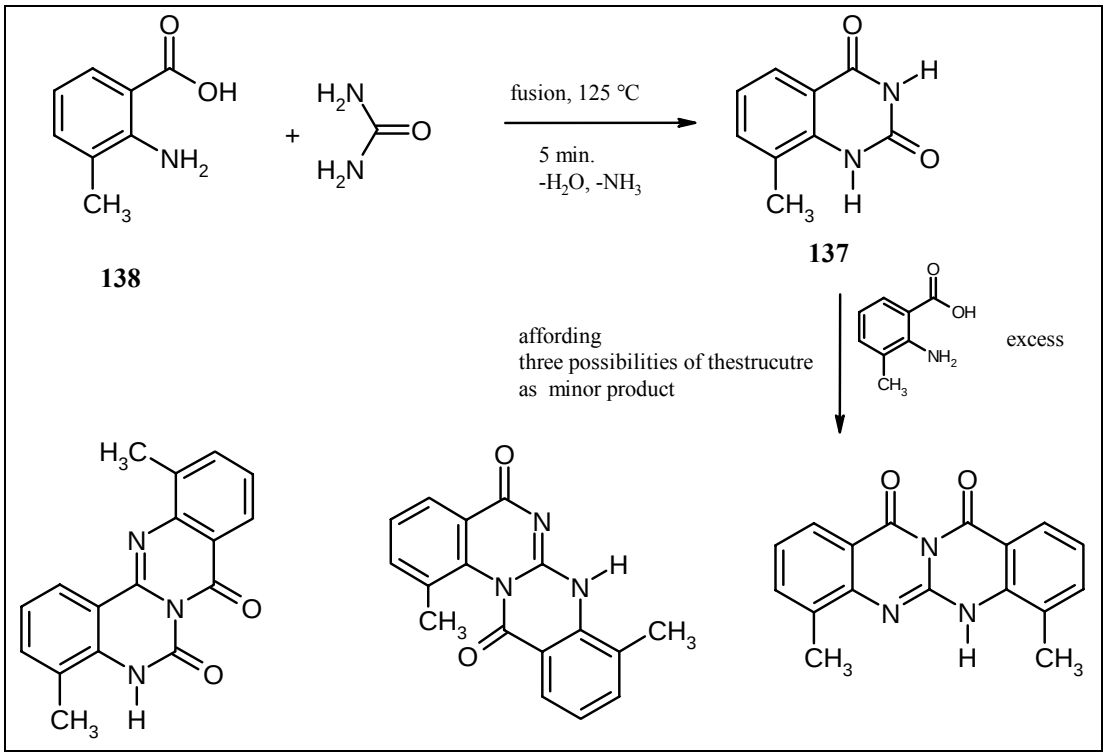


Figure 68: Synthesis of **151** by subsequent condensation of **149** with an excess of 3-methyl anthranilic acid

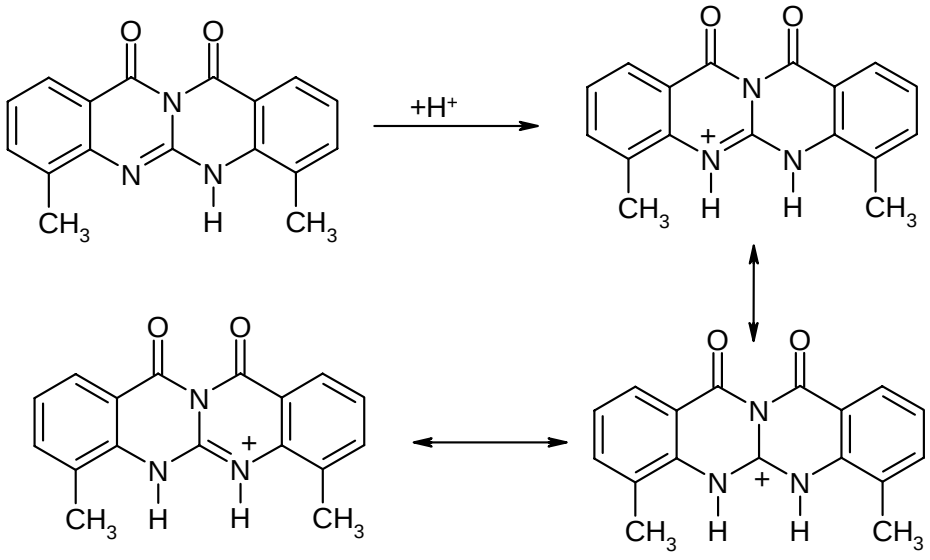
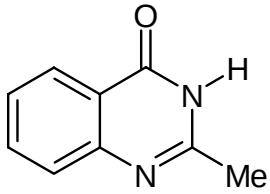


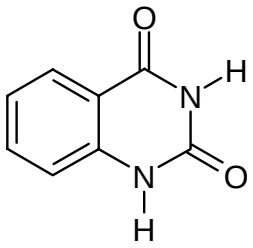
Figure 69: Mesomeric forms of protonated **152**

Only one signal (δ 159) in the ^{13}C NMR spectrum of the side product is in the expected range for an amide carbonyl and shows an HMBC coupling with a *peri* proton (δ 7.96). The other candidates at δ 146.5 and 146.1 do not show long-range couplings and differ strongly from shifts in related compounds: The corresponding

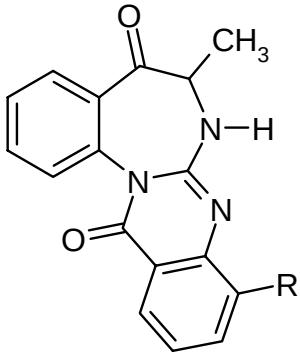
CO signals in **154**, **148** or **149** are in the range of δ 150-152[193], and also shift predictions using computer programs have not been helpful[122]. The $\Delta^{13,13a}$azomethine bond in **151** should influence its neighbourhood similarly as a carbonyl group, however, much weaker as the comparison with **153** indicates. The strongly deshielded methyl signal (δ 2.63) and the presence of *two* deepfield *peri*-protons (shifts as in **153**, **155** or **150**) cannot be explained readily by structure **151**, however, fit nicely on **151**. The structure and the origin of **151** show some similarity with the yeast pigment candidine[194].



153

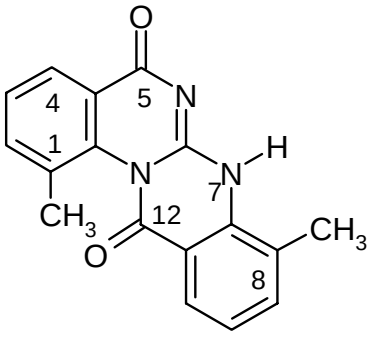


154



155a: R = H, **155b**: R = OH

The quinazoline-4-one structures are found in many plants and microbial metabolites as part of complex structures like tryptoquivaline[195], circumdatin F (**155a**) and G[196] (**155b**), tryptanthrine[197] (**63**), dipegine (**156**) and dipeginol[198], vasicinone and deoxyvasicinone[199] (**157**).



151

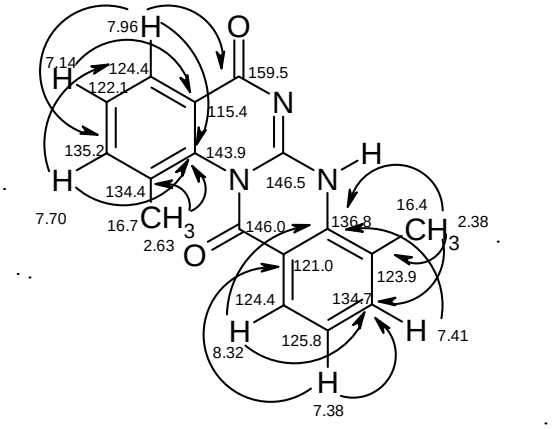
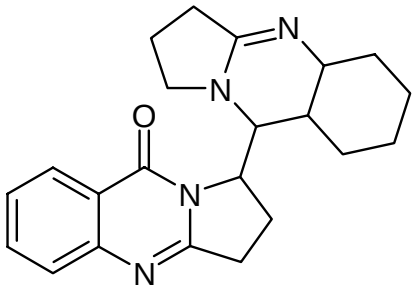
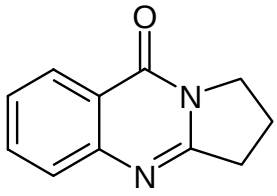


Figure 70: HMBC connectivities of **151**

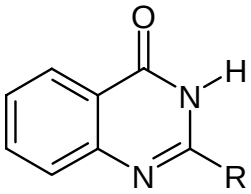


156

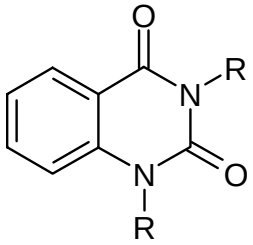


157

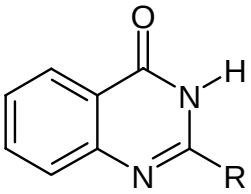
Also some simple quinazoline derivatives have been reported, e.g. 2-benzyl-3*H*-quinazolin-4-one[200] (**158**) or 1*H*-quinazoline-2,4-dione[201] (**159**) from plant sources, 2-acetyl-3*H*-quinazolin-4-one (**160**) and 2-(1-hydroxy-ethyl)-3*H*-quinazolin-4-one (**161**) from fungi[202]. From bacteria, however, only 2-methyl-3*H*-quinazolin-4-one (**162**), an inhibitor of the poly-(ADP-ribose)-synthetase, and 2,2-dimethyl-2,3-dihydro-1*H*-quinazolin-4-one (**68**) were isolated[193]. The related 2-benzyl-3*H*-quinazolin-4-one is biologically active as a specific inhibitor of serine protease and human leucocyte elastase[200], and 1,3-dimethyl-1*H*-quinazoline-2,4-dione (**163**) shows anti-inflammatory, analgesic and anticonvulsant properties[203].



158: R = Ph
162: R = CH_3



159: R = H
163: R = CH_3



160: R = $COCH_3$
161: R = CH (OH)CH_3

4.9 Strain Bio215

The crude extract of the North Sea bacterium Bio215 showed two spots, which gave a greenish-yellow UV fluorescence (366 nm) and a yellow colouration with anisaldehyde/sulphuric acid. In addition, several other absorbing UV bands at 254 gave different colours (from violet to blue) with the same spraying reagent. The extract possessed biological activity against *Bacillus subtilis*, *Escherichia coli*, *Mucor miehei* (Tü284), *Streptomyces viridochromogenes* (Tü57) and *Staphylococcus aureus*, as well as the algae *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*

Twelve 1 liter-Erlenmeyer flasks, each containing 250 ml of Luria-Bertani medium + 50% Sea water were inoculated with strain Bio215 and grown for 5 days at 28 °C while shaking at 95 rpm. The resulting intensive yellow culture served for the inoculation of a 25-liter jar fermenter for additional 5 days at 28 °C. The obtained ethyl acetate extract was applied to column chromatography on Sephadex LH-20 to give five fractions. After working up, the crude extract delivered indolyl-3-acetic acid methyl ester (**46b)**, *cis-cyclo*(prolyl-valyl) (**164)**, flazin (**166**), 1-(9H-β-carbolin-1-yl)-3-hydroxy-propan-1-one (**171**), *p*-hydroxylphenylacetic acid (**174**) and adenine (**175)**, in addition to other known compounds (Figure 71).

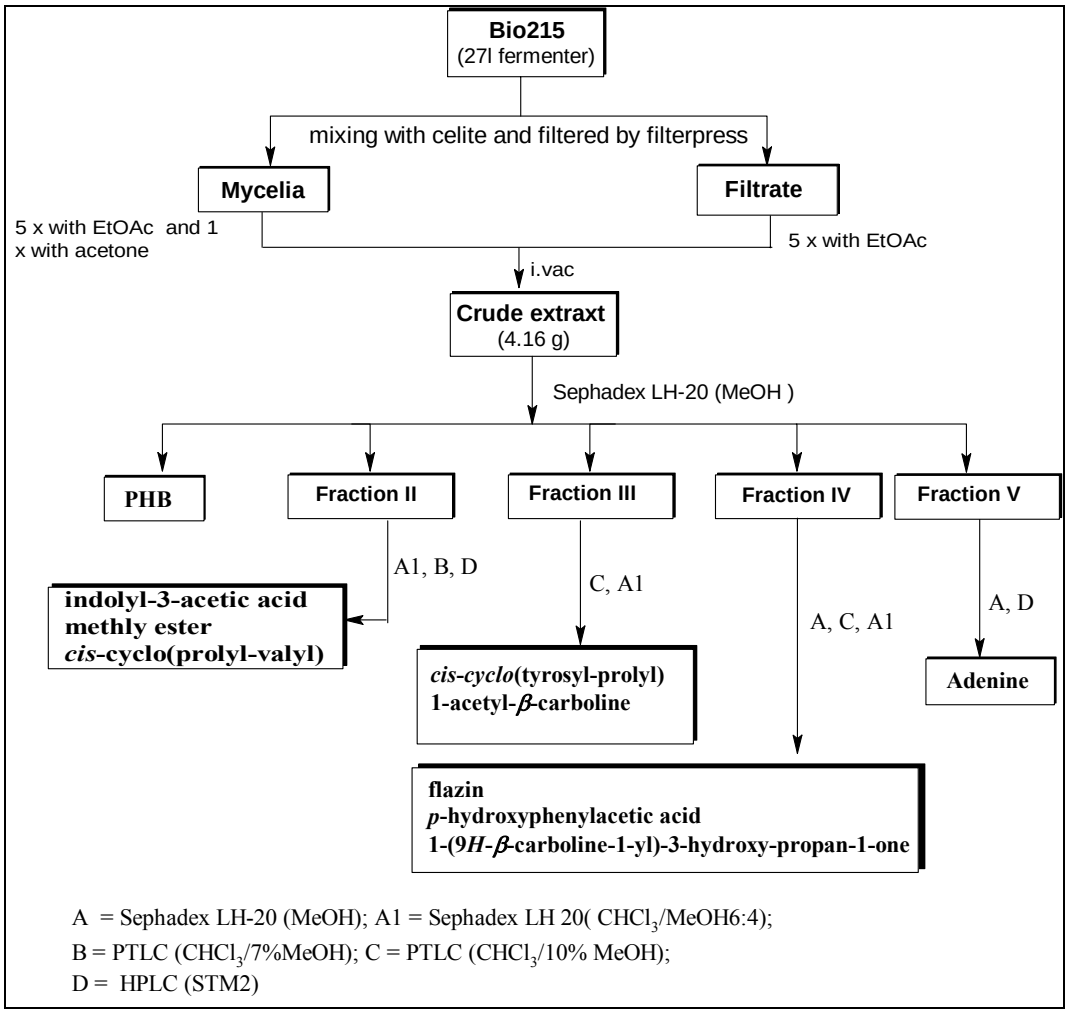


Figure 71: work-up procedure of strain Bio 215.

4.9.1 Indolyl-3-acetic acid methyl ester

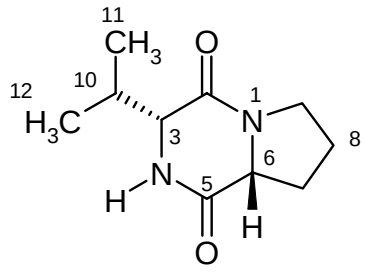
Compound **46b** was isolated as a colourless solid from fraction II after Sephadex LH-20, PTLC and HPLC. It showed an UV absorbing band which turned to orange/violet by anisaldehyde/sulphuric acid. By ^{1}H NMR and mass spectra and

comparison with reference data, the compound was identified as indolyl-3-acetic acid methyl ester (**46b)**.

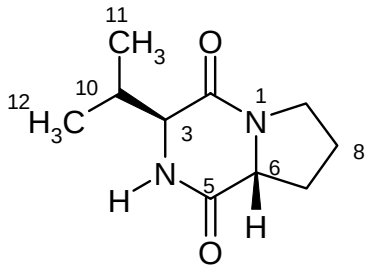
4.9.2 *cis-Cyclo*(Prolyl-Valyl)

Further investigation of fraction II led to the colourless solid **164**, which showed an UV absorbing band turning to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **164** exhibited two multiplets between δ 4.25 – 4.15 of two electron-deficient methines, one broad singlet of an acidic proton (NH) at δ 3.98, and a multiplet of two protons between δ 3.70-3.50. Several multiplets of five protons were shown between 2.65-1.85, two doublets each of two methyl groups at δ 1.07 and 0.91 to create an isopropyl system. The molecular weight of **164** was established as 196 Dalton using CI mass spectrum.

A search in AntiBase resulted in two possible structures, *cis-cyclo*(proly-valyl) (**164**) and *trans-cyclo*(proly-valyl) (**165**). Comparison the ^{1}H NMR data of both compounds **164** and **165** with authentic samples and literature[,204,205] established the compound as *cis-cyclo*(prolyl-valyl) (**164**). Compound **164** was reported as a specific *β*-glucosidase inhibitor. It was isolated previously from *Aspergillus* sp. F70609, various fungi and other natural sources[205]. Both isomers **164** and **165** were reported as secondary metabolites from the Caribbean sponge *Calyx* cf. *podatypa*[205].



164



165

4.9.3 Flazin

During the course of the screening of fraction IV, a yellow, greenish-yellow UV fluorescent (366 nm) solid **166** was isolated by PTLC followed by Sephadex LH-20.

The ^{1}H NMR spectrum exhibited a broad singlet of an acidic proton at δ 11.58, a deep field singlet at δ 8.83, which should be in a heteroaromatic ring (e.g. pyridine). In addition, four aromatic protons of an 1,2-disubstituted aromatic ring were ob-

served. Furthermore, two 1H doublets with a small coupling (<4 Hz) appeared at δ 7.41 and 6.68, which are characteristic for a 2,3-disubstituted five memberd heterocycle. Additionally, one 2H singlet of an oxymethylene group was exhibited at δ 4.64, along with a hydroxylic proton at δ 5.45.

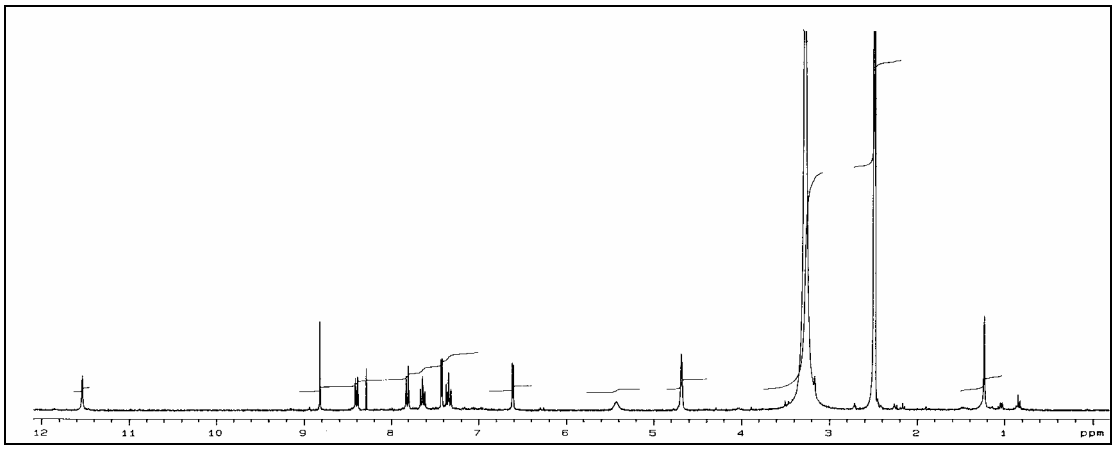


Figure 72: ^{1}H NMR spectrum ([D_6]DMSO, 300 MHz) of flazin (**166**)

The ^{13}C/APT NMR spectra displayed a CO group at δ 166.8 and eight quaternary and seven methine carbon signals in the aromatic region. In the aliphatic region, one signal appeared at δ 56.0 corresponding to the mentioned oxymethylene group. In accordance, partial structures were suggested as shown below (Figure 73), which were also obtained by H,H COSY and ^{1}J (2 D) spectra.

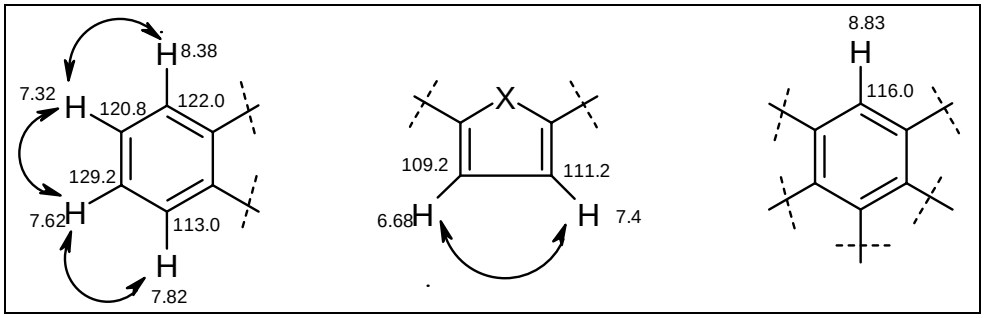


Figure 73: Partial structures of flazin (**166**) from H,H COSY; 2 D (^{1}J) correlations.

The ESI mass spectra of compound **166** established a molecular weight of 308 Dalton because of three *quasi*-molecular ions at *m/z* 353 ($[M + 2 Na - H]^+$, 331 ($[M + Na]^+$ and 309 ($[M + H]^+$) by (+)-ESI mode, and one ion peak at *m/z* 307 ($[M-H]^-$) by (-)-ESI mode. The molecular ion was further confirmed by EI MS, followed by fragmentation to give a base peak at *m/z* 264 due the expulsion of CO_2. This was indicative for the existence of a free carboxylic group. EIHR MS of **166** gave the corresponding molecular formula $C_{17}H_{12}N_2O_4$. This resulted in 13 double bond

equivalents as an indication of four aromatic rings. A search in AntiBase gave no results, pointing to a new natural product from microorganisms. However, according to the revealed physical and spectroscopic data of compound **166**, it showed a close structural similarity with *β*-carbolines, containing an additional carboxylic acid group and an attached hydroxymethyl-furan.

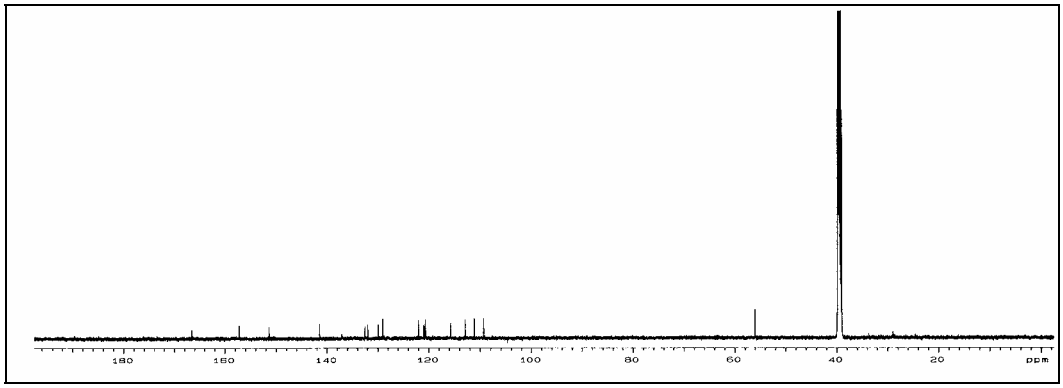


Figure 74: ^{13}C NMR spectrum ([D_6]DMSO, 150 MHz) of flazin (**166**).

The HMBC spectra confirmed the presence of the carboxyl group at position-3 of the *β*-carboline skeleton. In addition, the furan moiety was fixed at 1-position, although no correlation between the two rings was visible. However, the suggested structure was confirmed by comparison with the closely related perlolyrin (**167**). The last one had been isolated previously on our group[61]. It showed no correlation between the furan part and C-1 of the *β*-carboline skeleton as well. Hence the final structure of **166** was confirmed as 1-(5-hydroxymethyl-2-furyl)-*β*-carboline-3-carboxylic acid (flazin).

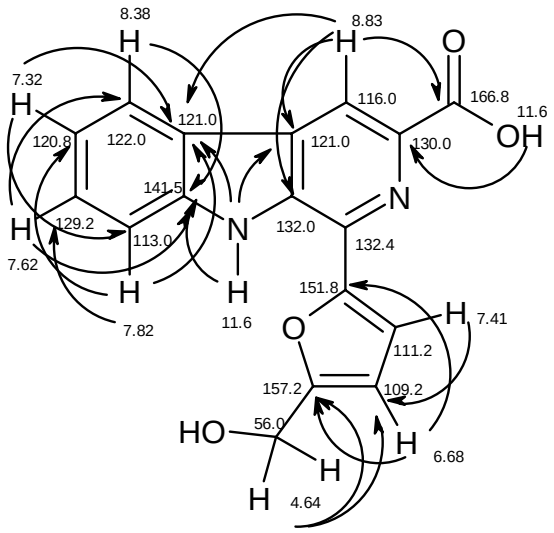
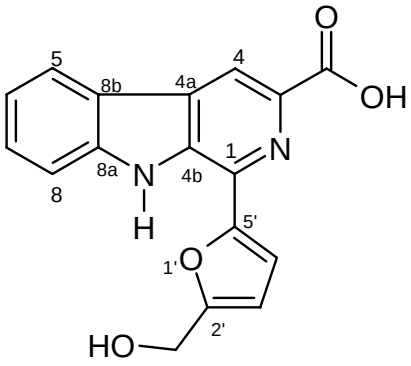
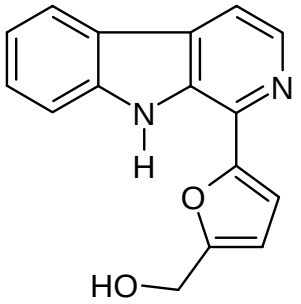


Figure 75: HMBC-connectivities of flazin (**166**)



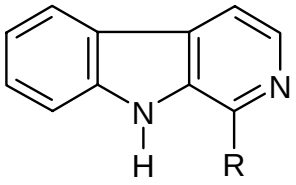
166



167

Flazin (**166**) and its analogue YS (perlolyrin) (**167**) were isolated previously as plant metabolite from soy sauce[206].Compound **166** was isolated first as plant metabolite by Higashi[207] from sake (Japanese rice wine) lees, Japanese rice vinegar, soy sauce and miso (fermented soy bean paste). Other β-carbolines occur in fungi, higher plants[215,208], marine animals[209], microorganisms[210], and fish powder[211]. Many of these alkaloids exhibited pharmacological effects[212] e.g. are enzyme inhibitors or antiviral agents[213]. β-carbolines are used as herbicide and fungicidal agents[214]. Of especial interests is their affinity to the benzodiazepine-receptor[212].

The simple β-carboline alkaloids[215] of the type **168** (R = aryl, alkenyl, alkyl, or other fragments) are originally constructed from tryptophane. For the synthesis of 1-substituted β-carboline, series of different methods were described[212], most of them depend on Bischler-Napieralski[216] or Pictet-Spengler [216]. Recently, there has been a new reaction created for the synthesis of 1-aryl-and-1-alkenyl β-carboline[212] from tryptophane (Figure 76). The carboxylic group at 3-position of flazin (**166**) is biosynthetically plausible. Starting with tryptophane, the carboxylic group can not be at 4-position, which gives us an additional explanation of the structure.



168

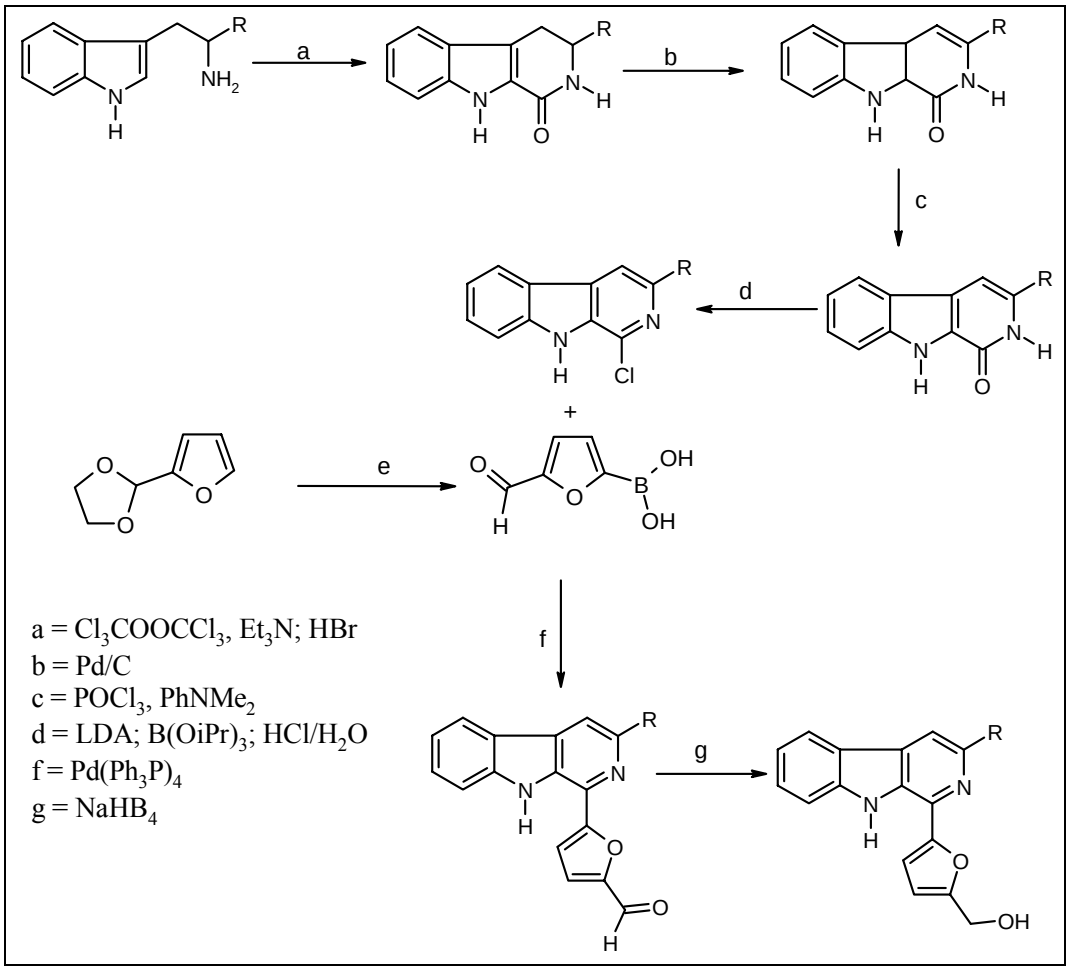


Figure 76: Synthetic reactions of flazin (**166**) and perlolyrin (**167**).

4.9.4 1-(9*H*-*β*-Carbolin-1-yl)-3-hydroxy-propan-1-one

Along with **166**, compound **171,** a second green fluorescent *β*-carboline was obtained from fraction IV with a slightly lower polarity.

The ^{1}H NMR spectrum exhibited six deep field aromatic protons, from which four are implied to construct an 1,2-disubstituted aromatic ring. The residual two are *ortho*-coupled protons at δ 8.49 and 8.38 present in a six-memberd heteroaromatic ring because of their observed small coupling constant (<5). In the aliphatic region, it showed an ethandiyl group located between two different substituents. The shift of the first methylene group at δ 4.05 (t) indicated oxygen, and the other at δ 3.55 (t) may be next to an sp^2 carbon atom or nitrogen. The aromatic pattern in the ^{1}H NMR spectrum of **171** is closely related to that observed in 1-acetyl-*β*-carboline (**81**).

The mass of **171** was established as 240 Dalton by EI mass spectrum, exhibiting an expulsion of an aldehydic group from the molecular ion to give a base peak at *m/z* 211. Two other peaks were observed at *m/z* 212 due to an expulsion of CO, and the

other at *m/z* 168 corresponding to the *β*-carboline skeleton (Figure 77). The molecular formula of **171** was deduced as $C_{14}H_{12}N_2O_2$ by EIHR MS.

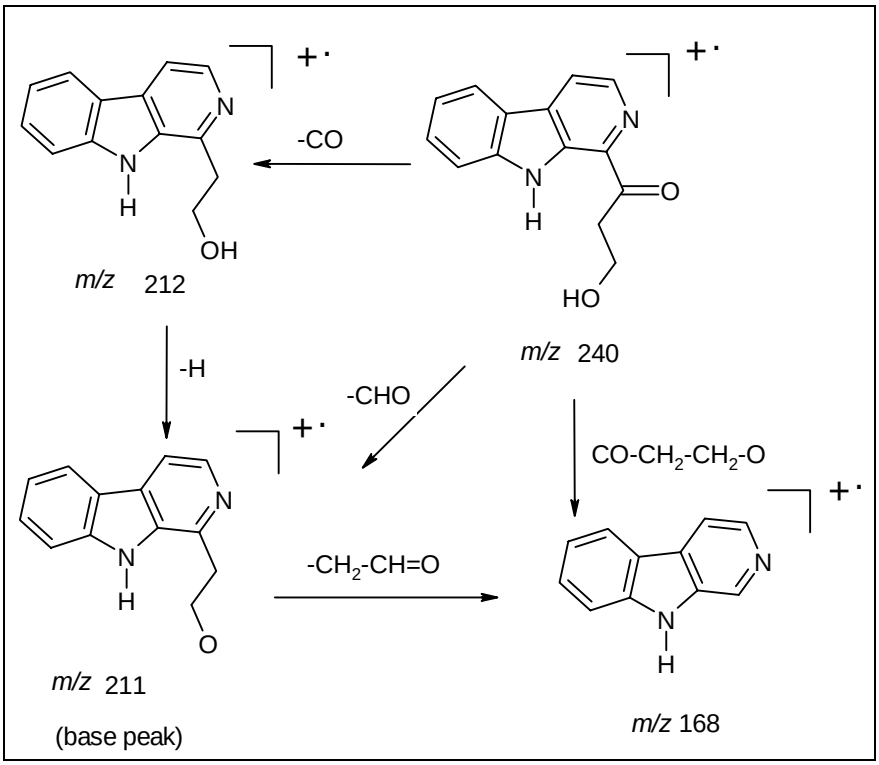
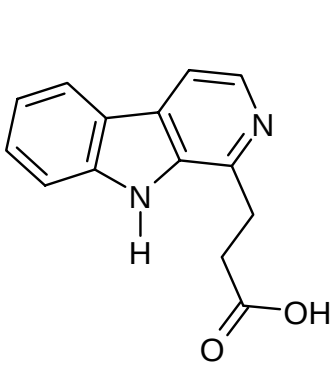


Figure 77: Proposed EI MS fragmentation pattern of 1-(9H-β-carbolin-1-yl)-3-hydroxy-propan-1-one (**171**)

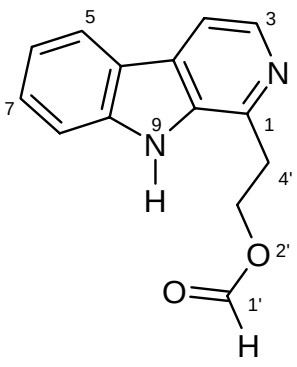
In agreement with the above spectral data as well as the molecular formula, three possible structures were projected, namely *β*-carboline-1-propionic acid (**169**), formic acid 2-(9H-*β*-carbolin-1-yl)-ethyl ester (**170**), and 1-(9H-*β*-carbolin-1-yl)-3-hydroxy-propan-1-one **(171**). As a search in AntiBase showed, compound **171** was known as a natural product from microorganisms, and a further search in the DNP led to compound **169**. However, compound **169** was excluded due to the lower δ value of its methylene group (δ 2.98) adjacent to the terminal carboxylic acid[217]. This was also supported by subjecting the compound to methylation with diazomethane, which brought no change in the spectroscopic data, thus excluding a free acid present in the compound. Compound **170** was also excluded as no aldehydic proton was observed in the ^{1}H NMR spectrum, despite of its observed fragmentation pattern, which pointed to the presence of an aldehyde moiety.

Hence, compound **171** is the only coincident structure with the above data, which was further confirmed by comparison with the literature[61] and with the closely related oxopropaline[218] G (**172**). The *β*-carboline **171** could be formed from

tryptamine and the terminal ketoaldehyde **173** *via* a Pictet-Spengler reaction. It is worth to mention that this ketoaldehyde, retin (**173**), is naturally known[23].



169



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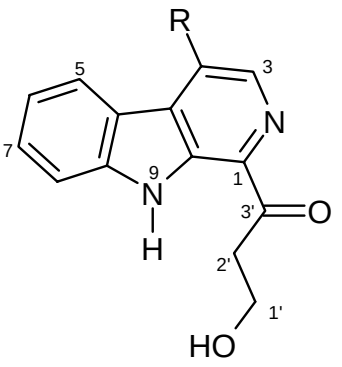
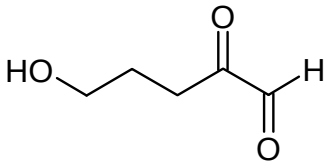


171: R = H
172: R = CH_3



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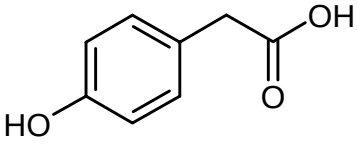
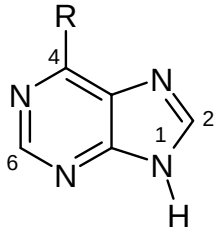


174



175: R = NH_2, **176**: R = H

4.9.5 *p*-Hydroxyphenyl acetic acid and Adenine; Angustmycin B

By further separation of fractions IV and V, two colourless solid compounds **174** and **175** were isolated. Both were UV absorbing, and the latter (**175**) turned to blue by anisaldehyde/sulphuric acid. Due to their chromatographic and spectroscopic data, compounds **174** and **175** were identified as *p*-hydroxyphenyl acetic acid and adenine (angustmycin B), respectively.

p-Hydroxyphenyl acetic acid was isolated as natural product by Power *et al*. from *Taraxacum* roots[219], and as a fungal metabolite[101] from *Ophiostoma crassivaginata*. Angustmycin B (**175**) was isolated first from the *Actinomycete* sp. 6 A-704 by Yüntsen *et al*.[220] as an antibiotic with *in vitro* activity against *Mycobacterium tuberclusis* 607 and *Mycobacteria phlei*. Additionally, it showed antiviral activity and pharmaceutical useful properties to extend the durability of whole blood preparates[24]. Adenine **175** can be easily synthesized from purine (**176**) using the Chichibabin reaction[221].

4.10 Strain Pic009

The crude extract of the marine bacterium sp. Pic009 showed several bands during the screening by TLC, which gave violet-blue colouration with anisaldehyde/sulphuric acid reagent and heating. Most of them exhibited UV absorption. Moreover, the extract exhibited activity against *Bacillus subtilis, Streptomyces viridochromogenes* (Tü57), *Escherichia coli* and *Mucor miehei* (Tü284). The fermentation was carried out using a 25-liter shaker culture at 28 °C for 4 days. After filtration and extraction with ethyl acetate, the crude extract was fractionated by Sephadex LH-20 column chromatography employing elution with methanol to yield four fractions. Further purification of these fractions led to *o*-hydroxyphenyl acetic acid (**177**), isoxanthohumol (**180**), uridine (**187**), in addition to other known compounds (Figure 78).

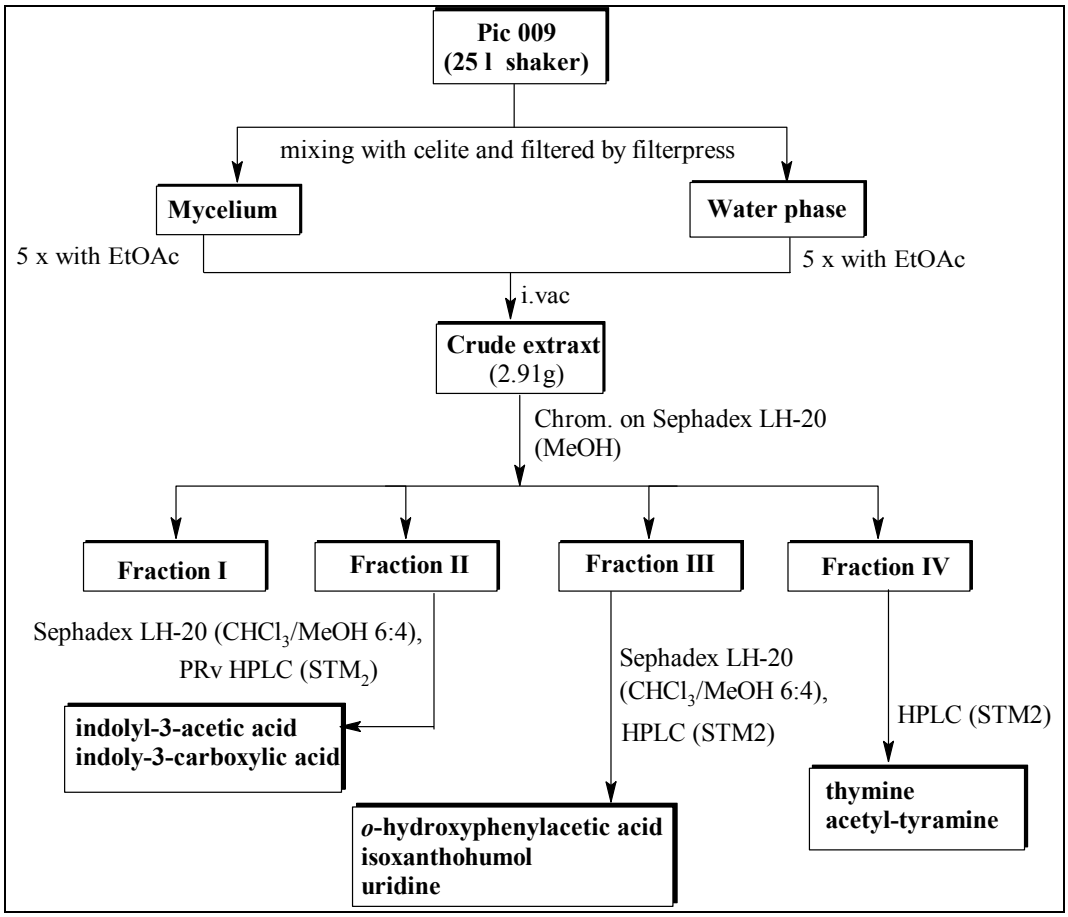


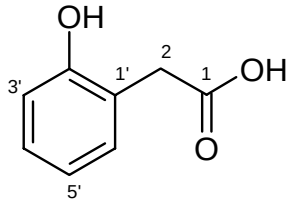
Figure 78: Work-up procedure of strain Pic009

4.10.1 *o*-Hydroxyphenyl acetic acid

Fraction III showed an UV absorbing middle polar spot from which **177**, a colourless solid was isolated by HPLC. The ^{1}H NMR spectrum displayed four proton signals of an 1,2-disubstituted aromatic ring. In addition, one 2H singlet of a methylene group was found at δ 3.60.

The EI mass spectrum delivered the molecular weight of **177** as 152 Dalton. The molecular ion exhibited two further fragments at *m/z* 134 and 106 due to elimination of H_2O and expulsion of acetic acid, respectively.

A search in AntiBase has led to *o*-hydroxy phenyl acetic acid (**177**). Compound **177** was further confirmed by comparison with the literature[222]. *o*-Hydroxyphenyl acetic acid (**177**) was found as a fungal metabolite e.g. from *Rhizoctonia leguminicola* and *Ophiostoma crassivaginata*[101] as well as plant metabolite e.g. from *Astilbe* sp.[24].



177

4.10.2 Isoxanthohumol

Compound **180** was isolated as colourless solid by applying HPLC to fraction III. It exhibited an UV absorbing band, which showed, however, no colour by staining with anisaldehyde/sulphuric acid. Compound **180** exhibited an UV fluorescent pale yellow band at 366 nm when exposed to ammonia. The ^{1}H NMR spectrum of **180** displayed two phenolic hydroxyl groups at δ 9.38, and 8.51, two doublets each with intensity of 2H at δ 7.39 and 6.88 as an indication of an 1,4-disubstituted aromatic ring. In addition, a deepfield 1H singlet at δ 6.26 could be due to an aromatic proton between two electron-donating OH-groups. Furthermore, an oxymethine proton gave a dd at δ 5.35 ($J_{2,3a}$ = 12.5, $J_{2,3b}$ = 3.5 Hz), which could be adjacent to an additional methylene group present as ABX between δ 2.93-2.61. A 1H triplet at δ 5.20 of an olefinic proton linked to a methylene group was observed as dd between δ 3.30-3.25. Furthermore a 3H singlet at δ 3.73 of a methoxy group were observed.

Finally, it showed two singlets of methyl groups at δ 1.61 and 1.57, which could be linked to an olefinic carbon. The partial structures derived thereof are given below (Figure 79).

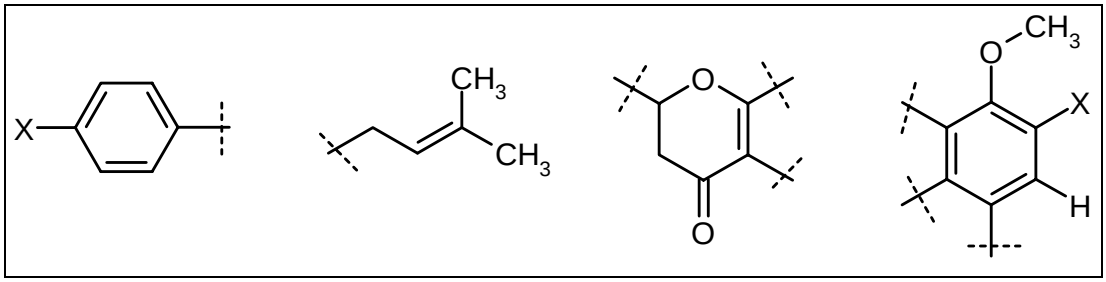


Figure 79: Partial structures of isoxanthohumol (**180**)

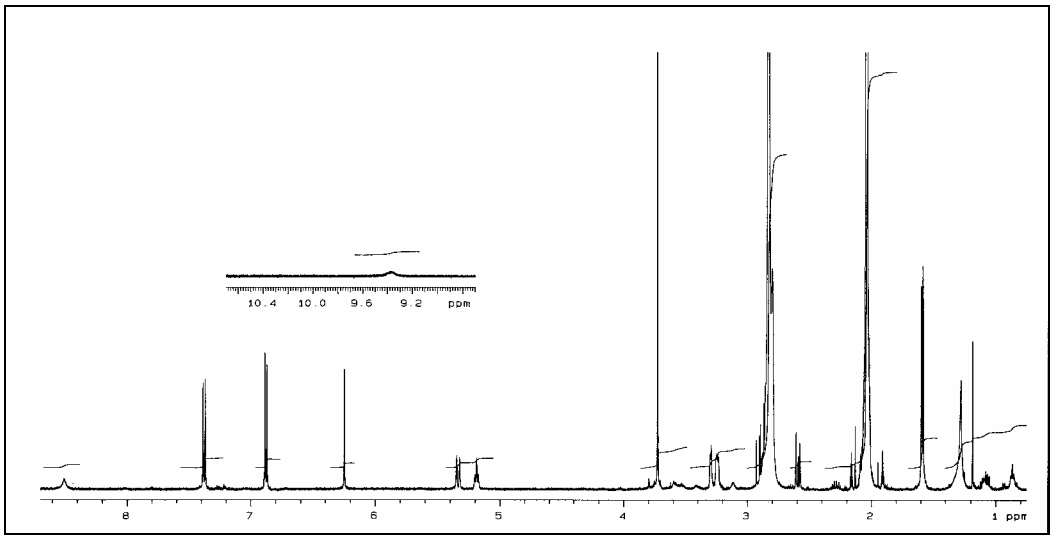


Figure 80: ^{1}H NMR spectrum ([D_6]acetone, 500 MHz) of isoxanthohumol (**180**)

Both positive and negative ESI mass spectra modes determined the molecular weight of compound **180** as 354 Dalton. The EI spectrum confirmed the molecular weight and gave some significant fragments (Figure 81), one of which at *m/z* 339 was due to the loss of a methyl group and one at *m/z* 299 was due to the expulsion of an isobutenyl group. Three other fragments (*m/z* 15, 43 and 55) showed the characteristic pattern of an C-prenylated compound[223]. High resolution of the molecular weight gave the corresponding molecular formula $C_{21}H_{22}O_5$.

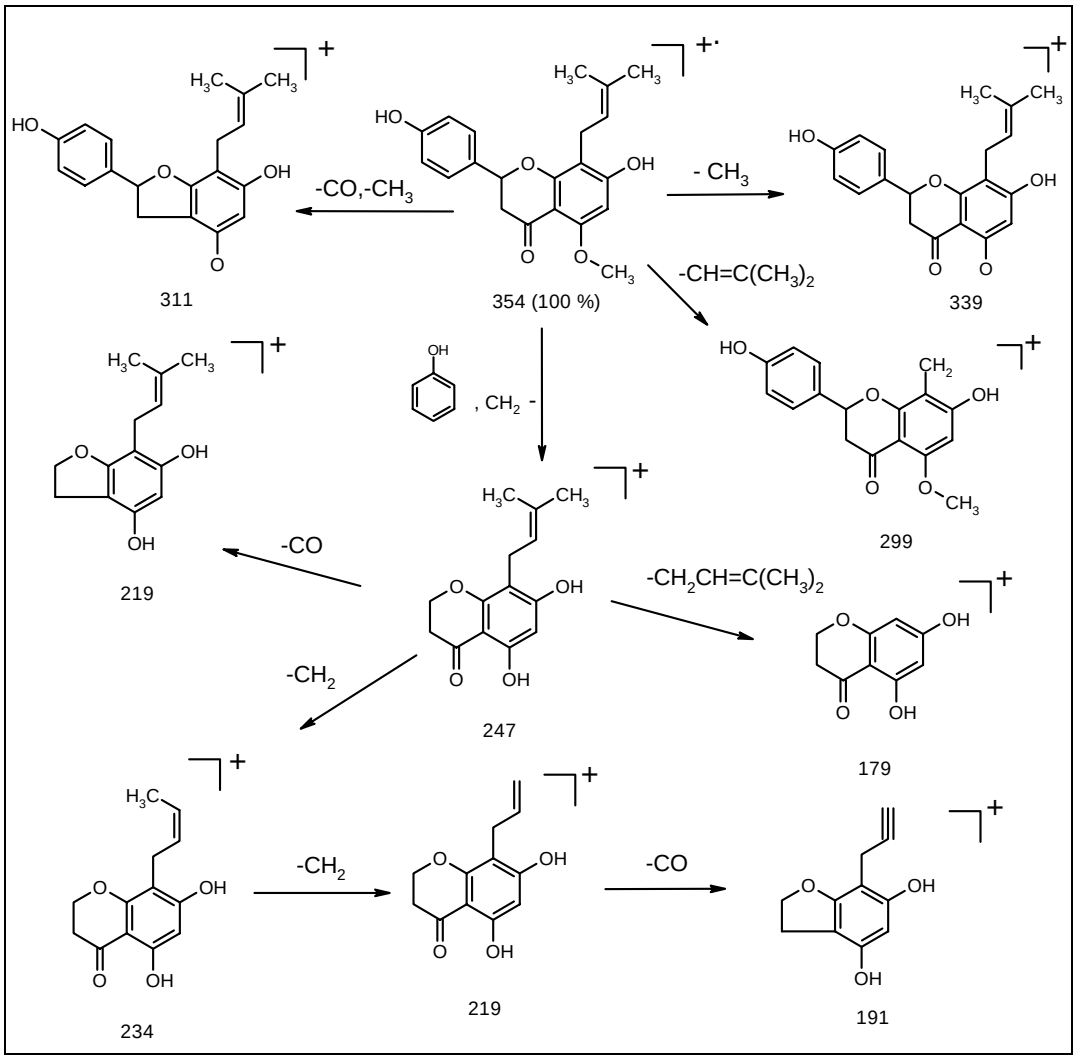
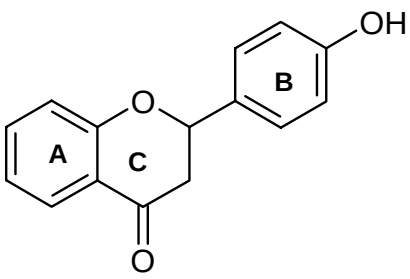


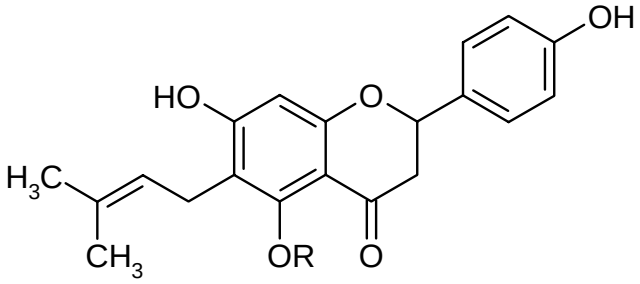
Figure 81: EI mass fragmentation pattern of isoxanthohumol (**180)**

Depending on the above revealed chromatographic, spectroscopic, molecular formula and UV properties of compound **180**, four possible flavanone (**178**) moieties were taken into account, namely 7,4'-dihydroxy-5-methoxy-6-(γ,γ-dimethylallyl)-flavanone (**179a**), 7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**180**), 7,5-dihydroxy-4'-methoxy-6-(γ,γ-dimethylallyl)-flavanone (**181**) and 7,5-dihydroxy-4'-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**182**).

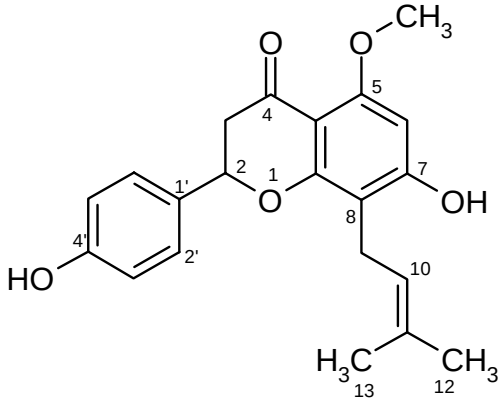
The latter compounds **181** and **182** were excluded due to their chelated *peri*-hydroxy group (5-OH) which normally displays a characteristic singlet at $\delta \geq 12$ [224]. A further search in DNP and CA resulted in isoxanthohumol (**180**), which was confirmed by direct comparison of ^{1}H NMR data with the literature[225,226,*]. The 6-prenyl isomer **179a** is not known as natural or synthetic product until now, although its analogue with a free hydroxy group at 5-position, 6-prenylnaringenin[225] (sophoraflavanone B, **179b**)[224] is a natural product.



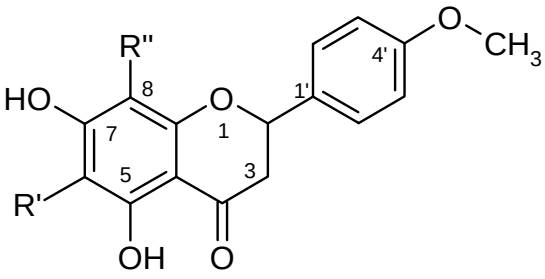
178



179a: R = CH_3, **179b**: R = H



180

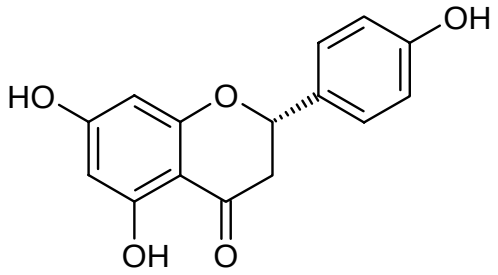


181: R' = CH_2-CH=$C(CH_3)_2$, R" = H
182: R' = H, R" = CH_2-CH=$C(CH_3)_2$

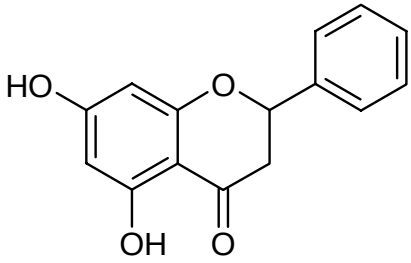
There are many 5,7-dihydroxyflavanones having 3,3-dimethylallyl (prenyl or (E)-3,7-dimethyl-2,6-octadienyl (geranyl) side chains at C-6 or C-8. In the NMR spectrum, the signals of 6-H and 8-H are very close together in 5,7-dihydroxyflavanone, and for example in naringenin (**183**), they are overlapping each other. However, they can be distinguished for instance by the Gibbs test[227], anomalous $AlCl_3$ induced UV shift[228], cyclized reaction[229]. Most flavonoid compounds can be identified based on their UV characteristics as follows:

- The flavanones containing a free C_5-OH in ring A show an UV absorbing deep purple spot with no change when exposed to NH_3. However, when C_5-OH is blocked, a blue UV fluorescence is observed.
- The free 4'-OH group in ring B is affected by NH_3 giving a yellow fluorescent spot under UV, e.g. in naringenin (**183)**. However, when it is blocked, no change by UV/NH_3 is observed, e.g. in pincocembrin[230] (**184**).
- When the 5-OH is occupied and 4'-OH is free, the compound shows a blue UV fluorescence which changes to yellow when exposed to NH_3, e.g. in sakuranin[231] (**185**). The latter case is the same as for **180**.

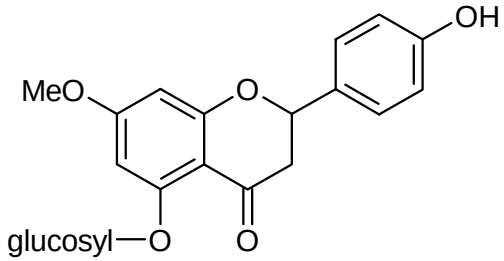
Isoxanthohumol (**180**) was isolated first in very little amount from the widely cultivated plant *Humulus lupulus L.* (*Cannabianacea*)[225] which contains also xanthohumol (**186**), the principle of hop resin. Compound **180** was isolated recently from the roots of *Sophora flavescens*[226]. Xanthohumol (**186**) and its isomer isoxanthohumol (**180**) are reported as anticarcinogenic, antifungal and antioxidant agents[232].



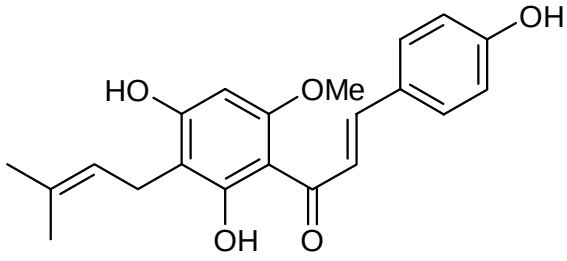
183



184



185

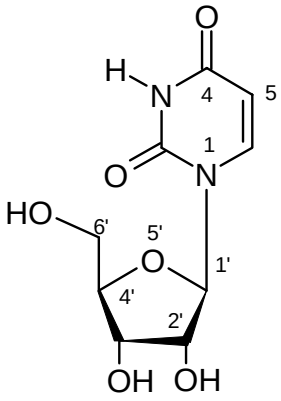


186

4.10.3 Uridine

Through further screening of fraction III by HPLC, compound **187** was isolated as a colourless solid, exhibiting an UV absorbing band which turned blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **187** displayed the two, uracil doublet, signals, however, with a slightly higher shift, in addition to a 1H doublet at δ 5.78 of an anomeric proton. Furthermore, three oxymethine protons were observed, two at δ 4.08, and the third one at δ .83. Moreover, a multiplet of an oxymethylene group was exhibited at δ 3.56, and two broad multiplets of 3 OH at δ 5.29 (OH) and 5.02 (2 OH) which disappeared by H/D exchange.

The molecular weight of **187** was confirmed as 244 Dalton by ESI and CI mass spectra. Comparison with authentic spectra and the literature[233,*] afforded uridine (**187**), which is widely distributed in nature in free state or in nucleic acid and can be produced by hydrolysis thereof [24].



187

4.11 Terrestrial *Streptomyces* sp. GW10/580

The ethyl acetate extract of the terrestrial *Streptomyces* sp. GW10/580 drew our attention due to the presence of a yellow-orange band during TLC, which turned to red by conc. sulphuric acid, one huge brown band after spraying by anisaldehyde/sulphuric acid, and many other absorbing spots. Moreover, the strain possessed a high activity against all of the tested microorganisms: *Mucor miehei* (Tü284), *Streptomyces viridochromogenes* (Tü57), *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and the algae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*. Therefore the strain was cultivated in two different ways as shaker culture and jar Fermenter.

* The compound reported in the literature was measured in DMF and gave a shift by Δδ 0.12 higher than the value measured here in DMSO

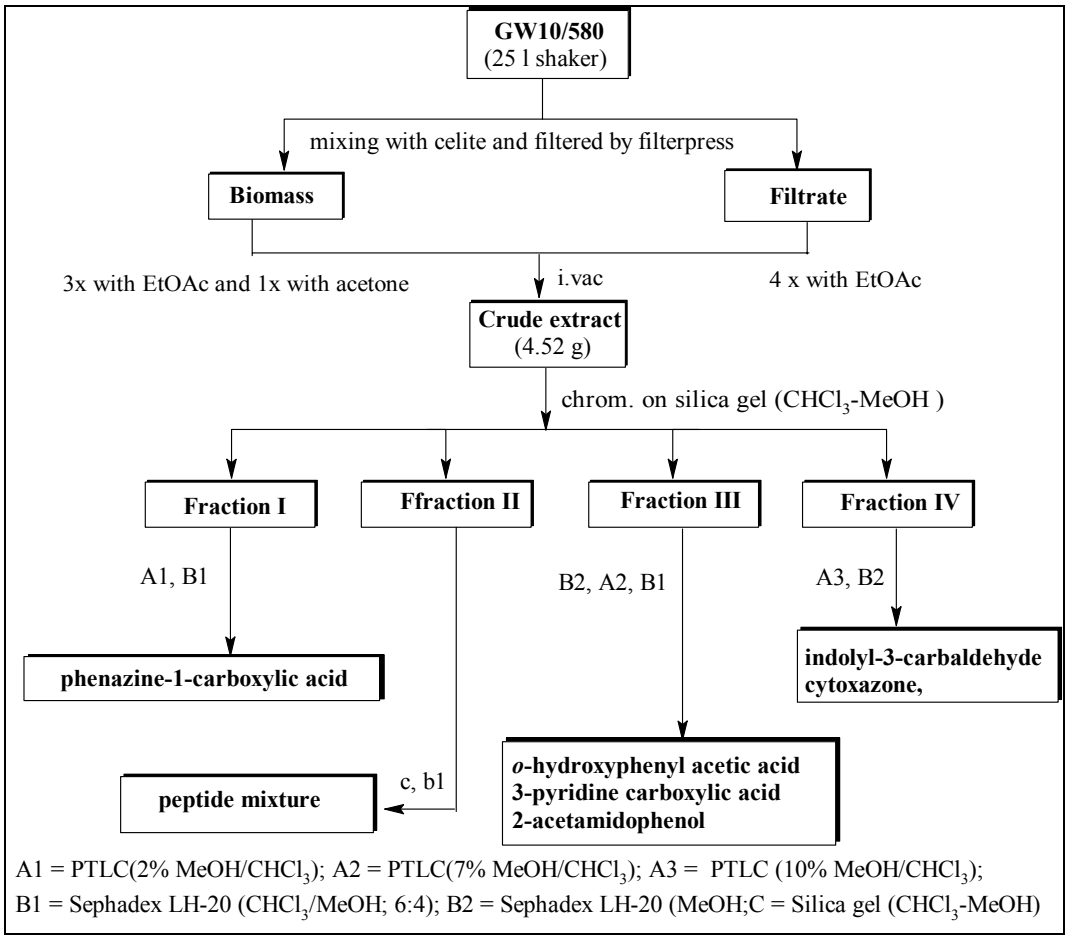


Figure 82: Work-up procedure of the terrestrial *Streptomyces* isolate GW10/580 (shaker)

A) Shaker culture

Well-grown agar cultures of terrestrial *Streptomyces* sp. GW10/580 served to inoculate 25 liters of M_2 medium. The flasks were incubated at 28 ºC while shaking with 110 rpm on a linear shaker for 3 days. The culture broth was filtered and extracted with ethyl acetate. The dark brown crude extract was applied to silica gel flash column chromatography, eluting with a chloroform-methanol gradient. Purification of fractions I-IV delivered phenazine carboxylic acid (**188**), surfactin C (**189**), pyridine carboxylic acid (**190**), 2-acetamidophenol (**192**), indolyl-3-carbaldehyde (**194**) and cytoxazone (**195**), in addition to other known compounds (Figure 82).

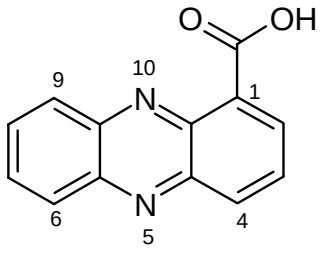
4.11.1 Phenazine-1- carboxylic acid; Tubermycin B

The first fast moving fraction I showed a yellowish-green non polar band which turned to red by sulphuric acid. A yellowish-green middle polar amorphous compound **188** was isolated by further applying the fraction to PTLC and Sephadex LH-

20. The ^{1}H NMR spectrum showed seven aromatic protons, the first four of them were exhibited as dd each of 1H at δ 8.92, 8.61, 8.52 and 8.41, and the residual three protons were shown as multiplet between δ 8.20-8.08. Hence, two aromatic 1,2-disubstituted and 1,2,3-trisubstituted rings could be derived. The displayed deep field shift pointed to their fusion with a hetero-aromatic ring.

The (+)-ESI mass spectrum of compound **188** fixed its molecular weight as 224 Dalton. The EI mass spectrum exhibited in addition to the molecular ion a base peak at *m/z* 180 resulting from the loss of a carboxy group, and the mass *m/z* 180 is indicative for the phenazine skeleton. A search in AntiBase led to phenazine-1-carboxylic acid (tubermycin B, **188**). The compound was further confirmed by comparing the data with authentic spectra and the literature[234].

Tubermycin B (**188**) was isolated previously from microorganisms *Pseudomonas*., *Streptomyces cinnamonensis*, *Streptomyces misakiensis* and *Actinomadura dassonvillei*. It exhibits a weak activity against Gram-positive bacteria, and a moderate activity against both *Mycobacterium tuberculosis* BCG and *Mycobacterium tuberculosis* H_{37}Rv (streptomycin resistant)[235].



188

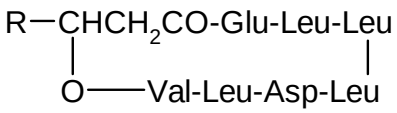
4.11.2 Surfactin C

Further separation of fraction II afforded a bulky zone of compound **189** which became brown after spraying with anisaldehyde/sulphuric and heating. Moreover, the compound showed a blue colour with the chlorine/*o*-anisidine reaction, however, was yellow with ninhydrin, as an indication of a cyclic or N-acylated peptide. The compound showed, however, no UV absorbance, referring to **189** as a non aromatic or olefinic compound which was isolated as a colourless solid by chromatography on silica gel and Sephadex LH-20. HPLC/MS analysis revealed it as a mixture of homologues.

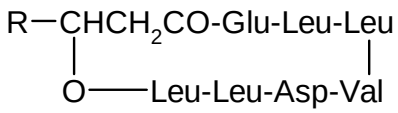
The ^{1}H and ^{13}C NMR spectra showed a bad resolution and most signals were in the aliphatic region, establishing the presence of an aliphatic long side chain. The ^{1}H NMR spectrum showed broad singlets of NH protons between δ 8.4-7.30 with an intensity of 6H and multiplets between δ 5.25-4.06 of 8H in α-position to nitrogen or oxygen. In the region between δ 2.88-1.15, it exhibited multiplets of methine and methylene protons with an intensity of 36 H. In addition, multiplets of 30H between δ 1.05-0.85 could be assigned as ten methyl groups. The ^{13}C NMR spectrum displayed 10 overlapped carbonyl signals between δ 178-169. Besides, 8 CH of oxy- and/or nitrogenous methines, which were displayed between δ 75-48. Overlapping methylene carbon signals (17 CH_2) were observed between δ 42~28, and possibly it showed two methine carbons between δ 27.32 and 24.22 of 2 isopropyl moieties. It exhibited numerous overlapped signals between δ 25~12, probably due to 10 CH_3 groups.

The ESI mass spectra of peptide **189** indicted a mixture of six homologues, the main of which showed *quasi*-molecular ions of $[M+H]^+$, $[M + Na]^+$ and $[M-H]^-$ at *m/z* 1036.7, 1058.8 and 1034.8, respectively. Therefore the molecular mass was established as 1036 Dalton.

By applying the above spectral data to AntiBase, 7 possible structures were revealed: Esperin, four surfactins C (**189),** daitocidin A1 and pumilacidin B. All are cyclic ß-hydroxyacyl peptides with 10 carbonyl groups, which fits well on the spectra and the TLC colour reactions. Pumilacidins were isolated in our research group previously by Huth[48]. They are described as a complex of antiviral antibiotics isolated from the culture broth of *Bacillus pumilus*[236]. All pumilacidins possessed no inhibitory effects against bacteria and fungi. Surfactins[237] (obtained from *Bacillus subtilis natto*) are most efficient biosurfactants, and exhibit potent antifungal, and antitumor activities against Ehrlich ascites carcinoma cells and inhibit fibrin clot formation, as well as cyclic adenosine 3',5'-monophosphate phosphodiesterase[238,239]. Both pumilacidins (**A**) and surfactins (**B**) are structurally closely related. They comprise the same amino acids, but they differ in the sequence of the amino acids.



A

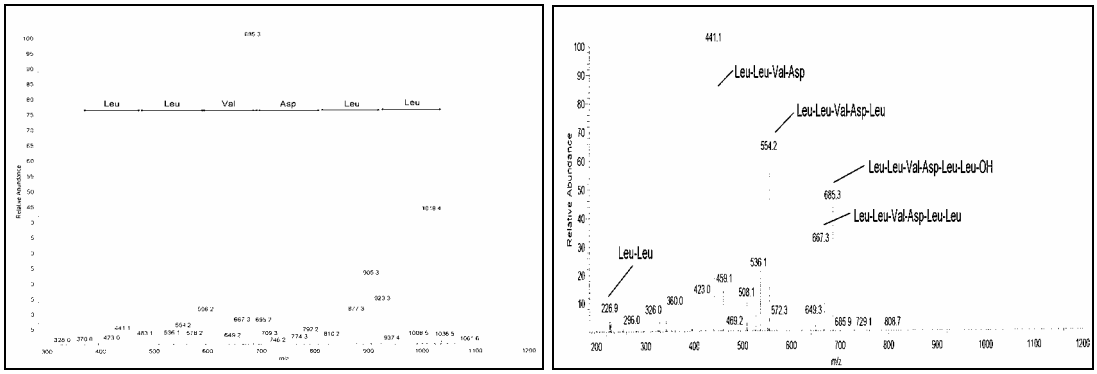


B

The structure of the peptide was elucidated by detailed MS studies. ESI MS^2 and MS^3 of 1036.70 $[M+H]^{2+}$ showed sequential losses of amino acids, confirming the sequence as Leu-Leu-Val-Asp-Leu-Leu (Figure 83). The observed intensive peak at *m/z* 685.3 in MS^2 confirmed the cleavage of the lactone bond and the loss of an hydroxyacyl glutamic acid fragment. MS sequencing of *m/z* 685 as above gave again the amino acid sequence Leu-Leu-Val-Asp-Leu-Leu-OH (Table 8)[240], thus confirming the surfactin C sequence with Leu as the first C-terminal amino acid, which is involved in the lactone ring. The fragment ions at *m/z* 370.0 [FA-Glu] (Table 8) and 339.4 $[FA\text{-}Glu - CO]^-$ (

Table 9) correspond to the β-hydroxy fatty acid (FA) linked to Glu. Additionally, most of the observed fragment ions were accompanied by the loss of CO and CO_2, which is attributed to the presence of Glu and Asp in the peptide skeleton of **189**.

In accordance, the whole sequence of the cyclic peptide directed to surfactin C (**189**), *cyclo*[FA-Glu-Leu-Leu-Val-Asp-Leu-Leu] **(**Figure 84**)**. The alternative structure of pumilacidine B (**A**) can be excluded. The structure of esperin (Figure 85) with its lactone between aspartic acid and the acyl residue was also excluded: MS^2 of esperin should show an intense peak at *m/z* 245, which was not observed.



a b

Figure 83: ESI MS^2 (a) and ESI MS^3 (b) fragmentation of 1036.7 $[M+H]^+$ of surfactin C (**189**)

Table 8: Fragment ions of surfactin C (**189**) observed in ESI MS^2 and MS^3 of $[M + H]^+$

MS^2	Sequence of amino acids	MS^3	Sequence of amino acids
370	FA-Glu	198.8	Leu-Leu – CO

483.1	FA-Glu-Leu	226.9	Leu-Leu
578.2	FA-Glu-Leu-Leu – H_2O	325.7	Asp-Leu-Leu
596.2	FA-Glu-Leu-Leu	709.3	$[M+H – Leu-Val-Asp]^+$
649.2	FA-Glu-Leu-Leu-Val - CO – H_2O	937.4	$[M+H – Val]^+$
667.3	FA-Glu-Leu-Leu-Val - CO	413.2	Val-Asp-Leu-Leu – CO
677.2	FA-Glu-Leu-Leu-Val – H_2O	423.0	Val-Asp-Leu-Leu – H_2O
695.2	FA-Glu-Leu-Leu-Val	441.1	Val-Asp-Leu-Leu
774.3	FA-Glu-Leu-Leu-Val-Asp – $2H_2O$	536.1	Leu-Val-Asp-Leu-Leu – H_2O
792.2	FA-Glu-Leu-Leu-Val-Asp – H_2O	554.2	Leu-Val-Asp-Leu-Leu
810.2	FA-Glu-Leu-Leu-Val-Asp	667.3	Leu-Leu-Val-Asp-Leu-Leu
877.3	FA-Glu-Leu-Leu-Val-Asp-Leu - H_2O – CO	685.3	Leu-Leu-Val-Asp-Leu-Leu-OH
905.3	FA-Glu-Leu-Leu-Val-Asp-Leu – H_2O		
923.3	FA-Glu-Leu-Leu-Val-Asp-Leu		
1018.4	FA-Glu-Leu-Leu-Val-Asp-Leu-Leu – H_2O		
1036.5	*cyclo*[FA-Glu-Leu-Leu-Val-Asp-Leu-Leu + $H]^+$		

Table 9: Fragment ions of **189** observed in MS^2 and MS^3 of $[M + Na]^+$ and $[M - H]^-$

MS^2& MS^3 [M +Na]	**Sequence of amino acids**	**MS^2&MS^3 [M-H]**	**Sequence of amino acids**
391.3	Val-Asp-Leu-Leu - CO – CO_2	339.4	FA-Glu - CO
435.2	Val-Asp-Leu-Leu -CO	452.5	FA-Glu-Leu - CO
463.2	Val-Asp-Leu-Leu	552.4	Leu-Val-Asp-Leu-Leu
481.2	Val-Asp-Leu-Leu-OH	665.6	Leu-Leu-Val-Asp-Leu-Leu-OH
594.4	Leu-Val-Asp-Leu-Leu-OH	692.7	FA-Glu-Leu-Leu-Val
618.4	FA-Glu-Leu-Leu	776.6	$[M-H -FA - H_2O]^-$
671.4	FA-Glu-Leu-Leu-Val - CO – H_2O	790.7	FA-Glu-Leu-Leu-Val-Asp - H_2O
707.4	Leu-Leu-Val-Asp-Leu-Leu-OH	794.6	$[M-H -FA]^-$
742.5	FA-Glu-Leu-Leu-Val-Asp –H_2O – CO – CO_2	859.7	FA-Glu-Leu-Leu-Val-Asp-Leu - CO_2 – H_2O
814.4	FA-Glu-Leu-Leu-Val-Asp – H_2O	903.7	FA-Glu-Leu-Leu-Val-Asp-Leu - H_2O
832.4	FA-Glu-Leu-Leu-Val-Asp	885.6	FA-Glu-Leu-Leu-Val-Asp-Leu - $2H_2O$
899.6	FA-Glu-Leu-Leu-Val-Asp-Leu - H_2O – CO	954.6	$[M-H - 2H_2O – CO_2]^-$
927.5	FA-Glu-Leu-Leu-Val-Asp-Leu –	998.8	$[M-H - 2H_2O]^-$

	H_2O		
945.5	FA-Glu-Leu-Leu-Val-Asp-Leu	1016.7	$[M-H - H_2O]^-$
986.7	FA-Glu-Leu-Leu-Val-Asp-Leu-Leu – CO – CO_2		
1030.6	FA-Glu-Leu-Leu-Val-Asp-Leu-Leu –CO		
1040.6	FA-Glu-Leu-Leu-Val-Asp-Leu-Leu – H_2O		
1058.7	*cyclo*[FA-Glu-Leu-Leu-Val-Asp-Leu-Leu]		

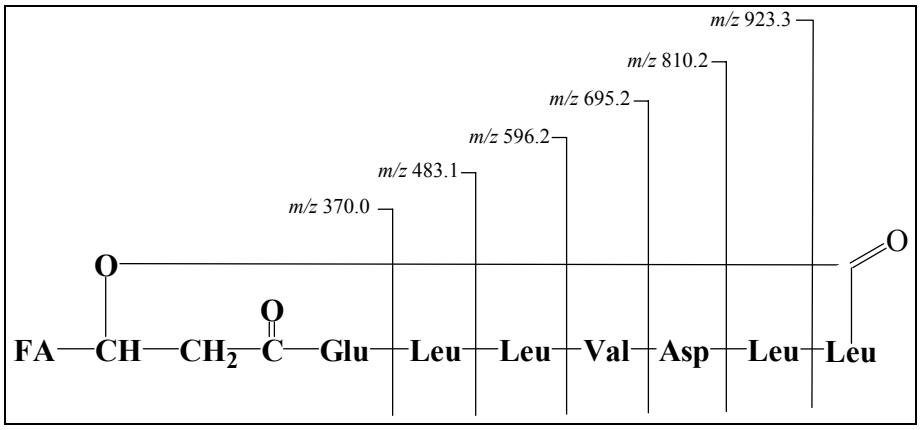


Figure 84: Fragment ions observed in $[M+H]^+$ mass spectrum of surfactin C (**189**)

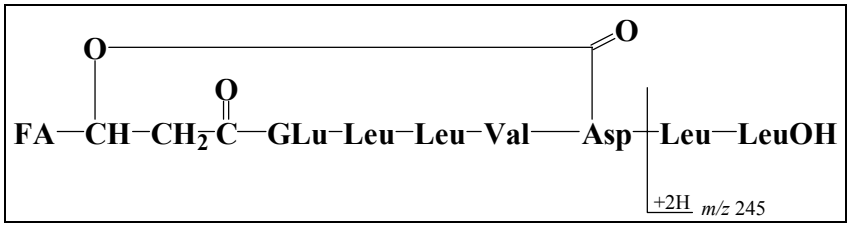
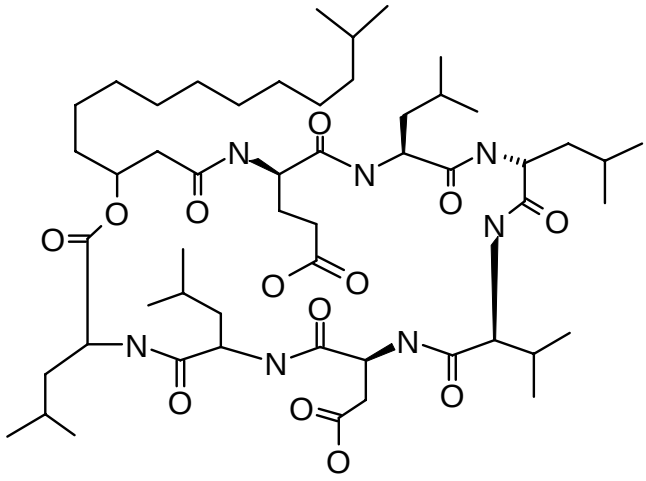


Figure 85: A characteristic ESI MS fragment should be existent in esperin.



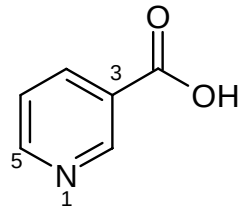
189

4.11.3 Pyridine-3-carboxylic acid; Nicotinic acid

Compound **190**, was obtained as a colourless solid from fraction III by application of PTLC and Sephadex LH-20. It showed a middle polar UV absorbing zone which turned to faint pink with Ehrlich's reagent.

The ^{1}H NMR spectrum displayed a broad singlet at δ 9.80 of an acidic proton. Moreover, a split (dd) deep field signal was observed at δ 9.14 (J = 0.9 Hz) which could be due to a *m*-coupled aromatic proton. In addition, three other dd protons with *o*-couplings were observed at δ 8.78, 8.31 and 7.52. Due to the observed high proton shifts, the compound must consists of a single hetero-aromatic ring.

The EI mass spectrum fixed the molecular weight as 123 Dalton. The odd mass established the existence of an odd number of nitrogen atoms. The fragment at *m/z* 78 is could be attributed to a pyridinium ion and the ion at *m/z* 45 is indicative for a carboxylic acid group. These spectral data are matching nicely with the ^{1}H NMR spectrum and allowed us to elucidate the compound as pyridine-3-carboxylic acid (**190**). Nicotinic acid (**190**) has been reported as a fungal metabolite from *Phycomyces blakesleeanu*[241] and is an important plant intermediate. It is used as vitamin, enzyme cofactor, vasodilator and antihyperglycemic agent and widely used in treatment of lipid disorders[24]. However, **190** was till now not known as bacterial secondary metabolite.



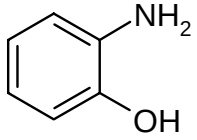
190

4.11.4 2-Acetamidophenol; 2-Hydroxyacetanilide

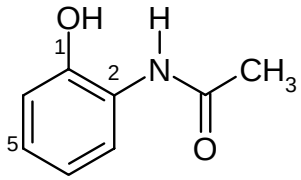
By further working up of fraction III, compound **192** was obtained as colourless solid, which turned to pink by Ehrlich's reagent during TLC. The ^{1}H NMR spectrum displayed a broad 1H singlet at δ 9.29 of a phenolic hydroxyl or amide proton. In addition, four aromatic signals of an 1,2-disubstituted aromatic moiety were observed at δ 7.43 (dd), 7.01 (t), 6.87 (dd) and 6.77 (t). Furthermore, the 3H singlet of a

methyl group was displayed at δ 2.20 which could be assigned as acetyl or S-methyl group.

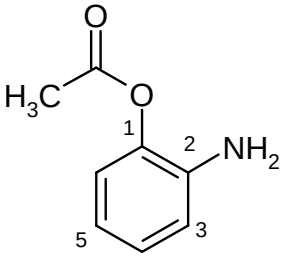
The EI mass spectrum established the molecular ion of compound **192** at 151 Dalton, followed by a base peak at *m/z* 109 corresponding to *o*-aminophenol (**191**) as a result from the loss of a ketene residue ($O=C=CH_2$). According to the outlined spectral data, two possible structures could be proposed: 2-acetamidophenol (**192**) and 2-amino-phenyl acetate (**193**). By searching the spectral data in AntiBase, 2-acetamidophenol **(192**) was established, and the latter was further confirmed by direct comparison with literature[242].



191



192

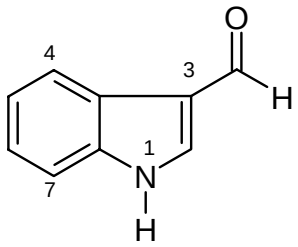


193

4.11.5 Indolyl-3-carbaldehyde

From fraction IV, compound **194** was obtained as colourless solid by PTLC and Sephadex LH-20, giving an UV absorbing spot during TLC which was stained to orange by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed a broad singlet of an H/D exchangeable proton at δ 11.19 of indolyl amino proton (NH). A 1H singlet at δ 10.01 was not exchangeable and could be assigned as an aldehyde proton. In addition, a doublet at δ 8.17 with a small coupling constant (J = 3.0 Hz) suggested a *m*-coupled proton. Moreover, four aromatic protons at δ 8.22 (d), 7.54 (d), and 7.23 (m, 2 H), indicated an 1,2-disubstituted aromatic ring. The ^{1}H NMR data pointed to an indole moiety substituted at 3-position.

The EI mass spectrum determined the molecular weight of compound **194** as 145 Dalton. The mass ion (*m/z* 145) showed a subsequent expulsion of H to give a base peak at *m/z* 144. This is indicative for an aldehyde group. A search in AntiBase fixed the compound as indolyl-3-carbaldehyde (**194**), which was finally confirmed by direct comparison with authentic spectra.



194

4.11.6 Cytoxazone

By further screening in fraction IV, compound **195** was obtained as polar colourless UV absorbing solid, which turned to violet by anisaldehyde/sulphuric acid and pink by Ehrlich's reagent during TLC. The ^{1}H NMR spectrum established the existence of an 1,4-disubstituted aromatic ring because of the presence of two doublets each of 2H at δ 7.25 and 6.95. In the aliphatic region, two oxygen- or nitrogen-connected methine protons were observed at δ 5.01 (d), and δ 4.81 (ddd). In addition, a singlet of a methoxy group at δ 3.78 (OCH_3-4'), and the ABX pattern of an oxymethylene group was observed between δ 3.21-3.18.

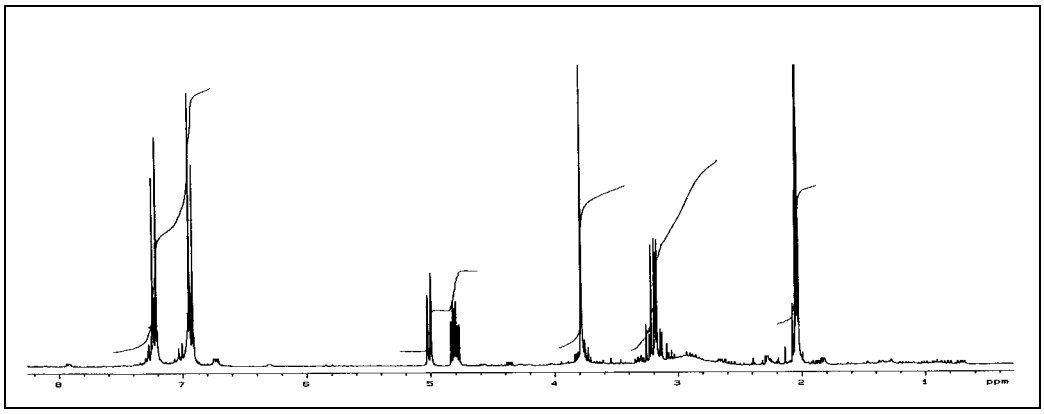
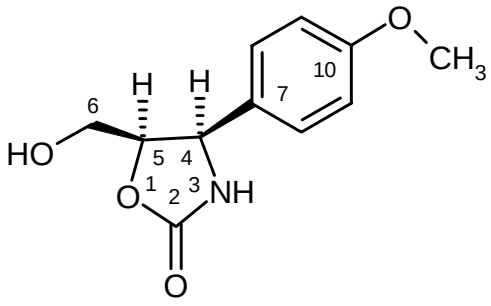


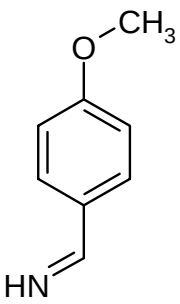
Figure 86: ^{1}H NMR spectrum ([D_6]acetone, 300 MHz) of cytoxazone (**195**)

The CI and EI mass spectra established the compound molecular weight as 223 Dalton. Further two fragments at *m/z* 192 and 135 were observed due to an expulsion of OCH_3, and the last one (*m/z* 135) was most likely attributed to the *p*-methoxy benzyl imine fragment (**196**), respectively. Another fragment at *m/z* 134 could be due to the *p*-methoxybenzonitrile ion (**197**). EIHR MS deduced the molecular formula as $C_{11}H_{13}NO_4$. A search in AntiBase using the above spectral data (and molecular formula) resulted in cytoxazone[243,244] (**195**), which was confirmed by comparison with the literature.

The cytoxazone structure (**195**) contains the rare 2-oxazolidinone ring. It was previously found in *Streptomyces* sp.[243] and shows a cytokine-modulating activity by inhibiting the signalling pathway of Th2 cells [244].



195



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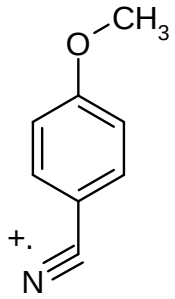


197

B. Fermenter

Due to the interesting microbial metabolites produced by the terrestrial *Streptomyces* GW10/580, the strain was additionally cultivated in a jar fermenter. It was cultured at 28 °C for 3 days on a rotary shaker (95 rpm) in 12 1 litre-Erlenmeyer each containing 250 ml of M_2 medium. The culture broth was used to inoculate a 22-liter jar fermenter which was kept at 28 °C for 72 hours. Working up of the resulting brown crude extract using TLC, non-UV absorbing middle polar bands were observed after staining to violet with anisaldehyde/sulphuric acid. The crude extract delivered the fractions II-III by flash column chromatography using a chloroform-methanol gradient. Further purification of the outlined fractions led to the colourless oily feigrisolide B (**198**), feigrisolide A (**202**) and feigrisolide C (**206**).

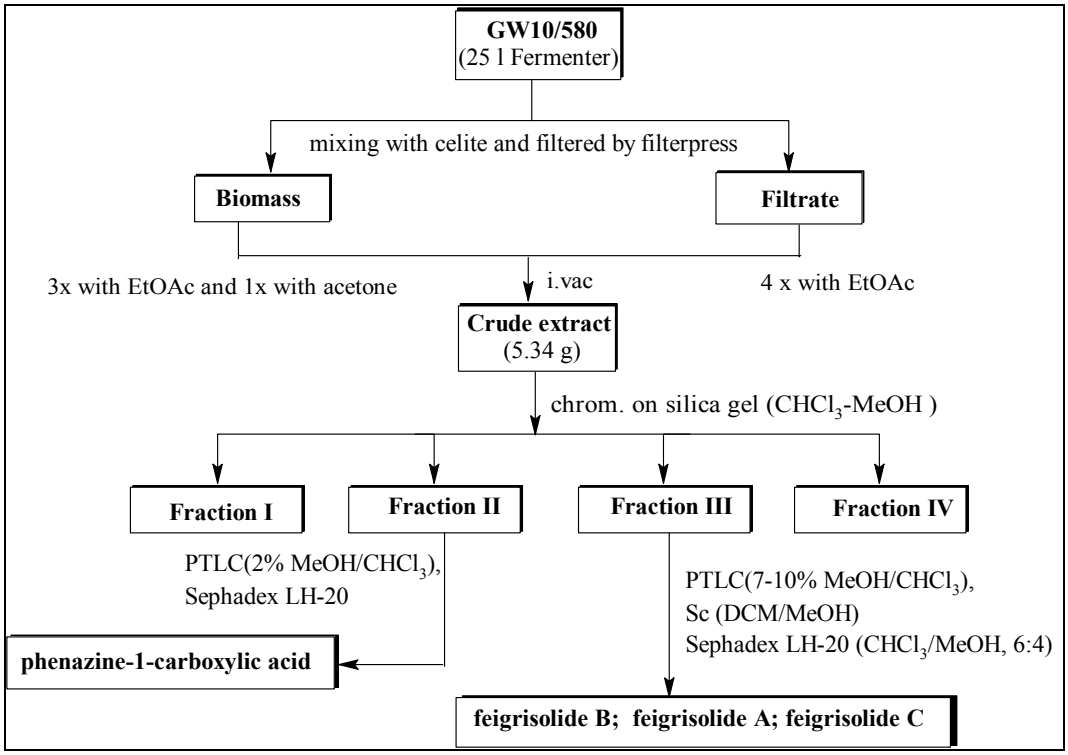


Figure 87: Work-up procedure for the terrestrial *Streptomyces* isolate GW10/580 (fermenter).

4.11.7 Feigrisolide B

Fraction III delivered a colourless oil using PTLC, silica gel column and Sephadex LH-20. The ^{1}H NMR spectrum showed a broad OH signal (2H) at δ 6.90. In addition, three oxymethine multiplets were displayed between δ 4.19-3.77. Moreover, a doublet of quartet was seen at δ 2.45 due to a methine proton linked probably to sp^2 carbon (e.g. a carbonyl group). Three additional multiplets of 8H between δ 2.05-1.35 were exhibited, which could be attributed to four methylene groups. The complex splitting of the methylene protons was possibly due to their inclusion in a ring. Furthermore, a doublet at δ 1.14 and triplet at δ 0.78 each of 3H, indicated a $CH\text{-}CH_3$, and a terminal ethyl group, respectively.

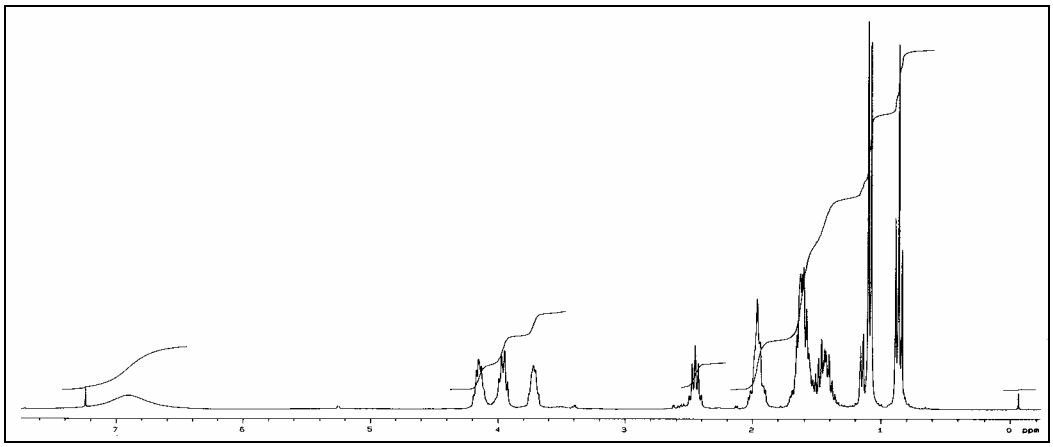
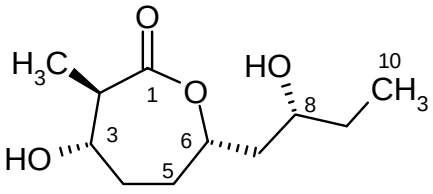
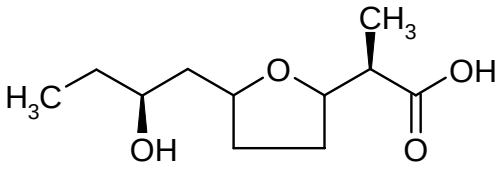


Figure 88: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of feigrisolide B (**198**)

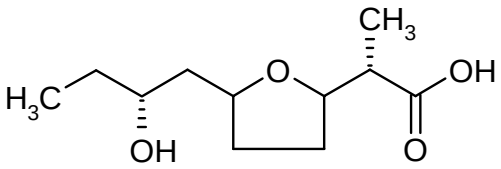
The ^{13}C/APT NMR spectra established eleven carbon signals, from which one was due to an ester, amide or carboxylic acid group, three oxygenated methine carbons and a fourth methine attached to an sp^2 carbon. Furthermore, four methylene groups at δ 40.7, 30.6, 29.7 and 28.9 were displayed, and two methyl carbons at δ 13.6 and 10.0. The molecular weight of **198** was determined as 216 Dalton by (+)-ESI mass spectrum. By applying the above spectral data to AntiBase, four possible structures were displayed: Feigrisolide B (**198)**, (+)-homononactic acid (**199**), (-)-homononactic acid (**200**), and (-)-2-*epi*-homononactic acid (**201**).



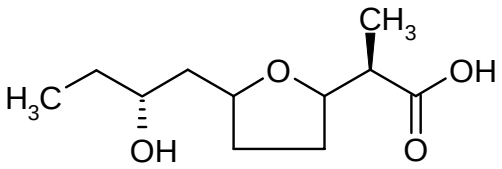
198



199



200



201

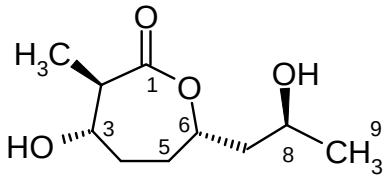
The free acids **199**, **200**, **201** were distinguished from the lactone isomer feigrisolide (**198**) by subjecting the compound to methylation with diazomethane. As no change occurred, the structure was fixed as **198,** which was further confirmed by direct comparison with the literature[245].

4.11.8 Feigrisolide A

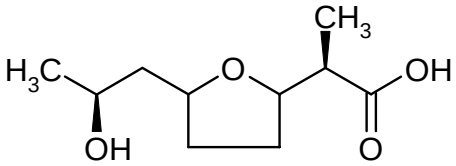
Along with **198**, compound **202** was obtained from fraction III as colourless oil. It showed the same absorbing and chromatographic properties as compound **198** pointing also to a structural similarity.

The ^{1}H NMR spectrum of **202** showed close structural similarities with **198,** except that in the side chain the signal of the ethyl group at C-8 in **198** was replaced by two doublet methyl groups at δ 1.21 and 1.16 to construct an isopropyl group. This modification was supported by the ESI mass spectrum, which fixed the molecular weight of **202** as 202 Dalton and is by 14 au (CH_2) lower than that of **198**.

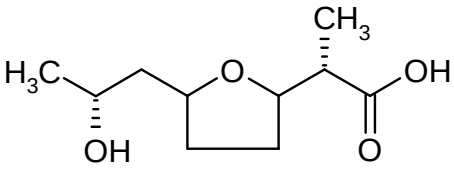
A search in AntiBase resulted again in four possible structures: Feigrisolide A[245] (**202**), (+)-nonactic acid (**203**), (-)-nonactic acid (**204**) and (-)-2-*epi*-nonactic acid (**205**). However, compounds **203**, **204**, and **205** were excluded as above due to their missing reaction with diazomethane. Feigrisolide A (**202**) was further established by comparison with the literature[245].



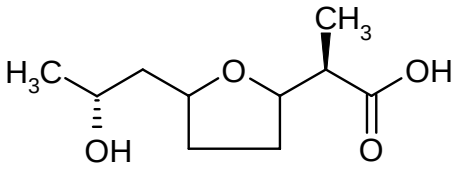
202



203



204



205

Unpredictably, the ESI mass spectra of feigrisolide B (**198**) and feigrisolide A (**202**) exhibited a pattern of signals with mass differences of 216 and 202, respectively. This seems to indicate higher polymers of the monomeric units **198** and **202**, respectively (Figure 89). This behaviour still needs further investigations.

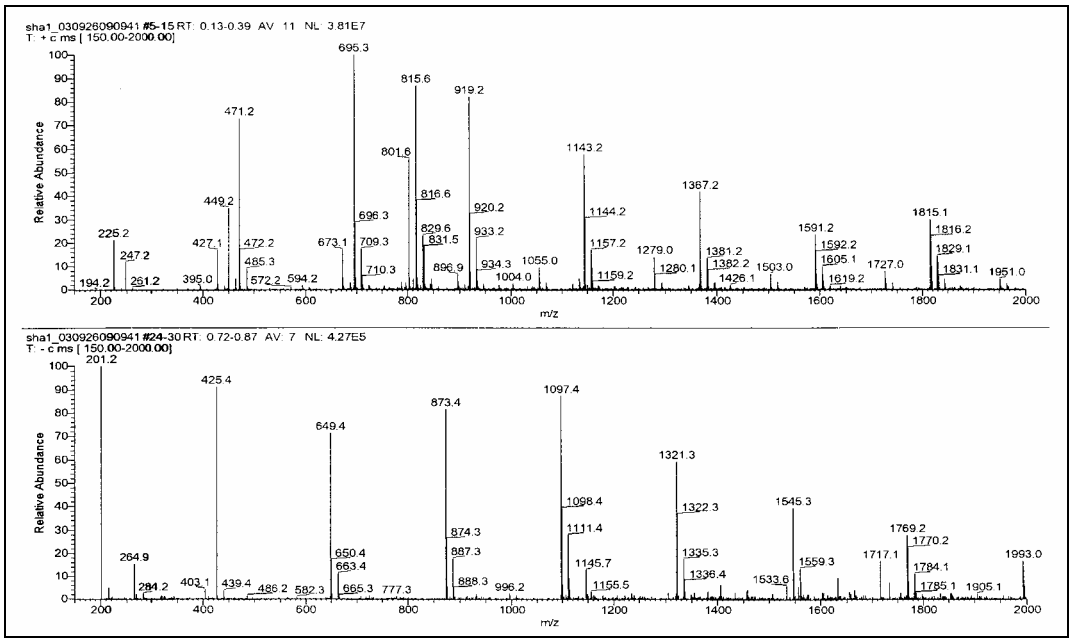


Figure 89: **(+)-** (top) and (-)-ESI mass spectra (bottom) of feigrisolide A (**202**)

4.11.9 Feigrisolide C

Compound **206** was also isolated as colourless oil by further separation of fraction III. It was slightly higher polar than the other two analogues **198** and **202**, but showed the same violet colour after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of compound **206** displayed subsequently close structural similarities to those of **198** and **202**. It displayed three multiplets at δ 4.90 (1H), 4.10-3.80 (4H) and 3.60 (1H), according to six oxymethine protons. In addition, a 2H multiplet at δ 2.40-2.50 of methine or methylene protons linked to sp^2 carbon was observed. Furthermore, a 4H multiplet of two methylene groups was found in the range of δ 1.90–2.00. The spectrum revealed additionally a 16H multiplet between δ 1.88-1.50 of eight methylene groups. Moreover, three doublets and one triplet each of 3H of methyl groups were exhibited, of which the last one at δ 0.90 was assigned to a terminal ethyl group.

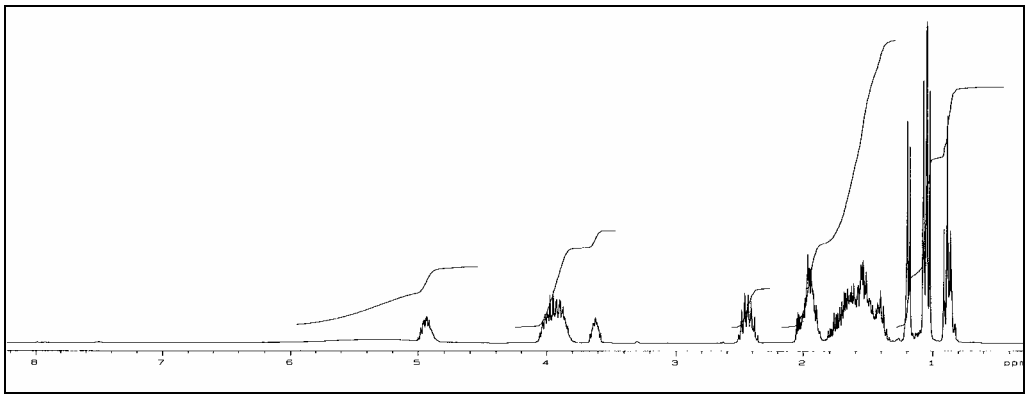
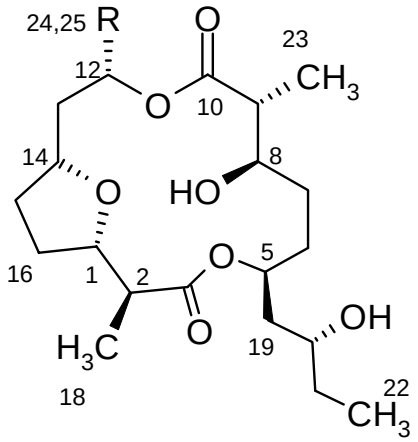


Figure 90: ^{1}H NMR spectrum ([D_6]acetone, 300 MHz) of feigrisolide C (**206**)

The ^{13}C NMR spectrum displayed 21 signals, among them two carbonyl signals at δ 178.5 and 174.2, eight sp^3 methine and six oxygenated carbons between δ 81-69. The two signals at δ 45.4 and 45.3 are probably due to groups adjacent to sp^2 carbons (e.g. C=O). Furthermore, seven methylene carbon signals were observed, from which two at δ 42.2 and 41.0 were characterised as two methylene groups located between two oxymethines carbons or linked to sp^2 carbons. The rest five methylene carbons between δ 31.0-28.2 were most likely present in a cyclic system. Finally, the spectrum displayed four carbon signals at high field corresponding to four methyl groups.

The molecular weight of **206** was fixed as 400 Dalton according to ESI mass spectrum. In accordance, the molecular formula $C_{21}H_{36}O_7$ was suggested. By applying the above spectroscopic data to AntiBase, feigrisolide C (**206**) was found, and established by comparing the data with the literature [245].



206: R = Me
207: R = Et

According to the ^{1}H NMR and ^{13}C NMR data, it could be suggested that the metabolites **198** and **202** may act as building blocks for **206** forming a 16-membered

bis-lactone. The hepta-lactones A (**202**), and B (**198**), in addition to the non-symmetric macrolides C (**206**) and D (**207**) were isolated by Y. Tang *et al.* from *Streptomyces griseus* and shown to exhibit antibacterial activity[245].

4.12 Marine *Streptomyces* sp. B 8335

The crude extract of the marine *Streptomyces* sp. B 8335 possessed high activity against *Bacillus subtilis, Escherichia coli*, moderate activity against *Streptomyces viridochromogenes* (Tü57) and weak activity against the fungus *Mucor miehei* (Tü284).

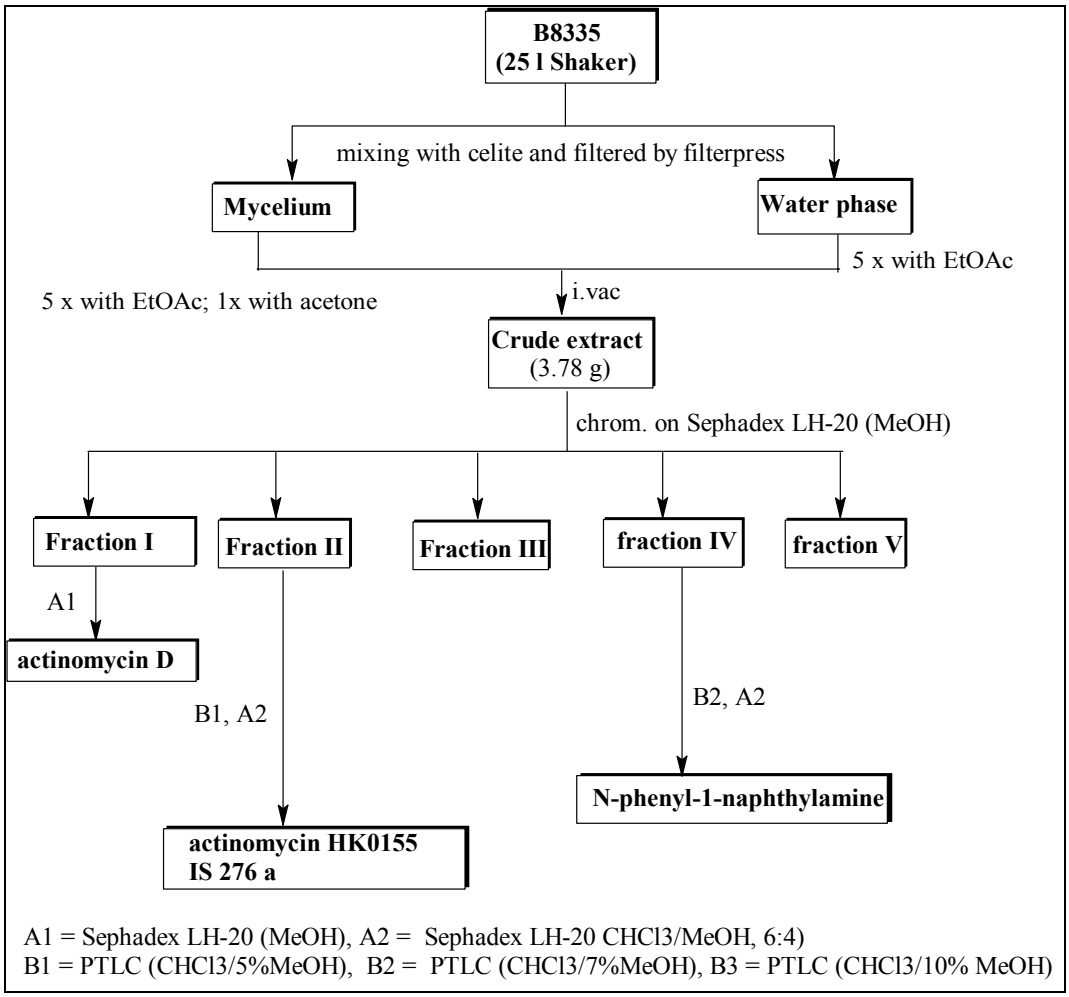


Figure 91: Work-up procedure for the marine *Streptomyces* sp. isolate B 8335.

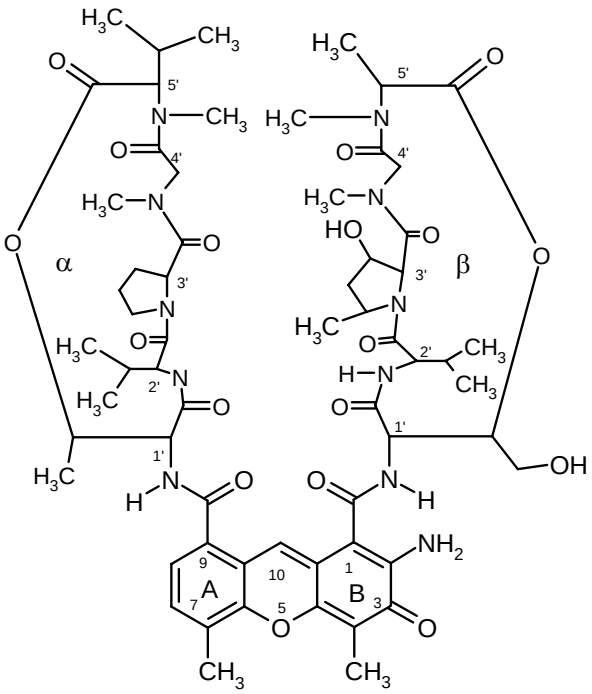
TLC of the primary extract showed two reddish-orange and one UV yellowish-green fluorescent bands at 366 nm, which showed no colour variation on treatment with sodium hydroxide. By treatment with anisaldehyde/sulphuric acid, the reddish-orange and yellowish-green bands changed to red and grey, respectively. This was indicative for actinomycin moiety in case of the first (reddish-orange). In addition, a

less polar UV absorbing band turned to violet/blue by treatment with anisaldehyde/sulphuric acid, was detected.

Cultivation of the microbial strain was carried out as 25-liter shaking culture for 4 days at 28 °C. The crude extract was defatted and applied to Sephadex LH-20 to give five fractions. Purification of fractions I-V led to isolation of actinomycin D (**130**), actinomycin HKI 0155 (**208**), in addition to a new actinomycin (still under elucidation). Along with other known compounds, N-phenyl-1-naphthylamine (**209a**) was isolated as new microbial metabolite from bacteria (Figure 91).

4.12.1 Actinomycin HKI 0155

From fraction II, compound **208** was obtained as a dark red powder by PTLC and Sephadex LH-20. It exhibited no colour change when treated with sodium hydroxide, referring most likely to an actinomycin moiety. The ^{1}H NMR pattern classified it as well as an actinomycin. The ESI mass spectra of compound **208** afforded a molecular weight of 1272 Dalton because it showed *quasi*-molecular ions at *m/z* 1295 ($[M + Na]^{+}$) and 1271 ($[M - H]^{-}$) in (+)-ESI and (-)-ESI modes, respectively. A search in AntiBase identified the compound **208** as actinomycin HKI 0155.



208

Compound **208** was isolated recently from a *Streptomyces* sp. and described as antibacterial agent against *Bacillus subtilis* ATCC 66333[246]. Actinomycin HKI-

0155 (**208**) shows a structural similarity to the ring-α in actinomycin D. In the ring β, threonin, however, is replaced by hydroxy-threonin, proline by 5-methyl-3-hydroxy-proline and N-methyl-valin by N-methyl-alanine.

4.12.2 N-Phenyl-1-naphthylamine, 1-Anilinonaphthalene

Working up of fraction IV led to isolation of compound **209a,** a colourless UV absorbing solid, which turned to violet with anisaldehyde/sulphuric acid and pink with Ehrlich's reagent. This referred possibly to a nitrogenous skeleton. It was isolated by applying the fraction to PTLC and Sephadex LH-20.

The ^{1}H NMR spectrum showed several multiplets in the aromatic region with an intensity of 12 protons, located in the range of δ 7.83-6.86 corresponding to at least three aromatic nuclei.

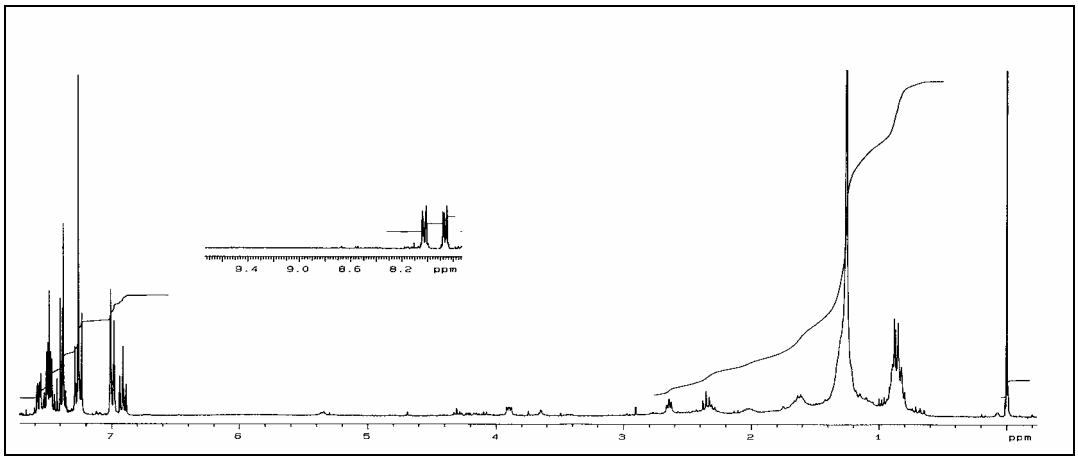
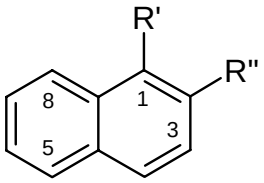


Figure 92: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of N-phenyl-1-naphthylamin (**209a**).

The ^{13}C/APT NMR spectra showed 16 carbon signals in the aromatic region, out of them 12 were methine and 4 quaternary carbons. The related shifts supported the assumption that two magnetically similar fused aromatic rings (naphthalene) are present, as well as one phenyl ring, and that both are connected *via* a heteroatom at either α- or β-position of the naphthalene skeleton. Based on these observations, four possible structures were suggested: **209a**, **209b**, **209c** and **209d**.



209a: R' = -NHPh, R" = H, **209b**: R' = -OPh, R" = H
209c: R' = H, R" = -NHPh, **209d**: R' = H, R" = -OPh

The molecular weight of compound **209a** was fixed as 219 Dalton. The odd mass of **209a** indicated a nitrogen atom and excluded thus the structures **209b** and **209d**. High resolution of the molecular ion afforded the molecular formula $C_{16}H_{13}N$.

A search in AntiBase resulted with no hits. This pointed to a new secondary metabolite from microoganisms. However, by searching in DNP, N-phenyl-1-naphthylamine (**209a**) was found and confirmed by direct comparison with an authentic sample and reference spectra[247].

N-Phenyl-1-naphthylamine (**209a**) is a constituent of *Eichhornia crassipes* and *Narcissus tazetta*, however, is also used as rubber vulcanisation accelerator, antioxidant, as well as an indicator in the analysis of organometallic reagents and could therefore be extracted as an artefact during workup. As it was possible to reproduce the isolation of **209a** from the marine *Streptomyces* sp. B3355 strictly avoiding all contact with plastic materials, this definitely ruled out an artificial origin of **209a**. Compound **209a** exhibited no biological activity[24].

4.13 Strain Hel59b

The ethyl acetate extract of the marine bacterium Hel59b attracted our attention during TLC due to the high number of metabolites which were stained to violet/orange or pink by spraying with anisaldehyde/sulphuric acid or Ehrlich's reagent. The strain showed also a moderate biological activity against *Bacillus subtilis*, *Escherichia coli, Streptomyces viridochromogenes* (Tü57), *Candida albicans*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Staphylococcus aureus*.

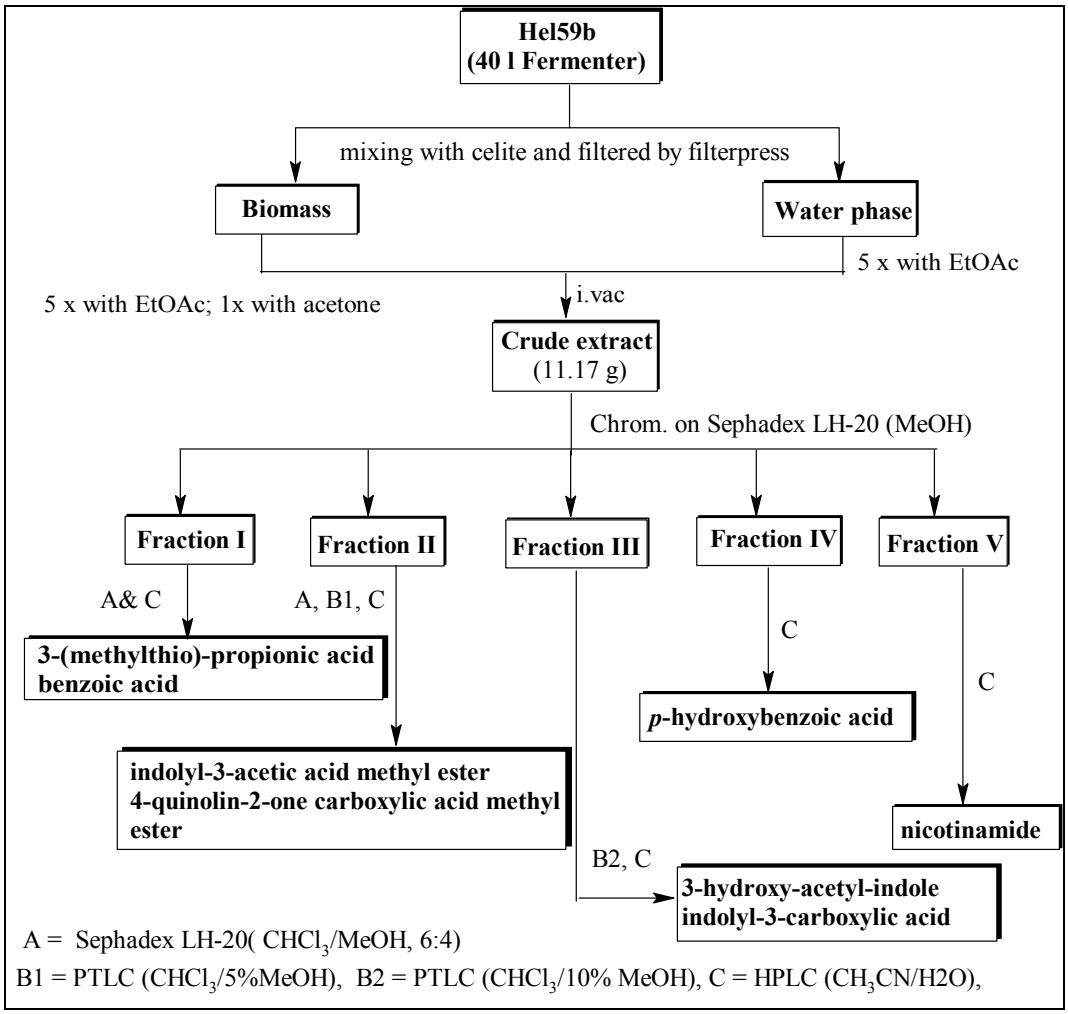


Figure 93: Work-up procedure of the marine bacteria isolate Hel 59b

Well-grown agar cultures of Hel59b served to inoculate 12 of 1 liter-Erlenmeyer flasks each containing 250 ml of LB medium. The resulting culture was used to inseed a 40-liter jar fermenter, which was held at 28 °C for additional 3 days. The culture broth was filtered and extracted with ethyl acetate, and the resulting brown crude extract was applied to Sephadex LH-20 column chromatography and finally eluted with a methanol gradient. Purification of fractions I-V delivered 3-(methylthio)-propanoic acid (**210**), benzoic acid (**211**), quinoline-2-one-4-carboxylic acid methyl ester (**212**), 3-hydroxy-acetyl-indole (**214**), 3-pyridinecarboxamide (nicotinamide, **215**), in addition to other known compounds (Figure 93).

4.13.1 3-(Methylthio)-propanoic acid and Benzoic acid

From the fast moving fraction I, compound **210** was obtained as colourless oil, which gave a dark brown spot after spraying with palladium(II)-chloride and heating, which referred to a sulphur compound. The compound was isolated by applying the fraction to Sephadex LH-20 and HPLC.

The ^{1}H NMR spectrum showed a broad singlet of an acidic proton at δ 9.25, perhaps of an carboxylic acid. In addition, two 2H triplets of two vicinal methylene groups were displayed at δ 2.77 and 2.68, constructing an ethandiyl group located between two magnetically different groups. Furthermore, at δ 2.14 a methyl singlet was observed which was connected either with an sp^2 carbon or a sulphur atom.

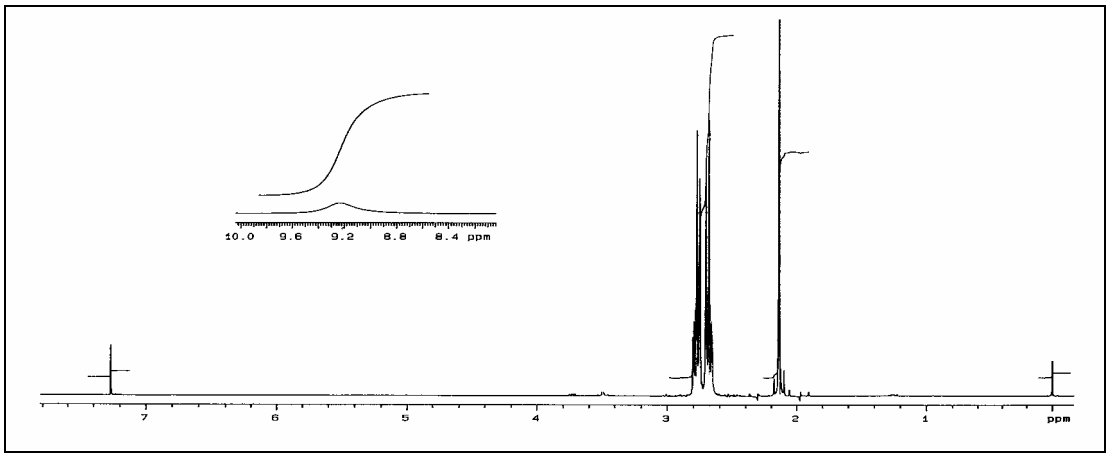
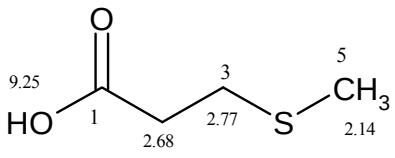
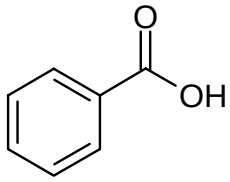


Figure 94: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-(methylthio)-propanoic acid (**210**).

The molecular weight of compound **210** was established as 120 Dalton using EI mass spectrum as base peak. An additional peak was detected at *m/z* 122.0 (5%), is attributed most likely to sulphur isotope (S^{34}). The molecular ion showed an expulsion of a methyl group to give two peaks at *m/z* 105 and 107 (isotope S^{34}). Additional, two fragments were observed at *m/z* 61.0 and 63, characteristic for the CH_2-S-CH_3 group. Furthermore, the spectrum showed a peak at *m/z* 59.0, characteristic for an acetic acid group. EIHR MS of compound **210** deduced the molecular formula as $C_4H_8O_2S$. A search in AntiBase identified the compound as 3-(methylthio)-propanoic acid (**210**), which was confirmed by comparison with literature[248]



210



211

Along with **210**, compound **211** was isolated as colourless, UV absorbing (254 nm) solid from fraction I by HPLC. Based on the chromatographic and spectroscopic characters, compound **211** was identified as benzoic acid.

Benzoic acid is widely spread in plants especially in essential oils, mostly in form of its esters, present in various flower oils and fruits and used in perfumery and flavourings. Benzoic acid is used a preservative in the food industry. Medicinally, benzoic acid used as antiseptic, expectorant, antifungal, antipyretic and keratolytic agent[24].

4.13.2 Quinoline-2-one-4-carboxylic acid methylester

Nitrogenous compounds are found in nature in various structural skeletons e.g. alkaloids, peptides, etc. In fraction II, compound **212** was found as an UV absorbing zone, which was stained to faint pink by anisaldehyde/sulphuric acid and Ehrlich's reagent. The colourless **212** was isolated by PTLC, Sephadex and HPLC.

The ^{1}H NMR spectrum exhibited a broad 1H singlet at δ 11.41 probably of a carboxylic acid or amide. In the aromatic region, it displayed a doublet of a *m*-coupled proton at δ 8.45, in addition to three multiplets at δ 8.28 (1H), 7.57 (1H), and 7.30 (2 H), pointing to an aromatic system containing two fused rings. The down field shift can be attributed to their existence in a heteroaromatic system. In addition, a methoxy singlet was found at δ 3.87.

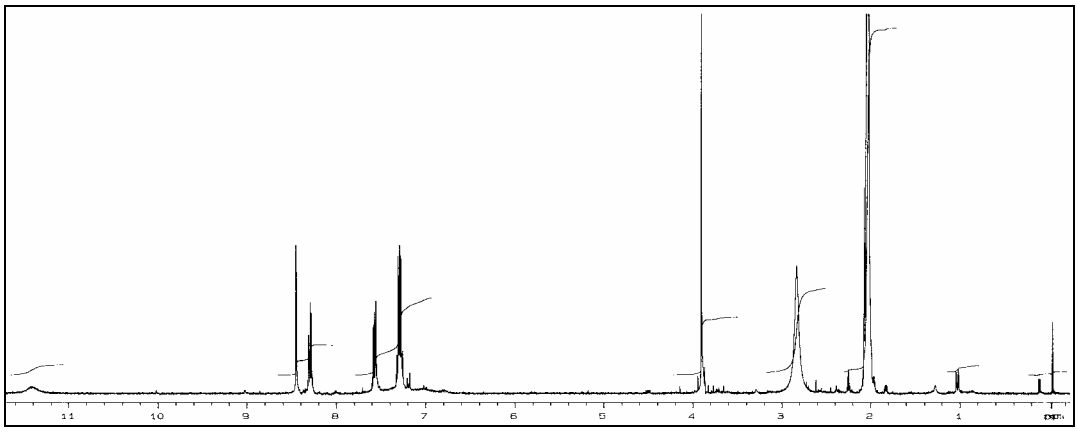


Figure 95: ^{1}H NMR spectrum ([D_6]acetone, 300 MHz) of quinoline-2-one-4-carboxylic acid methyl ester (**212**)

The molecular weight of **212** was determined as 203 Dalton by EI mass spectrum. The odd mass number confirmed the existence of a nitrogen atom. A base peak at *m/z* 144 was due to the loss of 59 corresponding to a carbomethoxy group, pointing to an ester moiety. An additional peak at *m/z* 116 can be explained by the successive expulsion of CO and COOMe (Figure 96).

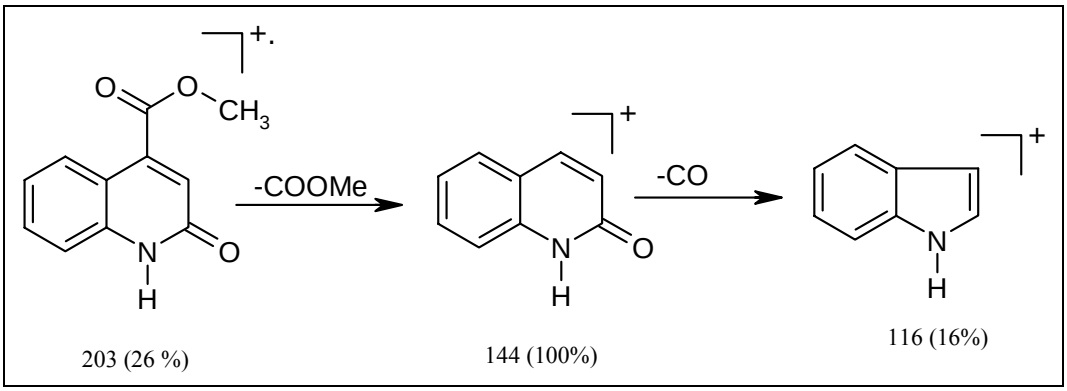
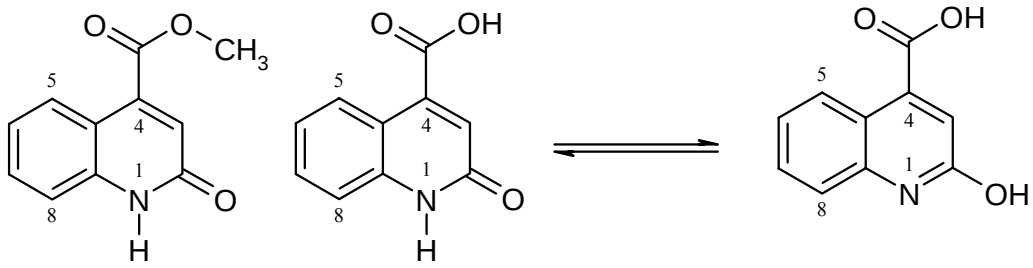


Figure 96: EI MS fragmentation pattern of quinoline-2-one-4-carboxylic acid methyl ester (**212**)

By applying the spectral data of compound **212** to AntiBase, no hits were found, pointing to a new natural product from microorganisms. A search in the DNP has revealed quinoline-2-one-4-carboxylic acid methyl ester (**212**) as the sole structure[249], a compound which is known from synthesis and as a natural product from *Papaver somniferum*[249]. The identity was confirmed by comparison with the literature.



212 **213**

The free 2-hydroxy-4-quinoline carboxylic acid (**213**) and its ester **212** occur in tautomeric forms. Quinoline derivatives are known as secondary metabolites from *Streptomycetes* (e.g. *Streptomyces griseoflavus*[250]) and show antibacterial activities or are inhibitors of dehydrogenases[250]. The esterfication of 2-hydroxy-4-quinoline carboxylic acid (**213**) to its methyl or ethyl derivatives is carried out smoothly with boron trifluoride etherate/alcohol[251].

4.13.3 3-Hydroxy-acetyl-indole

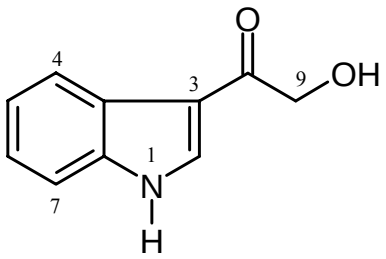
TLC of fraction III exhibited compound **214** as a middle polar UV absorbing band, which turned to orange when exposed to anisaldehyde/sulphuric acid. This established it certainly from our experience to be 3-substituted indole moiety conju-

gated with a carbonyl group. The compound was obtained as colourless solid by purification with HPLC.

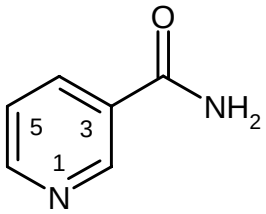
The ^{1}H NMR spectrum showed a broad singlet of an acidic proton at δ 11.18, three signals with intensity of 5H protons at δ 8.31 (d), 8.27 (m), 7.54 (m), 7.24 (m, 2 H). This resembled an indole skeleton substituted at 3-position. In the aliphatic region, a 2H singlet at δ 4.69 of an oxygenated methylene group attached to an sp^2 carbon was exhibited. The EI mass spectrum fixed the molecular weight of **212** as 175 Dalton. The molecular ion (*m/z* 175) showed an expulsion of methanol to give a base peak at *m/z* 144. Furthermore, it showed an expulsion of a carbonyl group to give a peak at *m/z* 116, which is characteristic for the indole fragment.

A search in AntiBase pointed to 3-hydroxy-acetyl-indole (**214**), and the latter was further confirmed by direct comparison with an authentic spectrum and the literature[61,252].

Compound **214** was isolated previously, in addition to several other indole derivatives, which could be responsible for the antifungal properties from myxobacteria *Archangium gephyra*[252]. 3-Hydroxy-acetyl-indole (**214**) was established as tryptophane metabolite from a staurosporine producer, *Streptomyces staurosporeus* [253], and was isolated from the fungus *Lactarius deliciosus*[254] and marine red algae [255].



214



215

4.13.4 3-Pyridinecarboxamide, Nicotinamide

During the screening of fraction V, compound **215** was obtained as a colourless solid. It exhibited a pink colouration with anisaldehyde/sulphuric acid and Ehrlich's reagent, which referred it as a nitrogenous compound. The ^{1}H NMR spectrum showed a *m*-coupled down field 1H doublet at δ 9.11 as in a heteroaromatic ring, two doublets of doublets each of 1H at δ 8.70 and 8.25, as well as a 1H multiplet at δ

7.50. In addition, two broad 1H singlets at δ 7.68 and 7.30 were observed. The spectra showed a close similarity to pyridine-3-carboxylic acid (**190**).

The molecular weight of compound **215** was fixed as 122 Dalton by EI mass spectrum. The molecular ion showed an ejection of 44 ($CONH_2$) giving a peak at *m/z* 78. The latter peak (*m/z* 78) was indicative for pyridinium ion. This fixed the structure as nicotinamide (**215**), which is a widespread metabolite in plants (e.g. rice), yeast and fungi, but has not been isolated from bacteria so far. Compound **215** is an important enzyme cofactor and used in the treatment of pellagra. It is commonly known as Vitamin B3 as well as PP-Factor[24].

4.14 Terrestrial *Streptomyces* sp. GW3/1786

TLC screening of the ethyl acetate extract from the terrestrial *Streptomyces* sp. GW3/1786 showed two yellow zones with a strong UV absorbance. The upper band showed a red colour reaction with sulphuric acid, while the lower one exhibited an orange fluorescence at 366 nm. The last band turned to red by sodium hydroxide and brown with sulphuric acid, as indication of *peri*-hydroxyquinones. In the biological screening, the extract showed high activity against all tested microorganisms: *Bacillus subtilis*, *Escherichia coli*, *Mucor miehei* (Tü284), *Candida albicans*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57), *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

The fermentation was carried out in a 25-liter shaker culture at 28 °C for 5 days. After filtration, both filtrate and biomass were extracted with Amberlite XAD-2 and ethyl acetate, respectively. The crude extract was fractionated by flash column chromatography employing gradient elution from dichloromethane to methanol, yielding four fractions. Working up of the fractions II-IV delivered four *peri*-hydroxyquinones; α-indomycinone (**286)**, β-indomycinone (**295)**), ε-indomycinone (**291)**) and saptomycin A (**297**) (see strain GW3/1130, page 228). Working up of fraction I led to isolation, the new secondary metabolite, phencomycin methyl ester (**217**).

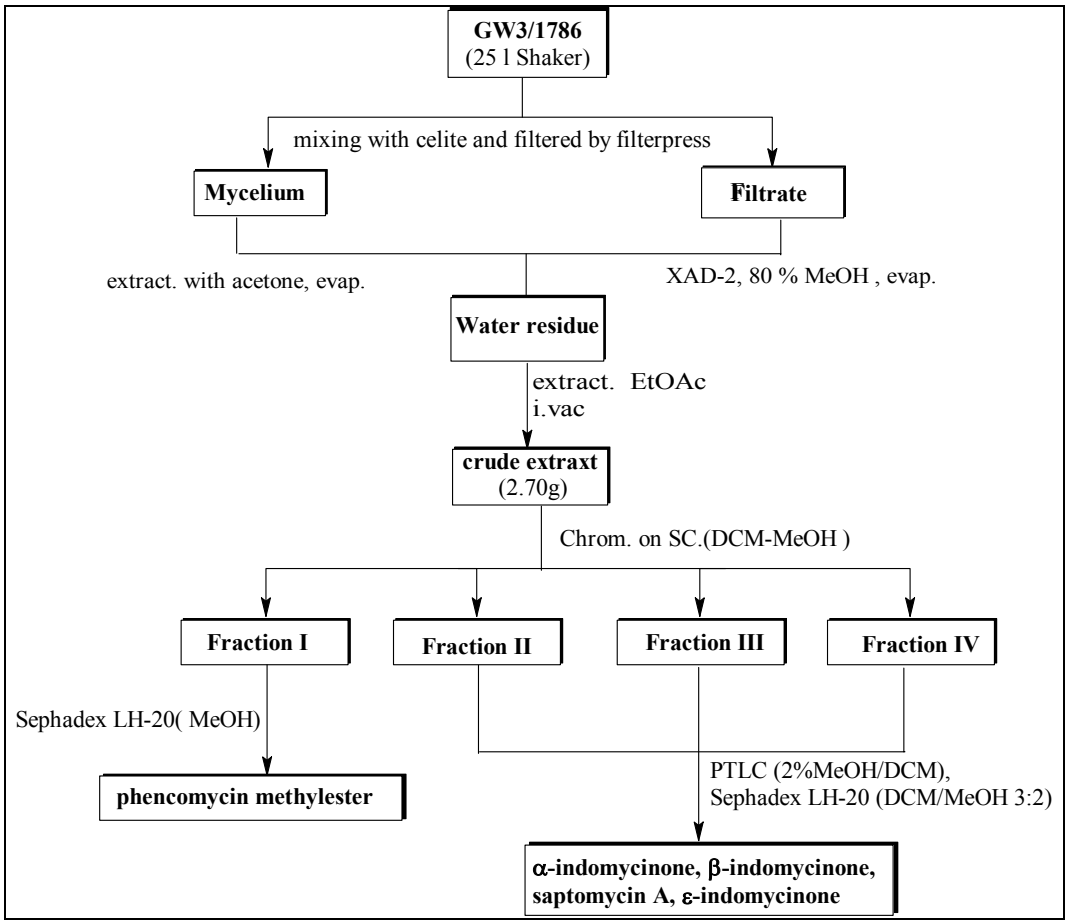


Figure 97: Work-up procedure of terrestrial *Streptomyces* sp. isolate GW3/1786 (shaker culture)

4.14.1 Phencomycin methyl ester

Compound **217** was obtained from fraction I by Sephadex LH-20 as a non-polar yellowish-green solid, which turned to red by anisaldehyde/sulphuric acid and showed no change with dilute sodium hydroxide. The ^{1}H NMR spectrum showed three deep field 1H dd signals of an 1,2,3-trisubstitued aromatic ring. In the aliphatic region, one 3H singlet at δ 4.15 could be assigned as an aromatic methyl ether or ester.

The ^{13}C NMR spectrum showed each three sp^2 methine and quaternary carbon signals and a CO signal of an acid derivative at δ 166.8. In the aliphatic region, only a methoxy carbon signal at δ 52.7 was found. The NMR pattern and the shifts were indicative for a phenazine skeleton with two symmetrical substituents.

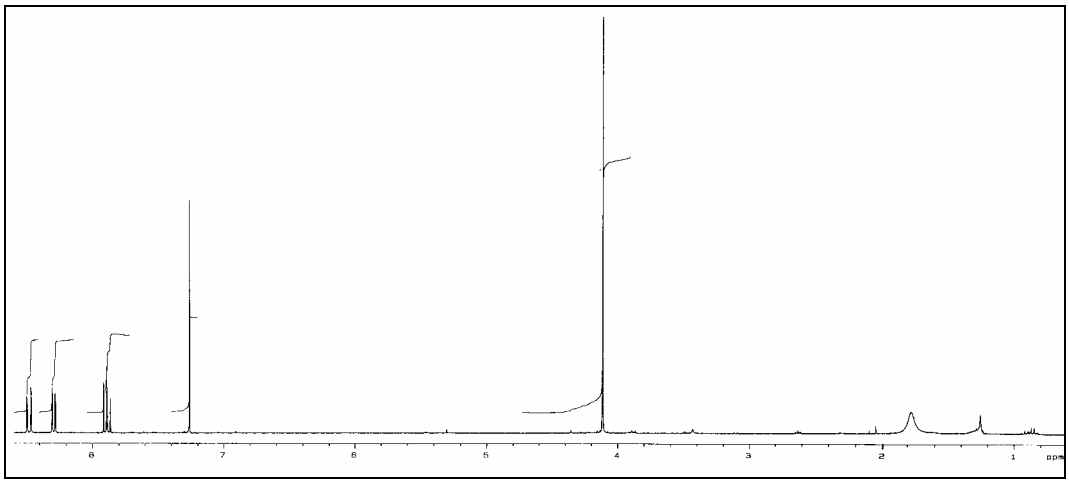


Figure 98: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of phencomycin methyl ester (**217**)

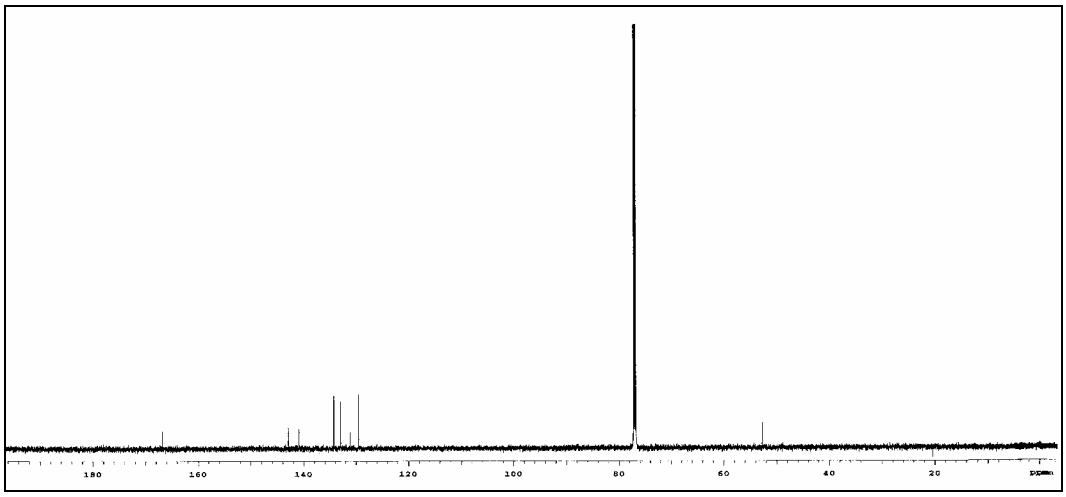
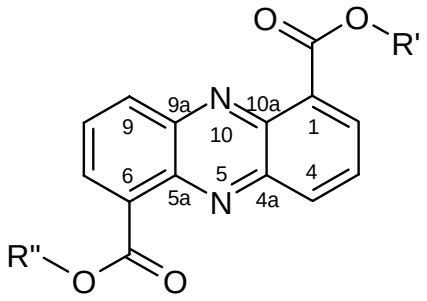


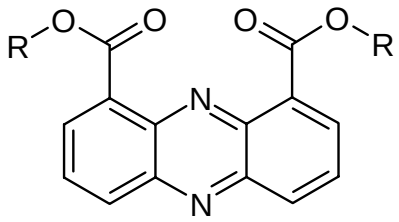
Figure 99: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of phencomycin methyl ester (**217**)

The EI mass spectrum fixed the molecular weight of compound **217** as 296 Dalton. The molecular ion (*m/z* 296) showed a loss of 31 (M-CH_3O) to give a fragment at *m/z* 265. The last fragment showed a further splitting to give a peak at *m/z* 238, which could be attributed to the expulsion of a carbonyl group. The fragment at *m/z* 238 is most likely indicative for phenazine carboxylic acid methyl ester (**223**).

On the basis of these spectral data, two possible Phenazine derivatives came into account, 1,6-dicarbomethoxy phenazine (**217**) and 1,9-dicarbomethoxy phenazine (**219**) as two symmetrical systems.



216: R' = CH_3, R" = H
217: R' = R" = CH_3
218: R' = R" = H

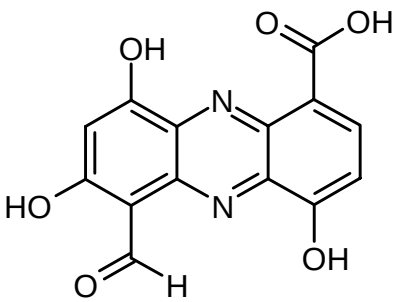


219: R = CH_3
220: R = H

Though the free acids **218** and **220** are naturally occurring in bacteria, their-methyl ester derivatives, **217** and **219** were not found in AntiBase. They were, however, reported as synthetic products, and by comparing the spectral data, the compound isolated here was elucidated as 1,6-dicarbomethoxy phenazine (**217**). The quaternary carbons, C-4a/9a and C-5a/10a present in compound **217** were reported to be at the same chemical shift (δ 143.15)[256], while in the measured data, they were distinguished as two values (142.9, 140.9).

The ester **217** is easily obtained by methylation of phencomycin (**216**)[256]. To exclude that **217** was formed as an artefact during isolation, the work-up was repeated carefully avoiding any methanol as solvent. Even under these conditions the ester was isolated again, indicating that **217** is a natural product but not artefact.

Phenazine 1,6-dicarboxylic acid (**218**) inhibits xanthine oxidase, shows antibiotic properties, and is an intermediate in the biosynthesis of lomofungin (**221**) produced by *Pseudomonas* and *Streptomyces* sp.[24,257]. Phencomycin (**216**) was isolated recently as a new antibiotic with antitumor activity as well from *Streptomyces* sp.[256] HIL Y-9031725. Dihydrophencomycin (**222a**) and its dimethyl ester (**222b**) were previously isolated in our group[258].



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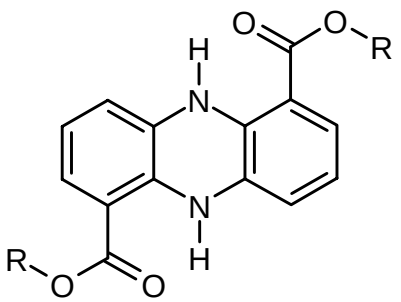


222a: R = H, **222b**: R = CH_3

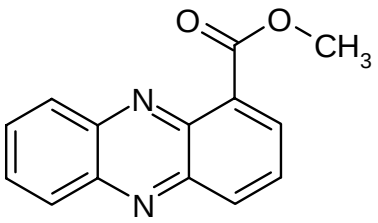
Fermenter

The structural diversity of the resulting microbial metabolites produced by the terrestrial *Streptomyces* sp. GW3/1786, as well as their high bio-activities, prompted us to repeat its cultivation in a 25-liter jar fermenter. However, working up and purification of the resulting extract delivered only 1-Phenazinecarboxylic acid methyl ester (**223**).

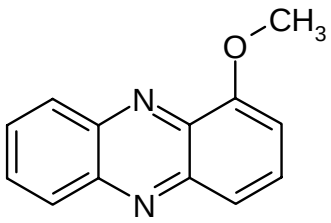
4.14.2 1-Phenazine carboxylic acid methyl ester

Compound **223** was isolated as yellowish-green solid from fraction 1 using cyclohexane-dichloromethane as eluent system and purified by Sephadex LH-20.

The ^{1}H NMR spectrum exhibited four deep field signals with an intensity of seven protons, form which one gave a dd at δ 8.42 and one a multiplet at δ 8.34, each with an intensity of 1 H. The residual signals were displayed as a 2H doublet at δ 8.27 and a 3H multiplet between δ 7.95-7.82. In the aliphatic region, it exhibited a 3H singlet at δ 4.15 which could be assigned as an aromatic methyl ether or ester. According to the ^{1}H NMR data, two Phenazine moieties were taken into account, phenazine-1-carboxylic acid methyl ester (**223**) and 1-methoxy-phenazine (**224**).



223



224

However, the EI mass spectrum fixed the molecular weight as 238 Dalton, thus confirming the structure as phenazine-1-carboxylic acid methyl ester (**223**). The mass spectrum showed a peak at *m/z* 207 due to an expulsion of a methoxy group. The latter fragment (207) showed a further splitting by expulsion of CO to give a peak at *m/z* 180 to establish the Phenazine skeleton. Phenazine-1-carboxylic acid methyl ester (**223**) was further established by comparison with the literature. Both tubermycin B (**188**) and its methyl ester **223** are antibiotically active against Gram-positive bacteria[235,24].

4.15 Marine *Streptomyces* sp. B2150

The ethyl acetate extract of the marine *Streptomyces* sp. B2150 displayed a high activity against *Mucor miehei* (Tü284) and *Candida albicans*, and moderate activity against the alga *Chlorella vulgaris*. During TLC, a green fluorescent band at 366 nm was observed which turned to yellow by anisaldehyde/sulphuric acid. In addition, other UV absorbing zones were exhibited, which turned mostly to violet-brown with anisaldehyde/sulphuric acid.

Well-grown agar cultures of B 2150 served to inoculate 12 of 1 liter-Erlenmeyer flasks each containing 250 ml of M_2^+ medium and cultivated for 3 days as shaking culture. The culture broth served to inoculate a 25-liter jar fermenter for further 3 days. After that, the culture broth was filtered and extracted by ethyl acetate giving a brown crude extract. Application of the crude extract to Sephadex LH-20 column chromatography and elution with methanol afforded five fractions. Purification of fractions I-IV delivered indolyl-3-lactic acid **(225),** flazin (**166**) and other known compounds.

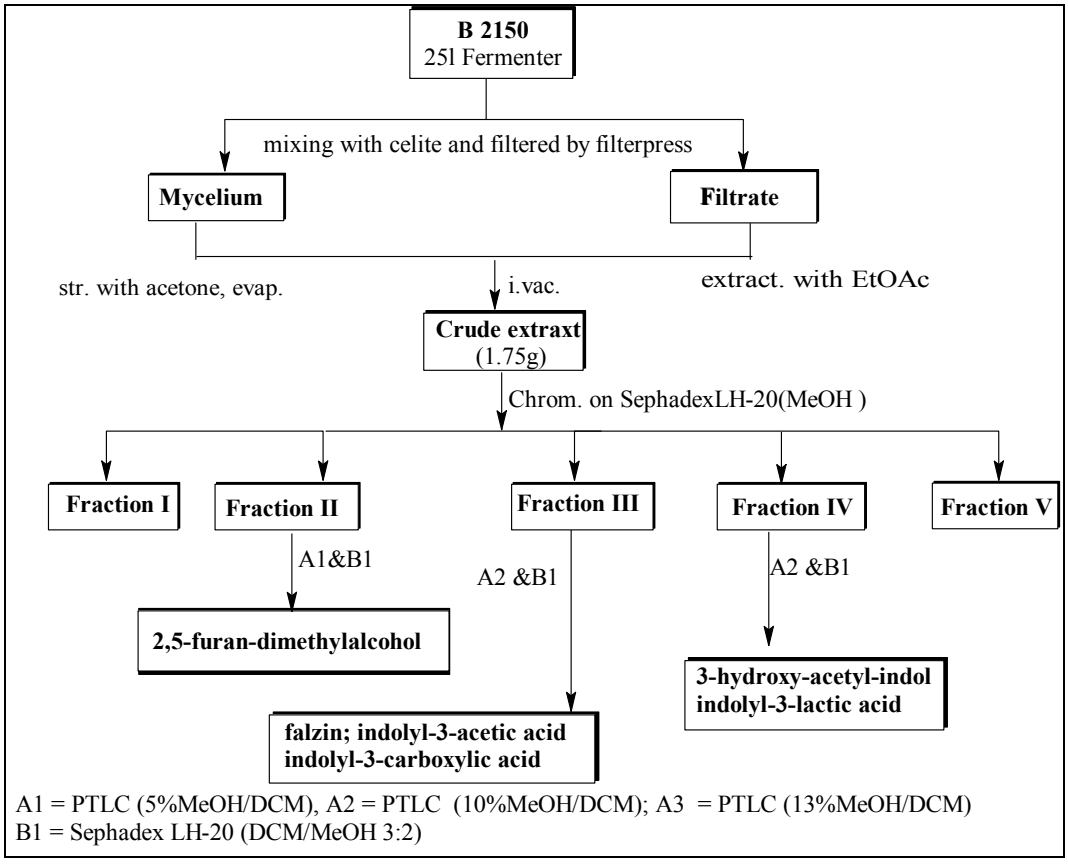
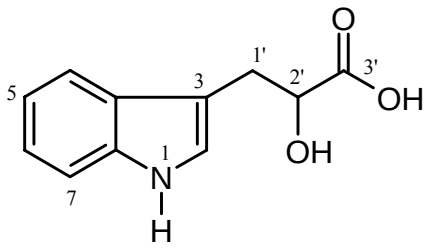


Figure 100: Work-up procedure of the marine *Streptomyces* isolate B 2150

4.15.1 Indolyl-3-lactic acid

Compound **225** was isolated as colourless solid from fraction IV. It exhibited UV absorbance and turned to violet by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **225** exhibited two broad 1H singlets of acidic protons at δ 10.75 and 8.31. In addition, five aromatic protons distinctive for a 3-substituted indole moiety were observed: Four of them at δ 7.52 (dd), 7.35 (dd), δ 7.02 (td), 7.98 (td) were characteristic for an 1,2-disubstituted aromatic ring. The fifth proton at δ 7.11 showed a small long-range coupling constant (^{3}J = 0.8 Hz). In the aliphatic region at δ 4.21, a dd signal of an oxygenated methine was displayed, forming an ABX system with a methylene group at δ 3.03 and 2.92, which was possibly adjacent to an sp^2 carbon.

The mass spectrum (EI MS) fixed the molecular weight of **225** as 205 Dalton. Furthermore, the mass ion (*m/z* 205) was fragmented giving a base peak at *m/z* 130, and the latter is characteristic for the indolyl-3-methylene ion. This confirmed the presence of a hydroxy acetic acid fragment (HO-CH-COOH), and hence a hydroxy propionic acid residue at position 3 of the indole skeleton. A search in AntiBase led to indolyl-3-lactic acid (**225**). This compound was confirmed by comparing the data with authentic spectra. Compound **225** is found frequently in bacteria, fungi and yeast[23] and exhibits activity against *Candida albicans*.



225

4.16 Terrestrial *Streptomyces* sp. GW12/3995

The ethyl acetate extract of a terrestrial *Streptomyces* sp. GW 12/3995 exhibited moderate antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Streptomyces viridochromogenes*. It had also antialgal activity against the micro-algae *Chlorella vulgaris* and *Chlorella sorokiniana*.

During TLC screening, two polar UV absorbing zones were observed, one band of which was stained to brown by treatment with anisaldehyde/sulphuric acid. The terrestrial *Streptomyces* sp. GW 12/2995 was cultivated on M_2 medium as 20-liter fermenter. Four fractions were isolated by using silica gel column chromatography and elution with dichloromethane-methanol. Further purification of fractions II-III led to 3,4-dihydroxybenzoic acid (**226**) and other known compounds (Figure 101)

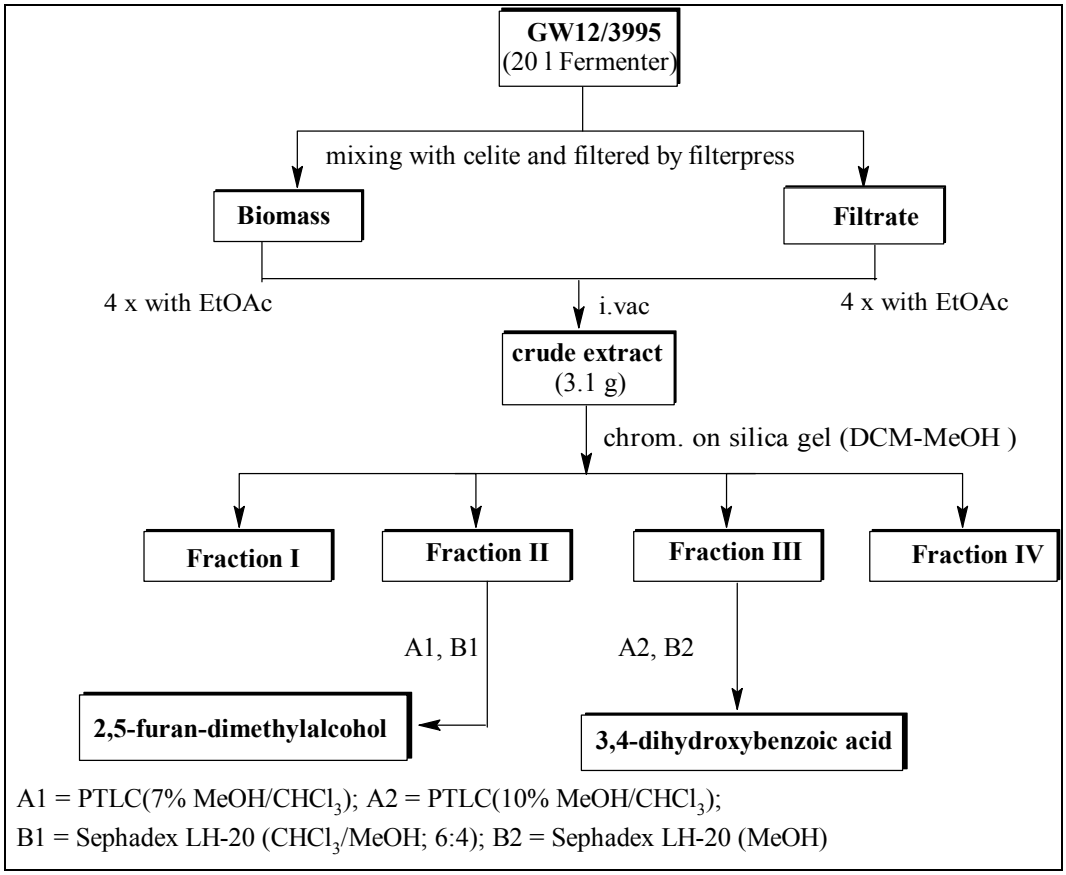


Figure 101: Work-up procedure of terrestrial *Streptomyces* isolate GW12/3995

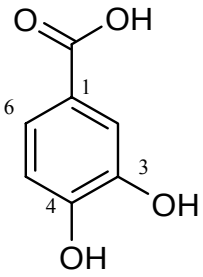
4.16.1 3,4-Dihydroxy benzoic acid

Compound **226**, was isolated as colourless solid from fraction III by PTLC followed by Sephadex LH-20. It exhibited no colour reaction with the spraying reagents, pointing to a nitrogen-free compound. The ^{1}H NMR spectrum of compound **226** showed three aromatic 1H signals, displayed as two *ortho*-protons (dd) at δ 7.37 and δ 6.77 and a third *m*-coupled proton at δ 7.42 delivering an 1,2,4-trisubstiuted aromatic system.

The ^{13}C/APT NMR spectra of compound **226** showed seven sp^2 carbon signals, out of them one at δ 174.2 due to a carboxylic acid derivative. In addition, three qua-

ternary carbons were displayed at δ 149.6, 145.5 and 128, the first two possibly being oxygenated. The other three signals were due to methine carbons. EI mass spectrum of **226** fixed its molecular weight as 154 Dalton. The loss of Δ*m/z* 17 indicated the expulsion of a hydroxyl group.

According to the revealed spectral data and the molecular weight, two possible structures were proposed: 3,4-dihydroxy benzoic acid (**226**) and 2,4-dihydroxy benzoic acid (**227**). The observed deep field of the *m*-coupled proton (δ 7.42) suggested its locality between the carboxylic and hydroxyl groups directing towards **226**. However, an existence of the *m*-coupled proton between two hydroxyl groups as in **227** should be shifted up field (δ < 7), which is not matched with the measured data. This pointed to 3,4-dihydroxy benzoic acid (**226**), which was further confirmed by 2 D long-range correlations (HMBC) as shown below.



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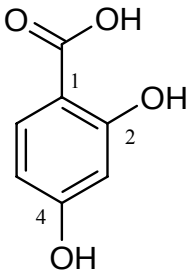

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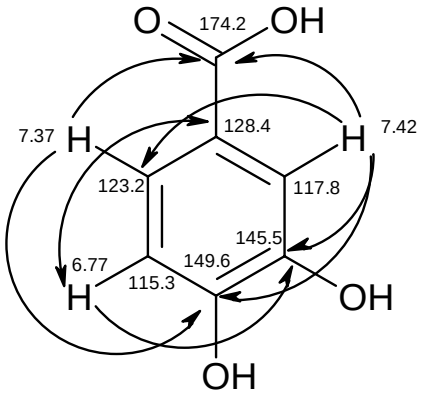


Figure 102: HMBC-correlations of 3,4-dihydroxy benzoic acid (**226**)

4.17 *Roseobacter* strains (DFL12, DFL38, DFL16, DFL27, DFL30)

The ethyl acetate extracts of various marine *Roseobacter* sp. (DFL12, DFL38, DFL16, DFL27 and DFL30) possessed rather weak antimicrobial activity, however,

drew our attention because of the distinguished dark red colour of their colonies. During TLC analysis, a dark red zone turned blue by treatment with sulphuric acid was detected, pointing to a carotinoid. Chemical screening of the various extracts by TLC and HPLC MS established identical metabolic patterns.

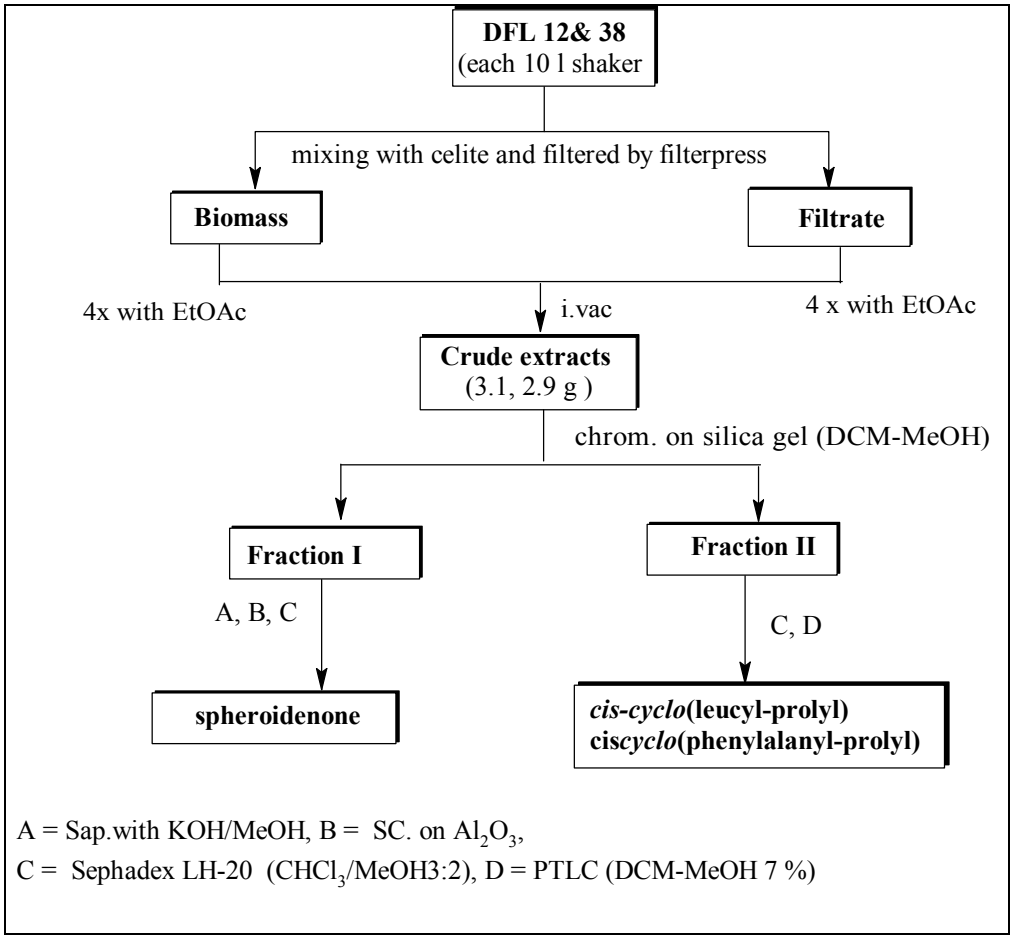


Figure 103: Work-up procedure of the *Roseobacter* sp. (isolates DFL12 and DFL38)

The strains (DFL 12 and DFL38) were each fermented as 10-liter shaking culture at 28 °C for 3 days. The resulting dark red culture broths were filtered and extracted and chromatographed using flash silica gel column and elution with dichloromethane-methanol gradient to give each two fractions. From fraction II, the known compounds *cis-cyclo*(leucyl-prolyl) (**119**), *cis-cyclo*(phenylalanyl-prolyl) (**40**), in addition to linoleic acid (**239**) were obtained.

4.17.1 Spheroidenone

Fraction I contained a red compound which turned to blue by treatment with sulphuric acid, pointing to a carotenoid. The compound was isolated by mild saponification using methanolic potassium hydroxide at room temperature[259], and the re-

sulting reaction mixture was extracted with ether. The ether extract was evaporated, followed by purification using column chromatography on inactivated aluminum oxide and Sephadex to give pure **230** as a dark red powder.

The UV spectrum showed a strong broad peak at λ_{max} 493 nm, in addition to three shoulders at λ_{max} 261, 304 and 382, establishing a polyene system with a conjugated carbonyl group. The molecular weight of compound **230** was established by (+)-ESI mass spectrum as 582 Dalton. HREI MS of **230** gave the corresponding molecular formula $C_{41}H_{58}O_2$ affording 13 double bond equivalents.

The ^{1}H NMR spectrum showed a *transoid* double bond (*J*~15.3 Hz) in conjugation with a carbonyl group due to the presence of two doublets at δ 7.52 and 6.72. In addition, overlapping olefinic proton signals with an intensity of 12H were exhibited between δ 6.68-6.18, in addition to a 1H doublet at δ 5.95. Furthermore, a 2H multiplet was displayed in the range of δ 5.17-5.09. In accordance, compound **230** possibly contained 17 olefinic protons. In the aliphatic region, it displayed at δ 3.22 a singlet of a methyl ether linked to sp^3 carbon. Furthermore, four singlets between δ 1.97~1.95 with an intensity of 12 protons and a 12H due to two singlets at δ 1.68 (3H) and 1.58 (9H) were indicating eight methyl groups linked to double bonds. The last singlet (1.32) is attributed to two methyl groups linked possibly to saturated carbon. The spectrum exhibited further two 4H multiplets between δ 2.10-2.03, which are assigned as of two methylene groups, probably adjacent to sp^2 carbons.

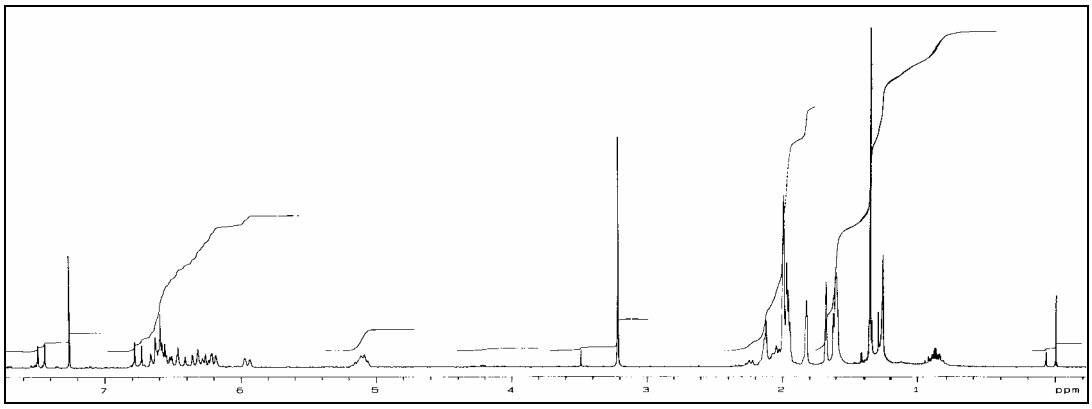


Figure 104: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of spheroidenone (**230**)

The ^{13}C/APT NMR spectra of compound **230** showed a deep field quaternary carbon signal at δ 203.0 of a carbonyl group conjugated with an olefinic or aromatic moiety. In addition, 17 olefinic methine signals and seven quaternary sp^2 carbons were displayed. In the aliphatic region, the spectrum showed a quaternary oxygen-

ated carbon at δ 81.6, and a methoxy signal at δ 52.2. Moreover, four methylene carbons were observed at δ ~ 26. Between δ 25.7~12.8, 10 methyl signals were found.

By a search in AntiBase and the DNP, four possible carotinoids were obtained: 1,2-dihydro-1-methoxy-ψ,ψ-caroten-4-one (**228**), rhodopinol (**229**), spheroidenone[24] (**230**) and thiothece 474 (**231**).

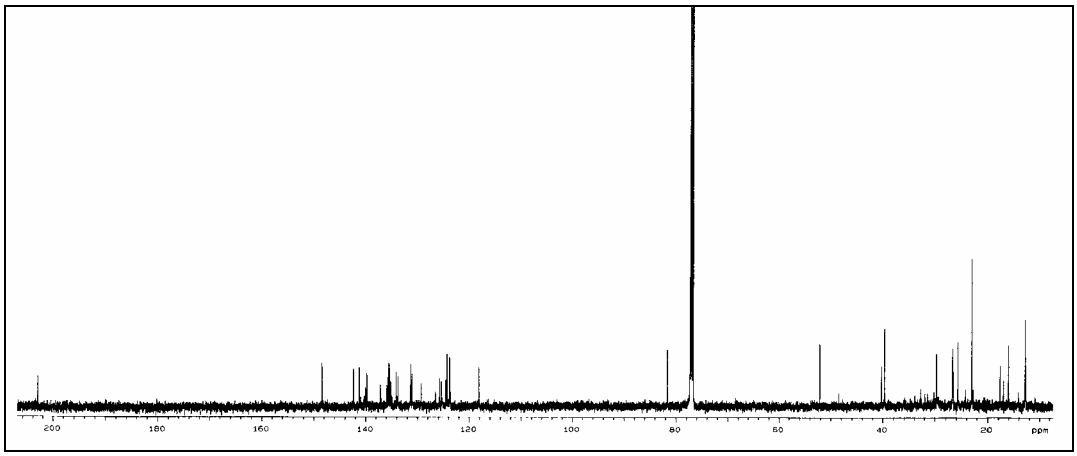
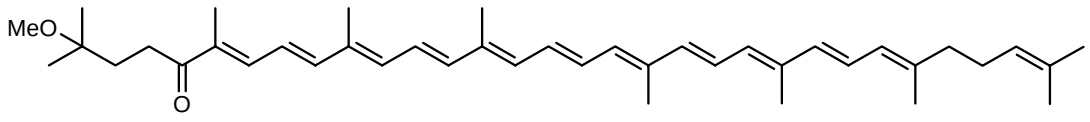


Figure 105: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of spheroidenone (**230**)

Compound **229** is an aldehyde and was excluded therefore. The carotenoids **228** and **231** are conjugated ketones, but don't have protons in α,β-position. In addition, the methylene groups in α,β-position to the carbonyl group in **228** and **231** were not displayed (normally found as two triplets). Therefore, compounds **228** and **231** were excluded, pointing to spheroidenone (**230**) as the sole consistent structure to the data. Furthermore, the fragment (**A**) of compound **230** was also confirmed by 2 D correlations. In addition, EI mass spectrum showed two characteristic fragments at *m/z* 73 and 69. The fragment (*m/z* 73) is due to a terminal tertiary methoxy group ([-$C(OCH_3)(CH_3)_2$), while the second one (*m/z* 69) is characteristic for a terminal isoprene group (-C_5H_9]), further establishing the spheroidenone (**230**).



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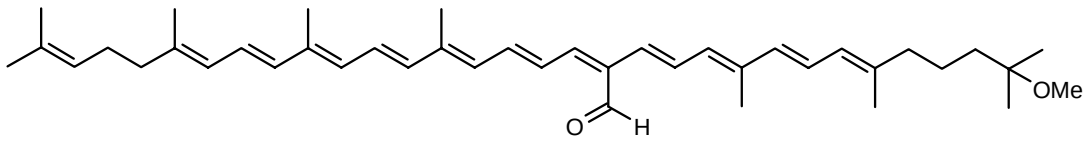
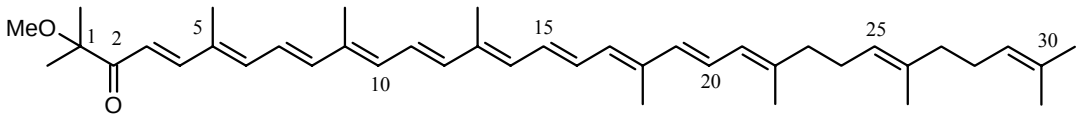


229



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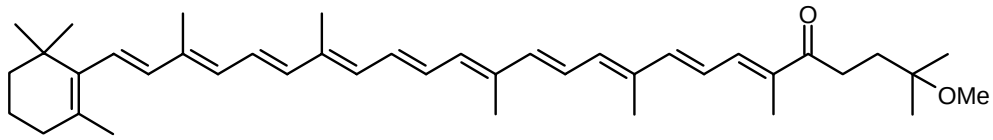

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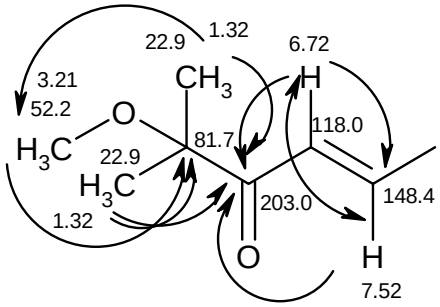


Figure 106: 2D correlation of partial structure (fragment A) of spheroidenone (**230**)

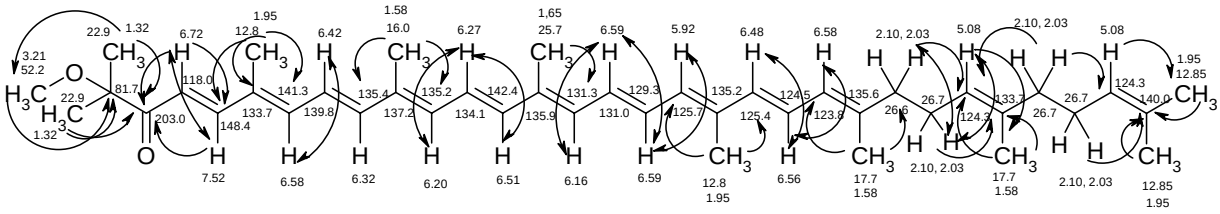


Figure 107: HH-COSY (H↔H) and HMBC (H → C) correlations of spheroidenone (**230**)

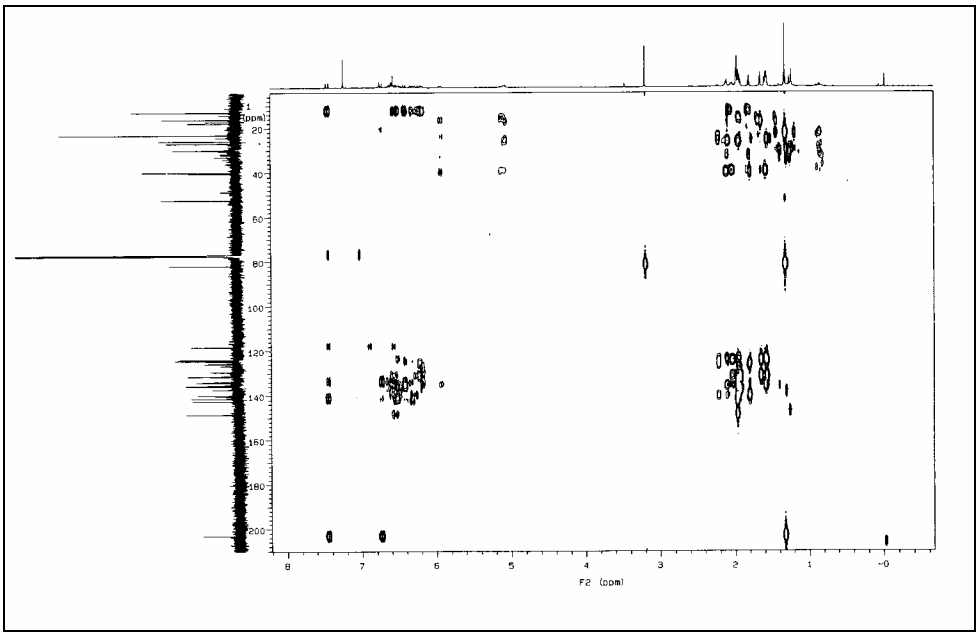


Figure 108: HMBC NMR spectra and its expanded sections ($CDCl_3$, 125 MHz) of spheroidenone (**230**)

Spheroidenone (**230**) was isolated previously from *Rhodobacter sphaeroides*, a member of the non-sulphur purple facultative[260] photosynthetic proteobacteria, capable to grow under a variety of cultivation conditions. Compound **230** was also obtained from the photosynthetic bacteria *Rhodopseudomonas spheroides* and *Erythrobacter* sp.[24,261]. It is structurally related to recently reported methoxylated ketocarotinoids from the purple non-sulphur bacteria *Rhodopseudomonas globiformis*. These bacteria belong to the *Athiorhodaceae* recently renamed as *Rhodopirillaceae*[262]. Additionally, *Rhodobacter denitrificans* (previously named *Erythrobacter* sp) synthesized spheroidenone as a major carotenoid under aerobic dark conditions. However, in illuminating under anaerobic conditions, many yellow pigments appeared e.g. 3,4-dihydrospheroidenone, while a considerable amount of spheroidenone disappeared. This pointed to a photo-reduction of the C=C double bond at the 3,4-position of spheroidenone[263].

Previous examination of drilling cores showed that the majority of the carotenes and carotenoids originally present in the various planktonic organisms and phototrophic bacteria are preserved in sediments and were found to act as indicators of trophic changes in Lake Lobsigen during the last 14000 years. Spheroidenone is one of such pigments present in the sedimental bacteria which were collected from deep sea[264].

Screening conclusion

During the course of the screening for further carotenoids in marine *Roseobacter* and other bacteria species, 18 bacterial isolates were selected. These strains showed a characteristic red/orange colouration during their growth on agar plates. The strains were compared with our five *Roseobacter* sp. (DFL16, DFL30, DFL27, DFL38 and DFL12) worked up previously. However, chemical screening of the selected strain extracts exhibited a different chromatographic behaviour (R_f, colour of bands (yellow~red) and no blue colours with sulphuric acid (mostly to brown). This indicated that carotinoides were not present in these strains and confirmed spheroidenone **(230**) as an taxonomic marker of *Roseobacter* sp. This conclusion was confirmed additionally by subjecting their extracts to HPLC MS analysis, which established their similarity.

Table 10: Comparison of the *Roseobacter* sp. isolates (DFL) with other bacteria sp. producing coloured metabolites on basis of TLC analysis.

Taxonomy	Strain Nr.	Agar plat colour	Colour change by H_2SO_4	Colour by TLC	Colour of the Extract
Roseob.	Hel 28	Red	-	Yellow/orange	Yellow/orange
Roseob.	Hel 38/983	Red	-	Yellow	Yellow/orange
Roseob.	Hel 42	orange	-	Yellow	Brown
Roseob.	Hel 28	Red	-	Orange	Red
Roseob.	DFL16	Red	Blue	Red	Red
Roseob.	DFL30	Red	Blue	Red	Red
Roseob.	DFL27	Red	Blue	Red	Red
Roseob.	DFL38	Red	Blue	Red	Red
Roseob.	DFL12	Red	Blue	Red	Red
Unknown	1241/Pic.	Red	-	Red	Red
Unknown	1261/Bio	Red	-	Yellow	Yellow
Unknown	1266/Bio	Red	-	Orange	Brown/red
Unknown	Bio215	Red	-	Red	Red/brown
G-posit.	Hel 31	Red	-	Orange	Brown
Unknown	Bio221	Orange/red	-	Red	Red/brown
Unknown	STO025	Red	-	Red	Red/brown
Unknown	1267/Bio	Red	-	Red	Red/brown

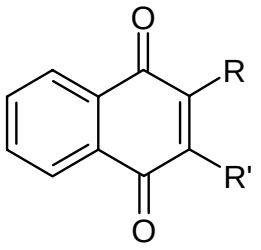
4.18 Terrestrial *Streptomyces* sp. GW10/1818

The ethyl acetate extract of the terrestrial *Streptomyces* sp. GW 10/1818 drew our interest due to its production of an UV absorbing band, which was stained to brown by anisaldehyde/sulphuric acid. Moreover, it possessed a high antifungal activity against *Mucor miehei* (Tü284). Therefore the strain was cultivated 25-liter as shaking culture (110 rpm) on M_2 medium for five days at 28 °C giving a grey/brown culture broth. Working up of the brown crude extract using flash silica gel chroma-

tography and elution by a dichloromethane/methanol-gradient afforded two fractions. Purification of fraction II led to hexahydromenaquinone MK-9 (**235**)

4.18.1 Hexahydromenaquinone MK-9 (II,III,VIII-H6)

Compound **235** was isolated as yellowish UV absorbing oil from fraction II using PTLC followed by Sephadex, which turned to brown by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum exhibited two characteristic 2H multiplets in the aromatic region at δ 8.08 and 7.69 which were similar as in 1,4-naphthoquinone (**232**) or phthalic acid esters (**233**) .



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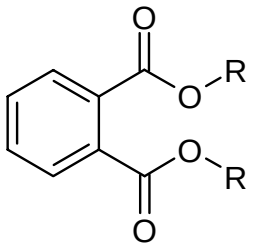

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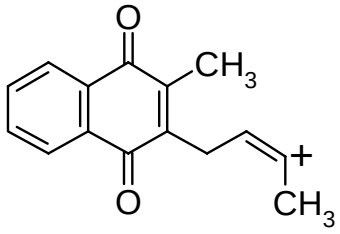


234

In addition, a 6H multiplet was displayed at δ 5.18-5.08 in a region characteristic for olefinic double bonds. In the aliphatic region, it showed a 2H doublet at δ 3.38 as for a methylene group flanked by two sp^2 carbons or attached to a heteroatom. Furthermore, 3H singlets were observed at δ 2.20, 1.78 and 1.69 of three methyl groups attached to olefinic carbon moieties. Moreover, a multiplet of 20 protons was exhibited in the range of δ 2.13-1.90, which is probably indicative for 10 methylene groups. It exhibited also a singlet of 15H at δ 1.61 characteristic for 5 methyl groups linked likewise to olefinic carbons. Additionally, a 3H multiplet was observed between δ 1.42-1.30, in addition to a broad singlet of 18H at δ 1.25 which can be assigned for for 6 methyl groups linked to sp^3 carbons. Finally, three doublets each of 3H between δ 0.88-0.78 (^{3}J = 6.2 Hz) are characteristic for three methyl groups of type -CH-CH_3.

The ^{13}C/APT NMR spectra of compound **235** exhibited two carbonyl signals at δ 185.5 and 184.5 which are characteristic for a 1,4-quinone moiety. In addition, twenty sp^2 carbon signals were observed in the range of δ 146.2-118.7, among them 10 quaternary carbon signals. In the aliphatic region, it showed additional 20 carbon signals between δ 42-24, according to 20 methylene groups. Furthermore, 14 carbon signals between δ 33-12 were attribute to CH and/or CH_3 groups.

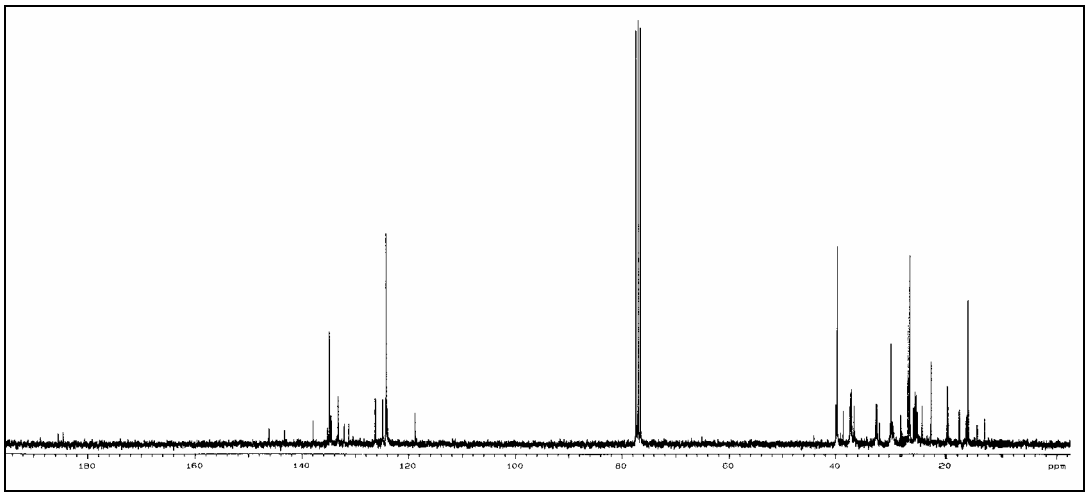
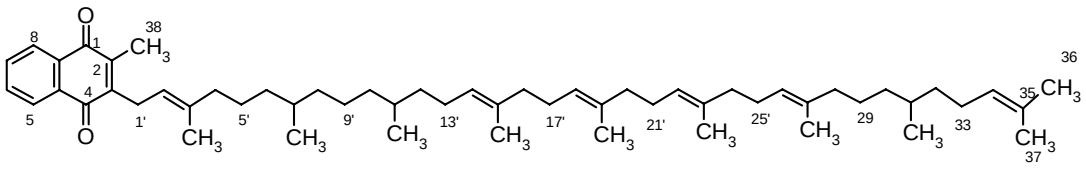


Figure 109: ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of hexahydromenaquinone MK-9 (II,III,VIII-H6) (**235**)

The molecular weight of compound **235** was established as 791 Dalton by the EI mass spectrum. Two further fragments were displayed at *m/z* 225 and *m/z* 69, of which the first one was interpreted as fragment **234** with a naphthoquinone moiety, and the second one seems to indicate a terminal isoprene group ($[C_5H_9]$).

Depending on the displayed spectral data and AntiBase searching, two possible structures were revealed, hexahydromenaquinone MK-9 (II,III,VIII-H6)[265-267] (**235**) and hexahydromenaquinone MK-9 (II,III,IX-H6)[265, 266] (**236**). However, based on the ^1H NMR spectrum, the observed three CH-CH_3 groups and the established terminal isoprene group, directed to hexahydromenaquinone MK-9 (II,III,VIII-H6) (**235**) but not to hexahydromenaquinone MK-9 (II,III,IX-H6) (**236**).



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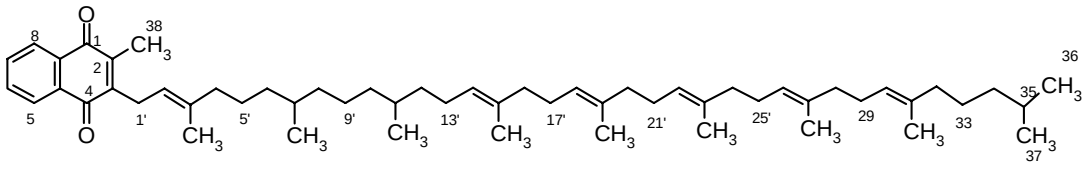


236

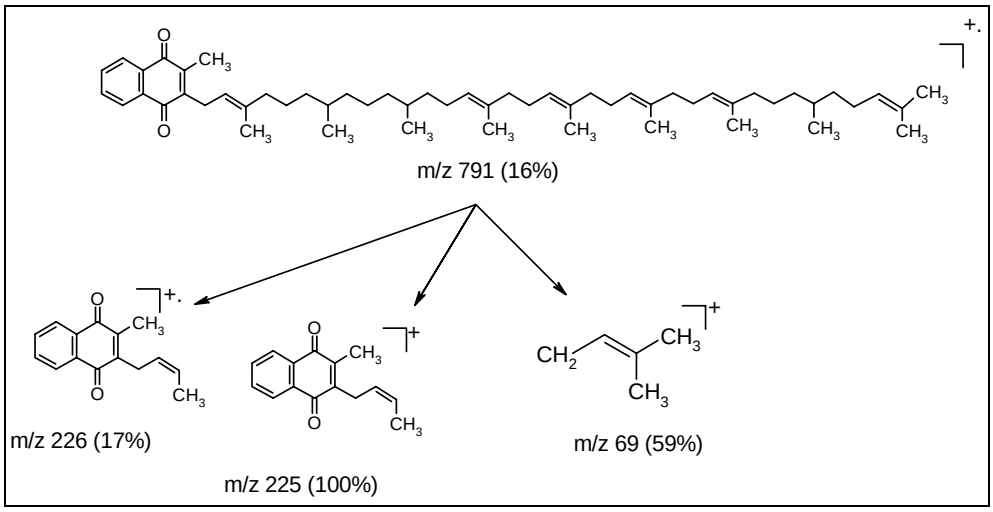


Figure110: EI fragmentation pattern of hexahydromenaquinone MK-9 (II,III,VIII-H6) (**235**)

4.19 Terrestrial *Streptomyces* sp. GW5/1749

During the screening route, the ethyl acetate extract produced by the terrestrial *Streptomyces* sp. GW 5/1749 attracted our attention because of its high antimicrobial activity against both Gram-positive and Gram-negative bacteria (*Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus*). Moreover, TLC of the extract showed an intensive yellow/orange UV absorbing band, which became brown by anisaldehyde/sulphuric acid.

The strain was inoculated on M_2 medium for five days at 28 °C as shaking culture (110 rpm). The resulting yellow-orange culture broth (20-liter) was filtered, and the biomass extracted with ethyl acetate and the filtrate with XAD-2. As the yellow substance was concentrated mainly in the biomass, it was worked up using silica gel chromatography to give the fractions I-III. Further purification of the fast moving fraction II led to the isolation of menaquinone MK-9 (II, III-H4) (**237**).

4.19.1 Menaquinone MK-9 (II, III-H4)

Compound **237** was isolated as an unpolar pale yellow UV absorbing oil, which was stained during TLC to brown by anisaldehyde/sulphuric acid and heating.

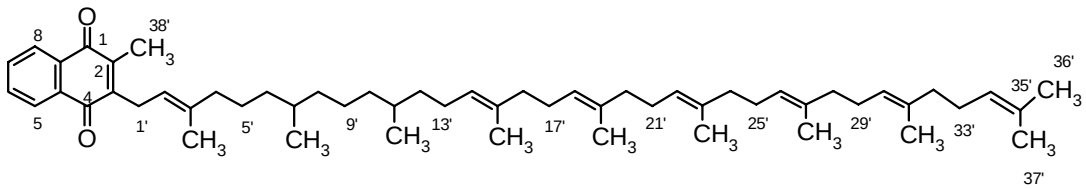
The ^{1}H NMR spectrum showed a similarity with that of **235**. It showed two 2H multiplets at δ 8.08 and δ 7.69 characteristic for the naphthoquinone moiety, 7H multiplet located between δ 5.18-5.08 of seven double bonds (-CH = C_q). In the aliphatic region, it exhibited a doublet of 2H at δ 3.38 of a methylene group flanked by two

sp^2 carbons, in addition to three singlets at δ 2.20, 1.78, and 1.69 of methyl groups attached to sp^2 carbons. Furthermore, a multiplet of 18 protons was exhibited in the range of δ 2.13-1.90, in addition to a singlet of 15H at δ 1.63 characteristic for 5 methyl groups linked to olefinic carbons. The spectrum displayed besides a 2H multiplet between δ 1.42-1.30, and a multiplet of additional 18 protons in the range of δ 1.42-1.18. Finally, a multiplet of 6 protons was observed between δ 0.91-0.82 due to two methyl groups of the type $CH\text{-}CH_3$.

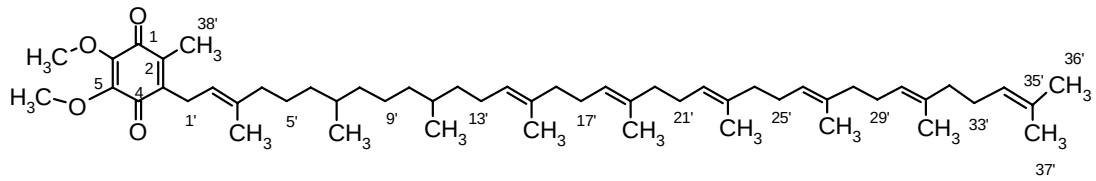
The ^{13}C /APT NMR spectra of compound **237** confirmed again its structural similarity with compound **235** (see the experimental part). The molecular weight of **237** was confirmed as 789 Dalton using of both CI and EI mass spectra. Two further fragments were observed in EI MS at *m/z* 606 and 225, of which the last (*m/z* 225) was assigned to fragment **234**. The terminal isoprene unit was also established due to the existence of a peak at *m/z* 69 as base peak.

By applying the above data to AntiBase, menaquinone MK-9 (II,III-H4) (**237**) was found as the sole coincident structure. The compound **237** was further established by direct comparison the spectral data with authentic spectra from the literature[265-267,48]. The menaquinone **237** shows a high structural similarity to ubiquinone-9 (**238**) except that the benzene ring of the quinone chromophore is replaced by two methoxy groups in **238**.

Vitamin K is the family name for a series of fat-soluble compounds which have a common 2-methyl-1,4-naphthoquinone nucleus but differ in the side chain structures at the 3-position. The first members have been discovered by Dam in the 1920s[268]. They are synthesised by plants and bacteria[269], and nearly most of the other microorganisms. In mammals, it is needed in the synthesis of proteins required for blood coagulation in the synthesis of prothrombin. Normally, it is produced by bacteria in the intestines, and hence dietary deficiency is extremely rare unless the intestines are heavily damaged. The menaquinones play an important role in the respiratory chain of bacteria.



237



238

4.20 Marine *Streptomyces* sp. B8904

The ethyl acetate extract of the marine *Streptomyces* isolate B8904 drew our attention due to three reasons. i) It showed a dark red aerial mycelium on the agar plate. ii) The crude extract exhibited a pronounced antibacterial activity against *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Bacillus subtilis, and Staphylococcus aureus*. iii) the TLC exhibited several coloured zones ranging between yellow and dark red, which turned to blue/violet with sodium hydroxide, as indication of *peri*-hydroxyquinones.

Fermentation of B 8904 was carried out in M_2^+ medium using two scales, a 26-liter jar fermenter and a 25-liter shaker culture.

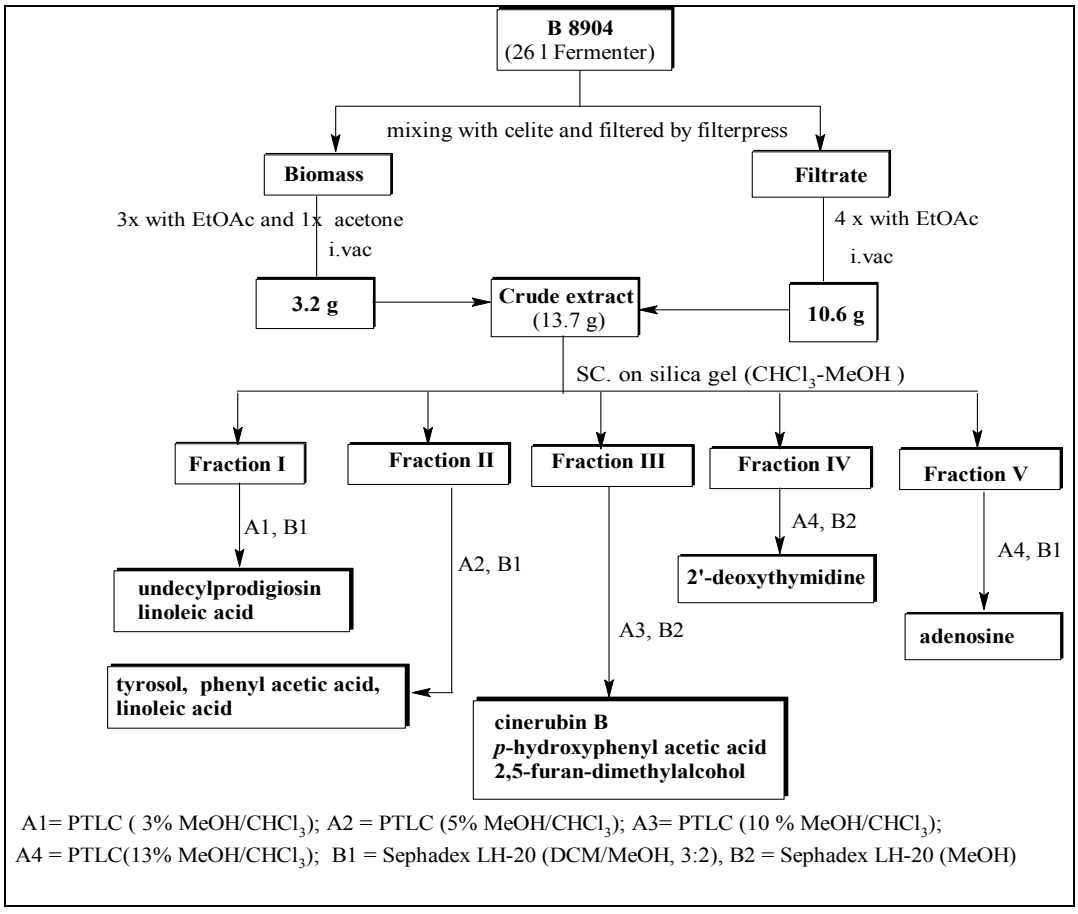


Figure 111: Work-up scheme of the marine *Streptomyces* isolate B 8904 (fermenter)

A: Fermenter

Twelve of 1liter-Erlenmyer flasks (each with 250 ml of M_2^+ medium) were inoculated with small pieces of well-grown agar plates of the producing strain B8904 for 7 days at 28 °C. The dark red broth was used to inseed a 23-liter jar fermenter which was kept for additional 5 days. After filtration and extraction (by ethyl acetate), the resulting extract was applied to flash silica gel chromatography and eluted with a chloroform-methanol gradient. As a result, five fractions were obtained. After working up and purification of the desired fractions, undecylprodigiosin (**128**), linoleic acid (**239**), and adenosine (**242**) were isolated in addition to other colourless known compounds (Figure 111).

4.20.1 Linoleic acid; (9Z,12Z)-9, 12-octadecanoic acid

During the screening of the fast moving fractions I-II, compound **239** was obtained as low polar colourless oil, exhibiting an UV absorbance and stained to blue by anisaldehyde/sulphuric acid. The observed blue colour reaction of compound **239** indicated most likely a terpene, steroid or fatty acid. The ^{1}H NMR spectrum exhibited a 1H broad singlet at δ 9.0 of a phenolic hydroxyl, amide or free aliphatic carboxylic acid group. In addition, a 4H multiplet was observed between δ 5.43-5.25, which could be assigned as two olefinic double bonds. Furthermore, a 2H triplet of methylene group adjacent most likely to sp^2 carbon was displayed at δ 2.78. Furthermore, a triplet of two magnetically equivalent methylene groups likely adjacent to sp^2 carbons was displayed at δ 2.52. Additionally, two multiplets were observed at δ 2.08 and 1.63, each of 4H as of two methylene groups, the first of them could be linked to sp^2 carbon. Moreover, a broad singlet of 10 protons was observed in the range of δ 1.42-1.23, pointing to a side chain of 5 methylene groups. At the end, a triplet of 3H was observed at δ 0.85.

The ^{13}C/APT NMR spectra of **239** exhibited only a carbonyl carbon at δ 180.1 of a quinone or aliphatic carboxylic acid. In addition, four sp^2 methine carbons of two olefinic double bonds were observed at δ 130.1, 129.9, 128.0, and 127.8. In the aliphatic region, 12 overlapping methylene carbons were observed between δ 31.5-22.5, besides one methyl carbon at δ 14.0.

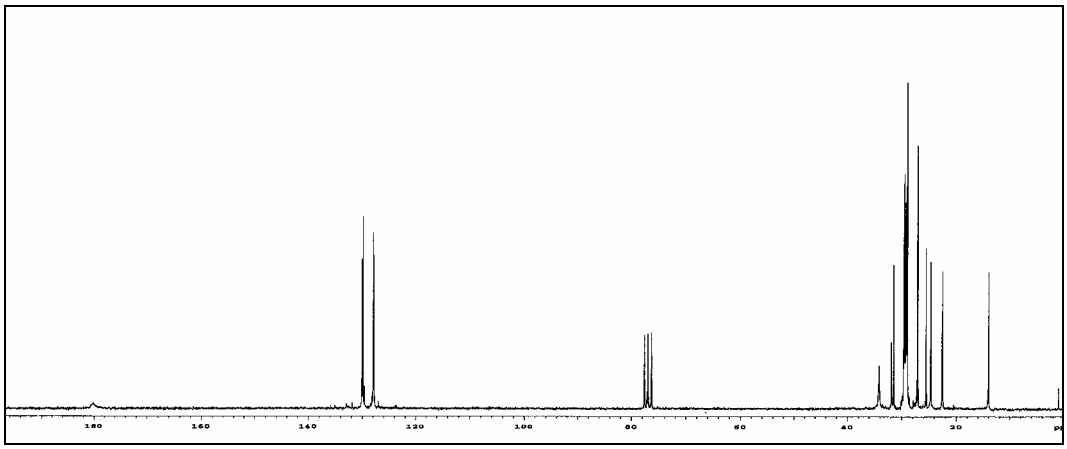


Figure 112: ^{13}C NMR spectrum ($CDCl_3$, 200 MHz) of linoleic acid (**239**)

The molecular weight of compound 238 was determined as 280 Dalton by EI mass spectrum. By applying the above data to AntiBase, three fatty acids were suggested: (9Z,12Z)-9,12-octadecanoic acid (linoleic acid, **239**), (10Z,13Z)-10,13-octadecanoic acid (**240**) and (11Z,14Z)-11, 14-octadecanoic acid (**241**). However, comparison of the spectra with an authentic one as well as the EI MS fragmentation patterns pointed to linoleic acid (**239**) (Figure 113)

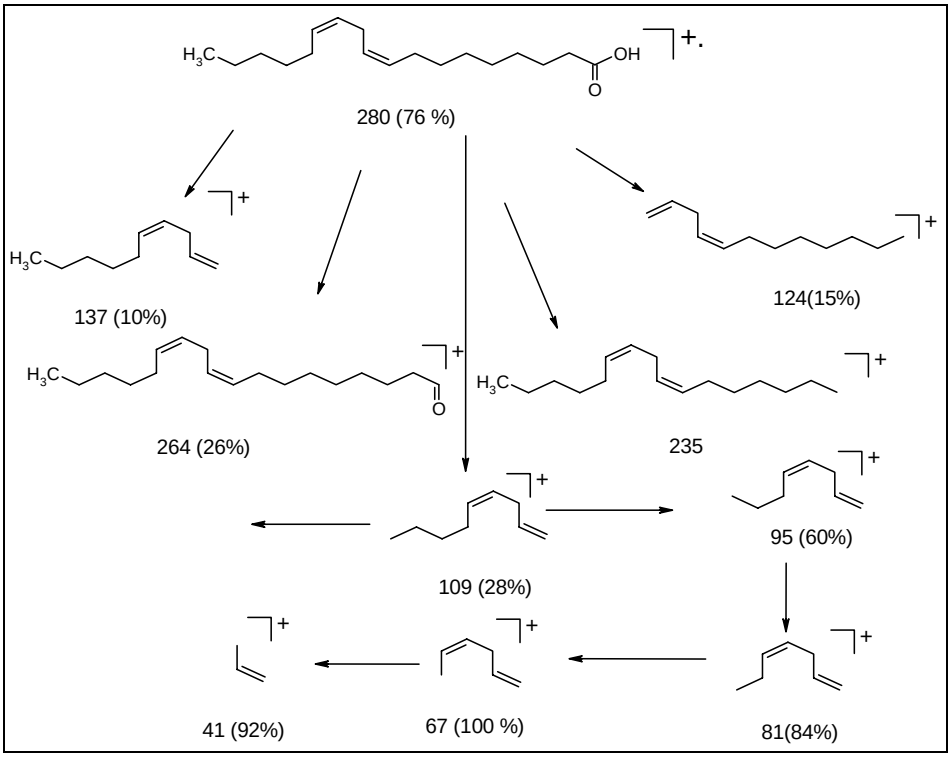
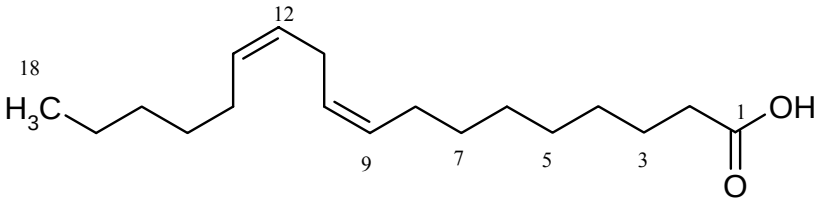


Figure 113: EI MS fragmentation pattern of linoleic acid (**239**)



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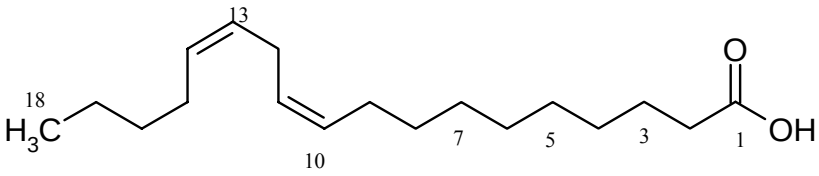

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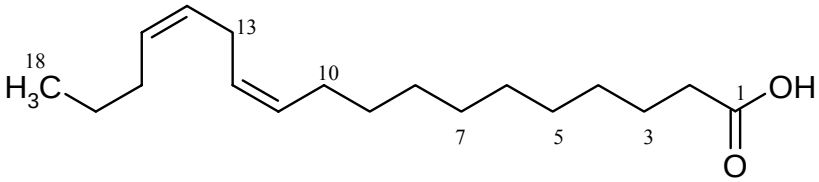


241

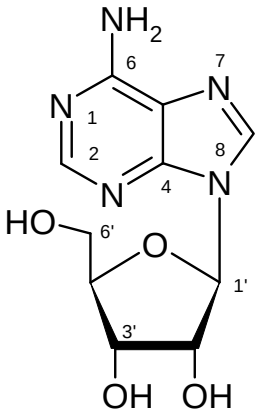
Linoleic acid (**239**) is known as a constituent of most vegetable and animal fats and is used biosynthetically as essential fatty acid for the production of prostaglandin, and was frequently isolated from marine brown algae[270]. Compound **239** was isolated also recently from a *Micrococcus* species using GC MS methods[271].

4.20.2 Adenosine

Compound **242** was obtained as colourless solid from fraction V. It showed a strong UV absorption, and was stained intensively greenish-blue by anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum showed two deep field singlets each of 1H at δ 8.34, and 8.12 of non-exchangeable protons and a broad singlet of two exchangeable protons at δ 7.33 (NH_2). This established an adenine pattern. In the sugar region, it showed a doublet at δ 5.81, most likely of an anomeric proton, besides a singlet of two acidic protons between δ 5.60-5.20 (2 OH). Furthermore, three additional oxymethine signals at δ 4.59, 4.17 and 3.95, besides an oxymethylene group giving an ABX systems at δ 3.65 and 3.55 were observed.

The molecular weight of compound **242** was established as 267 Dalton using EI mass spectrum. It showed additionally a base peak fragment at *m/z* 134, which could

be attributed to adenine ion. In agreement, a search in AntiBase resulted in the elucidation of compound **242** as adenosine. The compound was further confirmed by comparing its data with authentic spectra and the literature[48].



242

B) Shaker Culture

peri-Hydroxy-quinones are very common orange to red coloured microbial metabolites, and characterised by the bathochromic shift of their UV absorption in alkaline solution. According to our experience, coloured metabolites are preferably formed in shaker cultures rather than fermenters. Therefore, the strain B8904 was recultivated on a 25-liter scale for 7 days at 28 °C as shaker culture (110 rpm). After filtration, the dark red broth was extracted with ethyl acetate. The resulting extract was chromatographed on flash silica gel column using chloroform-methanol gradient. Further separation of the fractions containing the reddish-orange *peri*-hydroxyquinones (II-IV) delivered seven compounds: the known compounds, ζ-pyrromycinone (**243**), η-pyrromycinone **(244),** musettamycin (**246**), cinerubin B **(248**), in addition to the three new *peri*-hydroxyquinones, cinerubin M (**254**), islamomycin A (**257**) and islamomycin B (**258**) (Figure 114).

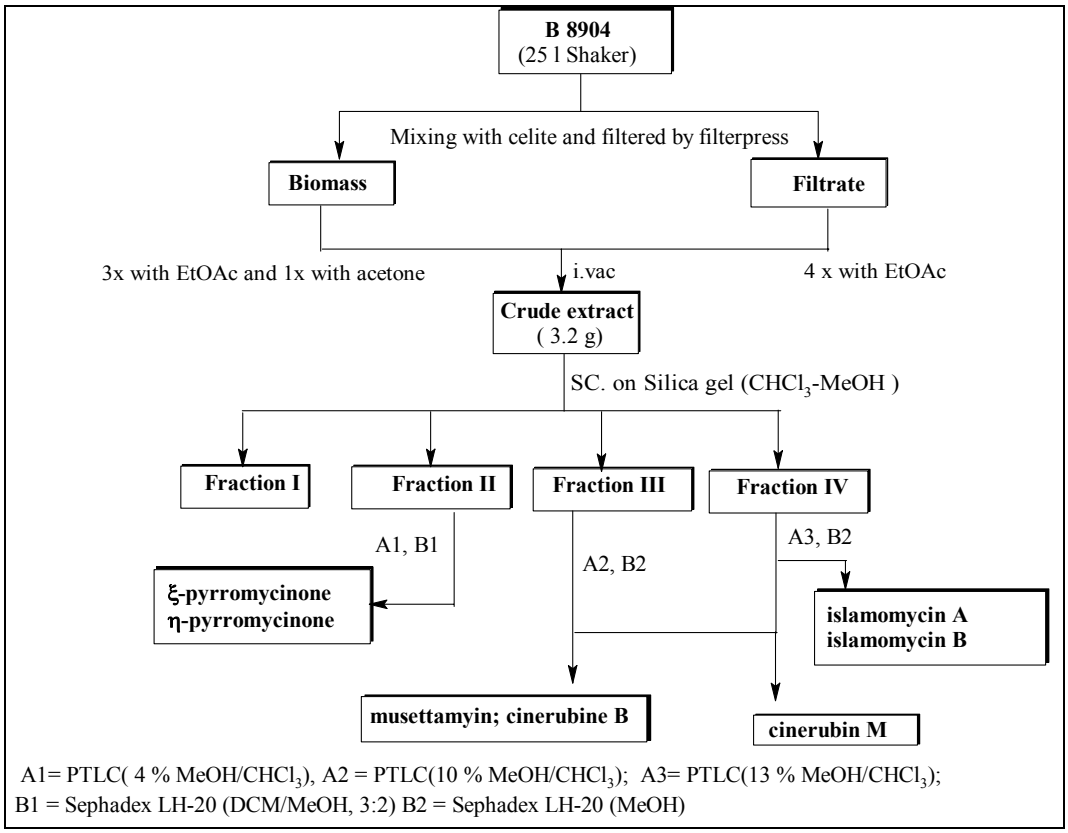


Figure 114: Work-up scheme of the marine *Streptomyces* isolate B 8904 (shaker)

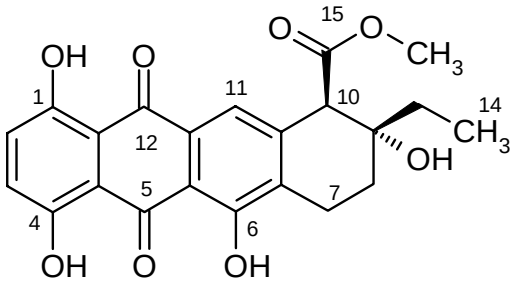
4.20.3 ζ-Pyrromycinone; Galirubinone C

During the work-up of the fast mobile fraction II, compound **243** was isolated as low polar and major product. It was isolated as red-orange solid by applying the fraction to PTLC and Sephadex. The compound showed an orange fluorescence zone under UV light at 366 nm which turned to violet by treating with sodium hydroxide as indication of a *peri*-hydroxyquinone.

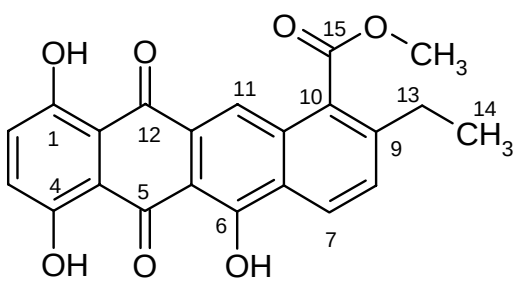
The ^{1}H NMR spectrum of **243** showed three singlets each of 1H at δ 13.02, 12.61, and 12.28, which are characteristic for *peri*-hydroxy groups in a quinone moiety. In the aromatic region, one singlet of 1H at δ 7.69, and two AB systems each of 1H at δ 7.29 and 7.28 were detected, as two *ortho*-coupled protons in 1,2,3,4-tetra-substituted phenolic ring. In addition, a 1H singlet was observed at δ 3.96, which could be assigned as oxymethine or as methine group flanked by two sp^2 carbons. One methoxy group was observed at δ 3.74 (s). Four multiplets were displayed (each of 1 H), of two vicinal methylene groups, at δ 3.07 (7-H_A), 2.86 (7-H_B), 2.30 (8-H_A) and 1.95 (8-H_B). The first methylene group (δ 3.07 and 2.86) should be next to a sp^2 carbon, while the other one (δ 2.30 and 1.95) should be next to an oxygenated qua-

ternary sp^3 carbon. Furthermore, a third methylene group was giving two multiplets between δ 1.70-1.58, which could be adjacent to a methyl group, observed as triplet at δ 1.09, to constitute an ethyl group.

The (-)-ESI mode exhibited two *quasi*-molecular ions at *m/z* 845 ([2 M +Na – 2 H]$^-$) and 411 ([M - H]$^-$), establishing the molecular weight of compound as 412 Dalton. It was further established by a CI mass spectrum which exhibited an ion peak at *m/z* 430 $[M + NH_4]^+$. Based on the above chromatographic and spectroscopic data of compound **243** and searching in AntiBase, one possible structure was suggested, ζ-pyrromycinone (**243**). The compound **243** was further confirmed by direct comparison of the spectral data with authentic one.



243



244

4.20.4 η-Pyrromycinone; Ciclacidine

Along with **243**, compound **244** was isolated from fraction II as a red-orange solid. It showed an orange UV fluorescence and turned to violet by treatment with sodium hydroxide, as indication of a *peri*-hydroxyquinone. The ^{1}H NMR spectrum of **244** showed three chelated *peri*-hydroxyl groups at δ 13.85, 13.12 and 12.32. In the aromatic region, it showed a deep field AB system at δ 8.53 and 7.61 indicating an 1,2,3,4-tetrasubstituted aromatic system. Additionally, a second AB system was observed at δ 7.29 of another 1,2,3,4-tetrasubstituted aromatic system. Moreover, one deep field of 1Hsinglet was observed at δ 8.27 due to a third aromatic ring. In the aliphatic region, a methoxy group was observed at δ 4.13. Finally, an ethyl group was established, due to the quartet of a methylene group at δ 2.85, and its vicinal triplet methyl group at δ 1.35. The observed down field shift of the last ethyl group referred to its link to an sp^2 carbon atom.

The molecular weight of compound **244** was established as 392 Dalton using EI MS. Applying the spectral data of compound **244** to AntiBase led to its identification

as η-pyrromycinone. The compound was further confirmed by comparison with authentic data.

4.20.5 Musettamycin

Additionally, from fraction III, compound **246** was obtained as a red-orange solid. The compound showed orange UV fluorescence and turned to violet by sodium hydroxide, as indication of another *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of compound **246** exhibited three *peri*-hydroxy groups at δ 12.93, 12.81 and 12.22. In the aromatic region, a singlet was observed at δ 7.68 indicating a penta-substituted aromatic ring. Furthermore, a 2H AB system was observed at δ 7.28 (2/3-H). In the sugar region, it showed three oxymethines at δ 5.51 (s br.), 5.28 (m), and 5.07 (d), establishing the presence of at least two anomeric protons. Eight additional oxymethines were exhibited between δ 4.47-3.61, containing a singlet at δ 4.13 (H-10). A methoxy group was observed at δ 3.70, an ethyl group was present in the compound because of the methyl triplet at δ 1.08 and the adjacent methylene signals at δ 1.72 and 1.51. Furthermore, two splitted multiplets of a methylene group were observed at δ 2.52 (8-H_{eq}) and 2.32 (8- H_{ax}). This led to establish the pyrromycinone chromophore. The spectrum exhibited a singlet of 6 protons at δ 2.16, assigning two equivalent methyl groups linked most likely to a heteroatom ($N(CH_3)_2$). Moreover, a 2H multiplet of a methylene group was observed at δ 2.09-1.94 as present e.g. in desoxy sugars. Two methyl doublets were observed at δ 1.27 and 1.18.

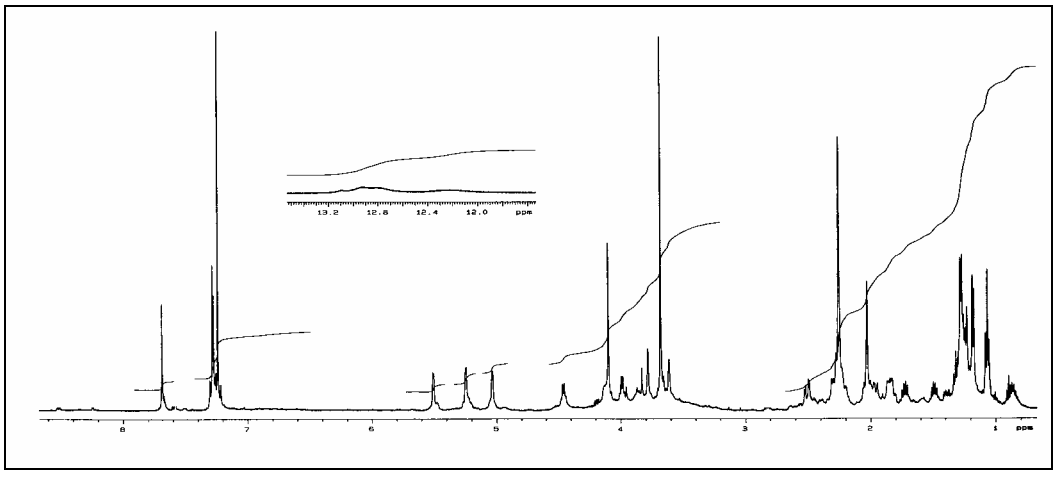
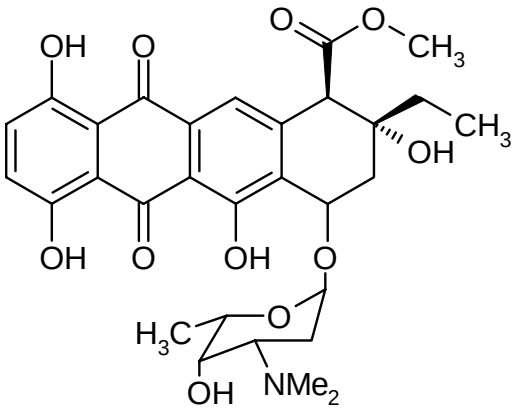


Figure 115: ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of musettamycin (**246**)

The ^{13}C NMR spectrum of compound **246** exhibited 35 carbon signals. Two quinone carbonyl carbons were observed at δ 190.7 (C-5), and 185.8 (C-12), besides the carbonyl of an acid derivative at δ 171.3 (C-15), three phenolic C atoms at δ 162.3, 158.4 and 157.9, and further 6 quaternary and 3 sp^2 methine carbon atoms, establishing the aromatic part of pyrromycinone chromophor. In the sugar region, two methine carbons were observed at δ 101.6 and 99.1 and assigned as two anomeric carbons. This pointed to the presence of two sugar moieties. Also, eight methine carbons were observed between δ 74.1-61.5 which were assigned as oxy- and /or nitrogenous methine carbons. Additionally, a quaternary sp^3 oxy-carbon was observed at δ 71.7. A further methine carbon was observed at δ 57.2, which could be assigned as nitrogenous methine or flanked by two sp^2 carbons. The methyl ester carbon of pyrromycinone chromophore was established at δ 52.5, and a carbon signal of two equivalent methyl carbons was observed at δ 42.9 corresponding to NMe_2. Furthermore, it exhibited four methylene carbons at δ 33.8, 32.2, 29.3 and 27.0, from which the first two carbons were established of C-8 and C-13 of the pyrromycinone moiety. Finally, three methyl carbons were observed at δ 17.9, 16.0 and 6.7, from which the last carbon (δ 6.7) is characteristic for C-14 in the pyrromycinone skeleton. The first two carbon signals (δ 17.9 and 16.0) were assigned as two methyl carbons present in the sugar moieties. Compound **246** has the skeleton of ζ-pyrromycinone **(243)**, except that the methylene group at position-7 (normally at δ 20.7) is replaced by CH-O observed at δ 65.3.

The mass spectrum of compound **246** was fixed as 715 Dalton by ESI mass spectra. (+)-ESI MS^2 of the molecular ion led to a base peak at *m/z* 586 as a result from the loss of 2-deoxyfucose (**A**) to afford pyrromycin (**245**). The latter one (**245**) showed a further fragmentation due to the expulsion of rhodosamine (**B**), followed by aromatisation by loss of two water molecules to give a peak at *m/z* 393.1 ([M- (2-deoxyfucose + rhodosamin + 2 H_2O) + H]$^+$) (Figure 116).



245

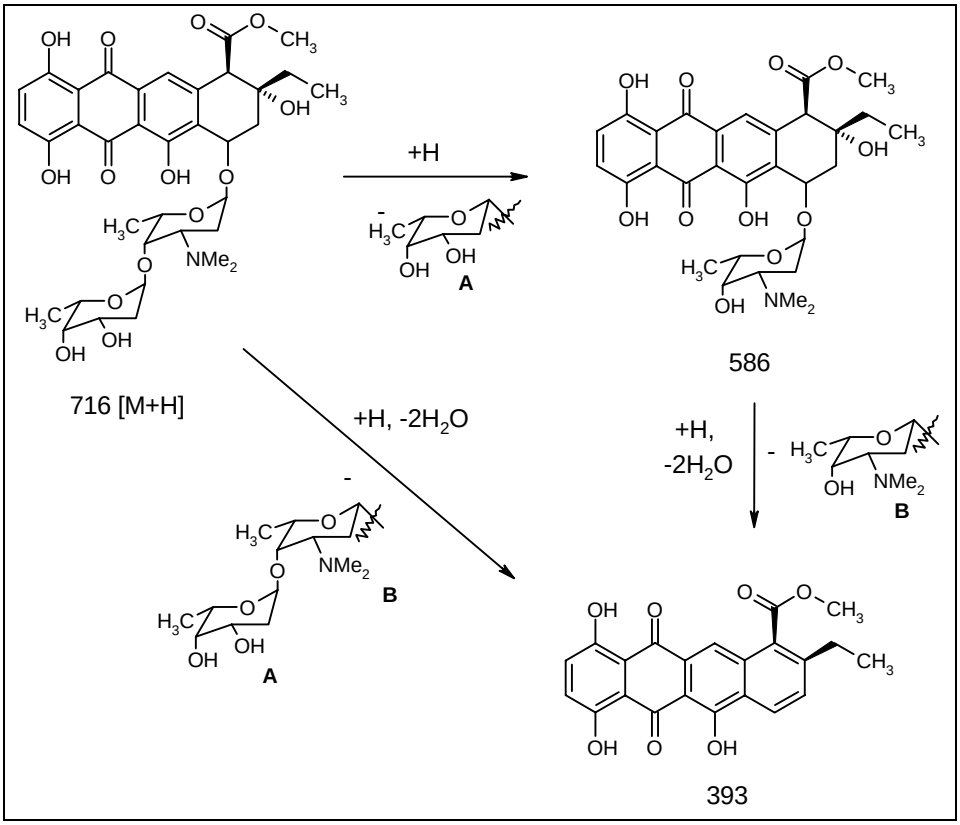
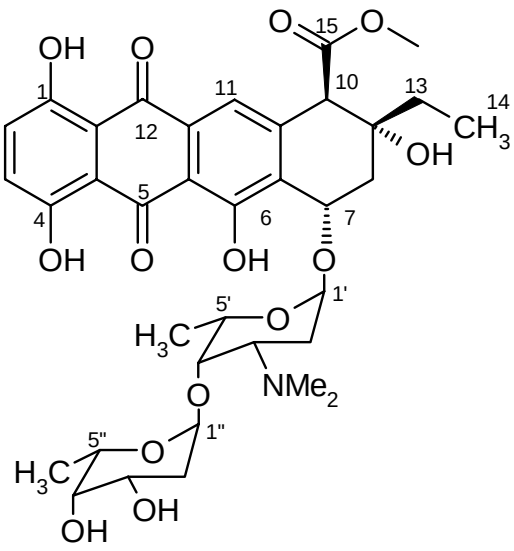
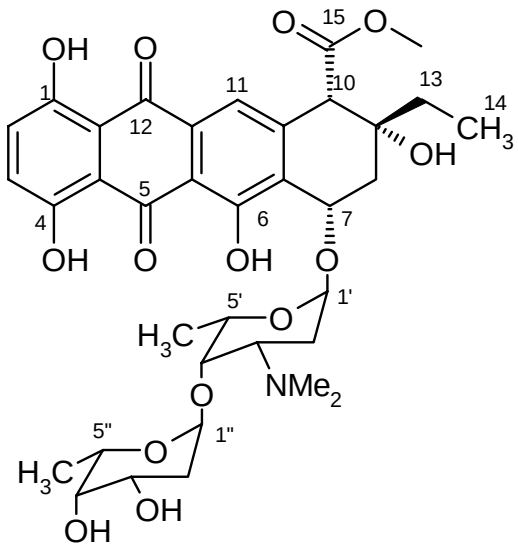


Figure 116: ESI fragmentation pattern of musettamycin (**246)**

By means of the detailed spectroscopic data, and a search in AntiBase, two possible compounds were suggested: Musettamycin (**246**) and collinemycin (10-*epi*-musettamycin, **247**)



246



247

Based on the spectral data of both isomers **246** and **247**, the compound isolated here was identified as musettamycin[272] (**246**): This is attributed to the up field shift of H-10 (δ 4.00) and the down field shift of the methoxy group to δ 3.88 in collinemycin (**247**)[272, 273]. In contrast, musettamycin (**246**) showed an up field shift of the methoxy group (δ 3.69) and a down field shift of H-10 (δ 4.10) as in the spectrum reported here.

It is still under debate if collinemycin (**247**) is a natural product or an artefact obtained by epimerisation of musettamycin (**246**) during the extraction procedure. Both compounds **246** and **247** are characterised by their antitumor and high antimicrobial activities, especially against Gram-positive bacteria. These types of compounds are present in a complex known as bohemic acid complex, where pyrromycinone is the basic constituent[272,273].

4.20.6 Cinerubin B; Ryemycin B

An additional red-orange UV fluorescent major product **248** was obtained from fraction III as red-orange solid, which was more polar than **246**. It exhibited a colour change to violet by sodium hydroxide, pointing to a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of compound **248** showed a close similarity with **246**. Three *peri*-hydroxy groups of the pyrromycinone moiety were observed at δ 12.97, 12.83 and 12.25. In addition, the 1H singlet of an aromatic proton at δ 7.73 (11-H), and two AB protons were observed at δ 7.32 and 7.28 (2,3-H), as part of the pyrromycinone skeleton. Also, the ester methoxy group and the 10-H singlet were present

at δ 3.70 and 4.13, respectively. Moreover, an ethyl group was established due to a triplet at δ 1.09 and a methylene group at δ 1.87 and 1.52. Besides, 8-H_2 protons were observed between δ 2.48-2.32. This pointed to **248**, containing the same pyrromycinone substituted at position-7. In the sugar region, it displayed four oxymethines at δ 5.51 (s br), 5.28 (m), 5.19 (d) and 5.13 (m). The proton displayed at δ 5.28 is characteristic for 7-H in pyrromycinone. In addition, three methyl groups were observed as three doublets at δ 1.37, 1.30 and 1.25. This pointed to the presence of three sugar moieties in compound **248**, one more than in **246**. In addition, a 6H singlet of two equivalent N methyl groups was displayed at δ 2.16. Furthermore, seven hydroxymethines were displayed between δ 4.81~3.72, in addition to a methylene doublet at δ 2.60 linked most likely to sp^2 carbon. A further multiplet of a methylene group was exhibited at δ 2.09 (2"-H_2). Finally, it showed a multiplet of a third methylene group at δ 1.87-1.60

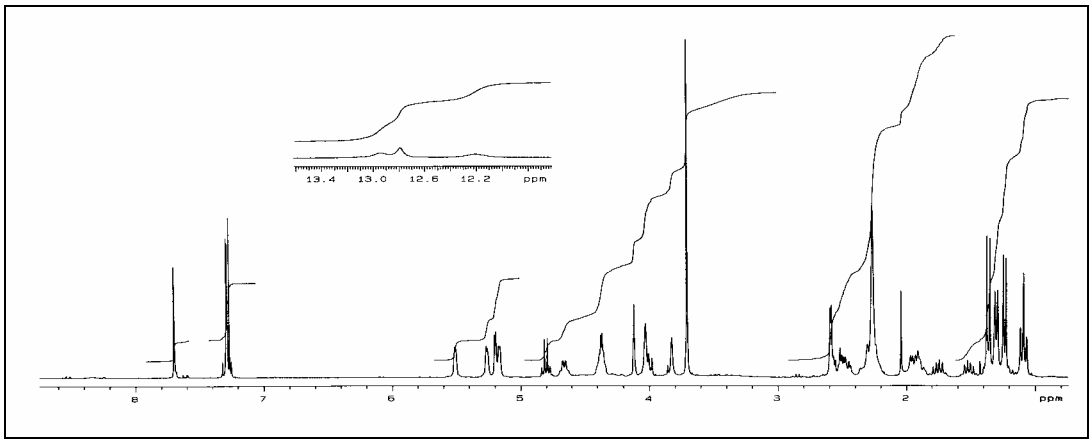
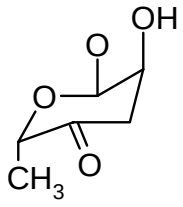


Figure 117: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of cinerubin B (**248**)

The ^{13}C/APT NMR spectrum of compound **248** showed an aliphatic ketone carbonyl at δ 208.2, in addition to the two carbonyls of the pyrromycinone moiety, the aromatic carbon signals of the pyrromycinone skeleton were also visible. Three anomeric methine carbons pointed to the presence of three sugar moieties. Moreover, nine methine and one quaternary sp^3 carbon atoms connected to oxygen were found. Furthermore, five methylene carbons were shown, corresponding to the ^{1}H NMR data. Besides, a signal at δ 42.9 with double intensity was found indicating two magnetically equivalent carbons linked to a heteroatom ($N(CH_3)_2$). At the end, four methyl carbon signals were shown at δ 17.9, 16.17, 16.0 and 6.7, of which the last

one is characteristic for C-14 in the pyrromycinone skeleton. The other methyl carbons were due to the sugar methyls.

In accordance, compound **248** had a pyrromycinone parent chromophore with three sugar moieties attached at 7-position, which could be rhodosamine and 2-deoxyfucose as in musettamycin (**246**). The third one contained a carbonyl group as in cinerulose (**C**).



C

The ESI mass spectrum of compound **248** fixed its molecular weight as 825 Dalton. The ESI MS^2 fragmentation patterns lead to two peaks at *m/z* 586 (M - [cinerulose B + 2-deoxfucoe] + H) and 393 ([M - (cinerulose B+2-deoxyfucose + rhodosamin + 2 H_2O) + H]) as shown below (Figure 118).

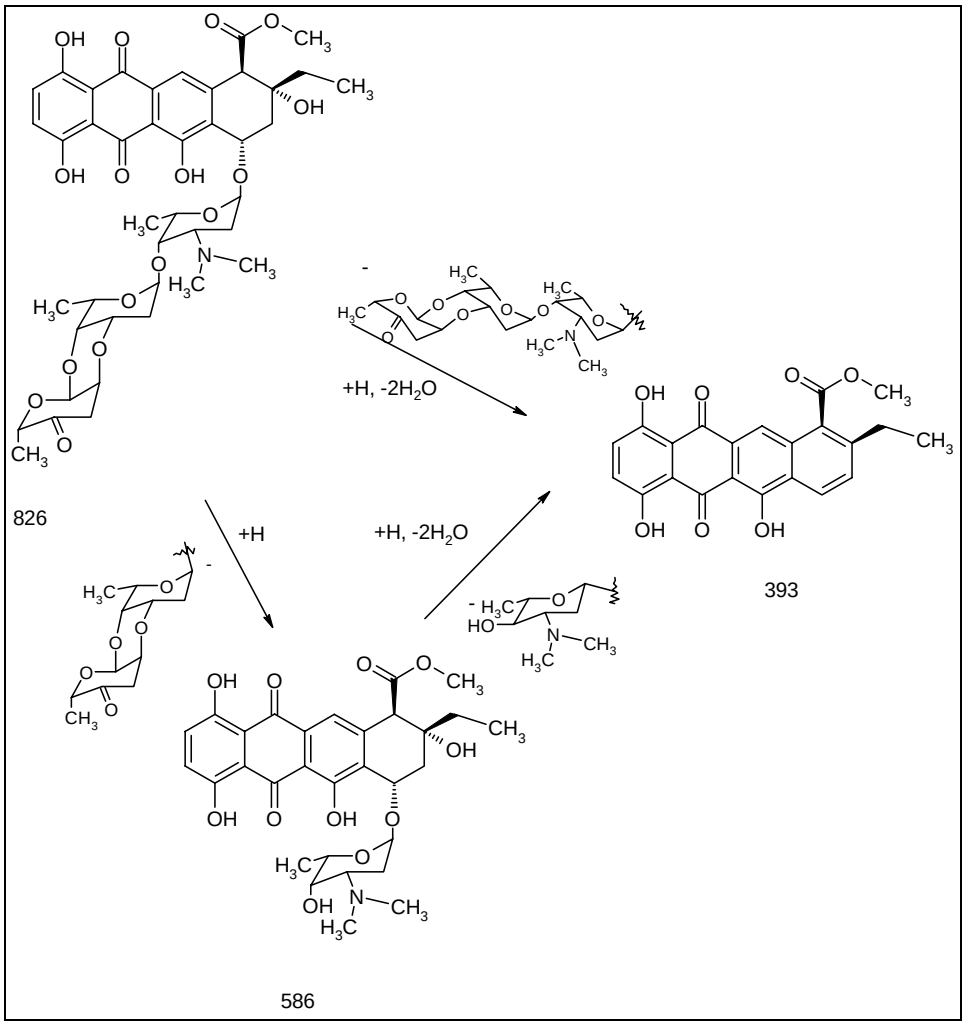


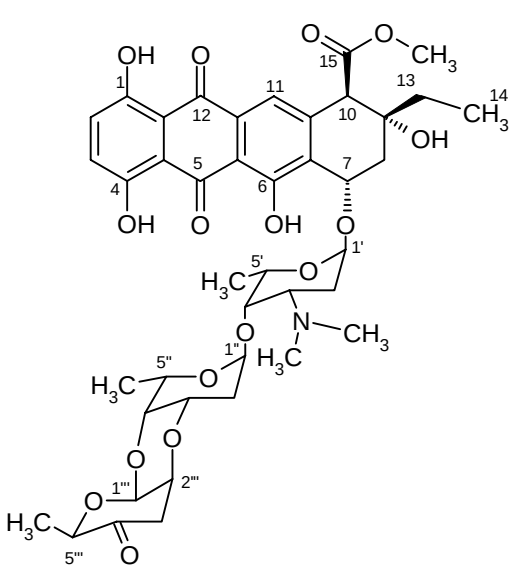
Figure 118: ESI MS fragmentation pattern of cinerubin B (**248**)

A search in AntiBase using the above spectral data resulted in three structures: Cinerubin B (**248**), spartanamicin A (**249**) and pyrraculomycin (**250**). However, the last one (**250**) showed a conjugated carbonyl group with an olefinic double bond which shifts the carbonyl group normally up field (< 203). In addition, the spectra exhibited no olefinic double bond, and hence compound **250** was excluded.

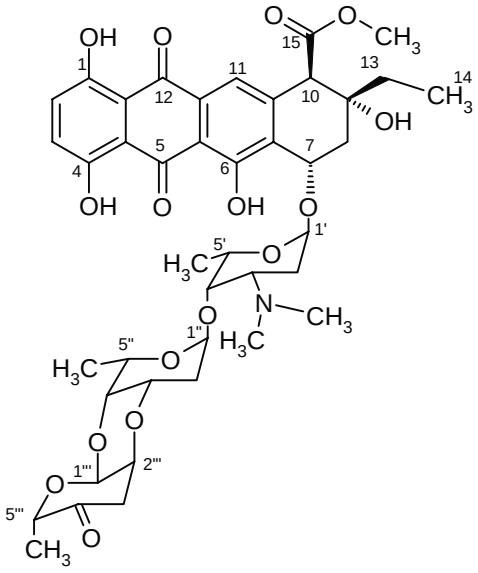
Spartanamicin A[274] (**249**) and cinerubin B (**248**) are configurational isomers isolated from *Micronospora* sp. They differ only in the stereochemistry of the methyl group at 5'''-position, where it is equatorial (H-5'''axial at δ 4.37) in cinerubin B and axial in spartanamicin A[274] (**249**) (H-5''' equatorial at δ 4.62). By comparing literature data[48] of both compounds **248** and **249** with the present values, the compound was elucidated as cinerubin B (**248**). The difference in stereochemistry of both compounds could be the reason for their different biological activities. Cinerubin B (**248**) is characterised by its pronounced activity against Gram-positive bacteria, viruses

and tumours[24], while spartanamicin A (**249**) exhibits a cytotoxic and antifungal activity[24].

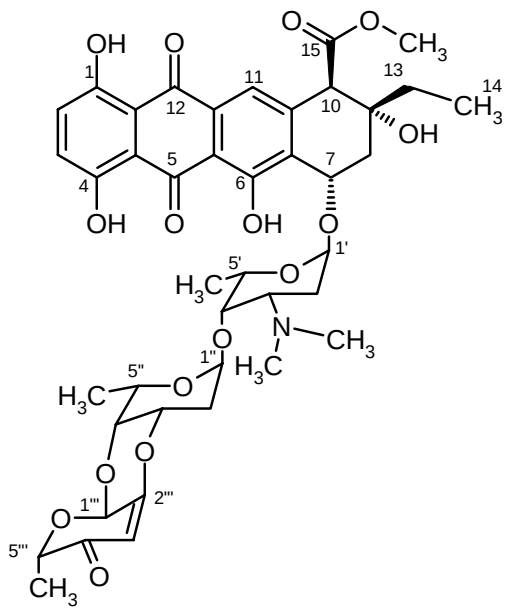
Cinerubins B (**248**) and A were isolated and identified previously from different *Streptomyces* sp. and described by Ettlinger *et al*. as red/orange antibiotics[275]. The aglycone of the cinerubins was identified previously by Brockmann *et al.* as η-pyrromycinone. Cinerubin B was isolated frequently from bacteria e.g. *Streptomyces antibioticus*, *Streptomyces griseorubiginosus*, *Streptomyces galilaeus* and *Streptomyces niveorubes*.



248



249



250

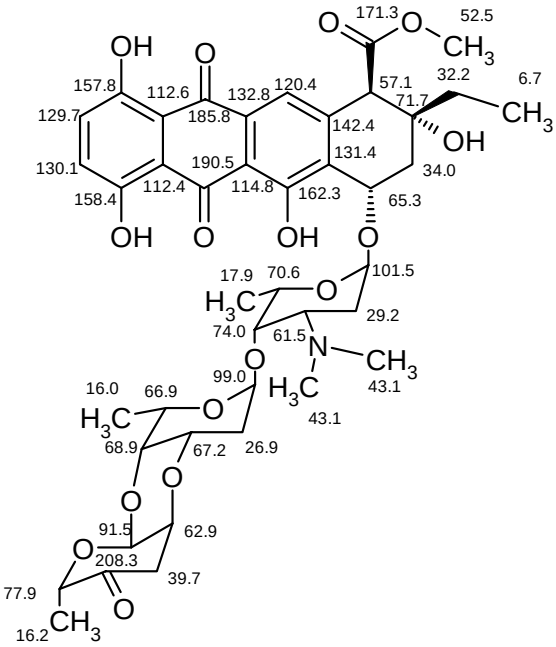


Figure 119: ^{13}C NMR values of cinerubin B (**248**)

4.20.7 Cinerubin M

Further screening of fractions III-IV gave compound **254** as a red solid minor component. It exhibited a characteristic colour change from red to violet by sodium hydroxide, as a sign of a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of **254** exhibited a high similarity in its pattern to cinerubin B (**248**). It displayed the same three chelated hydroxy groups (δ 12.94, 12.81, and 12.22). Moreover, an 1,2,3,4-tetrasubstituted aromatic ring was exhibited because of the *ortho*-coupled protons at δ 7.33, 7.30 in addition to the *peri*-proton at C-11 (δ 7.70). In addition, the methyl ester group present at position-10 was found at δ 3.69 as singlet. The H-10 proton was displayed also at δ 4.13, in addition to the terminal methyl triplet (δ 1.08), confirming the ethyl group present at C-9 of ζ-pyrromycinone. Also the methylene group at 8-position was exhibited as two multiplets (δ 2.48, 2.38), while the methylene group at 7-position in ζ-pyrromycinone (**243**) was exchanged by CH-O, which gave a signal at δ 5.25, as in cinerubin B (**248**). In the sugar and aliphatic regions, three hydroxy methine protons, were exhibited at δ 5.45, 5.17 and 5.08, referring to the presence of three anomeric sugar protons attached at 7-position of the pyrromycinone moiety (as in cinerubin B). In addition, the three methyl groups (as doublets) present in the sugar-moieties were exhibited (δ1.37, 6'''-H_3; 1.30, 6' -H_3 and 1.25, 6''-H_3). This pointed to a structural similarity of both chromophores and sugar moieties with cinerubin B (**248**). However, the N,N-dimethyl group of rhodosamine (at 3'-position) was absent and replaced by a methyl singlet, displayed at δ 2.36 in compound **254**, with down field shifted, in comparison with those of N,N-dimethyl groups **(**δ 2.19) present in cinerubin B (**248**).

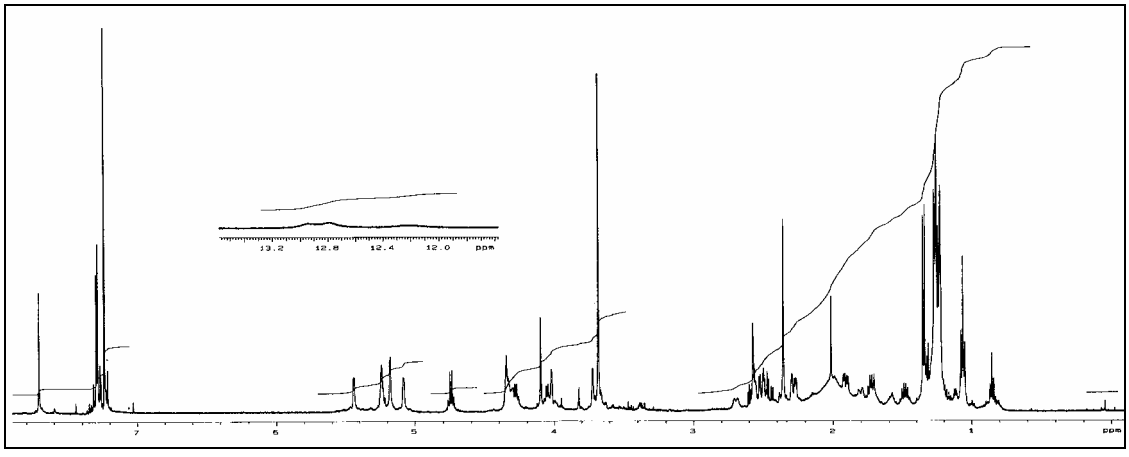
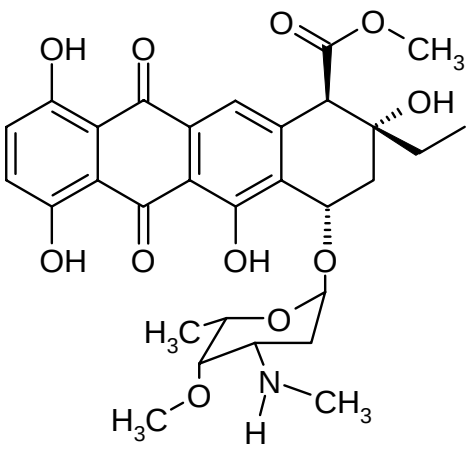
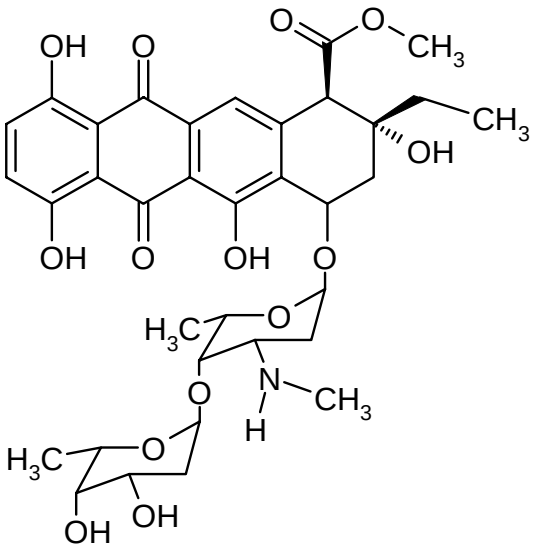


Figure 120: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of cinerubin M (**254**)

This singlet character of the HN-Me group at 3'-position was established during the comparison with an authentic spectrum of (7S, 9R, 10R)-pyrromycin X[276] (**251**), at which the methyl singlet was detected up field at δ 2.12. Additionally, the methyl singlet present at 3'-position in Schaunardimycin[277] (**252**) was established at δ 2.35 corresponding to the measured value in compound **254**.



251



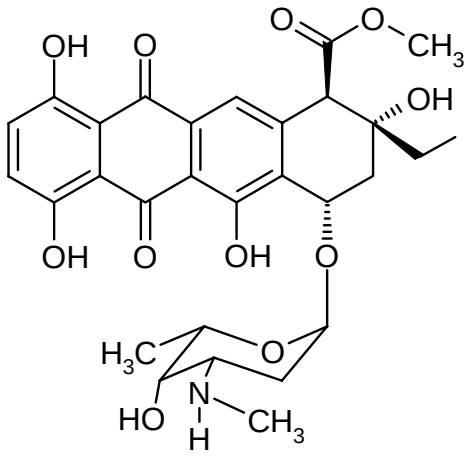
252

The ^{13}C NMR spectrum of compound **254** showed the same carbon values in both the chromophor (ζ-pyrromycinone) and the three sugar moieties, as in cinerubin B (Table 11). The sole difference was found at C-3' and its $NHCH_3$ substituent. Both NH-$\underline{C}H_3$ and C-3' were shifted up-field to δ 40.7 and 54.6, respectively, while in the case of cinerubin B (**248**), they were found at δ 43.2 and 61.5, respectively.

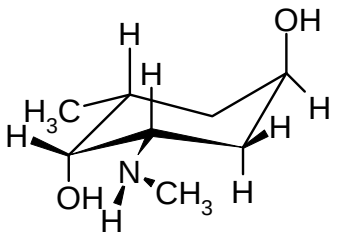
Table 11: Comparison of ^{13}C NMR spectral data of cinerubin M (**254**) and cinerubin B(**248**).

Position	Cin. B **(248)**	Cin. M**(254)**	Position	Cin. B **(248)**	Cin. M **(254)**
	δ_C	δ_C		δ_C	δ_C
1	157.7	157.9	15-O<u>C</u>H$_3$	52.5	52.6
2	129.7	129.7	1'	101.5	100.5
3	130.0	130.1	2'	29.2	29.7
4	158.3	158.4	3'	**61.5**	**54.7**
4a	112.2	112.4	3'-N(<u>C</u>H$_3$)$_2$	**43.2**	**40.8**
5	190.5	190.5*	4'	74.0	74.0*
5a	114.7	114.9	5'	70.6	70.6*
6	162.2	162.2	6'	17.8	18.1
6a	131.4	131.4*	1"	99.0	99.0*
7	65.2	66.5	2"	26.9	27.0
8	33.7	32.6	3"	67.2	67.9
9	71.6	71.6	4"	68.2	68.2
10	57.1	57.2	5"	66.8	66.7
10a	142.3	142.3	6"	16.0	16.2
11	120.3	120.4	1"'	91.5	91.4
11a	132.7	132.9	2"'	62.9	63.0
12	185.6	185.6*	3"'	39.7	39.6
12a	112.4	112.4	4"'	208.3	208.2*
13	32.1	32.1	5"'	77.9	78.0
14	6.7	6.7	6"'	16.1	16.2
15	171.3	171.3			

The ESI mass spectrum of compound **254** established its molecular weight as 811 Dalton, which is 14 units lower than in cinerubin B (**248**) due to a missing methylene group. The ESI MS^2 fragmentation of compound **254** showed a fragment at *m/z* 571 which due to those of **253**, due most likely to the expulsion of cinerulose and 2-deoxyfucose simultaneously from the main structure of **254** ([M - cinerulose + 2-deoxyfucose) + H]$^+$). The compound **253** exhibited a structural similarity to pyrromycin (**245**) (*m/z* 585), with a lower methylene group. The corresponding fragment of **253** (*m/z* 571) showed a further expulsion of N-methyl daunosamine (**D**) and two water molecules to give the aromatised η-pyrromycinone (**244**) (*m/z* 393).

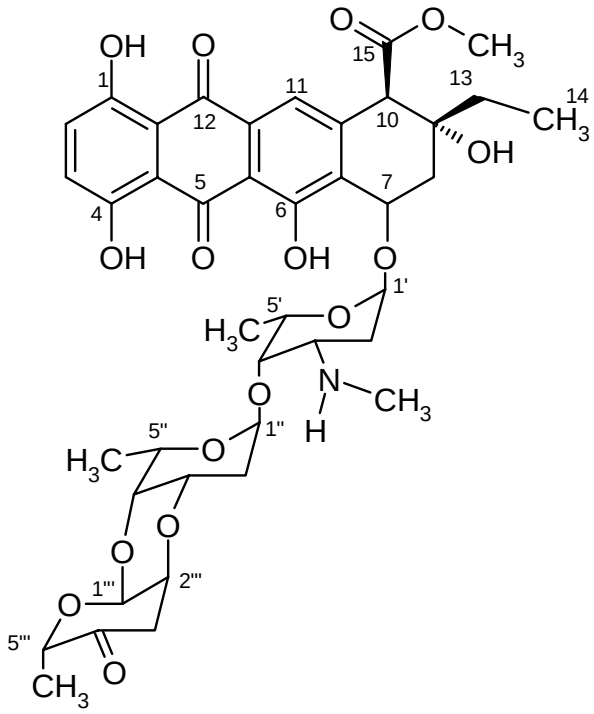


253



D

From the NMR and mass spectra and the observed fragmentation pattern, the structure could be assigned as 3'-N-desmethyl cinerubin B (**254**). The compound was not found in AntiBase, DNP and Chemical Abstract, and so, **254** is a new natural product, which was named cinerubin M (**254**).



254

4.20.8 Islamomycin A

Along with a thin band of cinerubin M (**254**) obtained from fraction IV, compound **257** was isolated as rather polar red compound. It turned to violet by treatment with sodium hydroxide, pointing to a further *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of **257** exhibited the same hydroxyquinone chromophore, ζ-pyrromycinone (**243**): It delivered three *peri*-hydroxy groups at δ 12.90 (2 OH) and 12.22 (OH), one methyl ester (δ 3.68), and three aromatic protons (δ 7.66, 7.25. and 7.23), two of them as AB signals (δ 7.25 and 7.23). In addition, a methyl triplet (δ 1.10) was detected, confirming the ethyl group at 9-position. The spectrum exhibited five hydroxy methines in the range of δ 5.63-5.10, at least three of which were anomeric protons. Furthermore, three methyl doublets (5'/5"/5"') were detected between δ 1.38-1.25, establishing the presence of three additional sugar units in compound **257**. However, the two magnetically equivalent methyl groups, $N(CH_3)_2$ in rhodosamine were not detected (they are normally at δ 2.20). In addition, 10 protons of 5 methylene groups were detected between δ 2.58-1.56.

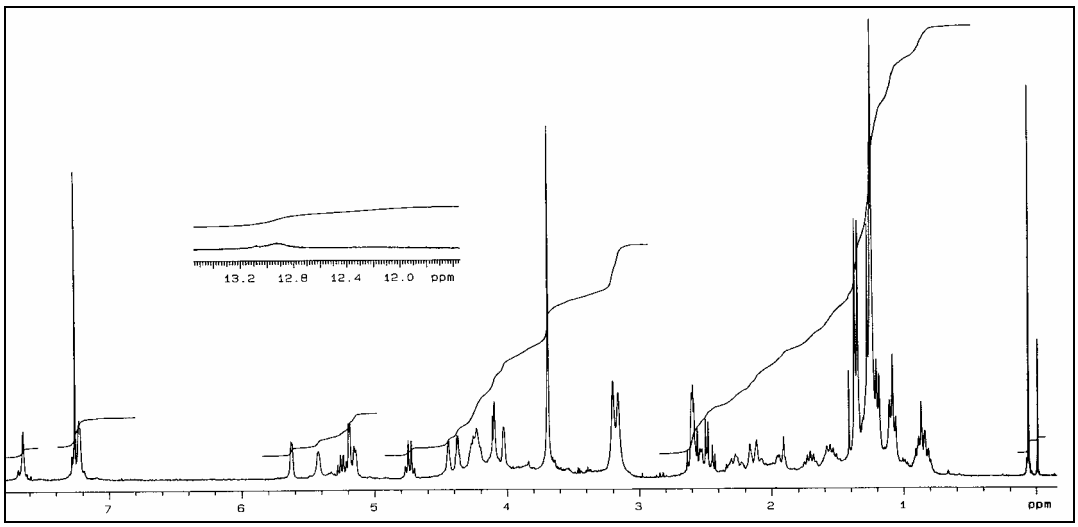


Figure 121: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of islamomycin A (**257**)

The ^{13}C/APT NMR spectra of compound **257** displayed 41~42 carbon signals which were classified into several categories i.e. one ketonic carbonyl at δ 207.8, characteristic to cinerulose B or A, four high-field carbonyls (δ 190.5, 185.6, 171.2 and 169.1), out of which two were quinone carbonyls (δ 190.5, 185.6), while the last two could be assigned as esters and/or amides. In addition of nine quaternary sp^2 carbons, three were assigned as oxygenated. Moreover, three sp^2 methine carbons, and twelve oxygenated sp^3 methine carbons (δ 100.0~63.1) were found, out of which three were assigned as anomeric carbons (δ 100.0, 99.1 and 91.3). Furthermore, one oxygenated sp^3 quaternary carbon (δ 70.8), one methoxy carbon (δ 52.5), five sp^3 methylene carbons (40.7, 39.6, 32.4, 31.9 and 26.5), and finally four methyl signals (δ 18.3, 16.2, 16.0 and 6.7) were detected.

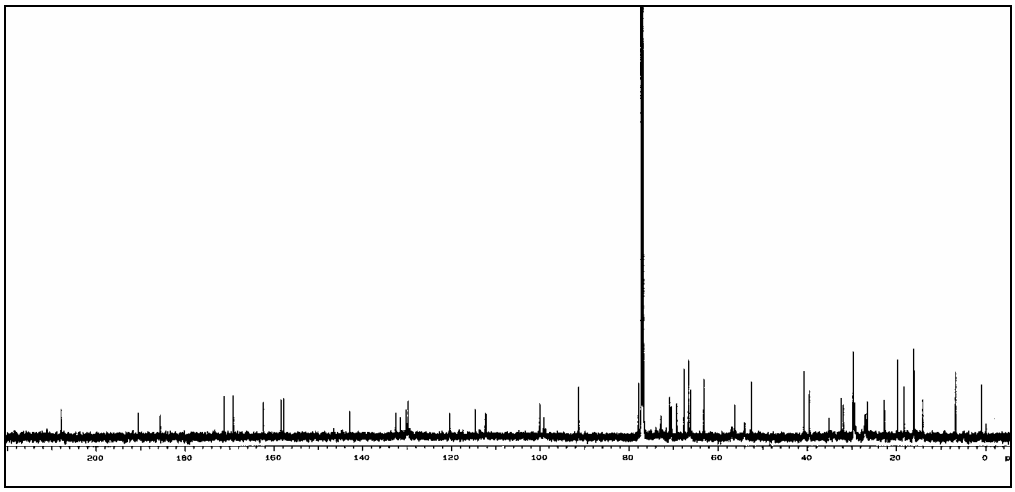


Figure 122: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of islamomycin A (**257**)

The anomeric proton at δ 91.3 is characteristic for cinerulose B, and hence cinerulose A (~99) possibility can be excluded[278]. The other two anomeric carbons (100.0 and 99.1) referred possibly to daunosamine, and 2-deoxyfucose sugar units, respectively.

The ESI mass spectra determined the molecular weight of **257** as 841 Dalton. Two *quasi*-molecular peaks were observed in (+)-ESI mode at *m/z* 1683 ($[2\ M + H]^+$, and 841 ($[M + H]^+$, while the (-)-ESI mode exhibited two *quasi* ion peaks at *m/z* 1704 ($[2\ M + Na - 3\ H]^-$) and 840 ($[M - H]^-$). The odd mass number of compound **257** pointed to the existence of at least one nitrogen. The (+)-ESI MS^2 of **257** exhibited a base fragment at *m/z* 602, which attributed mostly to a simultaneous loss of cinerulose B + 2-deoxyfucose from the parent compound. This established their direct attachment *via* an additional cyclic ring in between as in cinerubin M (**254**) and cinerubin B (**248**). A further fragment was observed at *m/z* 393 of η-pyrromycinone +H (**244**), as a result of the complete loss of the sugar units, followed by a loss of two water molecules during aromatisation (Figure 123).

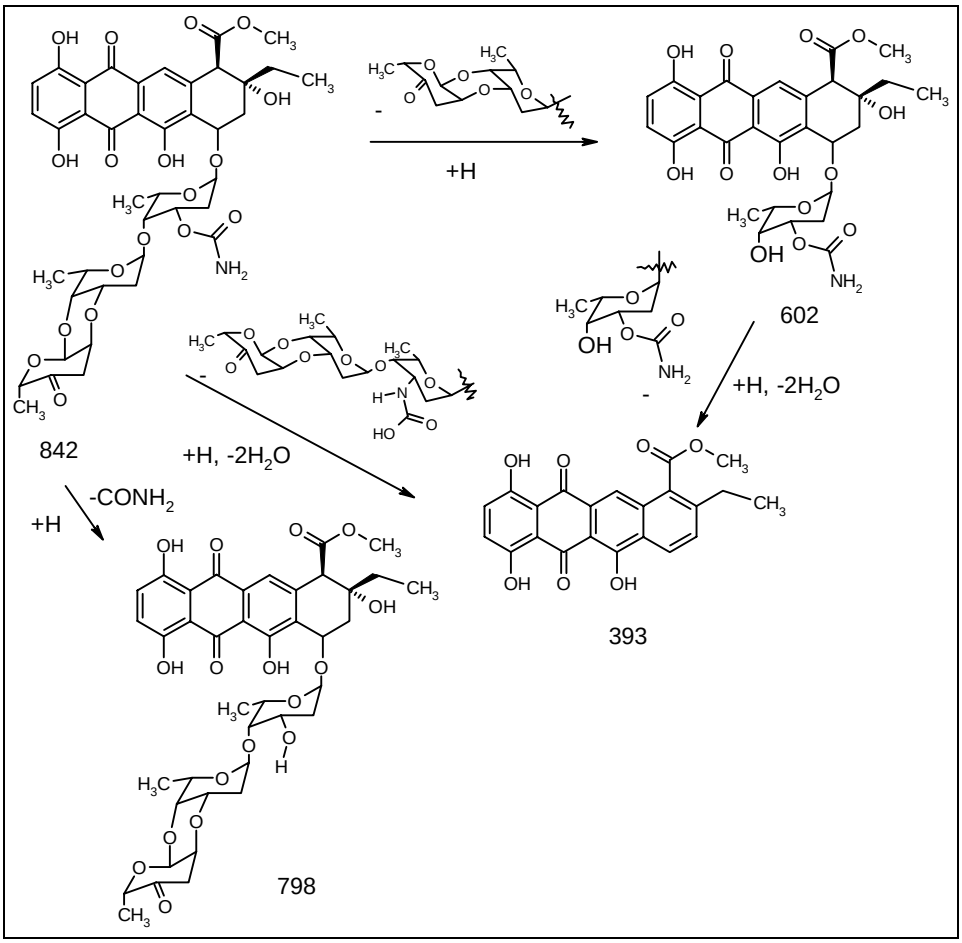
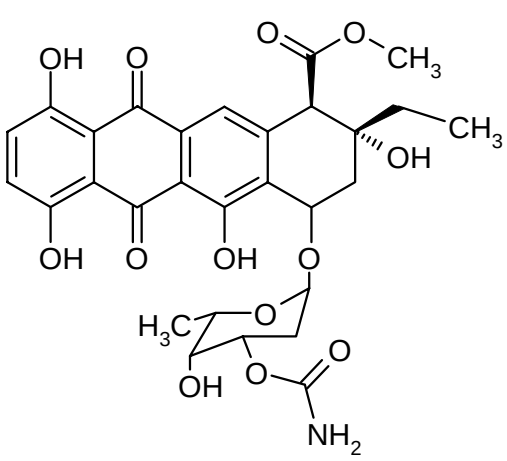
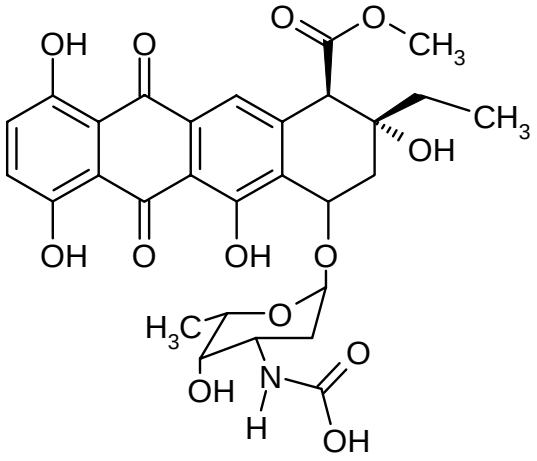


Figure 123: Suggested (+)-ESI MS^2 of islamomycin A (**257**)

Furthermore, a fragment was observed at *m/z* 798 due to the loss of *m/z* 44, which is indicative for either CO_2 or $CONH_2$. Methylation of compound was carried out **257** with diazomethane, however, the ESI mass spectrum of the resulting product exhibited no change, as an indication that no free carboxylic acid group was present. This established furthermore the presence of a free amide group linked to the sugar unit *via* oxygen (–O-CO-NH_2) (**255**), but not *via* nitrogen (–NHCOOH) (**256**). ESI HRMS of compound **257** (*m/z* 842.31241 (M + H) determined its molecular formula as $C_{41}H_{47}NO_{18}$.

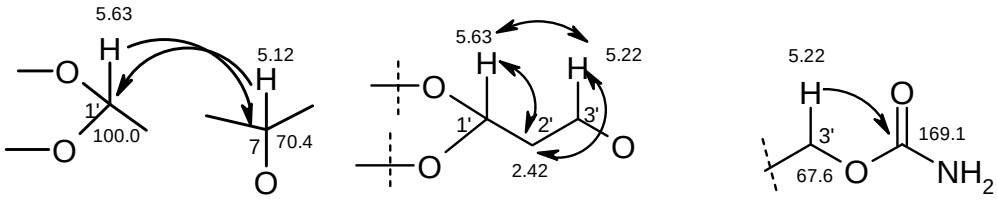


255

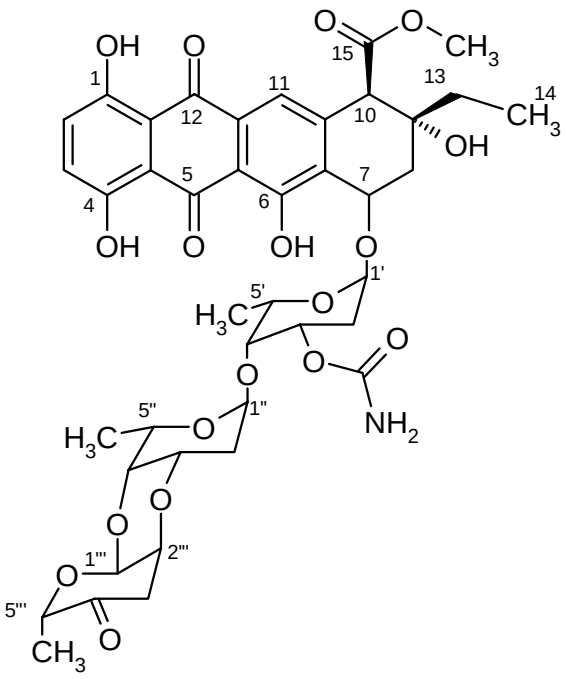


256

The HMBC correlations of compound **257** exhibited a long range coupling between the methine proton at δ 5.22 (3-H) and the carbonyl group at δ 169.1, proving the linkage between C-3' and $CONH_2$ *via* oxygen. In addition, the methylene protons (2.42) of C-2' (40.7), and their adjacent methine proton at δ 5.22 (H-3) were confirmed by H,H COSY correlation. The methylene group (C-2') exhibited an additional coupling with the anomeric proton at δ 5.63 (1'-H), confirming their direct linkage. The connection of the sugar rhodosamine to the pyrromycinone chromophore *via* C-7 was established because of the long rang coupling between the 7-H (δ 5.12) and the anomeric carbon C-1' (100.0).



Based on the connectivity observed in the HMBC and H,H COSY spectra, the other sugar fragments and the pyrromycinone moiety were established with connection at 7-position. This allowed the elucidation of compound **257** as an additional new hydroxy-glycosidic quinone, which was designated as islamomycin A (**257**)



257

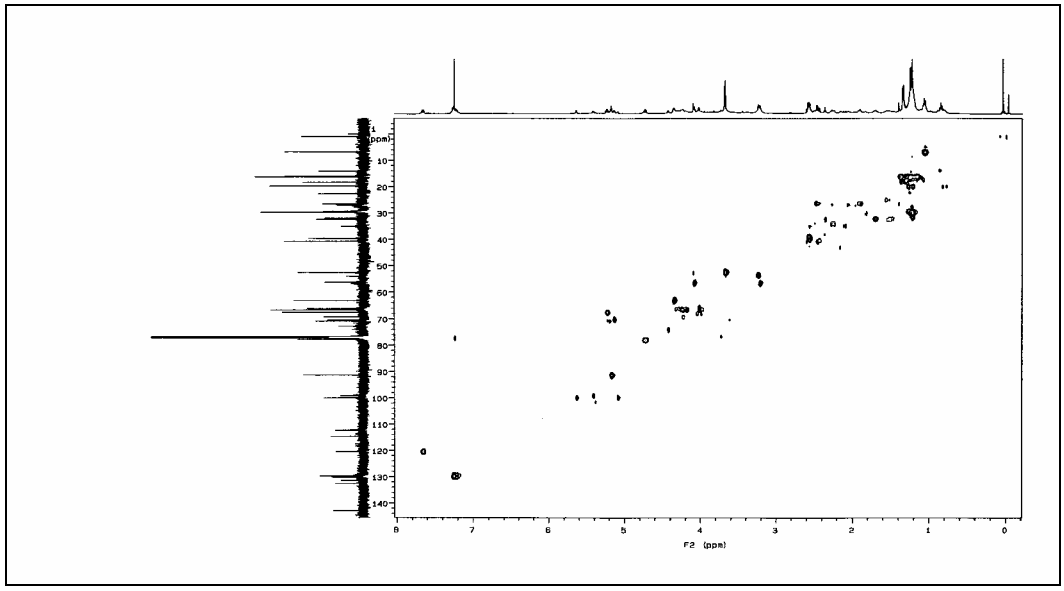


Figure 124: HMQC spectra ($CDCl_3$, F1 125 MHz, F2 499 MHz) of islamomycin A (**257**)

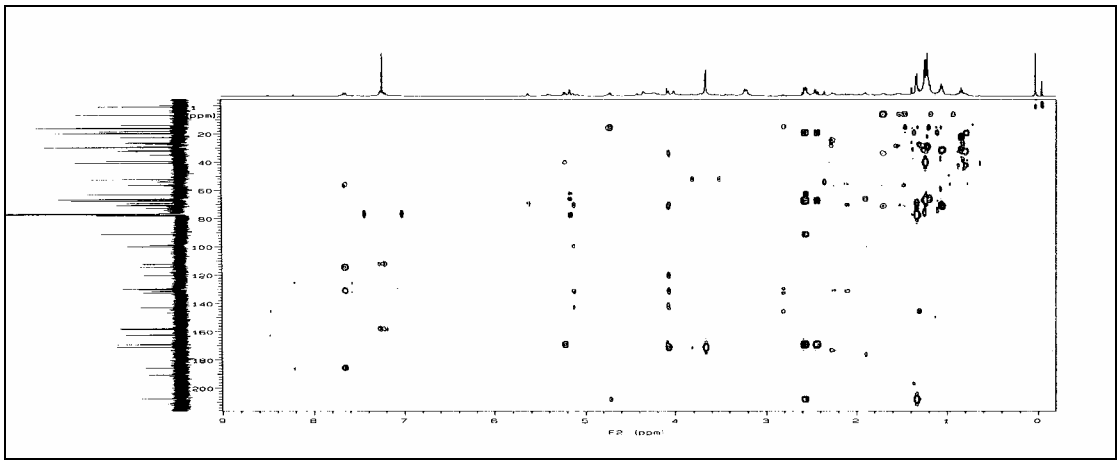


Figure 125: HMBC spectra ($CDCl_3$, F1 125 MHz, F2 499 MHz) of islamomycin A (**257**)

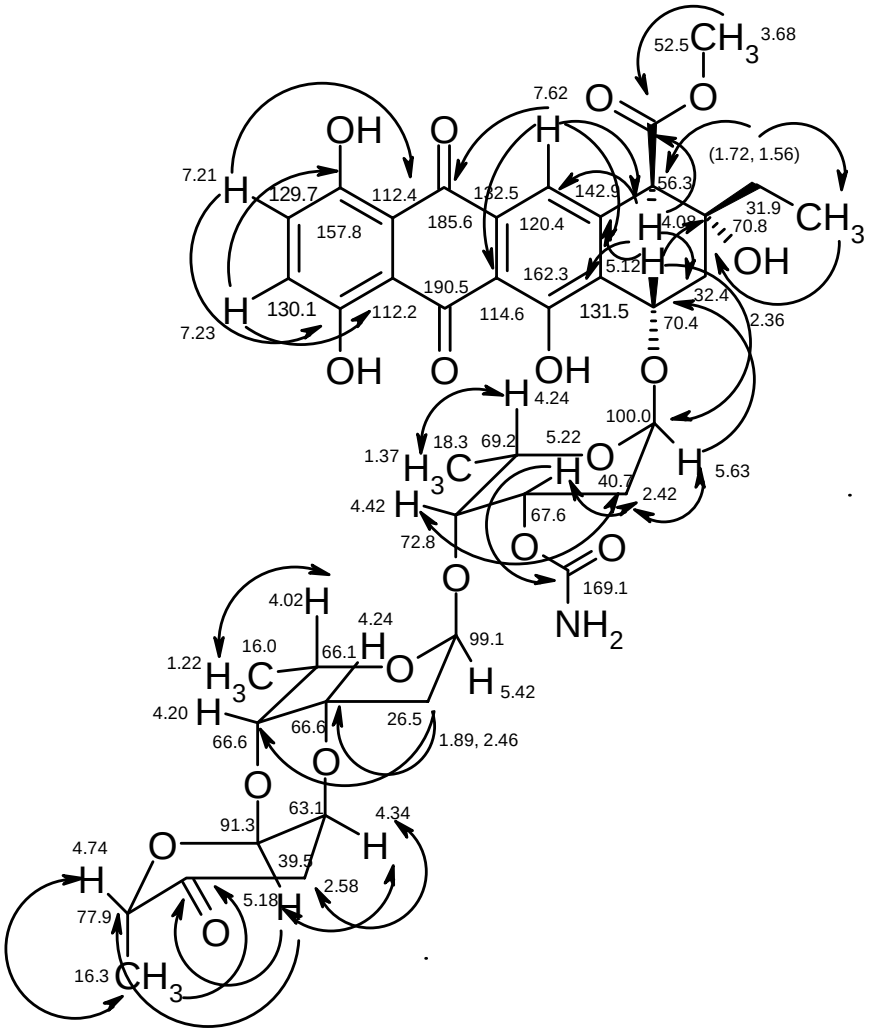
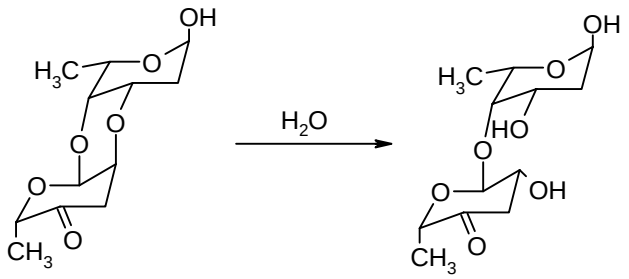


Figure 126: HMBC and H,H COSY correlations of islamomycin A (**257**)

4.20.9 Islamomycin B

In addition to islamomycin A (**257)**, compound **258** was found as a red polar minor product in fraction IV. It showed a colour change to violet by treatment with sodium hydroxide, pointing to another *peri*-hydroxy-quinone.

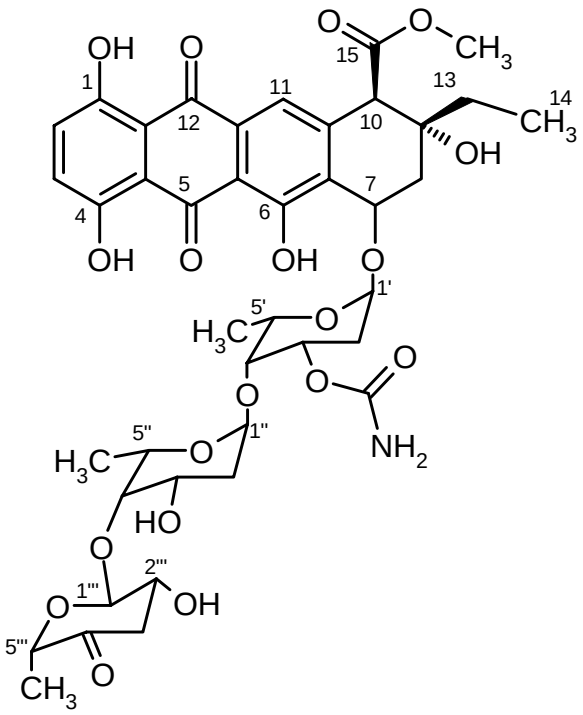
The ^{1}H NMR spectrum of **258** exhibited its close similarity to islamomycin A (**257**), containing the same pyrromycinone chromophore and ending with the same sugar units. The molecular weight of compound **258** was established as 859 Dalton using the ESI mass spectrum. The (+)-ESI mode exhibited two *quasi* ion peaks at *m/z* 1719 $[M + H]^+$ and 860 $[M + H]^+$. Similarly, the (-)-ESI mode displayed two other *quasi* ion peaks at *m/z* 1739 ($[2 M + Na – 3 H]^-$) and 858 ([M - H]). The derived molecular weight of **258** is 18 Dalton higher than in compound **257**, which could be attributed to a water molecule. The only part of the compound **257** that can add water is the cyclization between the last two sugars, cinerulose B + 2-deoxyfucose. This pointed to an additional structure analogue **258** of Islamomycin A.



m/z 258 m/z 276

The (+)-ESI MS^2 spectra showed a low intensity fragment at *m/z* 733 which could be attributed to the loss of the sugar moiety **C**, followed by the base peak at *m/z* 602 as in islamomycin A (**257)**. This established the same skeleton in both compounds **257** and **258**. ESI HRMS of compound **258** led to the corresponding molecular formula $C_{41}H_{49}NO_{19}$

Based on these data, compound **258** was established as an additional new analogue to islamomycin A (**257**) and designed as islamomycin B (**258**). Due to the insufficient amount of **258** and its low abundance, its other data could not be measured. The producing strain was subjected again to fermentation, working up and isolation. However, the obtained amount of compound **258** was still very little.



258

4.21 Terrestrial *Streptomyces* sp. GW10/1828

The terrestrial *Streptomyces* sp. isolate GW10/1828 attracted our interest because of its very well-grown black agar plates. Moreover, the crude extract exhibited a pronounced antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli* and *Streptomyces viridochromogenes* (Tü57), was antifungal against *Mucor miehei* (Tü284), and showed a moderate activity against the micro alga *Chlorella vulgaris*. The chemical screening of the crude extract using TLC showed interesting yellowish to green-orange zones, which turned to red by treatment with sodium hydroxide. Further absorbing bands were present which turned to violet-brown with anisaldehyde/sulphuric acid after heating.

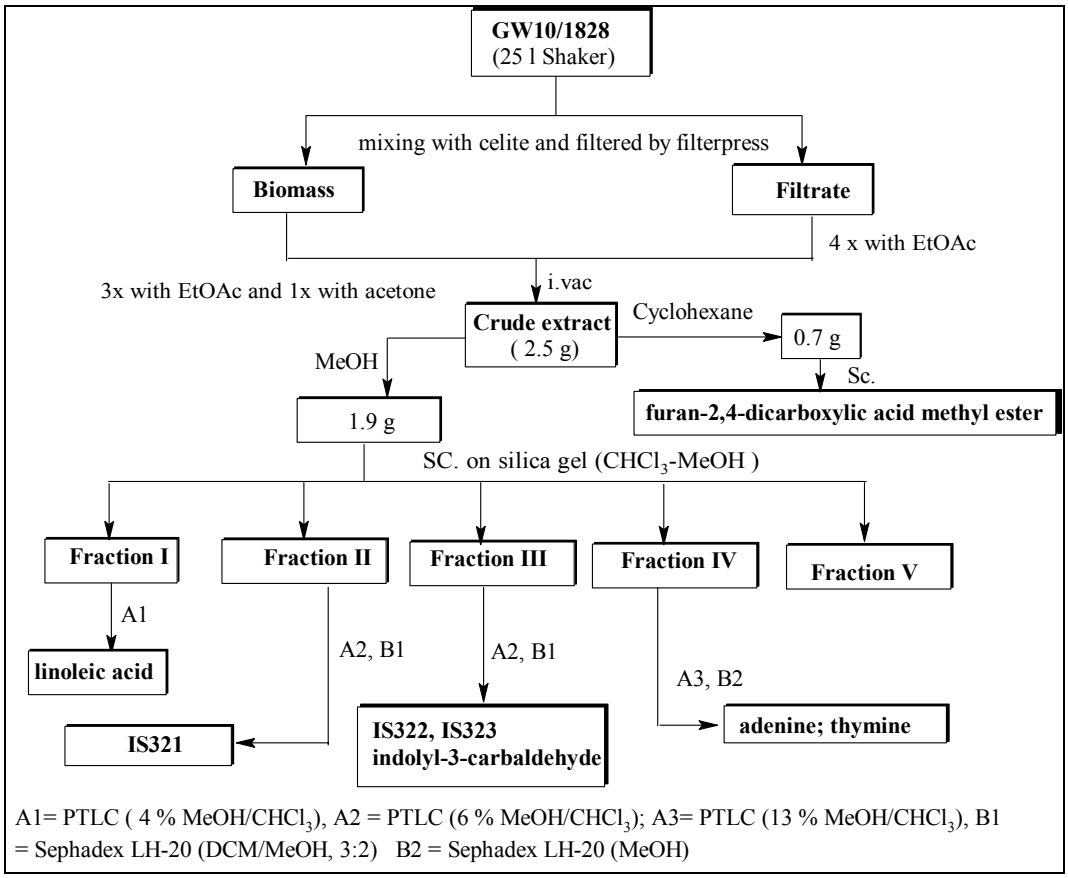


Figure 127: Work-up procedure of the terrestrial *Streptomyces* isolate GW10/1828 (shaker)

The strain was pre-cultured on agar plates with M_2 medium. 100 of 1 liter-Erlenmeyer flasks each containing 250 ml of M_2 medium were inoculated with the well-grown agar subcultures and incubated at 28 °C for 7 days on a shaker (110 rpm). The black culture broth was filtered and extracted with ethyl acetate. The resulting greenish-black crude extract was defatted. Both cyclohexane and methanol fractions were worked up separately. Purifications of the cyclohexane fraction led to isolation of the new furan-2,4-dicarboxylic acid dimethyl ester (**261**), while silica gel column chromatography of the methanol part delivered five fractions. Purifications of the fractions I-V led to the isolation of known metabolites. However, further purification of fraction II led to isolation of the new *peri*-hydroxyquinone, 2,9-dichloro-1,6,10-trihydroxy-7-methyl-naphthacene-5,12-dion (**269**).

4.21.1 Furan-2,4-dicarboxylic acid dimethyl ester

Purification of the cyclohexane fraction led to compound **261** as colourless solid, UV absorbing substance, which turned to brown by anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of compound **261** exhibited two aromatic

deep field singlets each of 1Hat δ 8.09 and 7.41, in addition, two singlets of two methoxy groups at δ 3.97 and 3.85 were detected. The deep field shift of the methoxy groups established them to be ether or ester groups linked to an aromatic system.

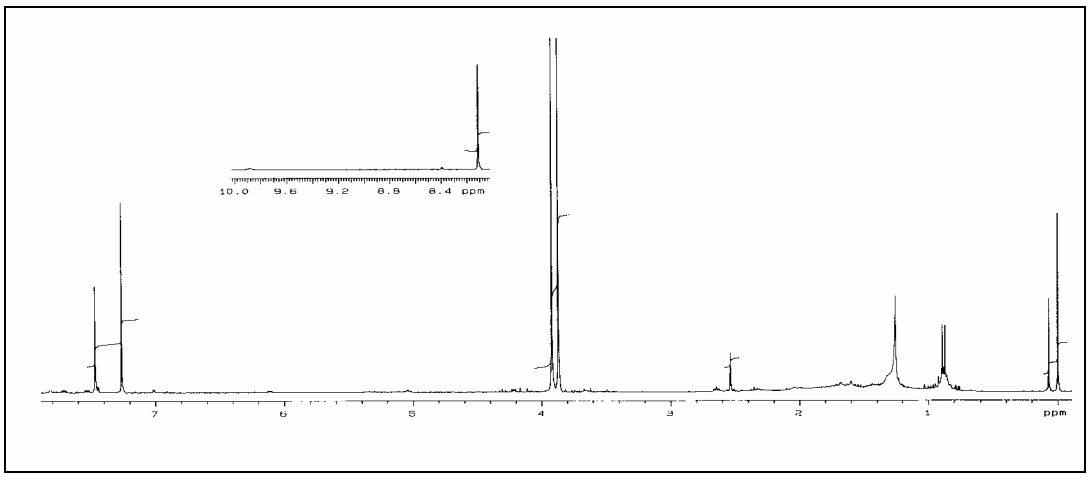


Figure 128: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of furan-2,4-dicarboxylic acid dimethyl ester (**261**)

The ^{13}C/APT NMR spectra displayed two quaternary carbon signals at δ 162.4, and 158.5, which could be two carbonyl groups of an acid, amide or ester. Two additional sp^2 quaternary carbons were observed at δ 145.5 and 123.0, the first of which could be assigned as an oxygenated carbon. Furthermore, two sp^2 methine carbons were detected at δ 150.1 and 117.4, of which the first could be assigned as β-oxymethine carbon present in conjugation with an electron withdrawing group (e.g. CO). At the end, two methoxy signals were exhibited at δ 52.5 and 52.0.

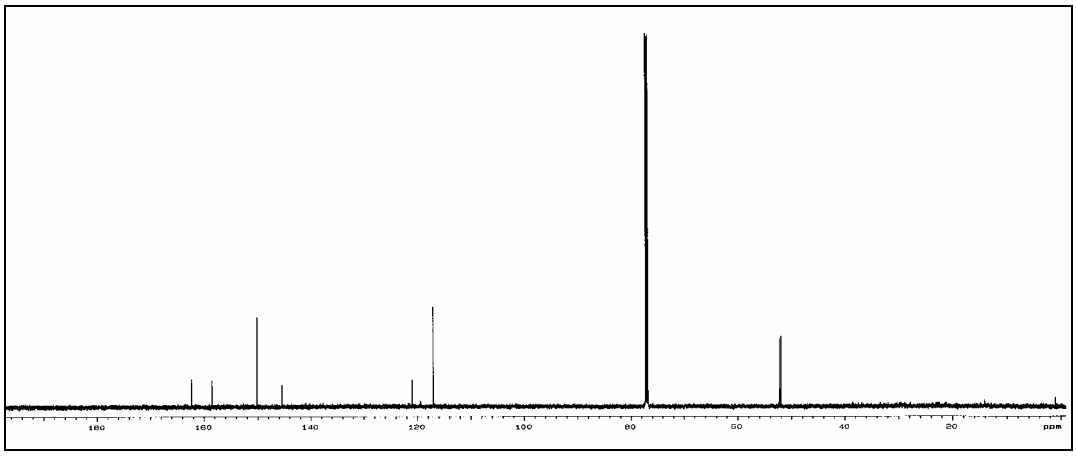
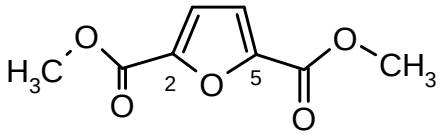


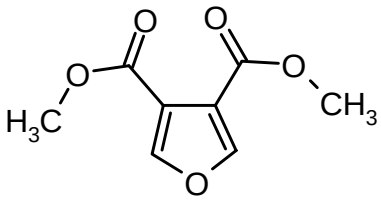
Figure 129: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of furan-2,4-dicarboxylic acid dimethyl ester (**261**).

The molecular weight of **261** was determined as 184 Dalton by EI mass spectrum. The molecule ion delivered two further fragments at *m/z* 153 and 121 because of the expulsion of one and two methoxy groups from the main structure **261**, respectively. Based on a search in AntiBase, no results were obtained. This pointed to **261** as new natural product.

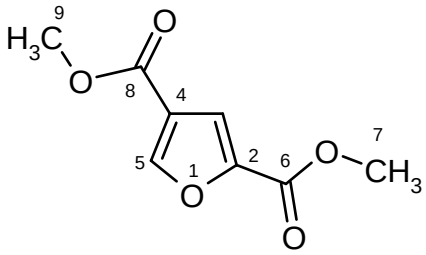
The corresponding molecular formula of **261**, $C_8H_8O_5$, was established using the Rule of 13[146]. In accordance, 5 double bond equivalents were delivered, which classified into: two carbonyls, two olefinic double bonds, and a ring containing oxygen, e.g. furan. Hence, three possible structures were suggested: furan-2,5-dicarboxylic acid dimethyl ester (**259**), furan-3,4-dicarboxylic acid dimethyl ester (**260**), and furan-2,4-dicarboxylic acid dimethyl ester (**261**), all three structures are unknown from nature.



259



260



261

The compounds **259** and **260** are having symmetrical skeletons, and accordingly, only one olefinic methine and one methoxy group should be displayed. Both compounds **259** and **260** are not matching with the spectra, and were excluded therefore. Hence, the only structure coinciding with the above data is furan-2,4-dicarboxylic acid dimethyl ester (**261**). In addition, compound **261** was compared with two other related compounds; furan-2,5-dicarboxylic acid (**262)** and furan-3-carboxylic acid (**263)**, at which the carbonyl values were found at δ 157 and 164, respectively [23].

The compound **261** was further established using 2 D correlations, where the two methyl groups at δ 3.97 and 3.85 showed a correlation with the corresponding ester carbonyls (158.5 and 162.4). They also showed long range coupling (^4J) with the quaternary carbons C-2 (145.5) and C-4 (121.0), respectively.

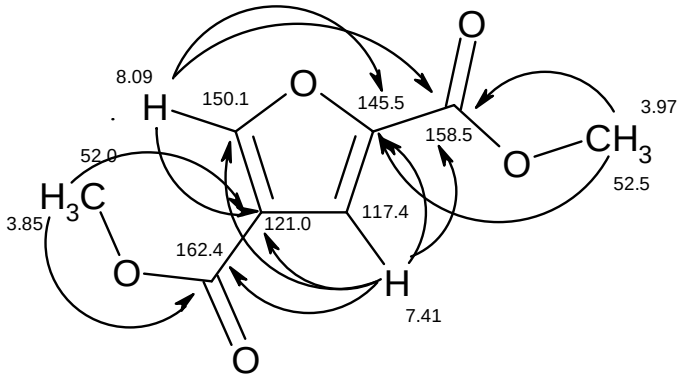
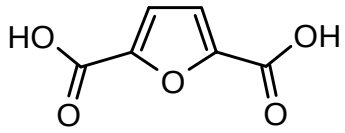
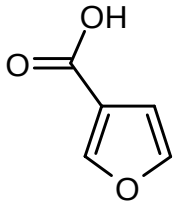


Figure 130: HMBC correlations of furan-2,4-dicarboxylic acid dimethyl ester (**261**)

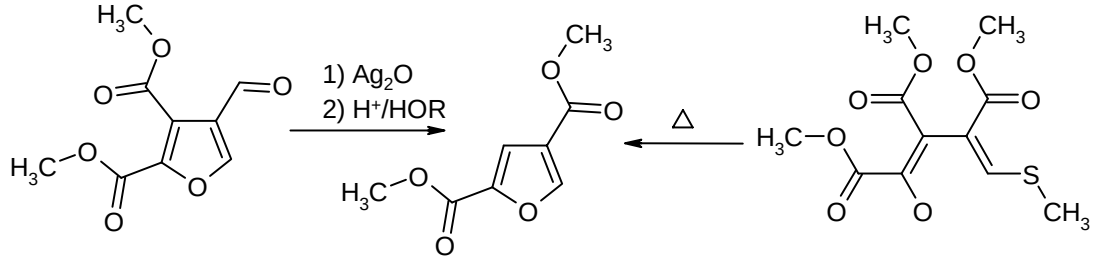


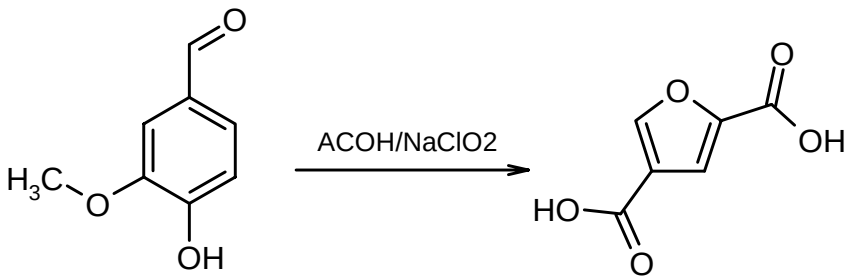
262



263

Compound **261** and the corresponding free carboxylic acid (**265**) are synthetically known[279]. The acid **265** was synthesized by Feist[280] during the treatment of methyl coumalate with potassium hydroxide. It was also obtained by Pearl *et al.*[281] by oxidation of vanillin (**264**).





264 **265**

4.21.2 2,9-Dichloro-1,6,10-trihydroxy-7-methyl-naphthacene-5,12-dion

Halogen compounds are relatively rare in nature and frequently characterised by their high bio-activities. Compound **269** was obtained as a minor secondary metabolite from the strain GW10/1828. It was isolated during screening of fraction II as yellowish-green solid, which turned to red by treatment with sodium hydroxide, as indication of a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of compound **269** exhibited two singlets of two *peri*-hydroxy groups at δ 12.25 and 11.97, and of a phenolic hydroxy group at δ 9.94. In the aromatic region, it exhibited two singlets at δ 8.35 and 6.98, and an AB system of two protons at δ 7.60 (^{3}J = 8.3 Hz), as of two *ortho*-coupled protons. Furthermore, the signal of a methyl group was detected at δ 2.52, which could be linked to an aromatic ring or a nitrogen atom.

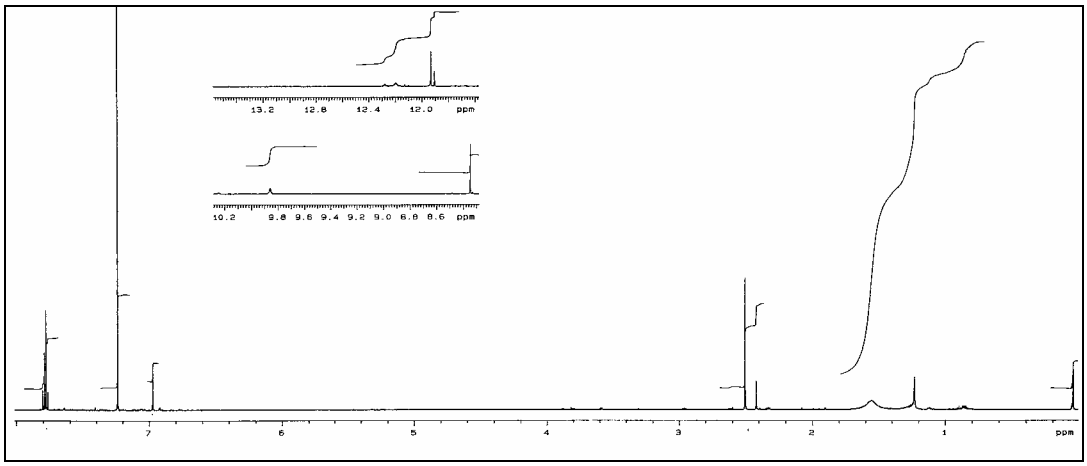


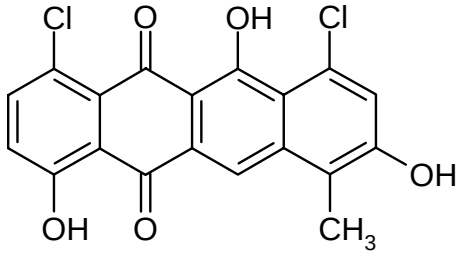
Figure 131:^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of 2,9-dichloro-1,6,10-trihydroxy-7-methyl-naphthacene-5,12-dion (**269**)

The compound **269** exhibited a molecular weight of *m/z* 389 Dalton in both ESI and EI mass spectra. The molecular ion consists of three peaks at *m/z* 288, 290 and 292 amu, characteristic for the existence of two chlorine atoms in compound **269** compound. An expulsion of one chlorine led to the presence of two peaks at *m/z* 354 (^{35}Cl) and 356 (^{37}Cl) in the EI mass spectrum[282]. An additional peak was observed at *m/z* 325 due to the expulsion of an aldehyde or a carbonyl group, followed by the loss of a proton. An additional expelled carbonyl group was detected to give a peak at *m/z* 297. This referred to the existence of two carbonyl groups. EI HRMS established the molecular formula of **269** as $C_{19}H_{10}Cl_2O_5$. Based on the above spectral

data, the compound **269** comprises 14 double bond equivalents, two of which are quinone carbonyls, and four rings are present with 8 double bonds. One of the rings contained two *ortho*-coupled protons, while each of the respite two rings afforded only one proton giving a singlet. One of these protons was obviously present in *peri*-position to a carbonyl (δ 8.35).

The ^{13}C NMR of compound **269** exhibited only the sp^2 methine and sp^3 methyl carbons because of its little amount. Four methine signals were exhibited at δ 137.7, 121.7, 121.6 and 119.4, in addition to a quaternary sp^2 oxygenated carbon at δ 141.0. In the aliphatic region, one methyl carbon was detected at δ 22.7.

Based on the revealed spectral data and the computerised values, six structure possibilities were recommended: the first two of which are 4,7-dichloro-1,6,9-trihydroxy-10-methyl-naphthacene-5,12-dione (**266**), and 4,10-dichloro-1,8,11-trihydroxy-6-methyl-naphthacene-5,12-dione (**267**). However, the *ortho*-coupled protons in compounds **266** and **267** should deliver two different δ values because of their neighbour to two different electronic groups, chloride and hydroxyl group, and hence these were excluded.



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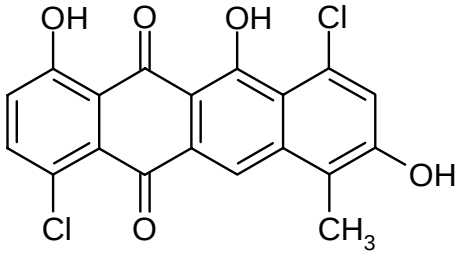
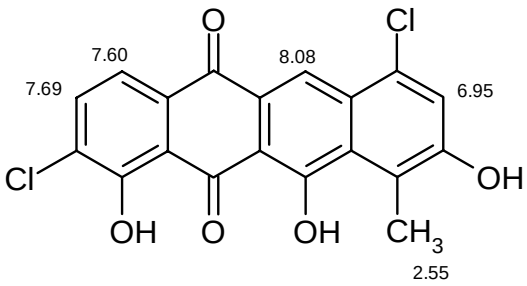


267

The other four suggested compounds, 2,7-dichloro-1,9,11-trihydroxy-10-methyl-naphthacene-5,12dione (**268**), 2,9-dichloro-1,6,10-trihydroxy-7-methyl-naphthacene-5,12dione (**269**), 2,9-dichloro-1,10,11-trihydroxy-7-methyl-naphthacene-5,12dione (**270**) and 2,9-dichloro-1,6,7-trihydroxy-10-methyl-naphthacene-5,12dione (**271**) showed good matching with the calculated δ values, especially in case of the *ortho*-coupled protons. Calculated and measured proton values in compound **269** were found to be identical. Compounds **268** and **270** showed a deviation in the δ_H values of the methyl group (in **270**), and the singlet aromatic protons singlets, and therefore are also excluded. Despite of the similar shift of the methyl group in compound **271**, the *peri*-proton (H-11, 8.02) exhibited an up field

shift. In accordance, the data are fitting best with structure **269**. M. Schiebel[82] in our group has described a related chloroquinone **272**.



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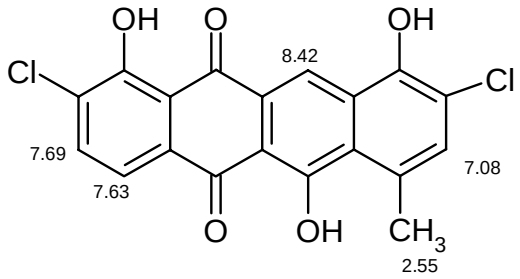

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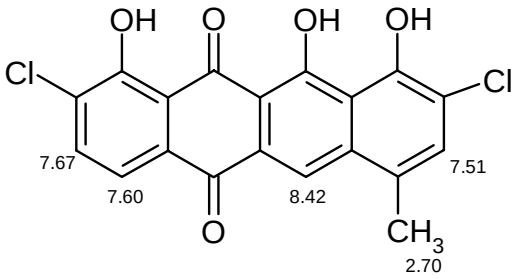

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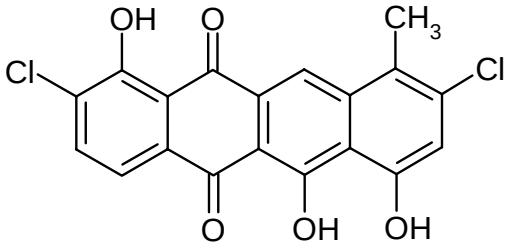

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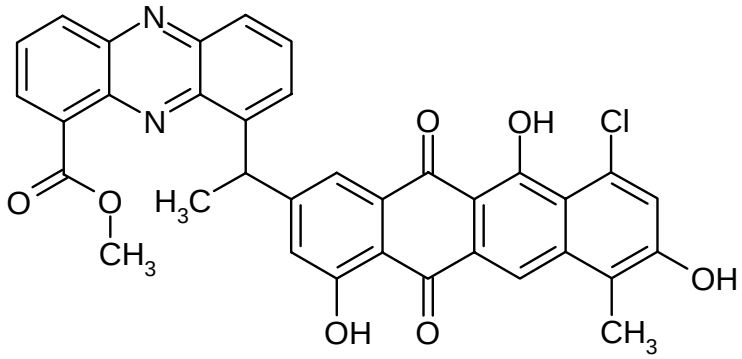


272

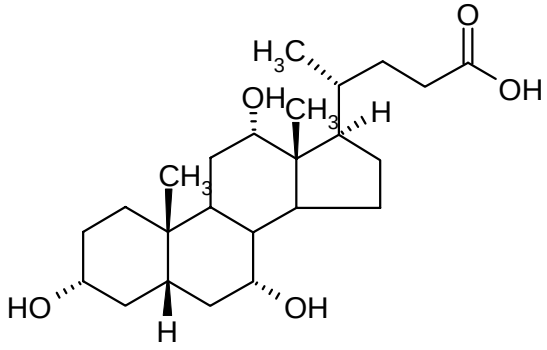
Optimization of the terrestrial *Streptomyces* sp. GW10/1828

Due to the high antimicrobial activity of the *Streptomyces* sp. isolate GW10/1828, and its interesting secondary metabolites, it was subjected to fermentation variations. Optimisation was carried out using four different nutrients (Bacto-peptone, Bennett's, M_1, and M_2 medium), as shaker culture at 28 °C for five days (at different pH values). TLC of the resulting extracts indicated that, both M_2 (pH 7.8) and Bacto-peptone media are optimal for the strain.

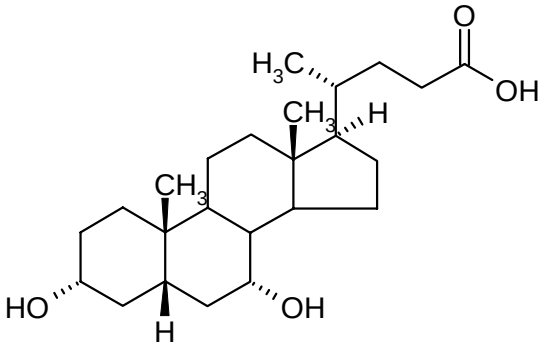
4.21.3 Cholic acid and Chenodeoxycholic acid

For large scale production, the strain was cultivated on 25-liter shaker culture using Bacto-pepton, resulting in a black culture broth. TLC of both biomass and water phase showed different patterns. However, the metabolites from the biomass were very little and neglected. The water phase was worked up by Patnam[283] in the group, giving two non-UV absorbing substances, which turned to blue with anisaldehyde/sulphuric acid. After isolation and purification, and on the basis of ^{1}H NMR and ESI mass spectra, they were identified as cholic acid[284] (**273**), with molecular weight of 408 Dalton and chenodeoxycholic acid[284] (**274**), with molecular weight of 392 Dalton.

Steroids are normally not produced by bacteria, however, may be derived from a fungal infection of the culture. Especially **273** and **274** are typical mammalian products, and they may also be introduced by the peptone medium. Therefore, chromatograms of the ethyl acetate extract of the medium were compared with the isolated steroids **273** and **274**, and showed identity. This was established by the comparison of the ESI mass spectra of both compounds with the isolated. This demonstrated that, **273** and **274** are contaminants from the culture medium, but are not produced by the microorganisms.



273



274

Therefore, a further cultivation using a 50-liter jar fermenter was carried out for 7 days in M_2 medium. The black culture broth was filtrated and extracted with ethyl acetate. TLC analysis of the extract exhibited two non-UV absorbing bands, which were stained to brown by spraying with anisaldehyde/sulphuric acid and heating. However, no coloured *peri*-hydroxyquinones were detected, pointing to different metabolic constituents.

The crude extract was applied to flash silica gel chromatography and eluted by a dichloromethane-methanol gradient to afford five fractions. Purification of the main fractions III-V led to isolation of the known metabolites, macrophorin D (**275**) and anicequol (**278**)

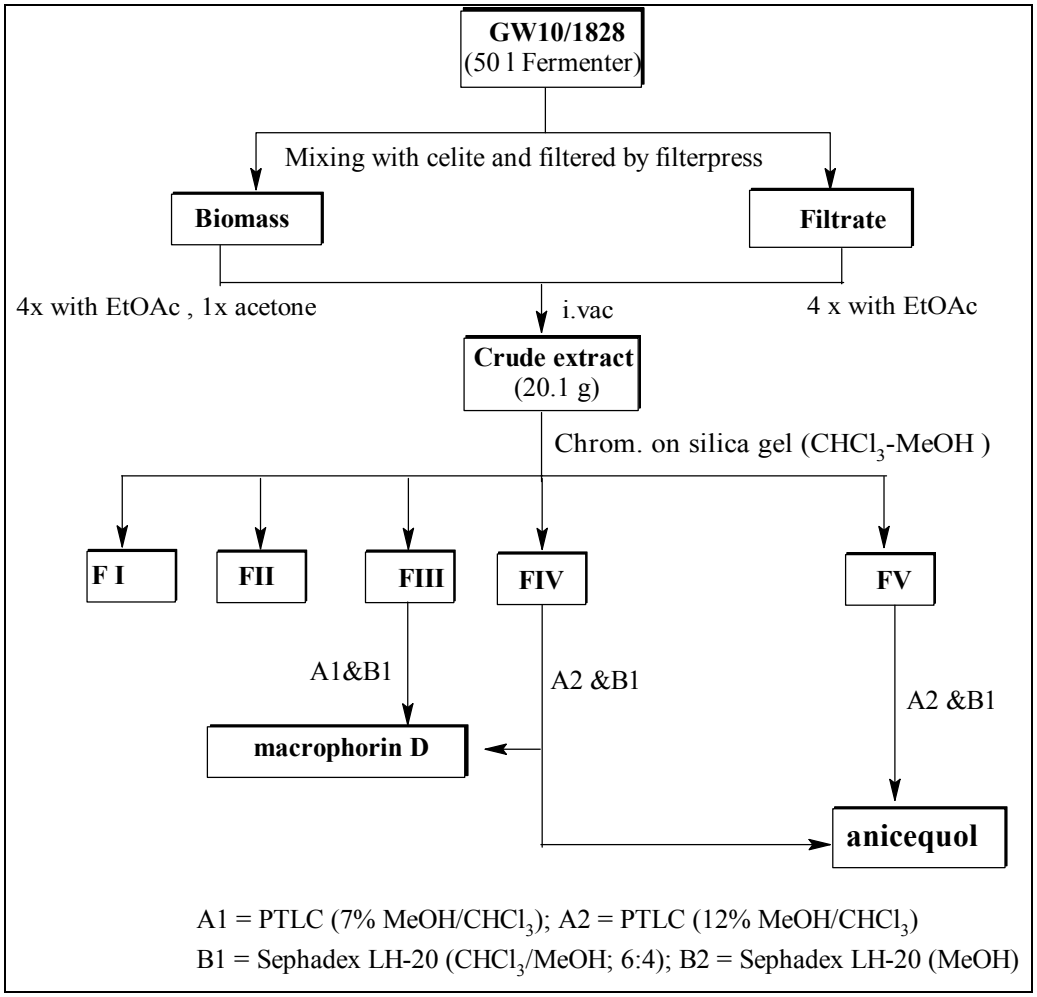


Figure 132: Work up procedure of terrestrial *Streptomyces* isolate GW10/1828 (fermenter)

4.21.4 Macrophorin D

Throughout the screening of both fractions III and IV, compound **275** was obtained as colourless semisolid. It showed a weak UV absorption, but exhibited a brown colour (later changing to blue) by spraying with anisaldehyde/sulphuric acid and heating.

The UV spectrum of **275** showed end absorption, as an indication of a missing aromatic system. The IR spectrum of compound **275** showed a band at ν 3426 cm^{-1} of hydroxyl or amino group. No further bands beyond 3000 were displayed. In the

region between ν 1736-1637, it showed bands of carbonyl groups. Moreover, it showed bands in the range of ν 1560-1511. At ν 1442-1382, several bands of methylene and methyl groups were detected

The ^{1}H NMR spectrum of compound **275** showed no aromatic signals, corresponding to the UV spectrum. However, a 1H singlet was observed at δ 5.87, and between δ 4.80-4.72 it exhibited multiplets of 2H, which could be due to olefinic protons (= CH_2) or oxymethines. Furthermore, three singlets of oxymethines were detected at δ 4.75, 4.60 and 4.51, in addition to a doublet of another oxymethine at δ 3.75. Furthermore, a 4H multiplet was observed between δ 2.81-2.61, as of two methylene groups linked to sp^2 carbons. Moreover, two protons were observed as dd at δ 2.28, and 2.25. Additionally, an unresolved multiplet of 5H was exhibited between δ 2.08-1.67, in addition to two other multiplets at δ 1.50 (2H) and 1.31 (1H). Three additional multiplets between δ 1.22-1.05 were detected, each with an intensity of 1 H. Finally, four methyl singlets were displayed at δ 1.31, 0.8, 0.7 and 0.6, of which the first one could be linked to sp^2 olefinic double bond or at quaternary sp^3 oxy-carbon.

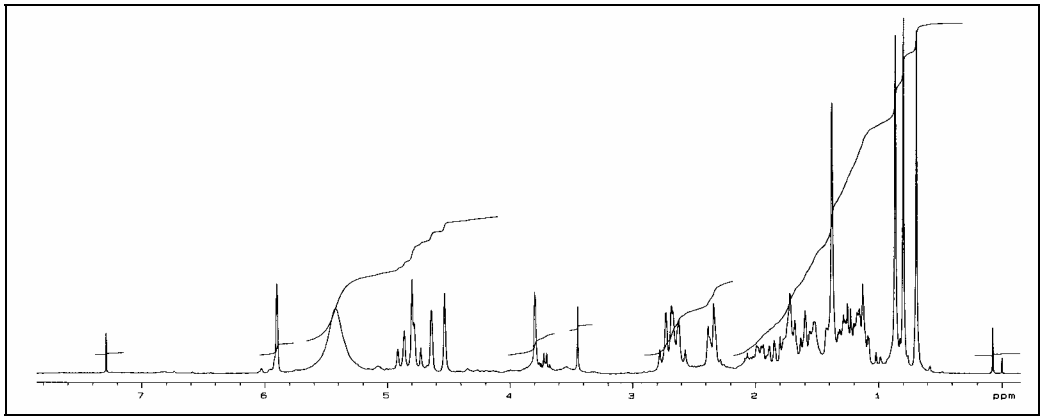


Figure 133: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of macrophorin D (**275**)

The ^{13}C/APT NMR spectra of compound **275** exhibited three quaternary carbons (δ 193.1, 174.9 and 170.8), which designed as carbonyl groups. The first carbonyl (δ 193.1) could be conjugated with a double bond, while the other two (δ 174.9 and 170.8) are carbonyl groups of acids, amides or esters. In addition, two quaternary sp^2 oxy-carbons in β-position to a carbonyl group were detected at δ 151.8 and 149.0. Moreover, one olefinic methine and a terminal sp^2 olefinic methylene carbons were found at δ 122.15 and δ 106.7(confirmed by HMQC), respectively. In the aliphatic

region, two quaternary oxygenated carbons were observed at δ 70.0 and 60.8. Additionally, four methine carbons were exhibited at δ 65.0, 60.6, 55.3 and 51.3, of which the first two could be oxygenated. Moreover, ten methylene carbons were detected, out of which the first two (δ 63.3, 60.8) are oxygenated. Furthermore, two sp^3 quaternary carbons were detected at δ 39.6 and 33.5. Four methyl carbon signals were observed at δ 33.5, 27.4, 21.6 and 14.4.

The ESI mass spectra of compound **275** determined its molecular weight as 504 Dalton, due to the presence of two *quasi* ion peaks at *m/z* 1031 $[2\ M + Na]^+$ and 527 $[M + Na]^+$ in (+)-ESI mode, and other two at *m/z* 1007 ([2 M - H]) and 503 $([M - H]^-$ in (-)-ESI mode.

Based on the revealed spectral data and a search in AntiBase two structures were suggested: Macrophorin D (**275**), a cyclohexenone epoxide having a sesquiterpene residue, and the closely related cyclohexenone with an opened epoxide ring containing a dilactone structure (**276)**.

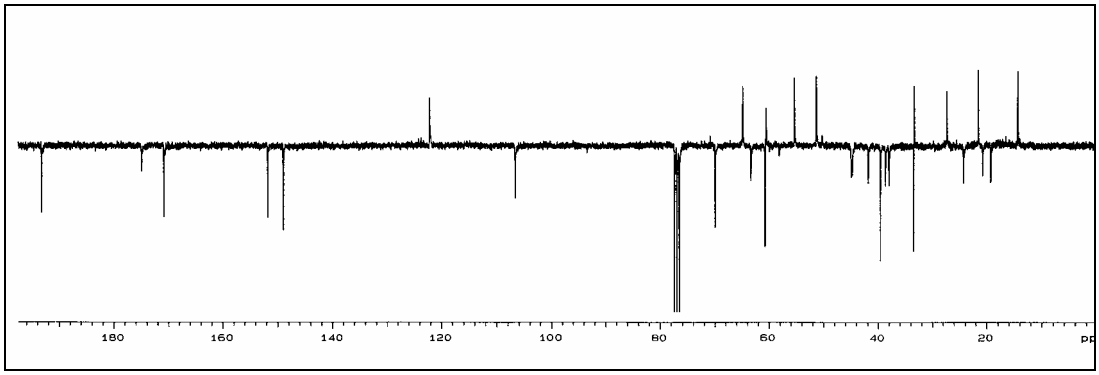
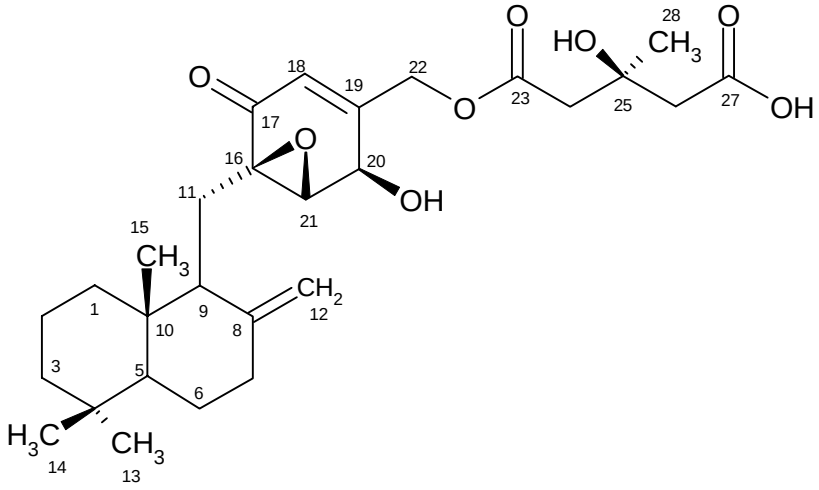


Figure 134: APT NMR spectrum ($CDCl_3$, 75 MHz) of macrophorin D (**275**)

According to the HMBC correlations (page 352), both compounds (**275**, **276**) exhibited the same correlations. However, the connecting correlation between the hydroxy methine at position-20 (δ_H 4.61) and its adjacent ester carbonyl group at position-27 (δ_C 174.9) was not detected, pointing to an open chain. To the best of our knowledge, both δ_H and δ_C values of epoxide methines are shifted normally upfield compared with those of the ring-opened hydoxymethines, and compound **276** was excluded therefore. Accordingly, comparison of δ_H and δ_C values with the literature of macrophorin D (**275**), led to establish it conclusively[285,286]. Further confirming of compound **275** was carried out by comparison with macrophorin A (**277**).



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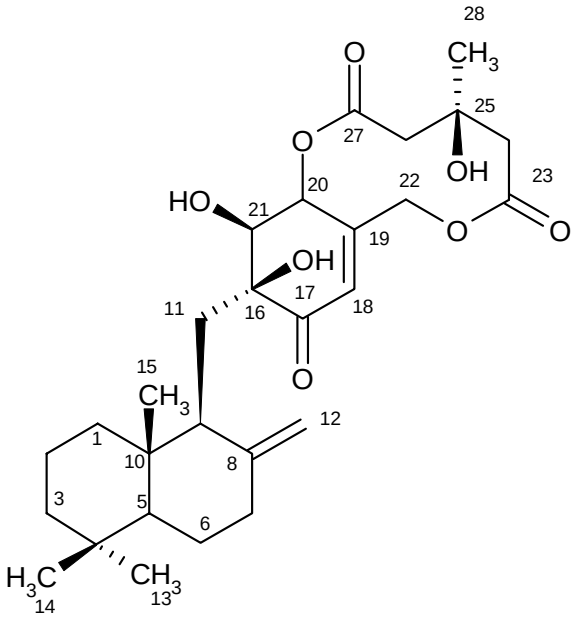

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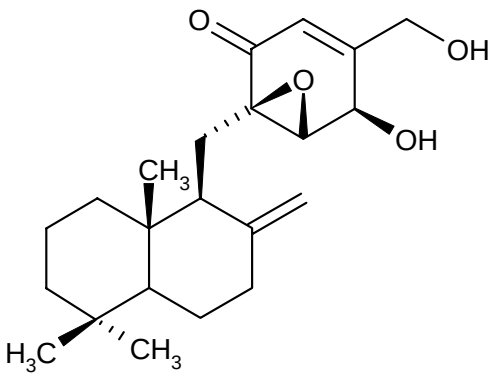


277

Macrophorin D (**275**) was isolated first in 1984 by Sassa *et al.*[285], as self-growth inhibitor of the fungus *Macrophoma* which infects apple. Recently, two new macrophorin derivatives were reported, namely 4'-oxomacrophorin A and 4'-oxomacrophorin D, are two immunomodulatory constituents from an ascomycete, *Eupenicillium crustaceum*[287]. They are the corresponding cyclohexendiones instead of cyclohexenones.

4.21.5 Anicequol

Continuous screening of fractions IV and V gave compound **278,** which showed related properties as **275**. It exhibited no UV absorbance, but was stained to brown

after spraying with anisaldehyde and heating. It was isolated as colourless solid during the purification of the fractions IV and V by PTLC and Sephadex.

The ^{1}H MNR spectrum of compound **278** showed two 1H dd signals between δ 5.19-5.17, constructing a transoid double bond (J = 15.3, 3.9 Hz). In addition, 4 hydroxymethines and two OH groups were detected between δ 4.85-3.35. Furthermore, one 2H multiplet was found at δ 2.52-2.40 of methine or methylene protons linked to sp^2 or oxy-sp^3 carbons, as well as two dd signals each of 1H at δ 2.30 and 2.14 of two methine protons present in a cyclic aliphatic ring. Four multiplets of 5 protons were exhibited between δ 1.91-1.58, due to methylene and methine protons linked probably to sp^2 carbons. Multiplets of 7H were observed between δ 1.40-1.20, as methine and methylene protons. Additionally, two singlet methyl groups appeared at δ 1.93 and 1.05, of which the first one could be linked to sp^2 carbon, as e.g. acetyl group. In addition, five methyl doublet were found at δ 1.01 and 0.81-70 ($J \approx 6.6$ Hz).

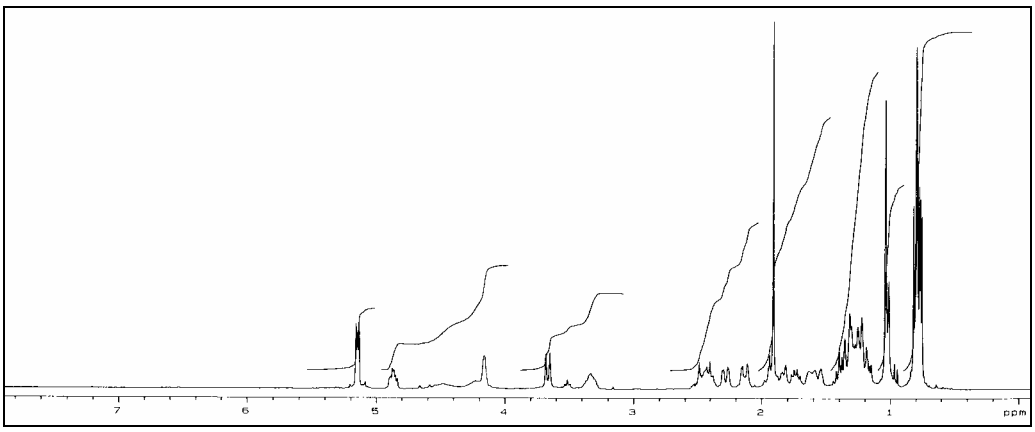


Figure 135: ^{1}H NMR spectrum ([D_6]DMSO, 300 MHz) of anicequol (**278**)

The ^{13}C/APT NMR spectra as well as ^{1}J correlations (HMQC) of compound **278** established the presence of 30 carbons, pointing most likely to a triterpene skeleton. It showed two carbonyl carbons at δ 210.2 and 169.3. The first carbonyl (210.2) could be located between two sp^3 carbons forming a ketone, while the other one could be of an ester, acid or amide. Furthermore, two olefinic methine carbons were observed at δ 135.2 and 131.7. In the aliphatic region, four oxymethine carbons were detected at δ 78.3, 74.5, 68.4 and 66.3. Moreover, eight other sp^3 methine carbons were shown between δ 59.0-32.4. Additionally, five methylene carbons were displayed between δ 47.7-29.5, in addition to two sp^3 quaternary carbons at δ 42.6 and 40.3 (confirmed by HMQC). Finally, 7 methyl carbons were detected between δ 21.1~14.8.

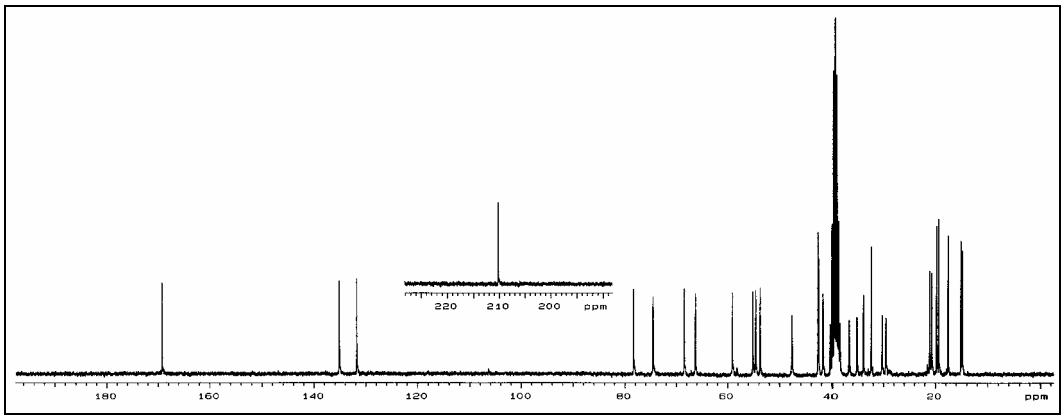
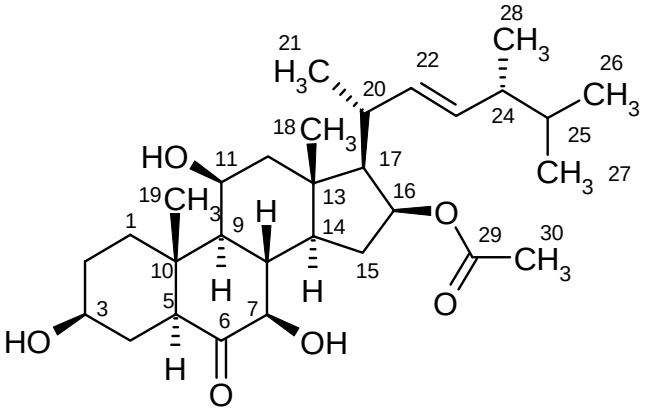


Figure 136: ^{13}C NMR spectrum ([D_6]DMSO, 75 MHz) of anicequol (**278**)

ESI mass spectra established the molecular weight of compound **278** as 504 Dalton by (+)-ESI and (-)-ESI modes. A search in AntiBase based on the above spectral data resulted in anicequol (**278**). The compound was further confirmed by comparison with the literature[288,289]] and 2 D spectral data.

Anicequol was obtained first from *Penicillium aurantiogriseum* Dierckx TP-F0213 [288], as an inhibitor for anchorage-independent growth of tumor cells. It was recently reported as a novel neuritogenic compound from *Acremonium* sp[289] TF-0356. Triterpenes or steroids are unusual metabolites from bacteria, while they are produced mainly by fungi. This indicated that, compounds **275** and **278**, could be produced by an unidentified fungus growing as contaminant, or that the producing strain GW10/1828 itself is a fungus and not a *Streptomycete*.



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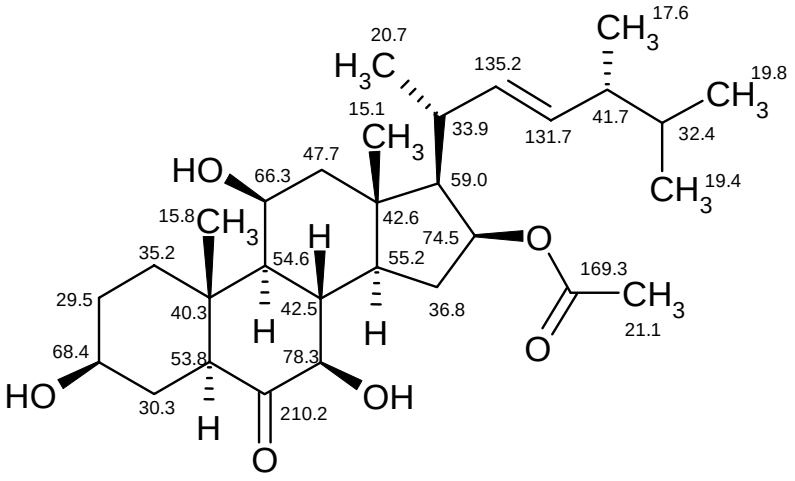


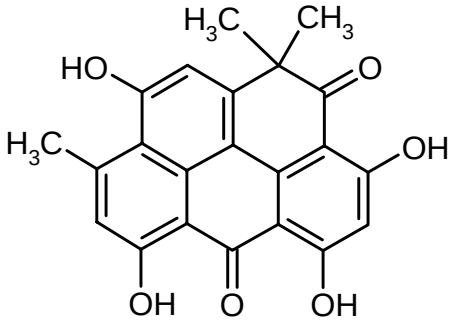
Figure 137: ^{13}C NMR shifts of anicequol (**278**)

4.21.6 Resistomycin

A fourth trial to isolate the chlorine constituents was carried by re-cultivation of the strain GW10/1828 as 25-liter shaker culture using M_2 medium (4 days). By purification of the strain extract, a yellow middle polar band was obtained. It exhibited no colour change by sodium hydroxide, and was therefore not a *peri*-hydroxyquinone. It turned, however, to reddish-brown by treatment with sulphuric acid, and showed an orange-yellow UV fluorescence at 366 nm. Further purification of the respective fraction led to isolation of compound **279** as a yellow solid.

The ^{1}H NMR spectrum of compound **279** revealed three proton singlets at δ 14.55, 14.36 and 14.07, which are characteristic to three chelated hydroxyl groups. In addition, a broad 1H singlet at δ 11.40 of phenolic hydroxyl group, and three 1H singlets in the aromatic region at δ 7.21, 7.01 and 6.31 were observed. Furthermore, a 3H singlet at δ 2.90 of a methyl group attached to an aromatic ring was found. Besides, the spectrum showed a singlet at δ 1.56 with the relative intensity of 6 which accounts for two magnetically equivalent methyl groups.

The molecular weight of **279** was determined by ESI mass spectra in the positive mode as 376 Dalton by the ions at *m/z* 775 $[2\ M + Na]^+$ and 377 $[M + H]^+$. This was also confirmed in the negative ion mode by *m/z* 375 $[M - H]^-$. A search in AntiBase delivered resistomycin, as the only possibility of compound **279**. Comparison of the proton NMR data of **279** with the authentic sample confirmed the identity with resistomycin (**279**).



279

Resistomycin (**279**) was firstly isolated from the culture of *Streptomyces resistomycificus* by Brockmann and Schmidt-Kastner[290]. The structure of the compound was established by classical methods, and on the basis of chemical and spectral data together with x-ray crystallographic analyses[291,292]. Sung-Woo *et al.*[293] reported that resistomycin had an antitumoral effect on proliferation and morphology of the human breast tumour cell line MF-7. Furthermore, resistomycin is active against Gram-positive bacteria and preferentially inhibited RNA synthesis in *Bacillus subtilis*[294]. Two closely related metabolites, resistoflavine and 1-hydroxy-1-nor-resistomycin have been isolated in our group from marine *Streptomyces sp.* B8005[295]. Resistomycin (**279**) belongs to the acetogenins, and is built from acetyl and malonyl units. Resistomycin is characterised by its RNA polymerase inhibitory and antibacterial activity against Gram-positive microorganisms and *mycobacteria*[24].

4.22 Terrestrial *Streptomyces* sp. GW50/1568

The ethyl acetate extract of a terrestrial *Streptomyces* sp. GW50/1568 exhibited moderate antibacterial and antifungal activities against *Streptomyces viridochromogenes* (Tü57), *Bacillus subtilis*, *Escherichia coli*, and *Mucor miehei* (Tü284). During the TLC screening, a highly polar non-UV absorbing band was detected, which turned to violet-black by treatment with anisaldehyde/sulphuric acid and heating. The strain cultivation was carried out in two different scales; a) 25-liter fermenter, and b) 25-liter shaker culture.

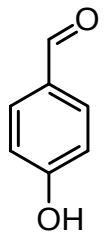
A: Fermenter

Well-grown sub-cultures of the strain GW50/1568 were inoculated into twelve 1 liter-Erlenmeyer flasks using M_2 medium for 5 days. The obtained faint yellow broth was further cultivated as 25-liter fermenter (for additional 4 days). After filtration

and extraction with ethyl acetate, a brown extract was obtained. By applying the extract to Sephadex LH-20 eluting with methanol, four fractions were delivered. Further purification of the fractions led to isolation of a low polar yellow solid (**512a**), *p*-hydroxy-benzaldehyde (**280**), and other known metabolites: Tryptophol (**44**), tyrosol (**50**), indolyl-3-carboxylic acid (**45**), indolyl-3-carbaldehyde (**194)**, *p*-hydroxy benzoic acid (**105a)**, uracil (**52**), thymine (**53**) and 2'-deoxyuridine (**96)**.

4.22.1 *p*-Hydroxy benzaldehyd

Screening of fraction III led to compound **280**, as a colourless solid with UV absorbance. Based on the chromatographic and spectroscopic data of compound **280** and search in AntiBase, it was identified as *p*-hydroxybenzaldehyd. Compound **280** was isolated frequently from aquatic organisms, e.g. by Fenical and McConnel[296] from the red seaweed *Dasya pedicellata* var. *stanfordiana* and has some antimicrobial activity against *Vibrio anguillarium*, *Candida albicans* and *Staphylococcus aureus*.



280

B. Shaker Culture

It was proposed to re-grow the terrestrial *Streptomyces* stain GW50/1568 because of the observed violet stained polar band with anisaldehyde/sulphuric acid, which was not observed in the fermenter. Hence, a 25-liter shaker culture of the strain GW50/1568 was performed for 7 days at 28 °C. Filtration of the culture broth and extraction with ethyl acetate led to a brown crude extract. By applying the extract to flash silica gel column chromatography, eluting with dichloromethane-methanol, with a gradual increasing of the polarity, 6 fractions were obtained. Purification of fraction VI led to isolation of oasomycin-A (**281**).

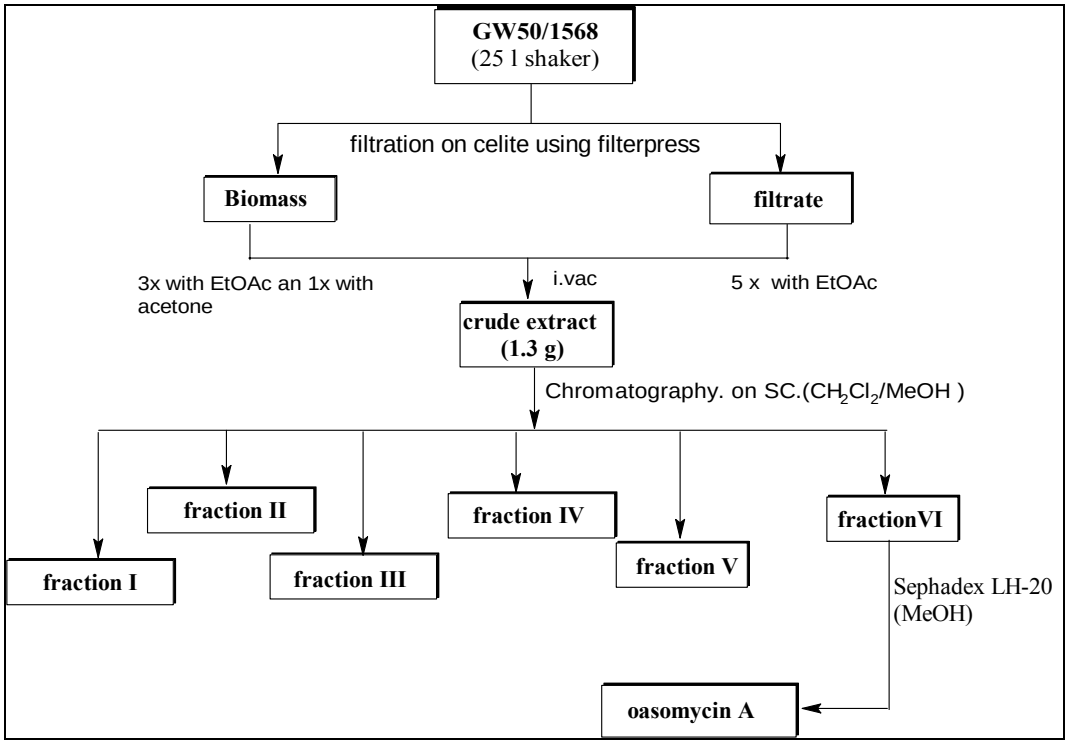


Figure 138: Work-up procedure of the terrestrial *Streptomyces* isolate GW50/1568 (shaker)

4.22.2 Oasomycin-A

Purification of fraction VI by applying to Sephadex LH-20 resulted in compound **281** as a colourless non UV absorbing solid, which turned to violet-black, red-pink and brown-grey with the spray reagents, anisaldehyde/sulphuric acid, vanillin/sulphuric acid and Ehrlich's reagent, respectively.

The ^{1}H NMR spectrum of compound **281** showed complex signal patterns in the olefinic and aliphatic regions. It showed a triplet of 1H at δ 6.69 (^3J = 6.3Hz), and seven olefinic protons were detected as multiplets in the range of δ 5.48-5.26. In addition, one multiplet of 1H at δ 5.00, and 14H between δ 4.48-3.25 were detected, which could be assigned as 15 oxymethines. Along with this, highly overlapped multiplets were exhibited in the range of δ 2.54-1.11 with an intensity of 22H, possibly due to eleven methylene groups. Moreover, two methyl singlets were observed at δ 1.77 and 1.54 linked to sp^2 carbons. Finally, seven methyl doublets were detected between δ 0.99-0.64.

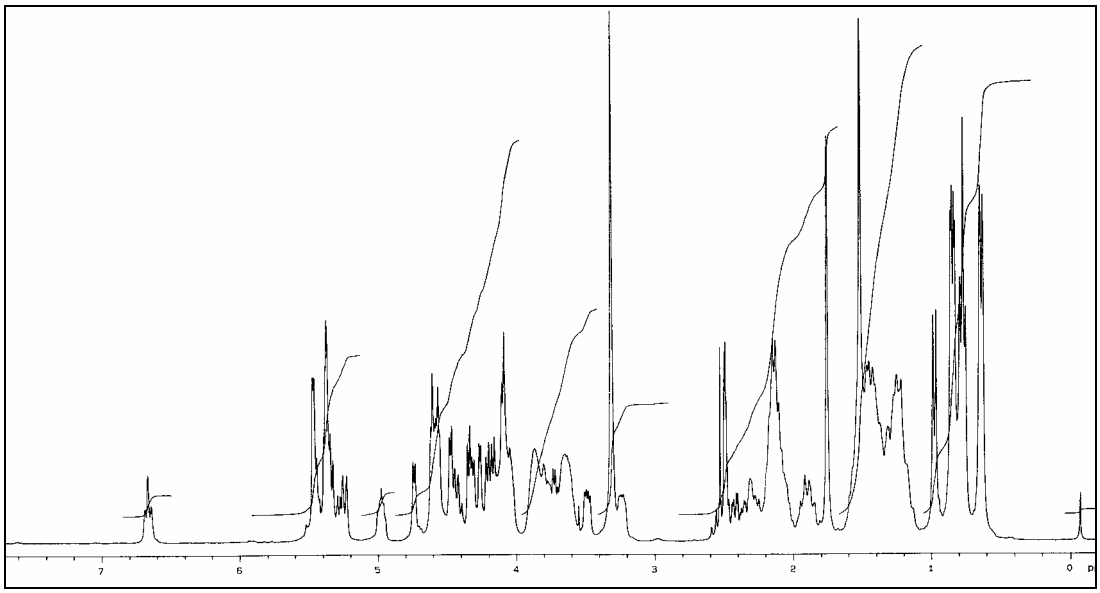


Figure 139: ^{1}H NMR spectrum ([D_6]DMSO, 300 MHz) of oasomycin A (**281**)

The ^{13}C/APT NMR spectra of compound **281** exhibited two quaternary carbonyl carbons at δ 176.7 and 166.4 of acids, esters or amides. Furthermore, ten sp^2 carbons were exhibited, from which 8 were methines, and two were quaternary carbons at δ 138.1 and 126.8. In accordance, there are two possibilities to locate the double bonds in **281**: (4 CH=CH + C_q=C_q or 3 R-CH=CH-R + 2 CH=C_q). Correspondingly with ^{1}H NMR, the last case was matched (3 R-CH = CH-R + 2 R-CH = C_q). In the aliphatic region, 15 oxymethine carbons were observed between δ 81.0~63.7. In addition, 12 methylene carbons appeared in the range of δ 45.7~24.8. Furthermore, 7 additional methine carbons of the type -CH-CH_3 were displayed between δ 42.2~34.3. Finally, 9 methyl carbons were detected between δ 16.8~9.4.

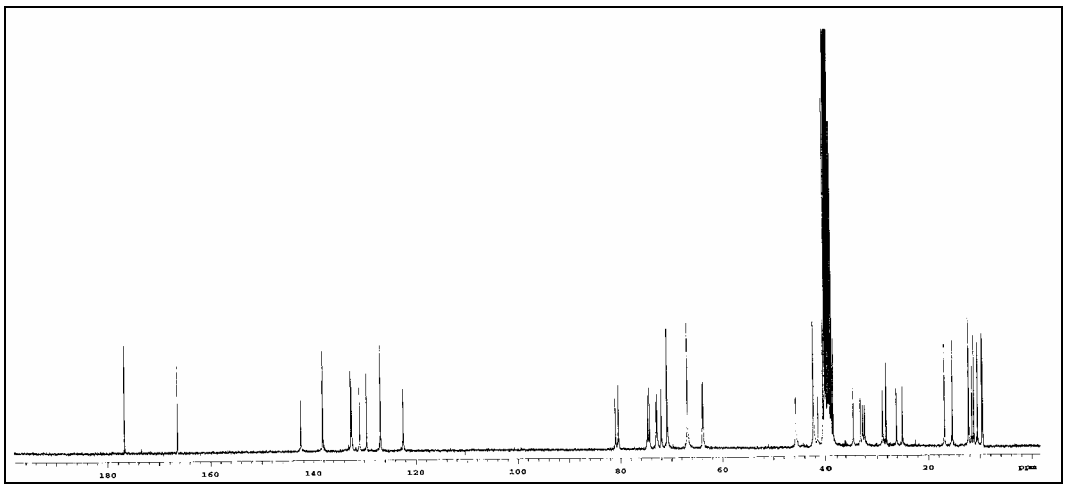
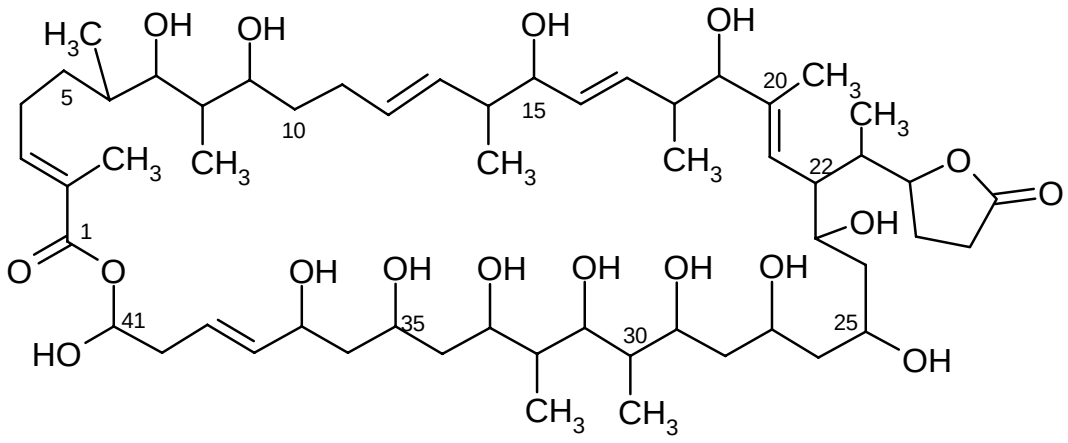


Figure 140: ^{13}C NMR ([D_6]DMSO, 75 MHz) of oasomycin A (**281**)

The molecular weight of compound **281** was established as 1027 Dalton by ESI mass spectra, due to the existence of an ion peak at *m/z* 1050.2 ($[M + Na]^+$) in (+) ESI mode, and 1026.1 ($[M - H]^-$) in the ESI negative mode. The molecular weight of compound was further determined by (+)-ESI giving a peak at *m/2z* 536.5 ($[M + Na]^+$). Based on the revealed chromatographic and spectral data of compound **281** and a search in AntiBase, it was elucidated as oasomycin A. The compound **281** was further confirmed by comparing the measured spectra with the literature [297].



281

Oasomycin A (**281**) was isolated first by Zeeck *et al.*[298] in 1992. The compound **281** belongs to the desertomycin family produced by *Actinomycetes*[299], secondary metabolites consisting of desertomycins A-D and oasomycins A-B (A **281**). Most of these metabolites bear a 42-membered macrolactone. Furthermore, the metabolites of the desertomycin family vary in the constitution of the side-chain located at either C-41 or C-43, as well as in the presence of an α-linked D-mannose moiety attached to the 22-OH. The macrolactones of the desertomycin family show close structural relationships to other macrolactone antibiotics. The largest ring size has been found in monazomycin[300] (48-membered), followed by the desertomycin family (44-and 42-membered), and antibiotic RP 63834[301] (41-membered).

The desertomycins exhibit broad antibacterial and selective antifungal activities[302], mainly against phytopathogenic fungi, while oasomycins A-B were found to be inactive in various antibacterial and antifungal assays. However, oasomycin A (**281**) showed inhibitory effects on *de novo* cholesterol biosynthesis as well as 50 % inhibition at a concentration of 10^{-5} M on the muscarine receptor, and weak antiprotozoal activity[298].

From the biosynthetic point of view, the metabolites in the desertomycin family bear interesting similarities. It is proposed that, these compounds are synthesized *via* the poly-ketide pathway with a polyketide starter unit, which is subsequently elongated by acetate and propionate moieties[303]. Studies concerning the biosynthetic origin of the oasomycins as an example of macrolactone biosynthesis are currently being carried out. The biosynthetic relationships of the desertomycin family as well as its biosynthesis was made by Mayer *et al*.[304,299] (Figure 141).

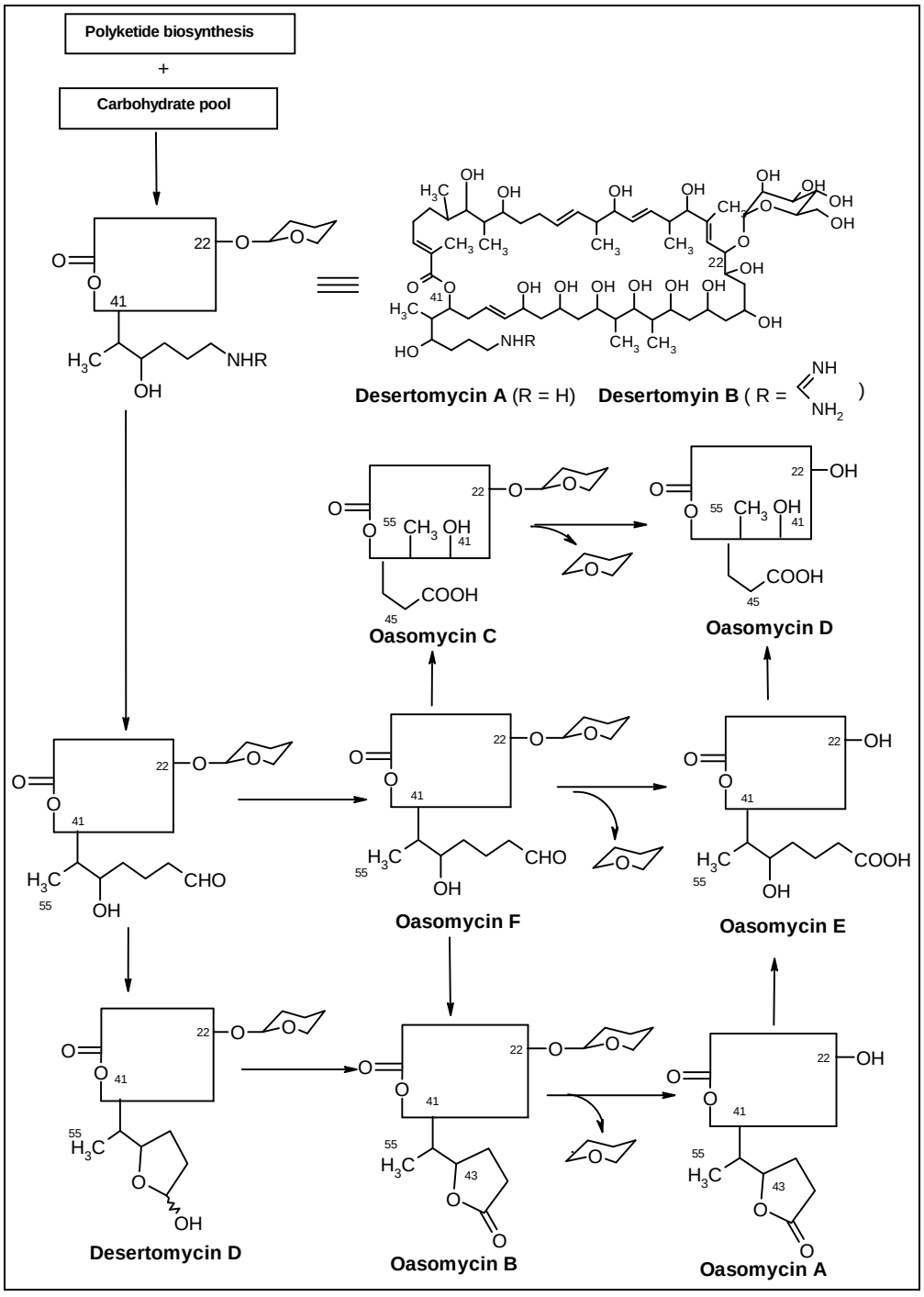


Figure 141: Biosynthetic relationships in the desertomycin family

4.23 Terrestrial *Streptomyces* sp. GW44/1492

The ethyl acetate extract of a terrestrial *Streptomyces* sp. isolate GW44/1492 exhibited a high antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Streptomyces viridochromogenes* (Tü57), and weak activity against *Escherichia coli, Mucor miehei* (Tü284) and *C. albicans*. Moreover, the TLC screening exhibited a middle polar yellow band, which turned to reddish brown by treatment with sulphuric acid, but not with NaOH. This band showed a yellowish-red UV fluorescence.

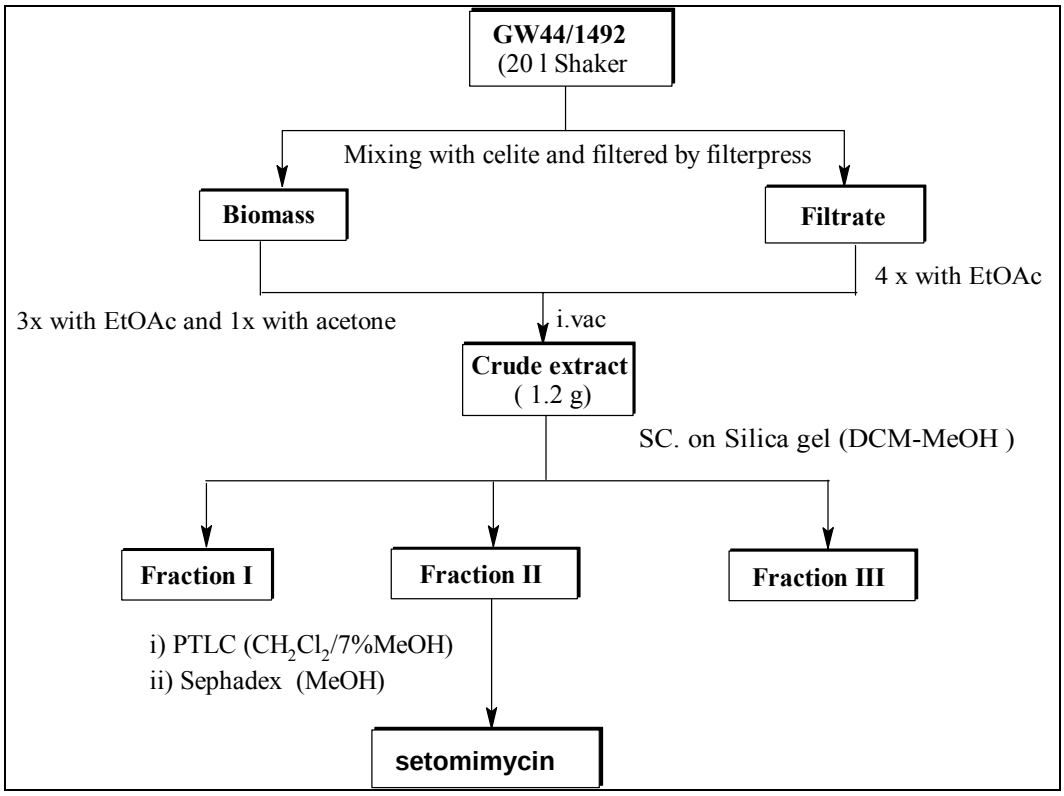


Figure 142: Work-up procedure of terrestrial *Streptomyces* isolate GW44/1492

So, 20-liter shaker culture (95 rpm) of the strain GW44/1492 were cultivated using M_2 medium (pH 7.8) for 4 days. The resulting yellow culture broth was filtered and extracted by ethyl acetate. The obtained brown extract was worked up on a middle pressure silica gel column, eluting with dichloromethane/methanol to get three fractions.

4.23.1 Setomimycin

Purification of the main yellow fraction II by PTLC and Sephadex led to isolation of compound **282**, as yellow solid. It was soluble in chloroform and dimethylsul-

foxide. During TLC, the compound showed an orange UV fluorescent zone at 366 nm, which turned brown after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of **282** showed four hydrogen-bound hydroxyl groups at δ 16.57, 16.51, 10.12 and 10.00, which were distinguished into two *peri*-hydroxy groups (δ 16.57, 16.51), and two phenolic (δ 10.12, 10.00). Additionally, a total of six aromatic hydrogens are indicative of two aromatic rings at δ 7.42 (t), 7.28 (t), 7.02 (d), 6.92 (d), 6.67 (d) and 6.26 (d) were detected, each with one hydroxyl group. Moreover, a 1H singlet at δ 6.27 could be corresponding to an olefinic α-proton conjugated with a carbonyl group. In addition, two singlets were exhibited at δ 4.03 and 3.72 of two oxymethines, or of two methines flanked by sp^2 carbons. Two doublets, each of 1H, were detected at δ 3.20 and 2.64. The magnitude of the coupling constant (^{2}J = 18.0 Hz) indicated these two hydrogens to be situated in a methylene group, in a ring system. Finally, four methyl singlets were detected at δ 2.02, 1.71, 1.42 and 1.25, the first three of which are linked to sp^2 carbons, e.g. acetyl groups (2.02 and 1.71), and an olefinic double bond (δ 1.42).

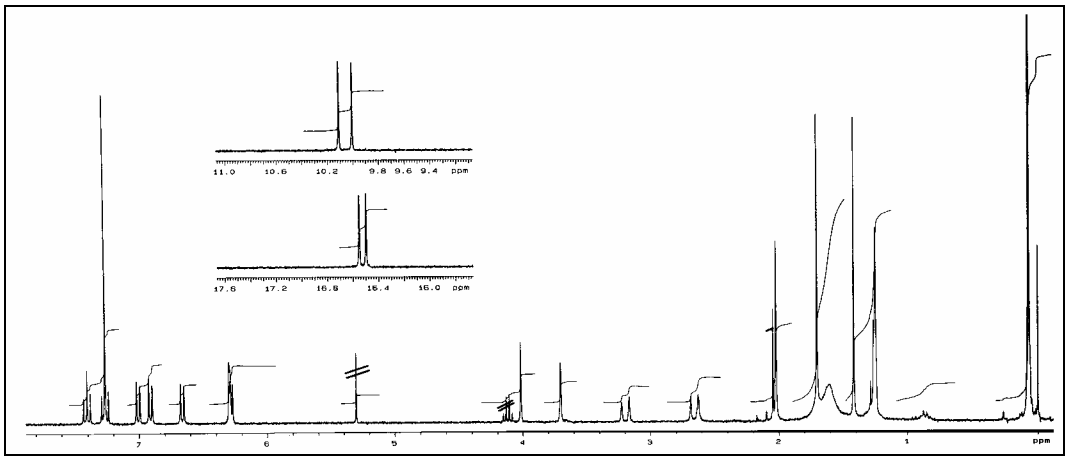


Figure 143: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of setomimycin (**282**)

The ^{13}C/APT NMR spectra of compound **282** exhibited 34~35 carbon signals classified in different categories: Four carbonyls were detected at δ 208.3, 203.2, 199.5 and 191.0, from which the first two are located between two sp^3 carbons, while the other two are most likely linked to sp^2 systems. Additionally, fifteen quaternary sp^2 carbons were detected in the range of δ 166.4~108.6, from which five are possibly oxygenated (δ 166.4, 164.8, 158.8, 158.5 and 158.0). Furthermore, seven sp^2 methine carbons were exhibited between δ 133.5~112.5. This pointing most likely to two aromatic rings of type 1,2,3-trisubstituted. The rest sp^2 methine carbon was de-

tected at 125.8, which present possibly at α-position to a carbonyl group. In the aliphatic region, one quaternary sp^3 oxy-carbon was detected at δ 72.1, in addition to two sp^3 methine carbons at δ 60.8 and 60.5, of oxymethines, or situated between sp^2 carbons. The spectrum exhibited finally, signals of one methylene and four methyl groups at δ 47.2, 33.9, 29.5, 28.2 and 23.0, respectively. The observed deep field shift of the methylene carbon is indicative for its location between two sp^2 carbon systems. The methyl carbons at δ 33.9, 29.5 and 28.2 are possibly linked to sp^2 carbons.

The molecular weight of compound **282** was established as 580 Dalton using ESI mass spectra, because of the presence of two *quasi*-molecular ions at *m/z* 1182.8 $[2\ M + Na]^+$ and 603 $[M + Na]^+$ in (+) ESI mode, and one at *m/z* 579 $[M - H]^-$ in (-) ESI mode. Based on the detailed spectral data of compound **282** and a search in AntiBase, it was elucidated as setomimycin. The compound **282** was confirmed by comparing the data with the literature[305]. Setomimycin (**282**) was further confirmed on the basis of 2 D correlations (page 360), as 9,9'-binathryl antibiotic.

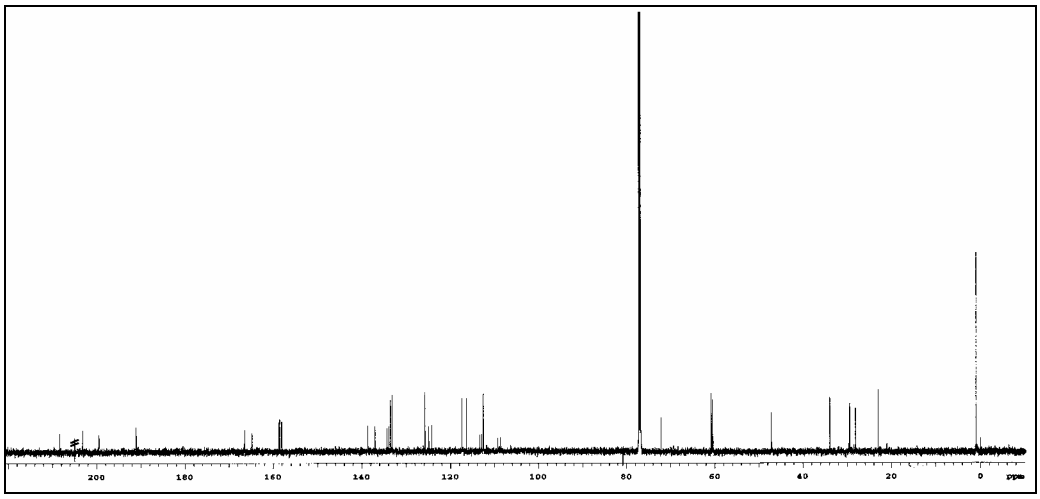
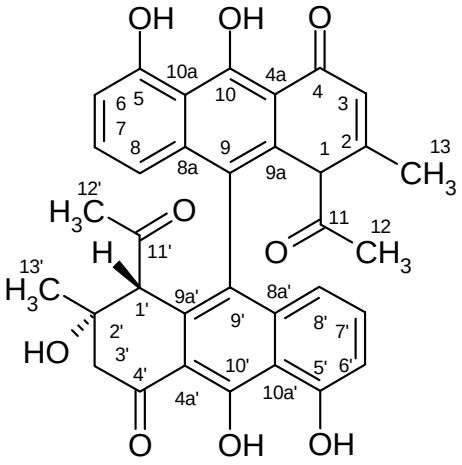


Figure 144: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of setomimycin (**282**)

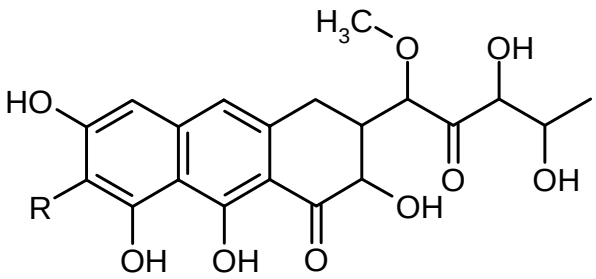
So far, a series of 2,2'-binathryl antibiotic pigments is known from *Streptomyces shiodaensis* namely the julimycins and julichromes, e.g. julichrome $Q_{5,6}$ (**285**). The basic carbon skeletons of julichrome $Q_{5,6}$ is quite similar to that of **282**, but non of the monomeric forms (Q_1 through Q_9) of julichromes is identical with setomimycin. In addition, setomimycin showed no colour change with sodium hydroxide referring to the absence of *peri*-hydroxy groups, while julichrome $Q_{5,6}$ is a *peri*-hydroxyquinone. Setomimycin (**282)** was isolated first in 1978 by Ômura *et al.*[306] from *Streptomyces pseudovenezuelae* as antibiotic, effective against Gram-positive

bacteria including *Mycobacteria* and Sarcoma-180 solid tumor in mice. It is worth to note that the chromophores of **282** resembles chromomycinone (**283**) and olivine (**284**), with the chromophores of the clinically important aureolic acid group of anti-tumor antibiotics.

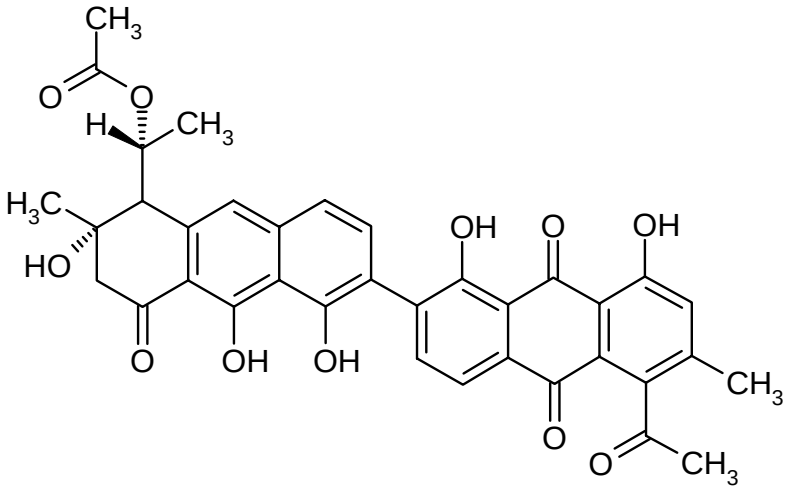
The biosynthesis of compound **282** is likely to be similar to those of julichromes. The carbon skeleton of **282** appears to be constructed by oxidative coupling of substituted hydroanthracene monomers, either prior to or after dehydration, and the hydroanthracene precursor may well be derived from polyacetate[305] (Figure 145).



282



283: R = CH_3, **284**: R = H



285

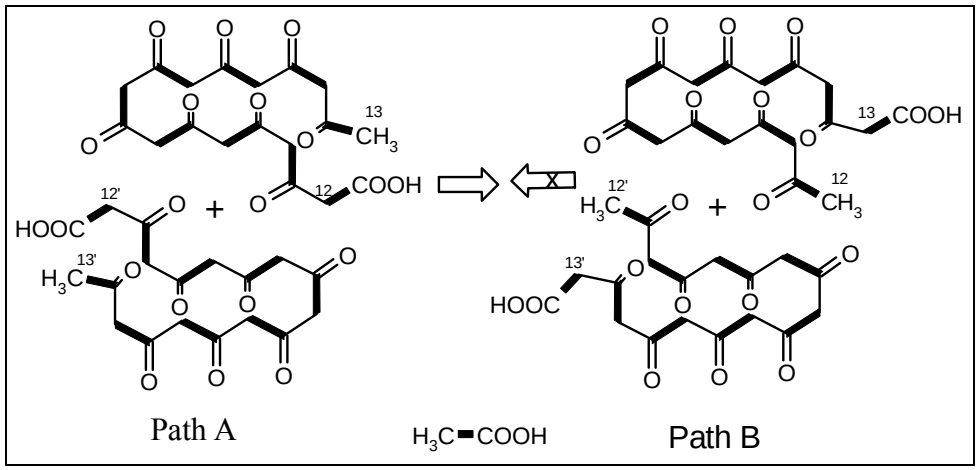


Figure 145: Possible biosynthetic origin of setomimycin (**282**)

4.24 Terrestrial *Streptomyces* sp. GW3/1130

The ethyl acetate extract of a terrestrial *Streptomyces* isolate GW 3/1130 was found to produce yellow and red compounds, which turned to red with sodium hydroxide, and brown with sulphuric acid, pointing to *peri*-hydroxyquinones. Moreover, it possessed high activity against *Mucor miehei* (Tü284), *Streptomyces viridochromogenes* (Tü57), and moderate activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*.

According to our experience, coloured compounds and especially quinones are produced better in shaker culture than in a fermenter. This was established also by cultivation of the strain GW3/1130 as 25-liter jar fermenter which delivered no *peri*-hydroxyquinone, but only the faint yellow 1-acetyl-*β*-carboline (**81**) as a blue UV fluorescent compound.

Therefore, 25-liter shaker culture of the strain GW3/1130 was cultivated on M_2 medium for 6 days at 28 °C. After filtration and extraction with ethyl acetate, the revealed yellow-red crude extract was applied to Sephadex LH-20, and gave four fractions. The main yellow metabolite fraction II was further purified by PTLC and Sephadex LH-20, giving six yellow solid *peri*-hydroxyquinones: Rubiflavinon C-1 (α-indomycinone, **286**), saptomycin F (**289**), ε-indomycinone (**291**), *β*-indomycinone (**295**), saptomycin A (**297**) and *γ*-indomycinone (**298**).

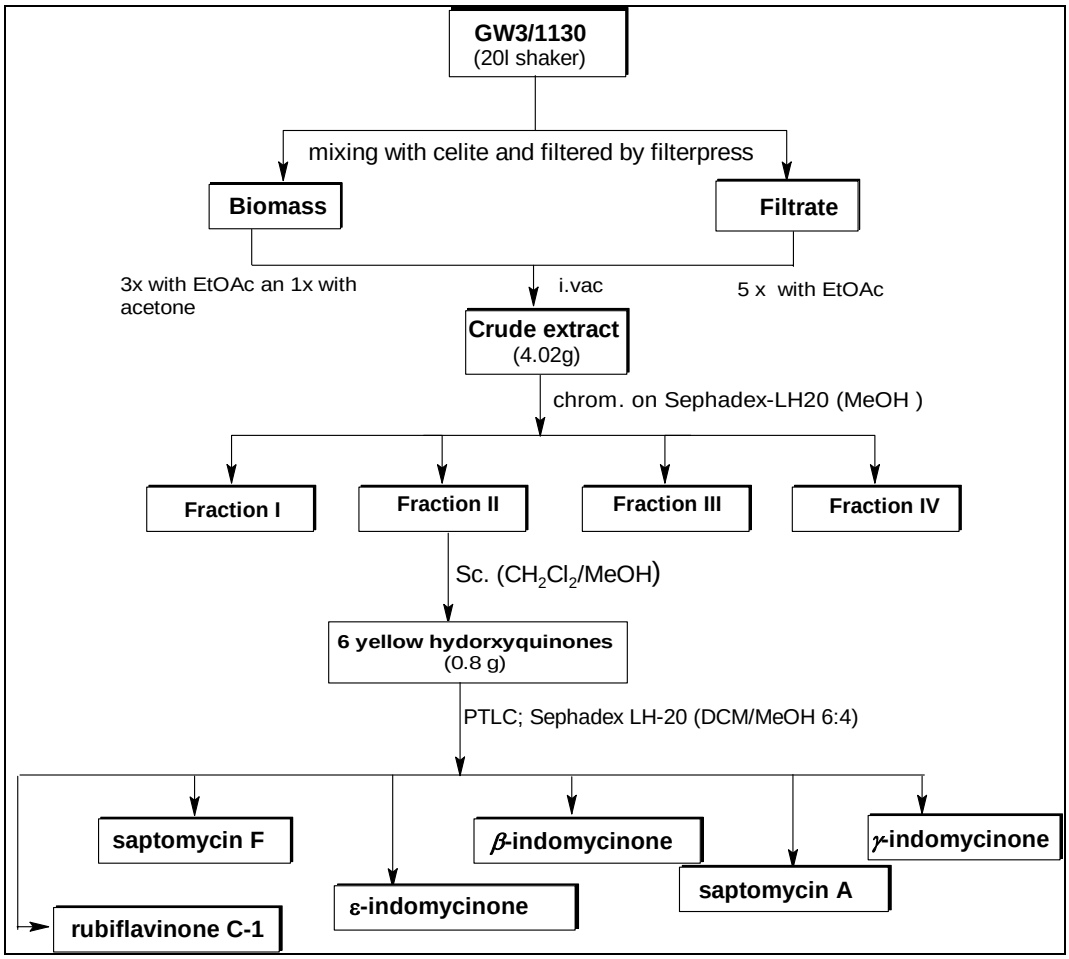


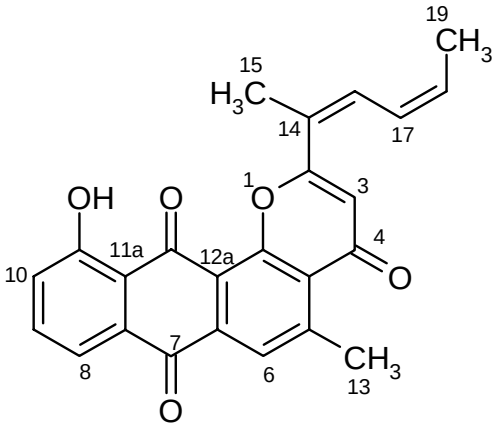
Figure 146: Work-up procedure of the terrestrial *Streptomyces* isolate GW3/1130 (shaker).

4.24.1 Rubiflavinon C-1; α-Indomycinone

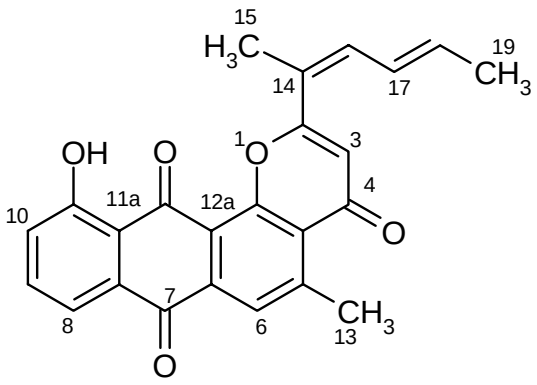
Compound **286** was obtained as a yellow major component from fraction II. It showed a reddish-orange UV fluorescence at 366 nm, and turned to red by treatment with NaOH, pointing to a *peri*-hydroxyquinone. The ^{1}H NMR spectrum of **286** displayed a 1H singlet of a *peri*-hydroxy group at δ 12.90. In the aromatic region, a 1H doublet was observed at δ 8.42, which could be assigned as an olefinic *cis* proton because of its high coupling constant (J~11.7 Hz). Furthermore, one singlet was detected at δ 8.03, which could be assigned as a proton in *peri*-position to a carbonyl group. Additionally, three protons of an 1,2,3-trisubstituted aromatic system were displayed at δ 7.82 (d), 7.68 (t) and 7.35 (d). Additional two multiplets each of 1H were observed at δ 6.54 and 6.08, constructing possibly an olefinic double bond. Moreover, a 1H singlet at δ 6.42, could be corresponding to an olefinic α-proton conjugated with a carbonyl group. One methyl singlet was detected at δ 2.98, which was possibly attached to an aromatic ring and located in *peri*-position to CO, or

bound as N-CH_3. Two other methyl groups were observed δ 2.16 and 2.06, the first of which was observed as a doublet, pointing to its presence in the form =CH-CH_3, while the other (δ 2.06) gave a singlet and was linked to a quaternary sp^2 carbon.

The (+) ESI mass spectrum established the molecular weight of **286** as 386 Dalton. It was further confirmed by EI mass spectrum, and the molecular ion showed an additional fragment at *m/z* 371 [M-Me], due to the expulsion of a methyl group. Based on the detailed spectral data of compound **286**, and a search in AntiBase, two possible structures were suggested; rubiflavinone C-1 (**286**), as *cis*-form and its *trans*-isomer, rubiflavinone C-2 (**287**). Comparing the measured data with the literature of both compounds[307,308] established the compound as rubiflavinone C-1 (**286**). The only difference between the two isomers **286** and **287** concerned the double bond protons at C-16 and C-18, where C-16 in compound **286** was displayed downfield (δ 8.42), however upfield in **287** (δ 7.96). In contrast, the signal of 18-H of **286** was detected upfield (δ 6.08), and downfield for **287** (δ 6.38) (Table 12).



286

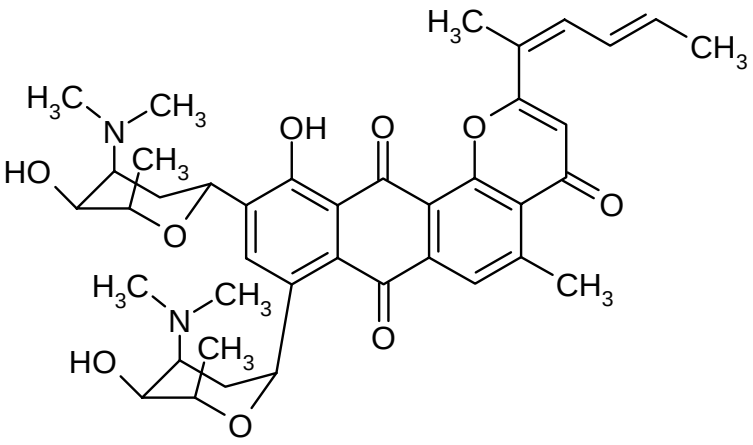


287

Rubiflavinone C-1 (**286**) is slowly isomerized into rubiflavinone C-2 (**287**), when left in $CDCl_3$ solution for some days. Thus (*Z*)-double bond between C-17 and C-18 in **286** was converted into the more stable (*E*)-form of **287**. This indicated that rubiflavinone C-2 (**287**) might be an artefact. Both compounds are investigated as antitumor agents[307]. Rubiflavinone C-1 is the aglycone of many biologically active compounds e.g. rubiflavin C-1 (**288**)[307].

Table 12: ^{1}H NMR data ($CDCl_3$) of rubiflavinone C-1 (**286**) and rubiflavinone C-2 (**287**) [*J* in Hz].

	rubiflavinone C-1 (**286**) exp.	rubiflavinoneC-1 (**286**)[307]	rubiflavinone C-2 (**287**)[307]
H-C (3)	6.42 (s)	6.45 (s)	6.42 (s)
H-C (6)	8.03 (d, *J* = 0.5)	8.05 (d, *J* = 0.5)	8.05 (d, *J* = 0.5)
H-C (8)	7.82 (dd, *J* = 2, 7.5)	7.84 (dd, *J* = 2, 7.5)	7.84 (dd, *J* = 2, 8)
H-C (9)	7.68 (t, *J* = 8)	7.69 (t, *J* = 8)	7.68 (t, *J* = 7.5)
H-C (10)	7.35 (dd, *J* = 2, 8)	7.35 (dd, *J* = 2, 8)	7.37 (dd, *J* = 2, 8)
HO-C 11)	12.90 (s)	13.3 (s)	13.0 (s)
CH_3 (13)	3.02 (d, *J* = 0.5)	3.02 (d, *J* = 0.5)	3.02 (d, *J* = 0.5)
CH_3 (15)	2.07 (br s)	2.07 (br s)	2.08 (br s)
H-C (16)	8.42 (br d, *J* = 12)	8.45 (br d, *J* = 12)	7.96 (br d, *J* = 11)
H-C (17)	6.54 (ddq, *J* = 12, 11,1)	6.54 (ddq, *J* = 12, 11,1)	6.59 (ddq, *J* = 11, 15,1)
H-C (18)	6.08 (dq, *J* = 11, 7)	6.10 (dq, *J* = 11, 7)	6.38 (dq, *J* = 15, 7)
CH_3 (19)	2.16 (dd, *J* = 7, 1)	2.21 (dd, *J* = 7, 1)	1.99 (br.d, *J* = 7)

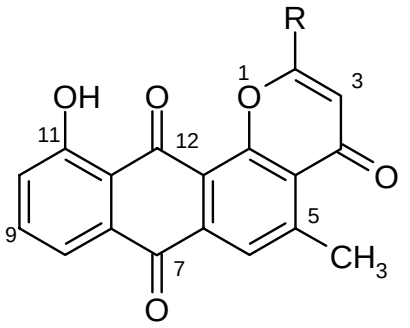


288

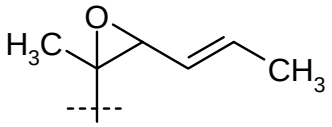
4.24.2 Saptomycin F

Along with **286,** compound **289** was obtained as a yellow amorphous minor product with a close structural similarity to **286**. The ^{1}H NMR spectrum showed the singlet of a *peri*-hydroxy group at δ 12.85, a 1H singlet at δ 8.08 (6-H), three correlated aromatic signals of a tri-substituted phenolic moiety (δ 7.82, 7.68 and 7.36), a 1H singlet at δ 6.53 (3-H), in addition to the singlet of a *peri*-methyl group at δ 2.98. This established the fragment **A** substituted at position-2 of the γ-pyrone. The fragment A is a common chromophore of many antibiotics[23]. The ^{1}H NMR spectrum displayed additionally, two olefinic multiplets at δ 6.05 and 5.42, in addition to a 1H doublet at δ 4.24 (^{3}J = 8.0 Hz), which could be assigned as oxymethine. Finally, two

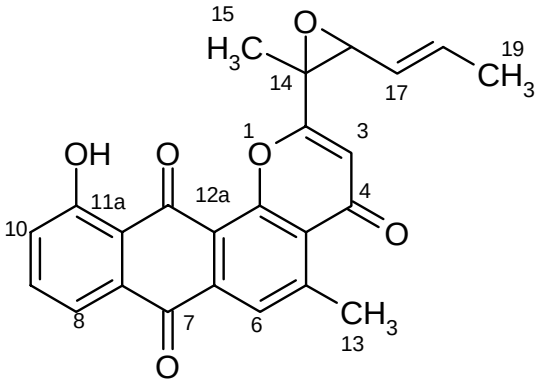
methyl groups were detected at δ 1.89 and δ 1.83, pointing to their linking to sp^2 carbons. The first of them was displayed as doublet, as indication of its linking to an olefinic methine (=CH-CH_3), while the other was observed as singlet. Based on the latter ^{1}H NMR data, the fragment **B** might be constructed. This is attributed to the blocked C-14, and the doublet proton at δ 4.24 should be linked to oxygen. But the up field shifting of this methine pointed to an epoxide, because hydroxy methines shifted normally down field (δ 5.01) as in spatomycin A (**297**). Hence, fragment **B** could be assigned.



A

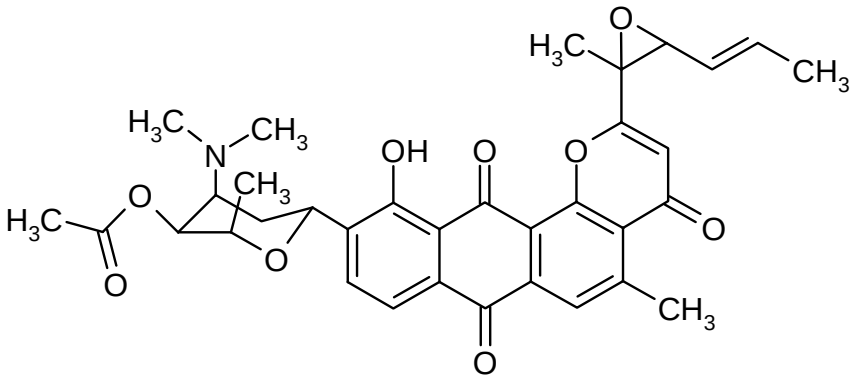


B



289

The (-)-ESI mass spectrum fixed the molecular weight of compound **289** as 402 Dalton. In accordance to a search in AntiBase, compound **289** was elucidated as saptomycin F[309]. Saptomycin F (**289**) is also observed as an aglycone of several antibiotics e.g. saptomycin-D (**290**), a family of compounds characterised by their antimicrobial and potent antitumor activities against human or murine tumor cell lines. In particular, saptomycin D (**290**) was the most effective component of all saptomycins. Saptomycins are closely related to the pluramycin antibiotics, which have remarkable antitumor activities[310].



290

4.24.3 ε-Indomycinone

Compound **291** was obtained as a yellow amorphous solid from fraction II. The compound exhibited a reddish-orange UV absorption. It turned to red by treatment with sodium hydroxide, as an indication of a *peri*-hydroxyquinone, possibly closely related to **286** and **289**. The UV absorption showed peaks at λ 447 and 423 nm, indicating the presence of a quinone chromophore, in addition to one peak at λ 270 nm, due to the presence of an aromatic system.

The IR spectra showed an absorption band due to a hydroxyl group at ν 3440 cm^{-1}, and three bands at ν 1736, 1720 and 1650 cm^{-1}, are characteristic for carbonyl groups. Several multiplets between ν 1582 and 1457, in addition to a band at ν 1380 cm^{-1}, are indicative for methyl groups.

The ^{1}H NMR spectrum of compound **291** showed a *peri*-hydroxy group at δ 12.92. Besides, the singlet of a *peri*-proton appeared at δ 8.08, in addition to three aromatic hydrogens with signals at δ 7.82, 7.68 and 7.37, which were indicative for a 1,2,3-trisubstituted aromatic ring. Moreover, a 1H singlet at δ 6.42 was observed, which could be assigned as α-olefinic proton conjugated with carbonyl group. Furthermore, the singlet of a *peri*-methyl group was detected at δ 3.02. Based on the ^{1}H NMR data above, the aglycone component **A** (substituted at C-2) might be established. In addition to the above spectral data, one aromatic dq signal of 1H was detected at δ 7.52 (^{3}J = 7.6 Hz), pointing possibly to an olefinic proton present in direct conjugation to an aromatic system. The observed splitting of the proton could be attributed to an attached methyl group, which was definitely observed as doublet at δ

2.05 (^3J = 7.6 Hz). Finally, one additional methyl singlet was detected at δ 1.98 which must be linked to an sp^2 carbon. This pointed to the fragment **B**.

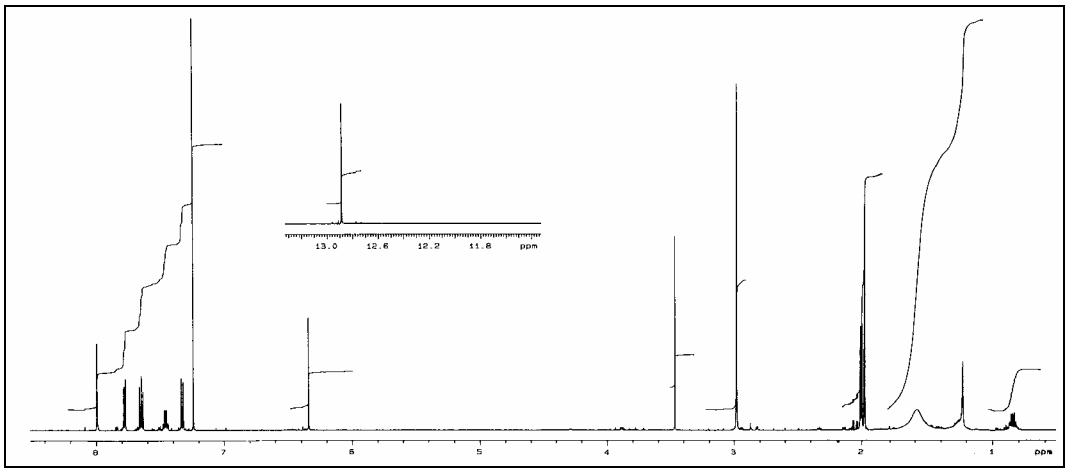


Figure 147: ^{1}H NMR ($CDCl_3$, 600 MHz) for ε-indomycinone (**291**)

The ^{13}C/APT NMR spectra of compound **291** exhibited ~22 carbons, which were classified into two categories i.e. nineteen sp^2 and three sp^3 methyl carbons. Three carbonyl carbons were detected at δ 187.4, 181.9 and 179.7, of which the first two are indicative of a quinone. According to their shift difference, one of them might have a *peri*-hydroxy group, while the third carbonyl is suggestive for γ-lactone. In addition, six sp^2 methine carbons are distinguishing for fragment **A**, while the sixth one at δ 134.5 is could be of the olefinic methine present in the double of fragment **B**. Additionally, ten quaternary sp^2 carbon signals were displayed, of which three oxygenated were detected at δ 164.1, 162.6 and 156.2. Finally, three methyl carbons were observed at δ 24.2, 15.0 and 12.18.

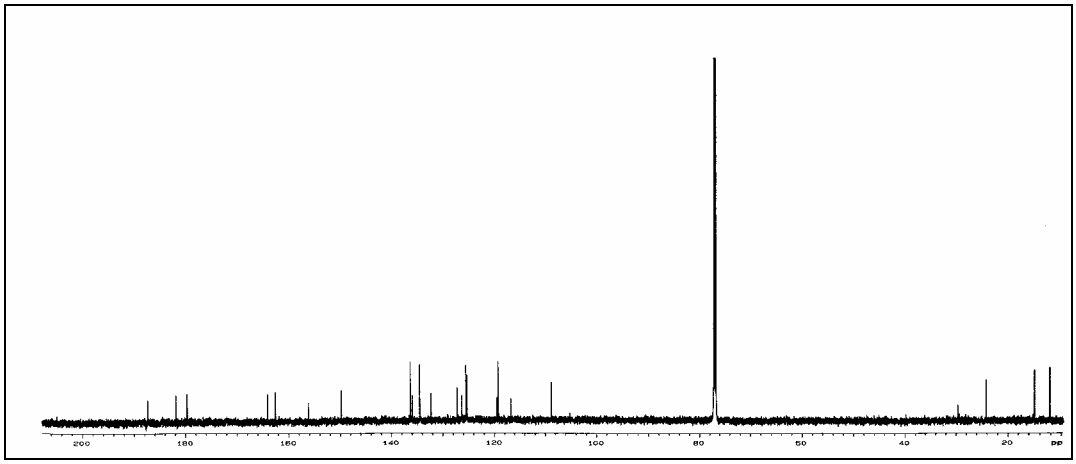


Figure 148: ^{13}C NMR spectrum ($CDCl_3$, 600 MHz) for ε-indomycinone (**291**)

The molecular weight of compound **291** was fixed as 360 Dalton by ESI and EI mass spectra. The EI mass spectrum exhibited a further fragment at *m/z* 281 ([M-C_6H_7]) as base peak (Figure 149). The EIHR MS established the molecular formula of compound **291** as $C_{22}H_{16}O_5$. A submission of the above spectral to AntiBase searching was without any results. This pointed to **291** as a new natural product.

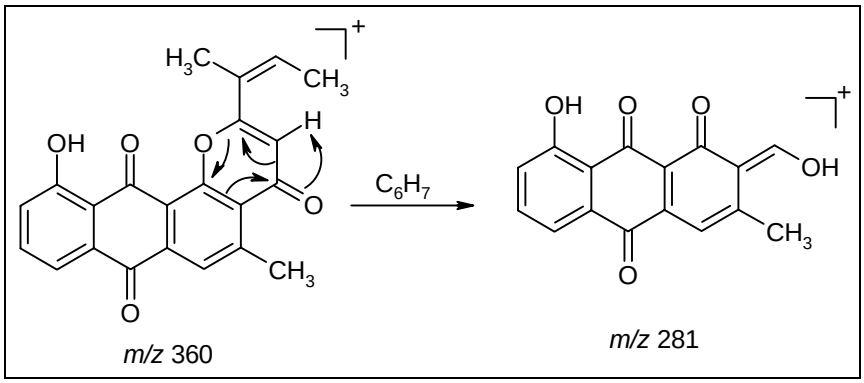


Figure 149: EI MS fragmentation pattern of ε-indomycinone (**291**)

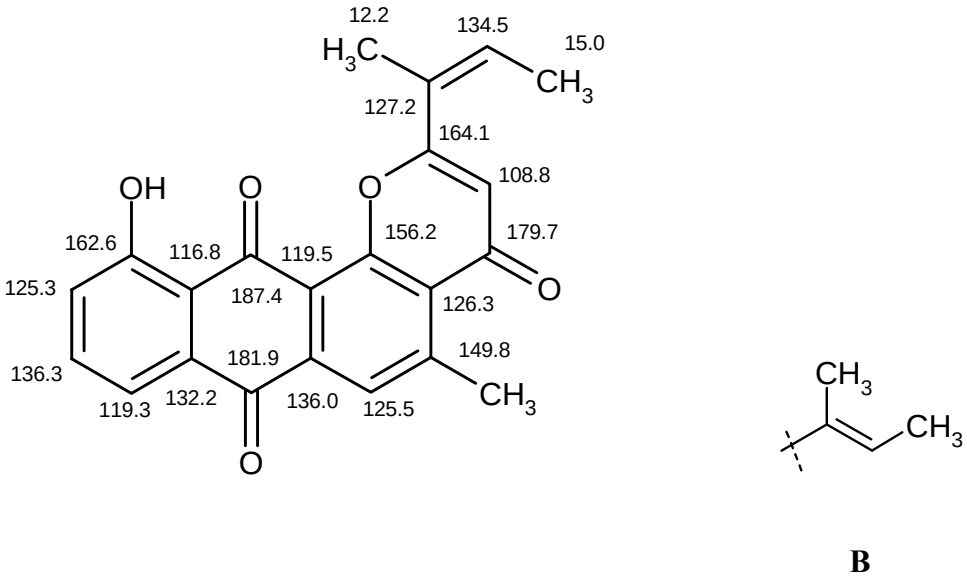
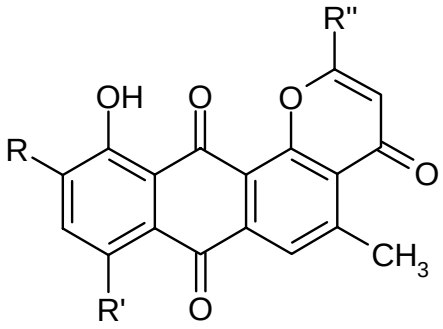
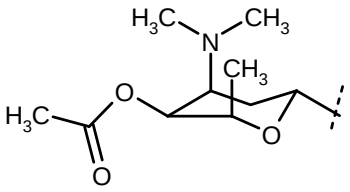
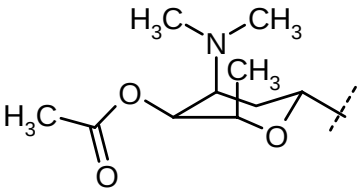
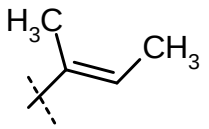
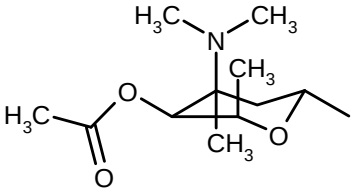
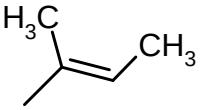
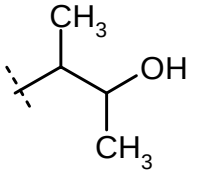


Figure 150: ^{13}C NMR sequence shifts of ε-indomycinone (**291)**

Based on the detailed spectral data, molecular formula and comparison with similar structural patterns from the literature[309], compound **291** was assigned as ε-indomycinone (**291**). The only diviation was detected in ^{13}C NMR of compound **291**, is the chemical shift of C-2 (δ 164.1), which normally exhibited at δ 172. The up-field chemical shift of C-2 in **291**, was due to its conjugation with the unsaturated fragment **B**, as in the case of saptomycin G (**293**)[309]. ε-Indomycinone (**291**) is found as an aglycone of many antitumor agents e.g. rubiflavin B (kidamycin) (**292**), sapto-

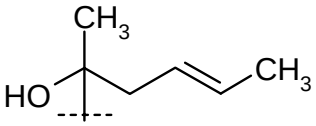
mycin G (**293**), and as a dehydrated analogous of the antiherpetic agent, AH-1763 IIa (**294**)[311].



	R	R'	R"
292			
293		H	
294	H	H	

4.24.4 *β*-Indomycinone

The yellow amorphous compound **295** was obtained as fourth *peri*-hydroxyquinone from fraction II. The ^{1}H NMR spectrum of **295** showed a close structural similarity to the previously mentioned quinones **286**, **289** and **291**. It showed singlets at δ 12.85 (OH) and δ 8.08 (H-6), three *ortho*-coupled aromatic protons at δ 7.83, 7.70 and 7.38, a 1H singlet at δ 6.56, and the singlet of a *peri*-methyl group at δ 3.02, establishing the fragment **A**. In addition, two multiplets of an olefinic double bond were detected at δ 5.74 and 5.38. In the aliphatic region, two methylene protons of an ABX system were detected at δ 2.91 and 2.78. Finally, two methyl groups linked to sp^2 carbons were displayed at δ 1.72 and 1.64, of which the first gave a singlet, and the second as doublet. So, the fragment **C** could be fixed.



C

The two fragments **A** and **C** were further confirmed on the basis of ^{13}C/APT NMR spectra. It showed two quinone carbonyls at δ 187.3, 181.6, and at δ 179.2 the signal of a γ-lactone. Furthermore, three quaternary sp^2 oxy-carbons at δ 171.9, 162.6 and 156.0 were found. Additionally, five quaternary sp^2 carbons were observed, in addition to seven sp^2 methines, from which five were of the aromatic part **A**, and the other two methine carbons were assigned as olefinic double bond. In the aliphatic region, it exhibited one quaternary sp^3 oxy-carbon and one methylene carbon in addition to three methyl carbon signals.

The molecular weight of compound **295** was established as 404 Dalton by CI and EI mass spectra. The EI MS of compound **295** showed two additional fragments at *m/z* 350 $[M\text{-}C_4H_6]^+$ and 349 $[M\text{-}C_4H_7]^+$. On the basis of the detailed spectral data of compound **295** and a search in AntiBase, it was identified as *β*-indomycinone[307,308].

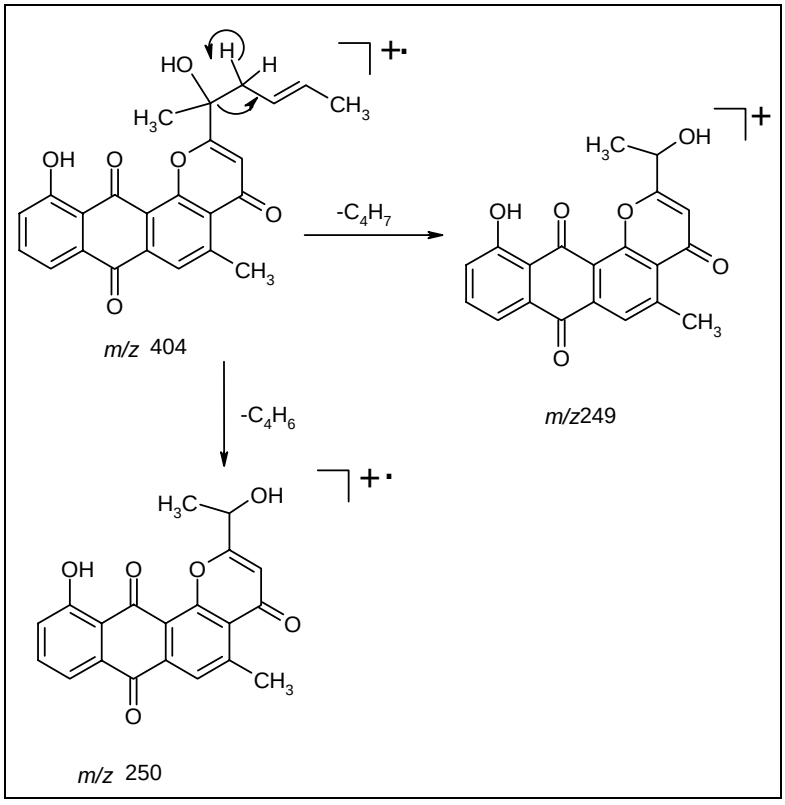
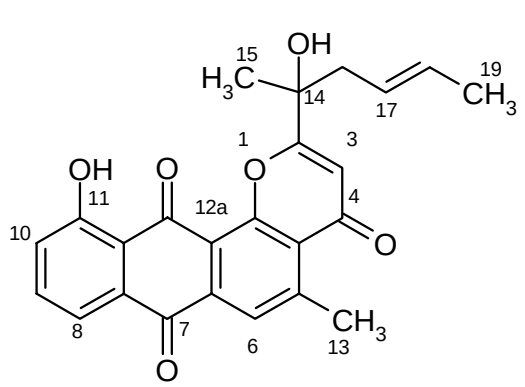
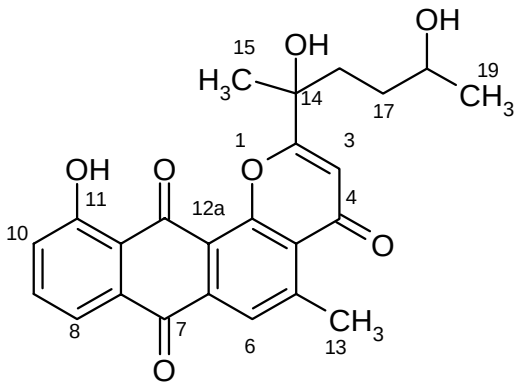


Figure 151: EI MS fragmentation pattern of *β*-indomycinone (**295**)



295



296

β-Indomycinone (**295**) belongs to the pluramycins, a group of structurally highly diverse reactive agents, possessing antimicrobial and anticancer activity[312]. *δ*-Indomycinone[313] (**296**) is a hydrated analogues of *β*-indomycinone (**295**).

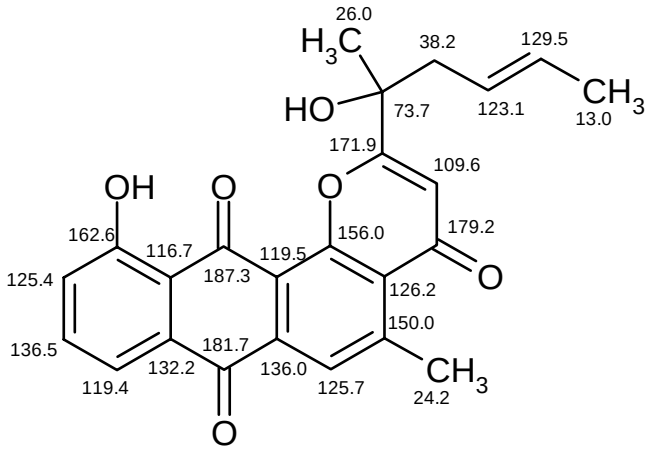


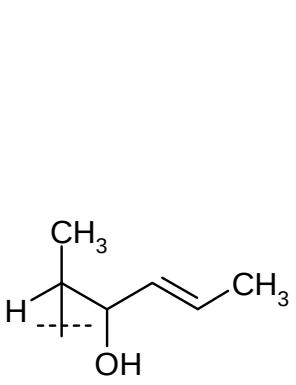
Figure 152: ^{13}C NMR shifts of *β*-indomycinone (**295**)

4.24.5 Saptomycin A

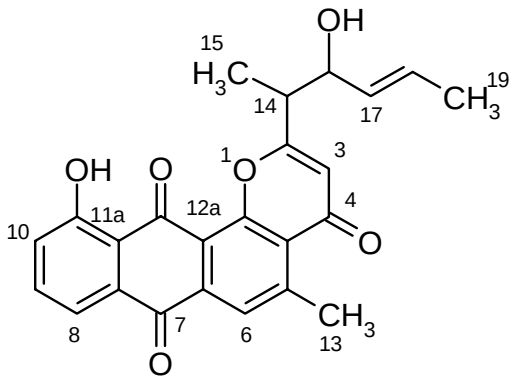
Compound **297**, a fifth *peri*-hydroxyquinone was obtained as a yellow solid from fraction II. It exhibited a close structural similarity to the hydroxyquinones mentioned above. The ^{1}H NMR spectrum showed nearly the same signal pattern as in saptomycin F (**289**). A singlet of a *peri*-hydroxy group at δ 12.64 was exhibited. In the aromatic region, a 1H singlet at δ 8.09, three aromatic protons at δ 7.83, 7.69 and 7.36 of an 1,2,3-trisubstituted aromatic ring, a singlet at δ 6.28 (3-H), in addition to the singlet of a *peri*-methyl group at δ 3.02 (13-H_3), served to construct the fragment **A**. In addition, two multiplets of an olefinic double bond were detected at δ 5.65 and 5.50. Moreover, two methine protons were displayed at δ 5.01 and 2.98, at which the first could be oxygenated, while the second was a methine flanked most likely by

two sp^2 carbons. Finally, two methyl doublets were detected at δ 1.71 and 1.45, of which the first could be attached to an sp^2 carbon, pointing to the possible fragment **D**.

Based on the ^{13}C/APT NMR spectra of compound **297**, the two suggested fragments **A** and **D** were connected as in *β*-indomycinone (**295**). The only exception was found in the aliphatic part **D** which displayed two methine carbons at δ 69.0 and 45.1, of which the first one could be assigned to an oxygenated sp^3 methine carbon. The molecular weight of compound **297** was fixed as 404 Dalton by CI mass spectrum. The corresponding molecular formula of compound **297** was designated as $C_{24}H_{20}O_6$, according to the Rule of 13[146]. Finally, based on the revealed spectral data and molecular formula of compound **297**, and search in AntiBase, directed to saptomycin A (**297**). This compound was further confirmed by comparing the data with the literature[309].



D



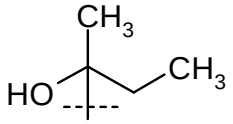
297

4.24.6 γ-Indomycinone

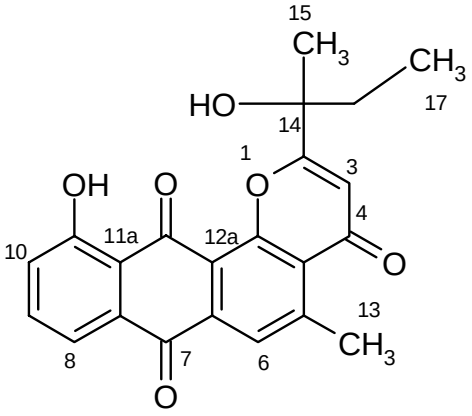
A final screening of fraction II resulted in compound **298**, as an additional yellow solid. It showed the same chromatographic properties as the *peri*-hydroxyquinones mentioned above. The ^{1}H NMR spectrum exhibited the singlet of a chelated OH group at δ 12.83, a 1H singlet at δ 8.09, of a *peri*-arotmatic proton to a carbonyl group. Three protons of an 1,2,3-trisubstituted phenolic moiety, a 1H singlet at δ 6.52 (3-H), in addition to the singlet of a *peri*-methyl group at δ 3.04, pointing to **298**, containing the same fragment **A**, substituted at 2-position. Moreover, two methylene protons, giving ABX systems, were observed at δ 2.10 and 1.98, methyl triplet at δ 0.97, to construct a terminal ethyl group. Finally, a singlet methyl

group was detected at δ 1.67, constructing fragment **E** [(1-hydroxy-1-methyl)-propyl].

The molecular weight of compound **298** was fixed as 378 Dalton using both CI and EI mass spectra. The EI mass spectrum of compound **298** showed further two fragments at *m/z* 350 and 349, as a result from the expulsion of carbonyl (CO) and aldehyde (CHO) groups, respectively. An additional peak was detected at *m/z* 281, pointing to the same fragment as in ε-indomycinone (**291**) (Figure 149). In accordance, and search in AntiBase led to the elucidation of compound **298** as γ-indomycinone[308] .



E



298

4.25 Terrestrial *Streptomyces* sp. GW22/3234

In the chemical screening, the terrestrial *Streptomyces* isolate GW22/3234 exhibited numerous UV absorbing bands, of which one showed a strongly greenish fluorescent band under UV, which turned to blue by anisaldehyde/sulphuric acid. Moreover, two low-polar red-orange bands were detected, which turned to blue/violet by treatment with sodium hydroxide, pointing to *peri*-hydroxyquinones, in addition to an UV absorbing band, which was stained to yellow-orange by Ehrlich's reagent. Additionally, the strain extract possessed high activities against all tested microoganisms i.e. *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus, Streptomyces viridochromogenes* (Tü57), *Mucor miehei* (Tü284), *Candida albicans, Chlorella vulgaris*, *Scenedesmus subspicatus,* and *Chlorella sorokiniana*.

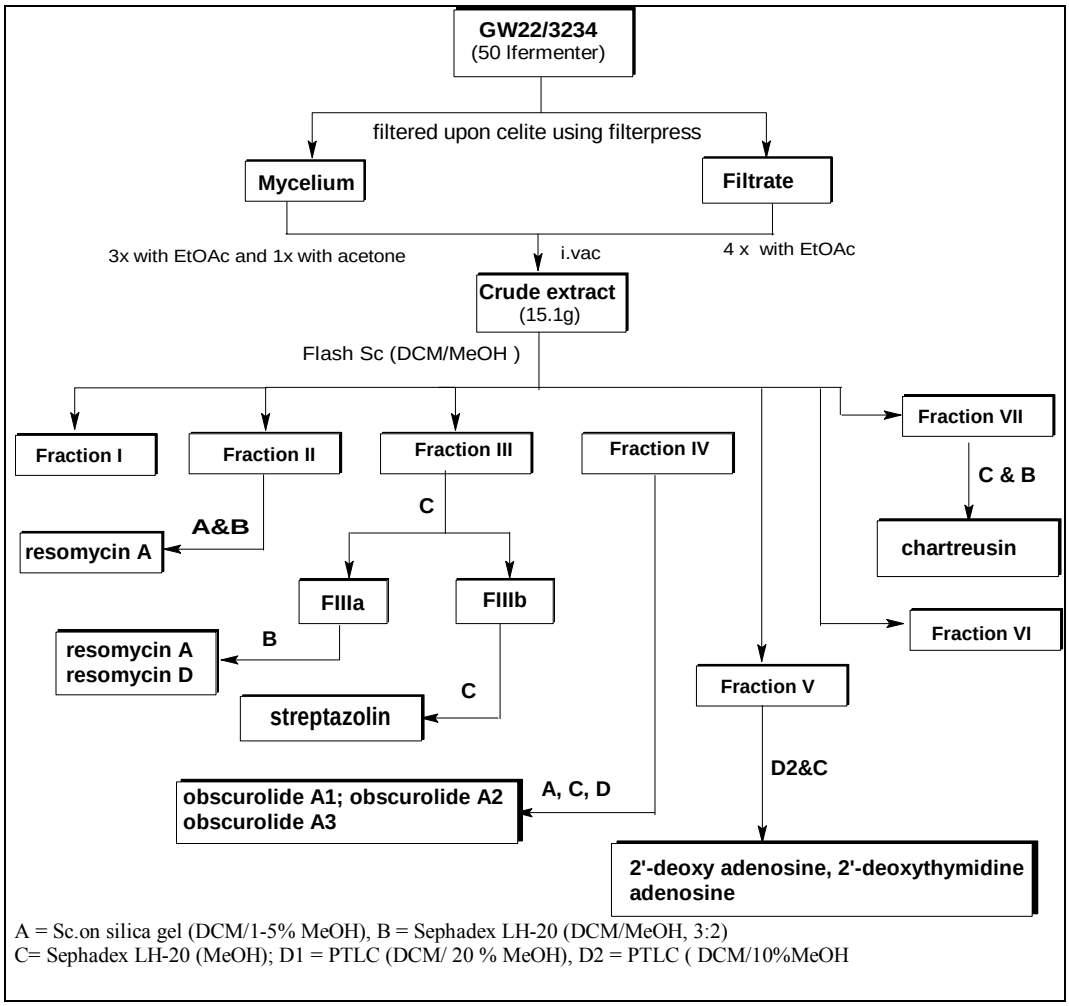


Figure 153: Work-up procedure for terrestrial *Streptomyces* isolate GW22/3234

The strain was cultivated on a 50-liter scale (jar fermenter) at 28 °C for 72 hours. After filtration and extraction with ethyl acetate, the brown extract was chromatographed using flash silica gel column, and eluted with dichloromethane-methanol, with a stepwise gradient of increasing polarity. As a result, seven fractions were obtained. Working up and purification of the fractions, led to the isolation of resomycin A (**299**), resomycin D (**302**), streptazolin (**303**), obscurolide A1 (**305**), obscurolide A2 (**306**), obscurolide A3 (**307**) and chartreusin **(308**). In addition to the previously discussed metabolites, furan-2,5-dimethylaclohol (**114**), phenyl acetic acid (**49a**), 2'-deoxyadenosine (**87**), 2'-deoxythymidine (**95**) and adenosine (**242**) were also detected.

4.25.1 Resomycin A

Compound **299** was obtained as a low polar orange amorphous solid from fraction II. It showed a yellow UV fluorescent band, which turned to violet by sodium hydroxide, pointing to a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of compound **299** showed two singlets of chelated hydroxy groups at δ 12.5 and 12.1. In the aromatic region, it showed aromatic proton signals of an 1,2,3-trisubstituted aromatic system, as well as a 1H singlet at δ 7.62. In the aliphatic region, it showed two methyl singlets at δ 3.75 and 1.42, of which the former could be assigned as methyl ester, and the latter was connected with a quaternary sp^3 oxy-carbon. Furthermore, a 1H broad singlet at δ 3.91, connected with an oxymethine carbon or located between two sp^2 carbons, was detected. Additionally, two methylene groups, split as four 1H double doublets, were observed at δ 3.06, 2.88, 2.34 and 1.92, which must be in a ring system.

The ^{13}C NMR spectrum of compound **299** showed ~21 carbon signals. Two carbonyl groups of a quinone at δ 192.8 and 181.6 with a shift difference Δδ 11.3, indicated that both hydroxyl groups must be at the same side of the chromophore. In addition, an ester carbonyl was detected at δ 171.6. Besides, two oxygenated quaternary sp^2 carbons were displayed at δ 162.5 and 161.0. Additionally, four sp^2 methine carbons and six sp^2 quaternary carbons, were exhibited. In the aliphatic region, one quaternary oxygenated carbon was displayed at δ 69.6, in addition to a methine carbon signal at δ 57.5 which could be located between two sp^2 carbons. Finally, two methylene and two methyl carbons were found, of which one was assigned as a methoxy group.

The molecular weight of compound **299** was established as 382 Dalton, based on the ESI mass spectra. Searches in AntiBase and DNP resulted in two diastereoisomers, resomycin A (**299**) [314] and resomycin B (**300**). However, the H,H COSY correlation, and direct comparison of the spectral data with a recent reference pointed to resomycin A (**299**) (Figure 155).

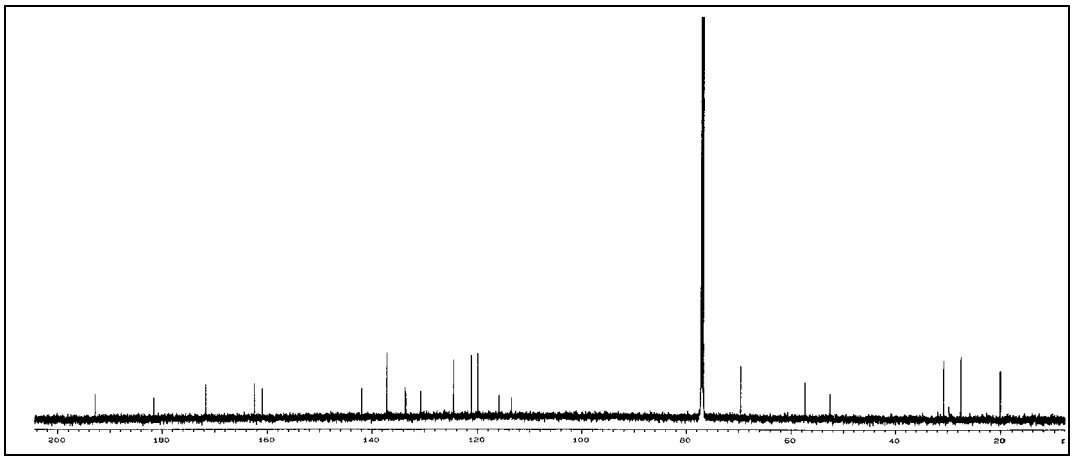


Figure 154: ^{13}C NMR Spectrum ($CDCl_3$, 150 MHz) for resomycin A (**299**)

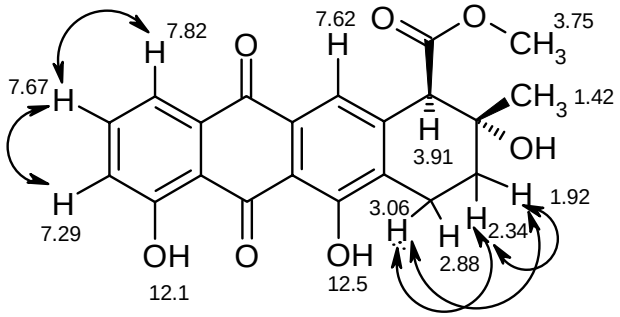
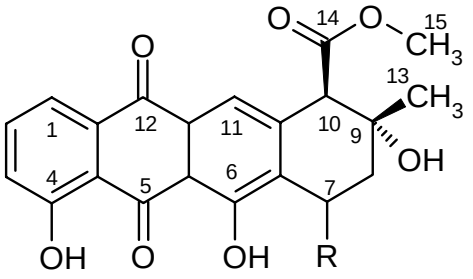
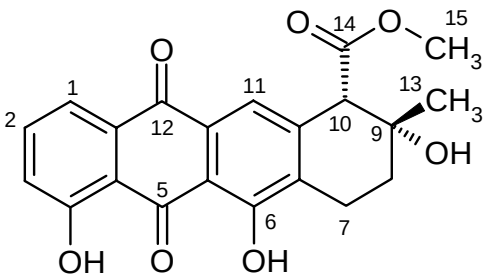


Figure 155: H,H COSY correlations of resomycin A (**299**)



299: R = H; **301**: R = OH



300

Resomycin A **299** was found as the chromophore of several anthracycline antibiotics[315,316] e.g. auramycinone (**301**). Resomycin A (**299**) was isolated in our group for the first time as a natural product[314], however, was known from synthesis[317] before.

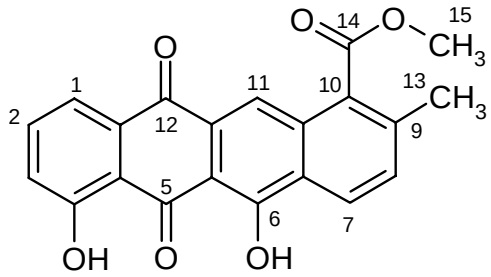
4.25.2 Resomycin D

Compound **302**, an additional orange solid minor product along with **299**, was isolated from fraction III. It turned to violet by sodium hydroxide, as a sign of a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of **302** showed a close structural similarity with resomycin A (**299**). Two chelated hydroxyl groups at δ 13.76 and 12.26 were displayed. Furthermore, two aromatic *ortho*-coupled protons of an 1,2,3,4-tetrasubstituted aromatic system, were exhibited at δ 8.50 and 7.56. Additionally, three aromatic protons of an 1,2,3-trisubstituted benzene system were detected at δ 7.88, 7.71 and 7.32. An additional deep field singlet of 1H at δ 8.26 was found, pointing to a possible third aromatic ring. In the aliphatic region, two methyl singlets were observed at δ 4.18 and 2.57, of which the first might be a methoxy group and the latter could be attached to sp^2 carbon or nitrogen.

(-)-ESI MS of **302** established the molecular weight as 362 Dalton, which is 20 au lower than for resomycin A (**299**). This pointed to a dehydrated and dehydrogenated product of **299** [resomycin A-(H_2O + H_2)]. Correspondingly, the methine singlet in resomycin A (10-H) was missed, as well as the two methylene protons of 7-H_2 and 8-H_2, giving a fully aromatized compound.

A search in AntiBase resulted in resomycin D[314] (**302**), a dehydrated structure from auramycinone (**301**). However, the isolate GW22/3234 did not produce **301** or other related compounds, which might act as a precursor of **302** during work-up. The latter is therefore not an artefact, but also a natural product. Resomycin D (**302**) was previously known as bisanhydro-auromycinone[318], a typical degradation product of anthracyclines formed during fermentation and workup.



302

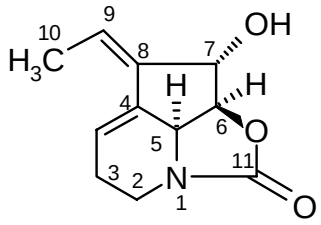
4.25.3 Streptazolin

Streptomyces sp. GW22/3234 provided a number of potentially useful natural products. During the screening of fraction IIIb, compound **303** was detected as UV absorbing band, which turned to orange by anisaldehyde/sulphuric acid, and to yellow by Ehrlich's reagent. It was isolated as colourless oil.

The ^{1}H NMR spectrum of compound **303** displayed two 1H multiplets of two olefinic protons at δ 6.08 and δ 5.92, in addition to three oxygenated or nitrogenous sp^3 methine protons at δ 4.65 (br, s), 4.6 (d) and 4.18 (d). A 2H multiplet of a methylene group was displayed at δ 3.28, which could be linked to nitrogen. Furthermore, an ABX-systems of an X-coupled methylene group was displayed at δ 2.38 and 2.13. Finally, a doublet of a methyl group, linked to an olefinic sp^2 carbon, was displayed at δ 1.75.

The ^{13}C/APT NMR spectra of compound **303** showed a quaternary carbon signal at δ 159.5, which might be due to an imide or oxamide group. In addition, four sp^2 carbon signals were detected, from which two were methines at δ 123.4 and 117.7, and the other two were quaternary at δ 142.8 and 137.9. Additionally, three sp^3 methine carbons were displayed at δ 82.0, 73.6 and 58.8, of which the first two could be oxygenated, while the third is possibly connected with nitrogen. Two methylene carbons were detected at δ 39.6 and 22.4, and a methyl carbon signal was observed at δ 14.5, which could be linked to sp^2 carbon.

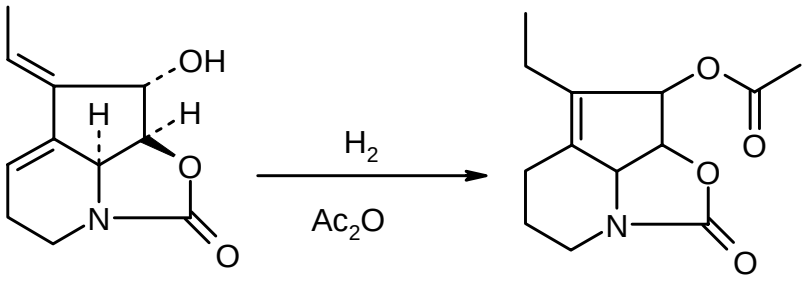
The (+)-ESI mass spectrum fixed the molecular weight of compound **303** as 207 Dalton. Based on the Rule of 13[146], the empirical molecular formula of compound **303** could be assigned as $C_{11}H_{13}NO_3$. On the basis of the spectral data of compound **303**, and searching in AntiBase, it was elucidated as streptazolin (**303**), which was further confirmed by comparison with literature data.



303

The antibiotic streptazolin (**303**) was isolated first from cultures of *Streptomyces viridochromogenes* strain Tü1678 in 1981[350]. Purification of compound **303** was rather difficult, because of its tendency to undergo partial polymerization in concentrated solution. In dilute solution at ambient temperature, however, streptazolin **303** proved to be stable for several days. The absolute configuration of **303** was elucidated by X-ray analysis[319], after reduction and acetylation to O-

acetyldihydrostreptozolin (**304**). Several syntheses of streptazolin (**303**) were carried out [320-322].



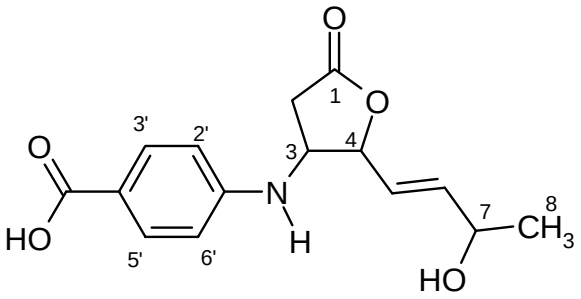
303 **304**

4.25.4 Obscurolide A1

Screening of fraction IV led to an UV absorbing middle polar broad pale yellow zone, which turned to blue by anisaldehyde/sulphuric acid, and to yellow by Ehrlich's reagent. Purification of the fraction using PTLC and Sephadex led to three faint yellow solids **305**, **306** and **307**. They exhibited a good solubility in acetone, methanol and dimethyl sulfoxide.

The ^{1}H NMR spectrum of compound **305** showed two 2H doublets of aromatic protons at δ 7.83 and 6.65, indicating an 1,4-disubstituted aromatic ring. In addition, a multiplet of two olefinic double bond protons was exhibited in the range of δ 5.75-6.01. Furthermore, three 1H multiplets were detected at δ 4.83, 4.31 and 4.17, and assigned as oxygenated methines. The ABX signal of a CH_2-CH fragment was at δ 3.07 and 2.42, indicated a methylene group embedded in a ring system. Finally, a doublet (with a slight further allyl splitting) of a methyl group at δ 1.15-1.22 was observed.

The molecular weight of compound **305** was fixed as 291 Dalton using (-)-ESI MS. Based on the detailed spectral data of compound **305,** and search in AntiBase, led to its elucidation as obscurolide A1 **(305**), which was further established by comparison with the literature[323].



305

4.25.5 Obscurolide A2

Compound **306** was obtained from fraction IV. The ^{1}H NMR spectrum indicated a close structural similarity with obscurolide A1 (**305**), except for the presence of a further 1H deep-field singlet at δ 9.85, which was not exchangeable and could be assigned as an aldehyde.

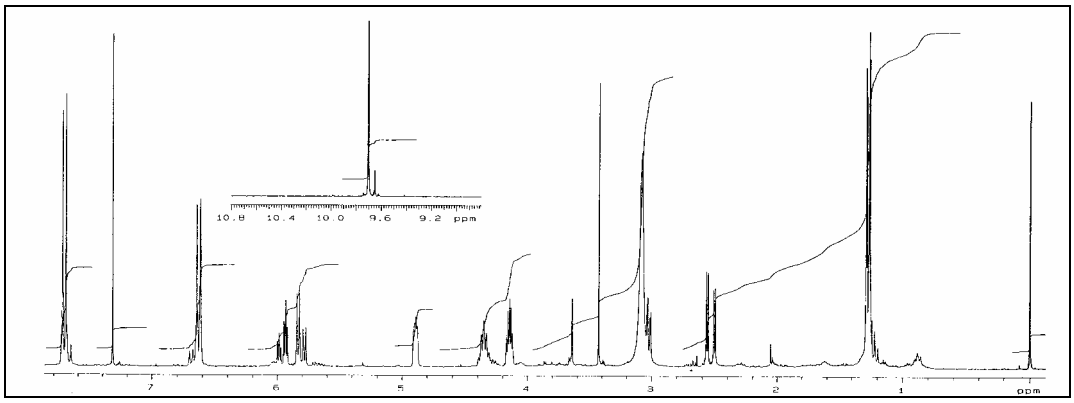
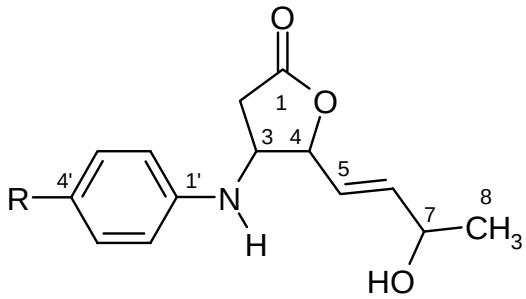


Figure 156: ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of obscurolide A2 (**306**)

EI mass spectrum of compound **306** delivered the mass of 275 Dalton. The molecule ion delivered a fragment at *m/z* 257, due to an elimination of water, in addition, two other peaks were observed at *m/z* 174 and 147 (Figure 157). A search in AntiBase led to interpretation of compound **306** as obscurolide A2 [323].



306: R = CHO, **307**: R = CH_2OH

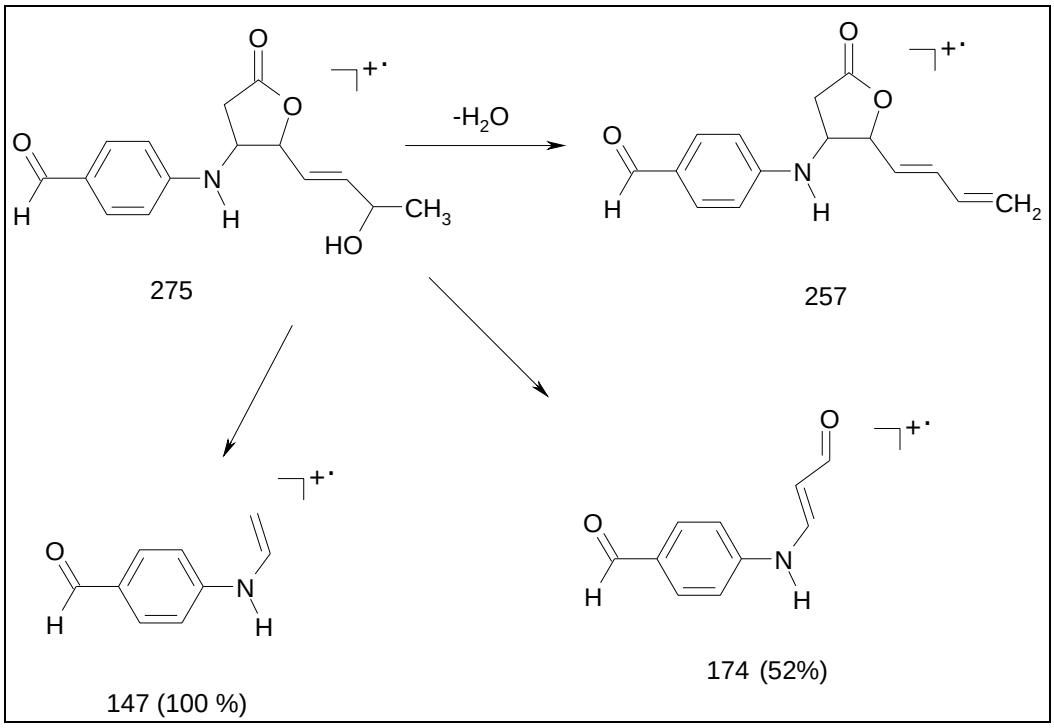


Figure 157: EI MS fragmentation pattern of obscurolide A2 (**306**)

4.25.6 Obscurolide A3

The ^{1}H NMR spectrum of compound **307** showed also a very close structural similarity to the two above mentioned compounds **305** and **306,** except that it exhibited a singlet of an oxymethylene group at δ 4.45, located at 4'-position of the benzene ring. The molecular weight of compound **307** was determined as 277 Dalton using ESI MS. Applying the spectral data of compound **307** to AntiBase led to its elucidation as obscurolide A3[323]. The obscurolides A1-A3 (**305-307**) were isolated first from the culture broth of *Streptomyces viridochromogenes* (strain Tü2580), as novel butyrolactone derivatives[324,325], with phosphodiesterase inhibitory activity.

4.25.7 Chartreusin

During the working up of fraction IV, compound **308** was detected as strongly green UV fluorescent band, which developed a blue colour by spraying with anisaldehyde/sulphuric acid and to reddish-brown with sulphuric acid red, however, it showed no colour change with sodium hydroxide. This pointed most likely to a *peri*-hydroxy xanthone moiety, present in linear or angular form (**A**, **B**, page 95). In the antimicrobial assay, it showed a high activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Streptomyces viridochromogenes* (Tü57). After

application the fraction to Sephadex LH-20, compound **308** was isolated as yellow-green solid.

The ^{1}H NMR spectrum showed a broad singlet at δ 11.55 of a *peri*-hydroxy group (6-OH). In the aromatic region, it revealed four 1H doublets at δ 8.12, 7.75, 7.65 and 7.54, and a 1H triplet at δ 7.75. This is an indicative for two aromatic rings, one of which is 1,2,3-trisubstituted, and the other is a 1,2,3,4-tetrasubstituted ring system, which was confirmed by the H,H COSY spectrum, to give the fragments **A** and **B** (Figure 159). In the sugar region, two 1H doublets were observed at δ 5.44 and 5.39, pointing most likely to anomeric protons. In addition, eight oxymethine protons appeared in the range of δ 4.83-3.57. Two methyl singlets were observed at δ 3.15 and 2.83, of which the first is assigned as methoxy linked to sp^3 carbon, while the latter is linked to an sp^2 carbon, present in *peri*-position to a carbonyl group. Finally, two methyl doublets were detected at δ 1.23 and 0.97, which are further suggestive for two sugar units.

The ^{13}C/APT NMR spectra of compound **308** showed that the compound comprised two main parts, an aromatic aglycone, and a sugar part. The spectra exhibited ~32 carbon signals.

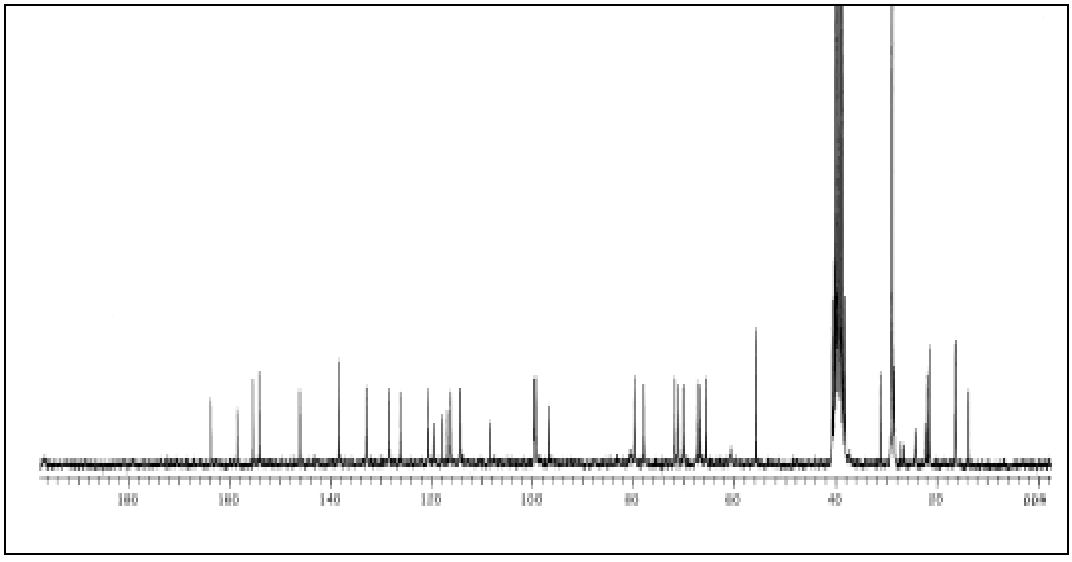


Figure158: ^{13}C NMR Spectrum ([D_6]DMSO, 50 MHz) of chartreusin (**308**)

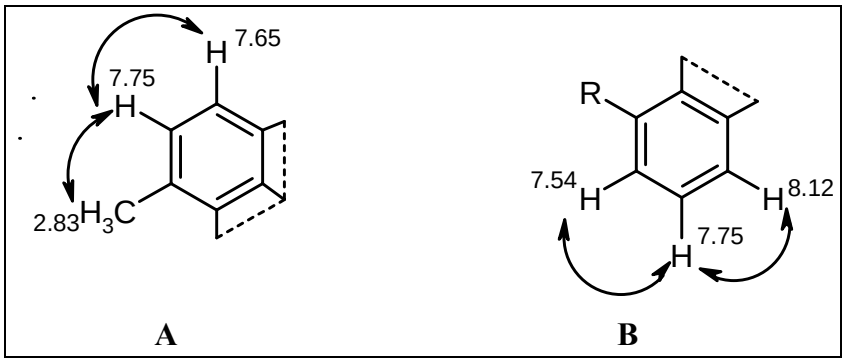
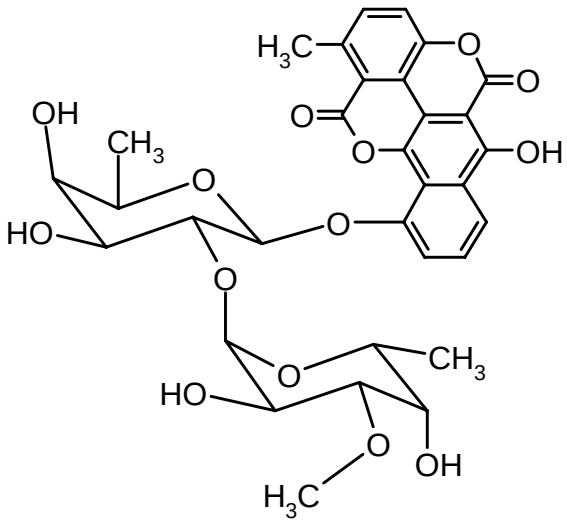


Figure 159: H,H COSY correlations of the aromatic part of chartreusin (**308**)

The molecular weight of compound **308** was determined as 640 Dalton, based on ESI mass spectra. According to the ESI MS^2 fragmentation (Figure 160), two sugar units were established as fucose and digitalose. Based on these data, and a search in AntiBase, compound **308** was elucidated as chartreusin, which was further confirmed by comparison with authentic spectra.



308

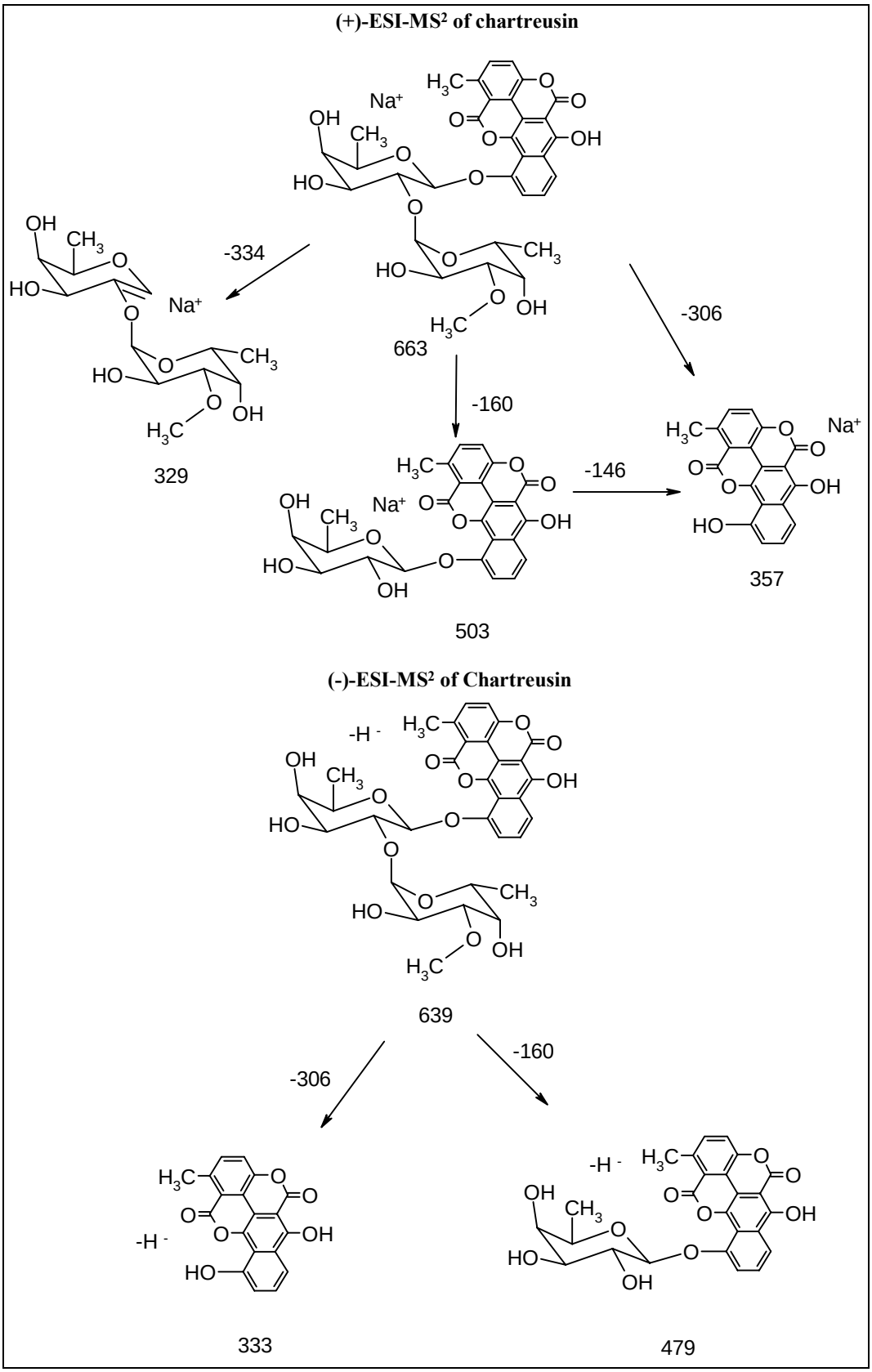
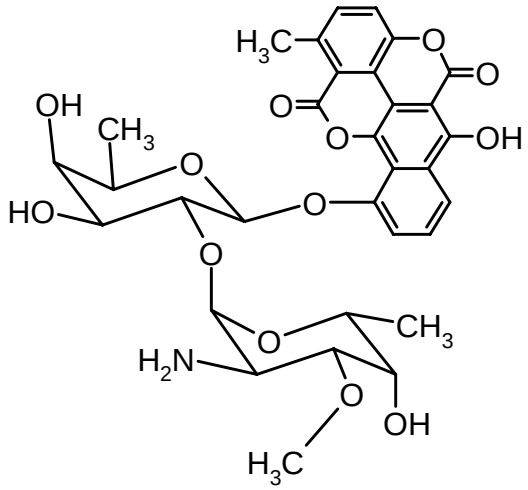


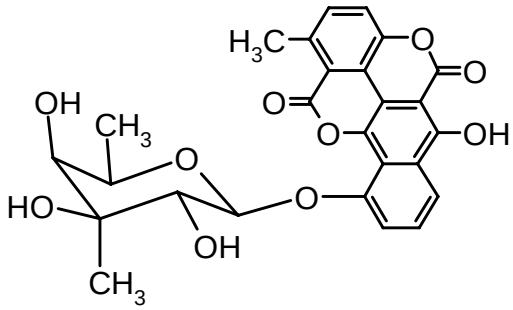
Figure 160: (+)-ESI MS^2& (-)-ESI MS^2 fragmentation patterns of chartreusin (**308**)

Chartreusin (**308**) was obtained first from *Streptomyces chartreusis* in 1953 [326], and was fully elucidated in 1964[327,328], as a glycosidic complex coumarin. Chartre-

usin (**308**) possessed antibiotic and antibacterial activity, and exhibited a significant antitumor effect against murine L1210, P388 leukemia and melanoma[329]. Until 1985, no chartreusin derivative with improvement antitumor activity had been reported. The replacement of the disaccharide moiety of chartreusin (**308)** by a neutral monosaccharide, as the only attempt to synthesize new derivatives, failed to develop a better compound than the original one[330].

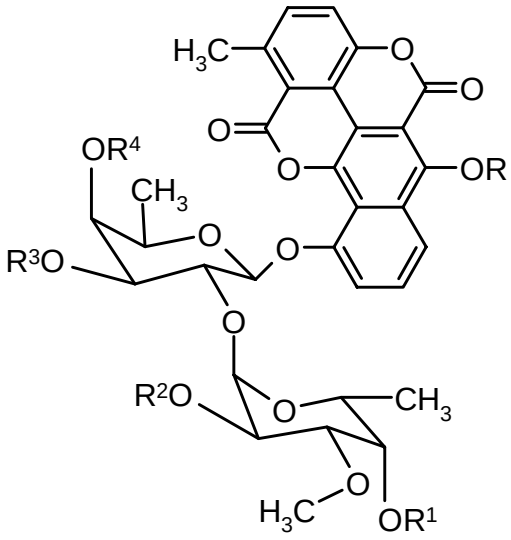


309



310

Recently, related antibiotics, elsamicin A (**309**) having an amino group on the sugar moiety, and elsamicin B (**310**), were isolated by Bristol-Myers group[331]. Elsamicin A (**309**) is more effective than chartreusin (**308**) against some murine tumors in ip-ip system[332].



311: $R^1 = R^2 = R^4 = H$, $R^3 = COCH_3$, **312**: $R^1 = R^3 = R^4 = H$, $R^2 = COCH_3$
313: $R^1 = H$, $R^2 = R^3 = R^4 = R^5 = COCH_3$, **314**: $R^1 = R^2 = R^3 = R^4 = R^5 = COCH_3$

Throughout a screening course in our research group of more than 1300 strains of terrestrial and marine *Streptomycetes*, chartreusin (**308**) was found only three times. It was accompanied by two trace components, the chartreusin monoacetates[333], **311** and **312**. Trials to obtain **311** and **312** by partial acetylation of **308** using various reaction conditions failed, and yielded only the tetra- and pentacetates[333] **313** and **314**, respectively.

The biosynthetic pathway of Chartreusin

Although simple coumarins are widespread throughout the plant kingdom, relatively few have been isolated from microoganisms[334]. The biosynthesis of plant coumarins proceeds *via* the shikimic acid pathway[335], while the microbial coumarins are derived *via* the acetate pathway[336].

Polyketide chain **A**, containing 22 carbon atoms, derived by head-to-tail condensation of acetate and malonate units, is condensed to a polycyclic aromatic intermediate **B** of the benzopyrene type. Oxidative cleavage of two rings, with elimination of three carbon atoms by decarboxylation, would generate the phenyl naphthalene dicarboxylic acid **C**. Rotation of the phenyl ring would orient the substituents to facilate formation of the dilactone **D** with labelling pattern observed in chartreusin aglycone. Attachment of sugars at the C-8 hydroxyl could take place at any stage after formation of the polyketide[337,338].

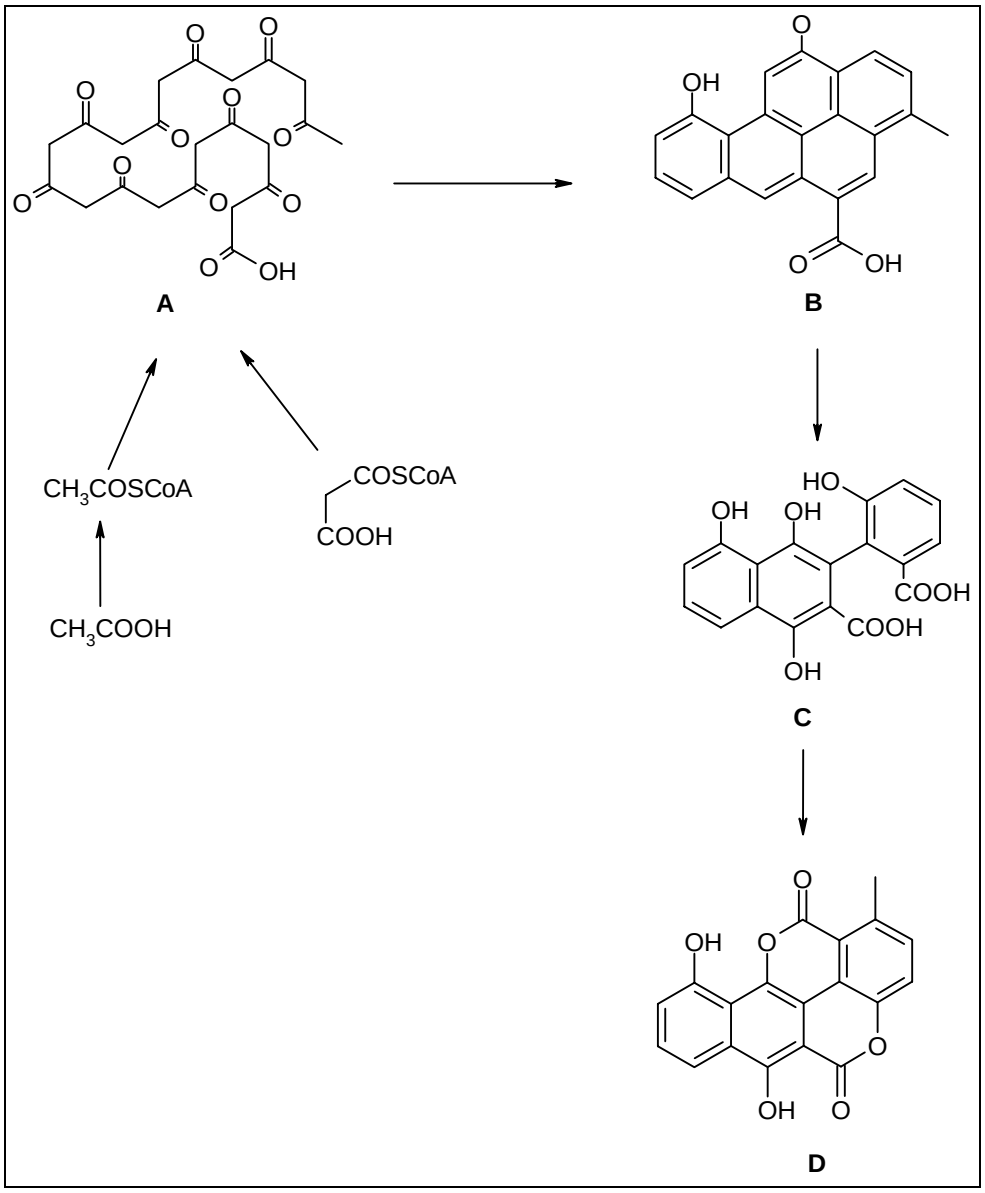


Figure 161: Suggested biogenesis of chartreusin aglycone (D)

4.26 1-Poly-3-hydroxybutyric acid (*sPHB) (*n > 50,000)

On treatment of fraction II from *Cytophaga marinoflava* isolate Am13,1 with methanol or acetonitrile/water, a white solid of compound **317** was precipitated. It gave a voluminous gel with chloroform or dichloromethane, which went only slowly into solution, and gave ductile foils on drying. Similar residues, were obtained from other marine bacteria e.g. *Alteromonas distincta* Hel69, Bio210 and Mcy4, as well as terrestrial *Streptomycetes* e.g. GW4/762 and GW22/503

The ^{1}H NMR spectra of all these samples were nearly identical and showed a sharp ABX signal of an oxygenated methine group, with the relative intensities of 1

at δ 5.26. In addition, the AB-part of a methylene group was observed at δ 2.60 and 2.48. Furthermore, a doublet methyl group was detected at δ 1.27.

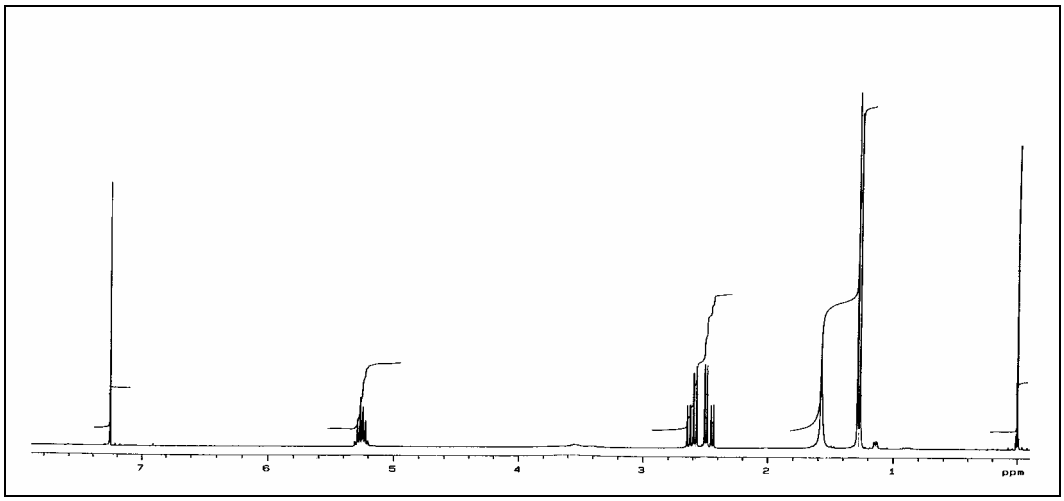
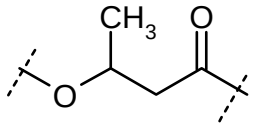


Figure 162: ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) of sPHB **(317)**

The ^{13}C NMR spectra of compound **317** showed three sp^3 carbon signals at δ 68.0, 40.4 and 19.8, in addition to the signal at δ 169.2 of a carbonyl group, which might be of an acid, ester or amide moiety, to give fragment **A**.



A

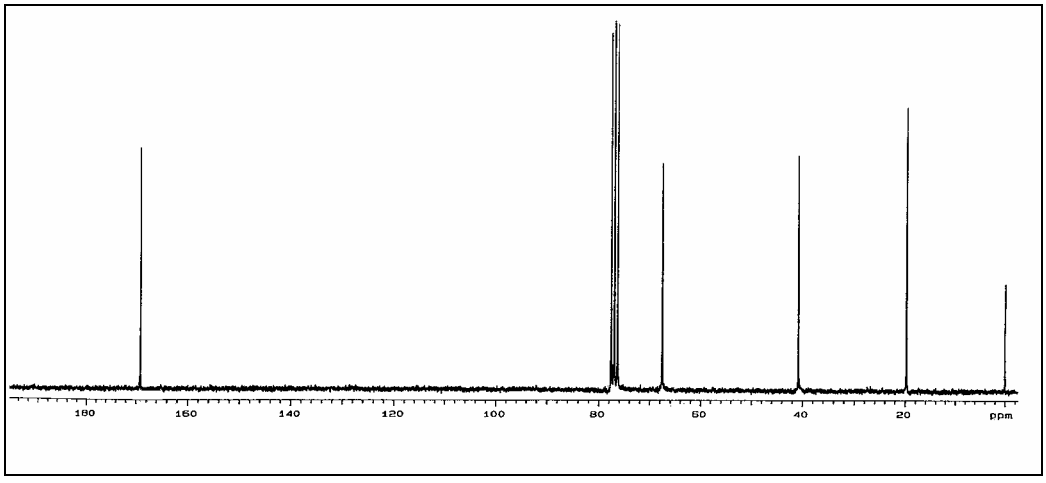


Figure 163: ^{13}C NMR spectrum ($CDCl_3$, 50 MHz) of sPHB (**317**)

The CI and EI mass spectra showed peaks with gradual decrease in intensities with Δm = 86 (Figure 164). The CI mass spectrum showed a peak at *m/z* 964 (11× 86 + NH_4) from strain Hel 69.

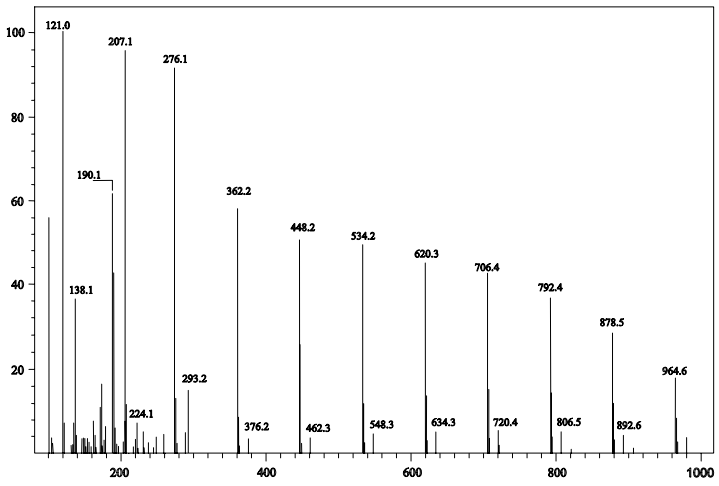


Figure 164: DCI mass spectrum (reactand gas NH_3) of sPHB (**317**, n > 50,000)

A search in AntiBase and DNP delivered two related butyrolides, the cyclodimeric DG-1[339] (**315**), from the *ascomycete Diplogelasinospora grovesii*, and pinnatifolide[340] (**316**), from the red alga *Laurencia pinnatifida* Lamour. Compound **316**, was previously obtained by acid treatment of PHB[341] (Figure 165).

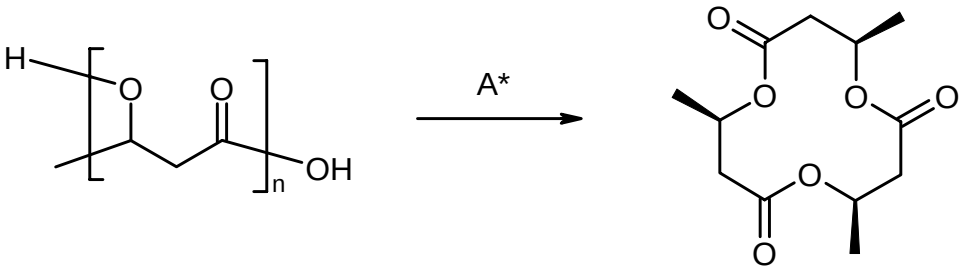
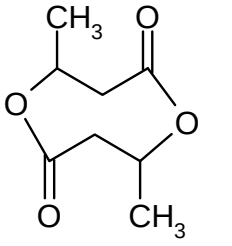
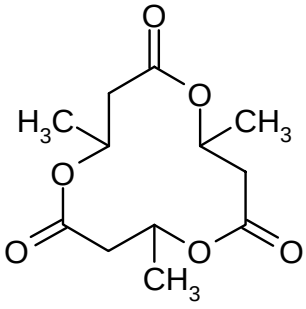


Figure 165: Acid-catalyzed *trans*-esterfication of higher or open-chain oligomers

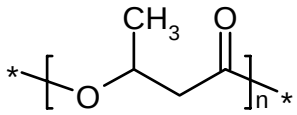


315



316

The products obtained from AM13,1 and various other strains are obviously oligomeric or polymeric *β*-hydroxybutyric acids. As the samples, however, did not show any molecular signal below 200.000 Dalton in the ESI or MALDI-TOF mass spectra, this pointed to a high polymer of *β*-hydroxybutyric acid, concluding it is a storage-PHB (sPHB) (**317**), according to the definition of Seebach[342]. The EI and CI mass signals are obviously formed by ring size reduction, similar as under acid catalysis (see above). An origin during work-up by partial hydrolysis of sPHB is less plausible, as the products were detectable in freshly harvested cultures, and did not have any unphysiological acid contact.



317 (n > 50,000)

Biosynthetically, sPHB is formed on the surface of so-called inclusion bodies by chain elongation. The inclusion bodies were electron-microscopically visible, and correspondingly sPHB was found[343]. Poly-(R)-*β*-hydroxybutyric acid is one of the most important biologically degradable and environmentally benign plastic materials[344]. It was described, isolated and identified from *Bacillus megaterium* by Lemoigne[345]. PHAs are synthesized by microorganisms under conditions of nutrient limitation[346]. The molecular weight values vary considerably, and are dependent on the microorganism, cultivation conditions, and method of isolation[347]. Typically, they lie between 100,000 and 750,000 g/mol, but they can be greater than one million[342].

4.26.1 Oligo-(ß-hydroxybutyric acid (cPHB; n = 8-30)

During the screening course of the ethyl acetate extract of the terrestrial *Streptomyces* sp. GW2/577, another white solid of **318** was precipitated by treatment with methanol. The chemical properties were similar to those of **317**, and also, the ^{1}H NMR spectra were nearly identical as for the latter. In contrast to **317**, however, the ESI and MALDI-TOF mass spectra of compound **318** displayed a typical signal pattern, as for a mixture of oligomers with molecular masses of (n × 86) + H_2O + Na^+, and a maximum at *m/z* 1933.7 Dalton (Figure 166).

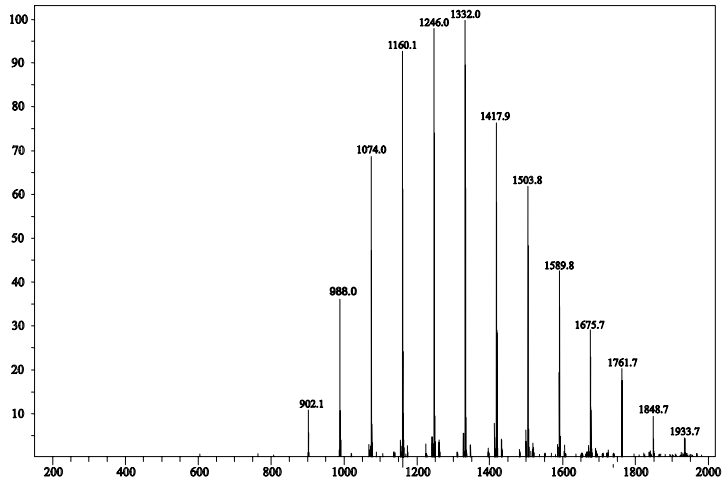
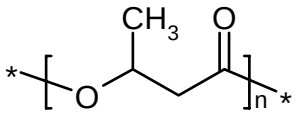


Figure 166: ESI MS of cPHB (**318**, n = 8-30).

Obviously, the product was a mixture of oligo-(*β*-hydroxybutyric acid) (**318**, cPHB), with n = 8-30. Accordingly, treatment with diazomethane gave cPHB methyl ester which exhibited a methoxy signal at δ 3.66 in the ^{1}H NMR spectrum. The intensity of the methoxy signal indicated the average chain length of cPHB (**318**) to be n ≈ 15 units.



318 (n = 8-30)

Low molecular weight PHBs were often found as constituents of prokaryotic and eukaryotic cell membranes, having sizes of 100-200 units. The function of these oligomers is still in debate, concerning the formation of ion channels or the stabilization of proteins. This group of polymers is therefore named as cPHB (c = channel-forming) (**318)**. To our knowledge, such short *β*-hydroxybutyric acid oligomers described here have not been isolated from any natural source before. They have just a chain length corresponding to the thickness of a cell membrane, and their pore or channel forming capabilities might be of interest.

The cPHB (**318)** producing strain GW2/577 did not show inclusion bodies in the exponential phase of growth under electronic microscope. Only in deteriorating cells, particles resembling inclusion bodies could be detected. They might be products of phase separation, containing cPHB (**318)**. It is unlikely therefore that cPHB (**318)** are intermediates of the sPHB (**317)** formation. This provides again a hint that low-

molecular weight cPHBs (**318)** may have another function than as storage material[343]. The small oligomers may play a role in the complexation of calcium or be involved in the formation of channels[342]. The real physiological importance of these compounds, however, remains still open.

5 Summary

Natural products are constantly the most successful source of drug leads. They continue to provide greater structural diversity than standard combinatorial chemistry and so they propose major opportunities for finding new and novel low molecular weight leading structures, that are active against a wide range of assay targets. Since, less than 10% of the world's biodiversity has been tested for biological activity, many useful natural lead compounds are in anticipation of discovery. In spite of this, the interest of drug discovery in natural products is presently decreasing.

Steadily over the years, the focus of interest has been shifted from plants towards the microorganisms e.g. bacteria as a new source of natural products. The production of bioactive metabolites by bacteria is preferred over plants and animals. This is attributed to: i) their ability of cultivation in any quantity and any condition, since, they are being found in surprisingly extreme environments i.e. polar ice, geothermal vents, dark cave-sand and deep-sea sites. ii) Bacteria are symbiotically important to animals and plants, and iii) most of natural antibiotic are attributed to bacteria and fungi.

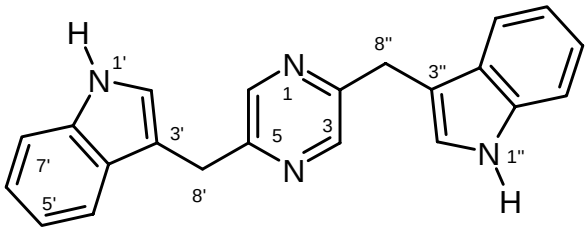
Through chemical and biological pre-screening, ten North Sea bacteria, eleven terrestrial *Streptomycetes*, and six marine-derived *Streptomycetes* strains were selected in this work for further investigations.

The bacteria were grown using standard conditions, for the work-up procedures solvent extractions, and chromatographic separations were applied. A fast identification of known metabolites (dereplication) was carried out by searching in AntiBase, the Dictionary of Natural Products (DNP) and Chemical Abstract (CA), using combined structural features from mass and ^{1}H NMR spectra. The structure elucidation of the new compounds was carried out by careful interpretation of mass, 1D and 2D NMR spectra.

On the stage of pure metabolites, antibacterial, antifungal, and cytotoxic activities, were measured in our group. Some further pharmacological tests were carried out by bioLeads in Heidelberg.

The marine bacteria strain Am13,1 (*Cytophaga marinoflava*) was selected because of its enormous number of metabolites, despite of its generally weak biological activity. Hence, 26 natural compounds were isolated and identified from a 25-liter jar

fermenter, among them six new natural products, 2-methylbutyramide (**35a**), 3-methylbutyramide (**35b**), indolyl-3-ethylisovaleramide (**42**), 2,5-bis(3-methenylindolyl)-pyrazine (**55**), pharacine (**73**), and *p*-hydroxyphenyl acetamide (**78**). In addition, the anti-yeast factor tryptanthrine (**63**) was isolated. Most metabolites produced by the strain were indole derivatives, substituted at 3-position. Beside others, the rare 2,2-dimethyl-2,3-dihydro-(1*H*)-quinazolin-4-on (**68**) was also identified.



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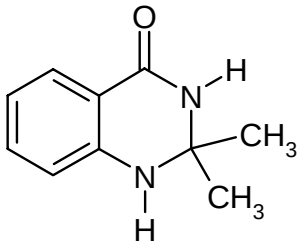
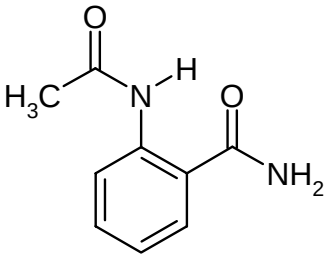
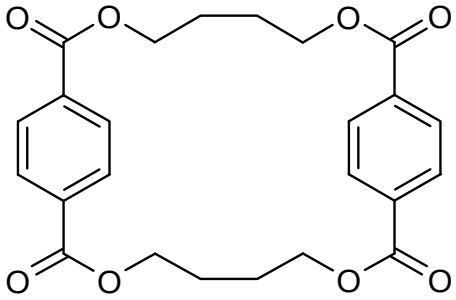


68

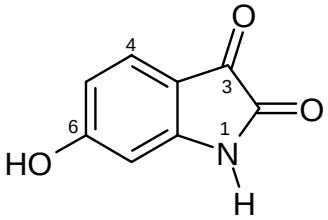


67

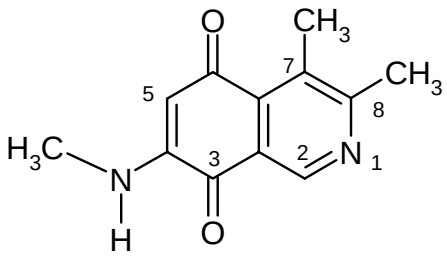


73

The marine *Streptomyces* strain B1848 exhibited high antifungal activity against *Mucor miehei* (Tü284) and *Candida albicans*. Numerous colourless compounds were isolated, among them the blue UV fluorescent 1-acetyl-*β*-carboline (**81**), in addition to three orange-dark red compounds, 6-hydroxy isatine (**93**), 4-methylamino-7,8-dimethyl-isoquinoline-3,6-dione (**97**) and 7-methylamino-3*H*-pyrrolo[2,3-c]isoquinoline-6,9-dione (**102**) were identified, out of them compounds **93** and **102** as novel natural products.



93



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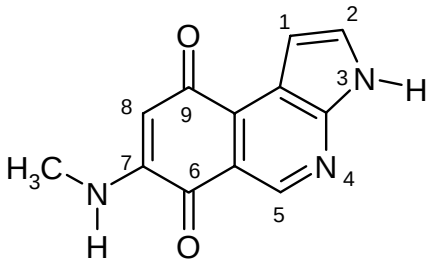
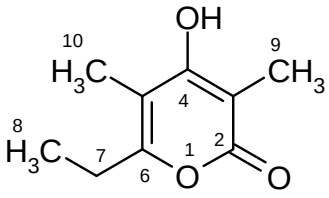


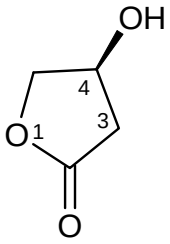
102

Isoquinoline-quinones are rare in nature, and especially from bacteria. They are occurred in the marine sponge *Cribrochalina* sp., and the bryozoan *Caulibugula intermis*. They are novel cytotoxic compounds, which showed activity against lymphocyte P388 leukaemia cells, and highly antibacterial, and fungicidal activity. The biosynthetic origin of these compounds is still unknown.

The terrestrial *Streptomyces* sp. isolate GW3/1538 produced numerous secondary metabolite, mostly five- and six-memberd heterocyclic rings, namely 6-ethyl-4-hydroxy-3,5-dimethyl-2-pyrone (**113**), 2,5-furandimethanol (**114**), 3-hydroxy-2-methyl-gamma-pyrone (**116**). In addition, the new bacterial metabolite, (S)-(+)-3,4-dihydroxy butanoic acid-γ-lactone (**122),** was identified.

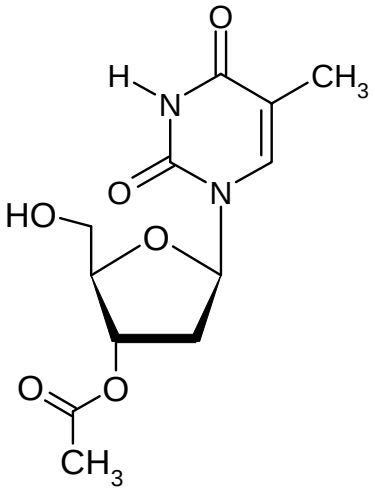


113



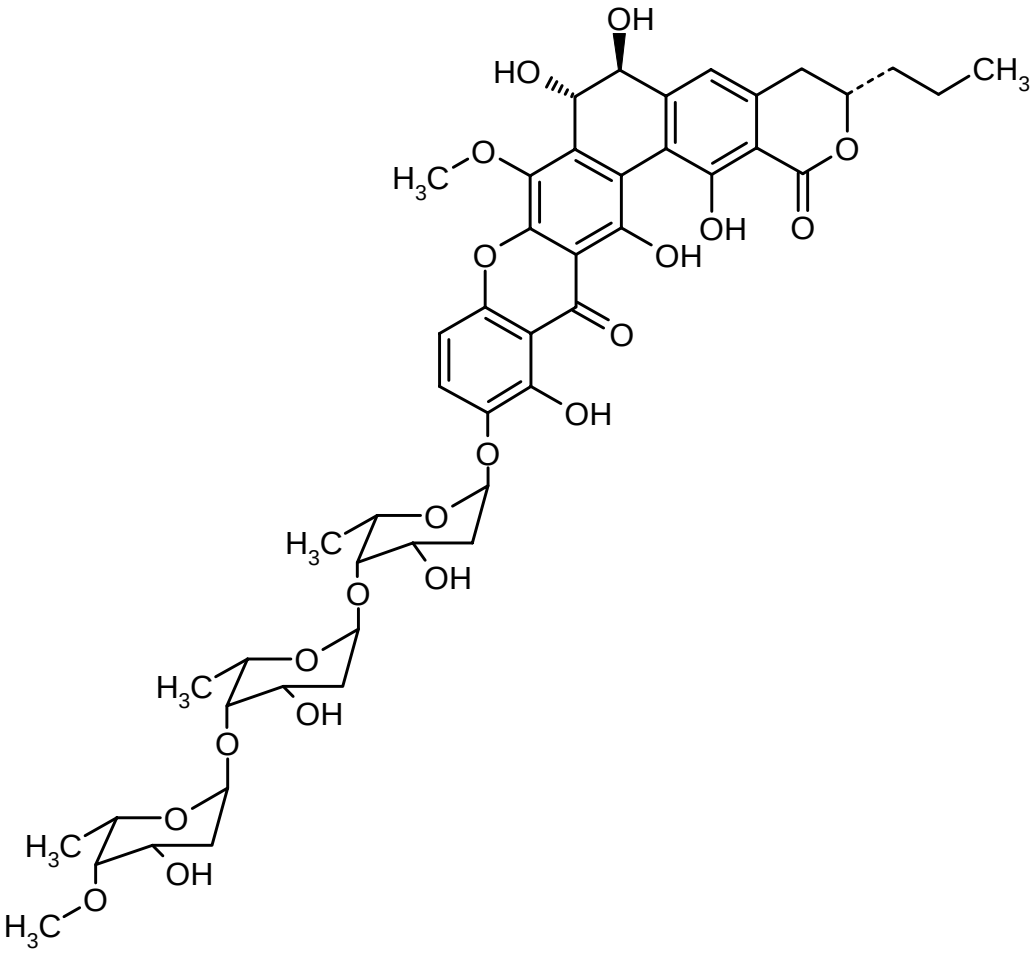
122

Working up of the ethyl acetate extract of the marine bacteria isolate Bio134 led to identification of the new nucleoside secondary metabolite, 3'-acetoxy-2'-deoxy-thymidine (**124**).

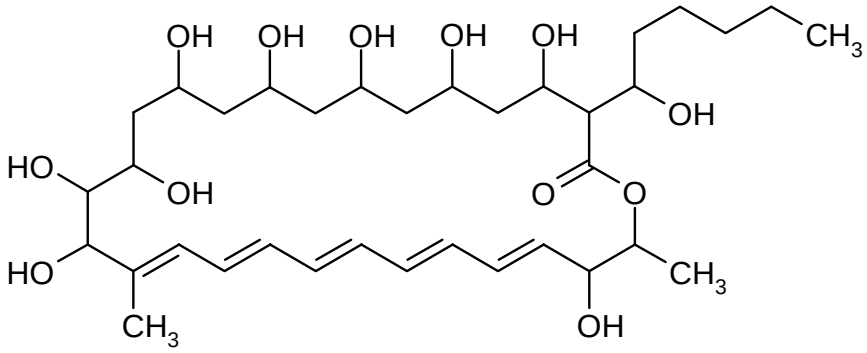


124

Four metabolites, actinomycin D (**130**), an actinomycin analogue, the antitumor active FD-594 (**135**), and the macrocyclic polyene antibiotic fungichromin (**138a**), were isolated from the highly bio-active extracts of the marine *Streptomyces* sp. isolate B7936.



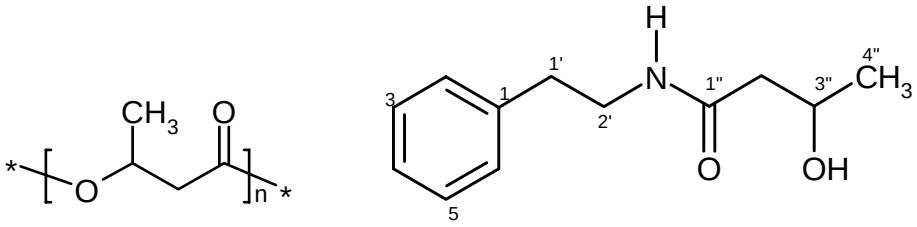
135



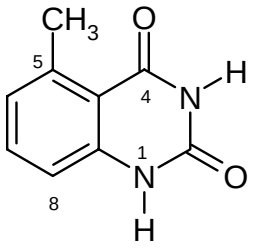
138a

During the working up of the antimicrobially highly active terrestrial *Streptomyces* sp. isolate GW2/577, four new natural products were found. cPHB (**318**) is the first natural oligo-(*ß*-hydroxybutyric acid) from bacteria, the others are, 3-hydroxy-N-phenethyl-butyramide (**145**), *p*-hydroxyphenethyl propionamide (**144**), and 5-methyl-1*H*-quinazoline-2,4-dione (**148**).

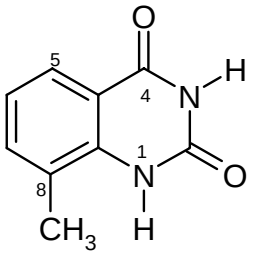
During trials to synthesize 3-hydroxy-N-phenethyl-butyramide (**145**) by heating of *ß*-phenethyl amine and sPHB (**317**), crotonic acid *ß*-phenylethyl amide (**147**) was obtained as a new product. The desired **145**, was finally obtained by deprotonation of *ß*-phenyl-ethylamine (**146**) with *n*-butyl-lithium, and successive reaction with a sPHB solution (**317**). For the structural confirmation of 5-methyl-1*H*-quinazoline-2,4-dione (**148**), the positional isomer 8-methyl-1*H*-quinazoline-2,4-dione (**149**) was synthesised. As a synthetic minor product, the quinazoline dimer, 4,12-dimethyl-5*H*-quinazolino[4,3-b]quinazoline-6,8-dione (**151**) was obtained, as a new synthetic product and confirmed by 2D correlations. Finally, N-(2-phenylethyl)-propionamide (**141**), and *p*-hydroxyphenethylacetamide (**142**) were detected, as potent antimicroalgal components from this strain.



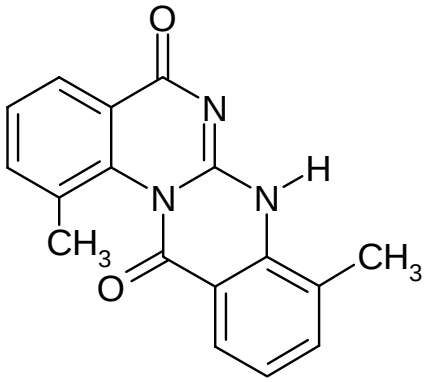
318 (n = 8-30) **145**



148

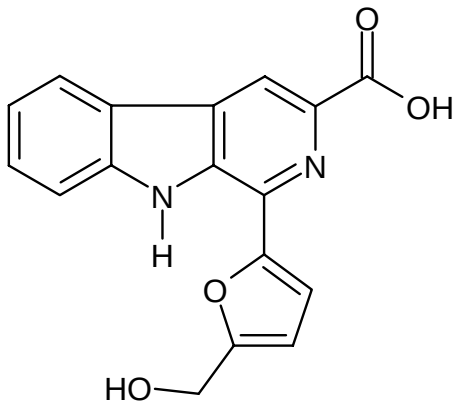


149

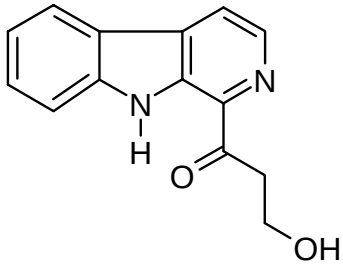


151

The North See bacterial isolate Bio215 exhibited yellowish-green fluorescent colonies on the agar plate. The ethyl acetate extract pronounced high antibacterial activity against the Gram-negative *Bacillus subtilis* and *Escherichia coli*, in addition to its antimicroalgal activity. Two greenish-yellow fluorescent *β*-carbolines, flazin (**166**) and 1-(9H-*β*-carbolin-1-yl)-3-hydroxy-propan-1-one (**171**) were identified. Flazin (**166**) is new from microorganisms, **171** had already been isolated in our research group.

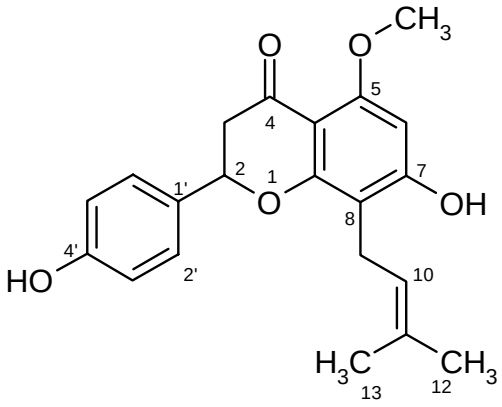


166



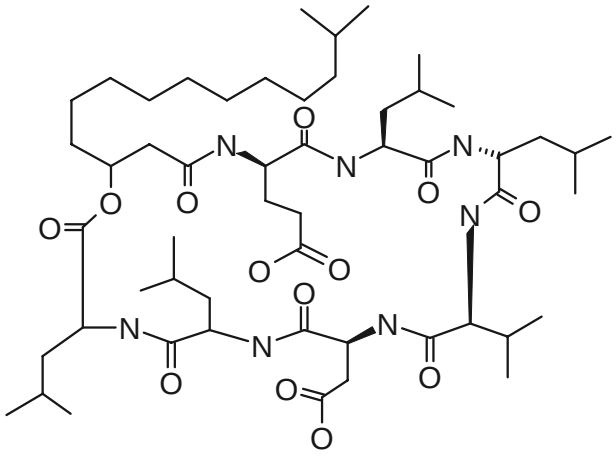
171

The North Sea marine bacterium, isolate Pic009 exhibited high activity against *Bacillus subtilis*. Working up of the strain extract, led to isolation of isoxanthohumol (**180**), as a new microbial metabolite. Xanthohumol (**186**) and its isomer isoxanthohumol (**180**), were reported as anticarcinogenic and antifungal agents, from the widely cultivated plant *Humulus lupulus L.* (*Cannabianacea*), and the roots of *Sophora flavescens*.

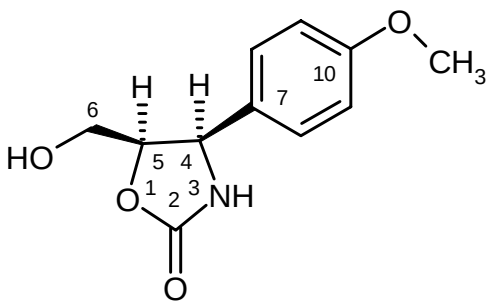


180

The terrestrial *Streptomyces* sp. isolate GW10/580 exhibited a vast number of metabolites, and its biological activity was high. A shaker culture led to the isolation phenazine-1-carboxylic acid (**188**), surfactin C (**189**), 3-pyridine carboxylic acid (**190**), and the antibiotic cytoxazone (**195**) with the rare 2-oxazolidinone ring. A fermenter culture led to isolation of feigrisolides A-C (**202**, **198**, **206**). The heptalactones A (**202**), B (**198**) and the non-symmetric macrolide C (**206**) exhibit antibacterial activity.



189



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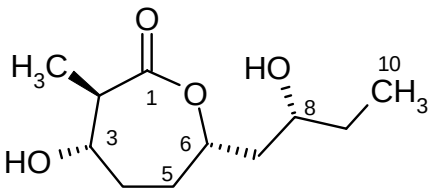

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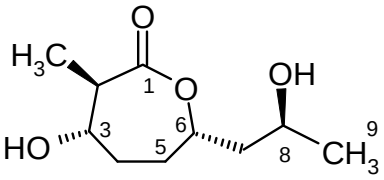

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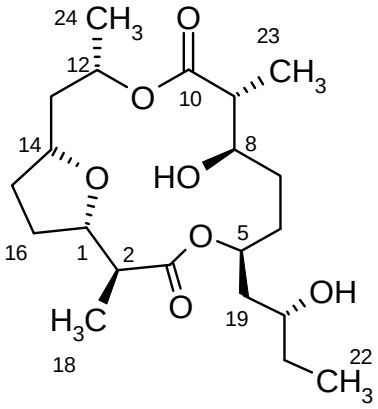
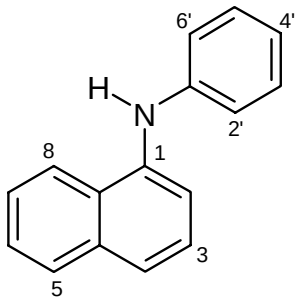


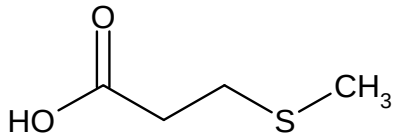
206

The marine *Streptomyces* B8335 exhibited a high antimicrobial activity against all tested microorganism. This was attributed to the presence of actinomyin D and another actinomycin, probably the recently discovered HKI 0155 (**208**). Working up of the low polar fraction gave N-phenyl-1-naphthylamine (**209a**), as new microbial metabolite. The formation of the latter compound was reproduced and **209a** confirmed as a natural product.



209a

Working up of the ethyl acetate extract of the marine bacteria isolate Hel59b led to identification of 3-(methylthio)propanoic acid (**210**), quinoline-2-one-4-carboxylic acid methylester (**212**), and 3-pyridinecarboxamide (**215**). The last two compounds **212** and **215**, are new natural products from microorganisms, characterised by their antibacterial activities. The compound **215** is used as dehydrogenase inhibitor.



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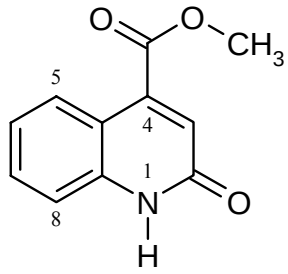
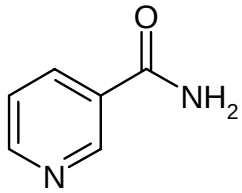
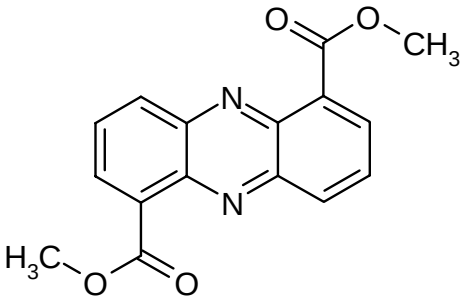


212



215

From the terrestrial *Streptomyces* sp. isolate GW3/1786, the new natural product, phencomycin methyl ester (**217**), was isolated from 25-liter shaking culture. However, 25-liter fermenter of the strain led to 1-phenazinecarboxylic acid methyl ester (**223**).



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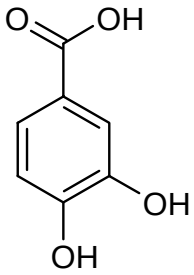
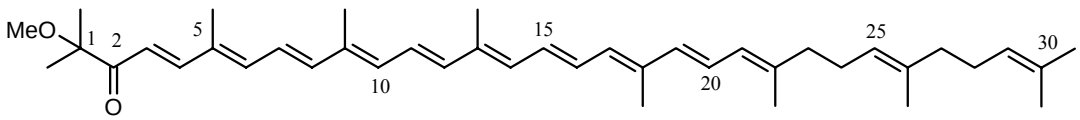


226

Despite of the exhibited antifungal activity against *Mucor miehei* (Tü284) and *Candida albicans* by the marine *Streptomyces* strain B2150, only indolyl-3-lactic acid (**225**) was isolated in addition to flazin (**166**), and some simple 3-substituted indole derivatives. Working up of the terrestrial *Streptomyces* sp. isolate GW12/3995, led only to 3,4-dihydroxybenzoic acid (**226**).

On the basis of the chemical (HPLC-MS, TLC), and biological screening of the isolates DFL16, DFL30, DFL27, DFL38 and DFL12, the carotenoid spheroidenone (**230**) was identified, as the main pigment of *Roseobacter* sp.

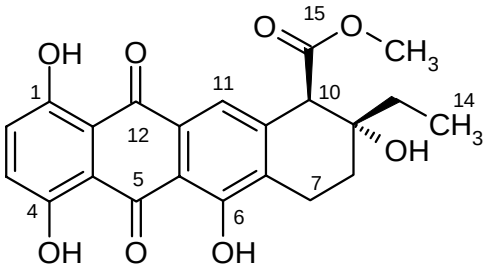


230

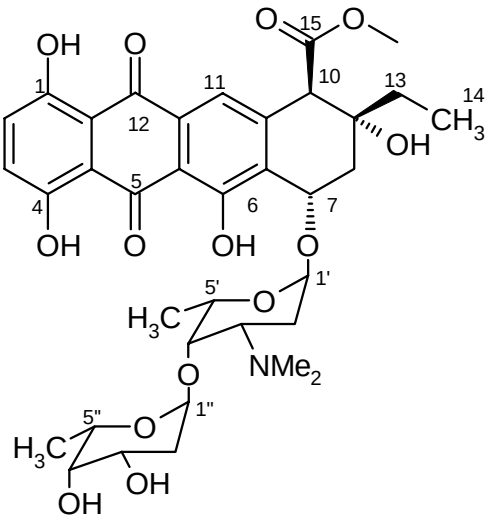
Investigation of the terrestrial *Streptomyces* sp. isolates GW10/1818 and GW5/1749 led to isolation of hexahydromenaquinone MK-9 (II,III,VIII-H6) (**235**) and menaquinone MK-9 (II,III-H4) (**237**).

Based on the high bioactivity and interest in the entire metabolic pattern, the marine *Streptomyces* sp. isolate B8904 was selected. Cultivation in a jar fermenter

led to linoleic acid (**239**) and the nucleoside adenosine (**242**), while a shaker culture led to isolation and identification of seven *peri*-hydroxyquinones, ζ-pyrromycinone (**243**), η-pyrromycinone (**244**), musettamycin (**246**), cinerubin B (**248**), cinerubin M (**254**), islamomycin A (**257**) and islamomycin B (**258**). The last three hydroxyquinones are new natural products. Islamomycins A-B (**257, 258**) comprise the rare carbamic acid group, which linked to the sugar *via* oxygen.

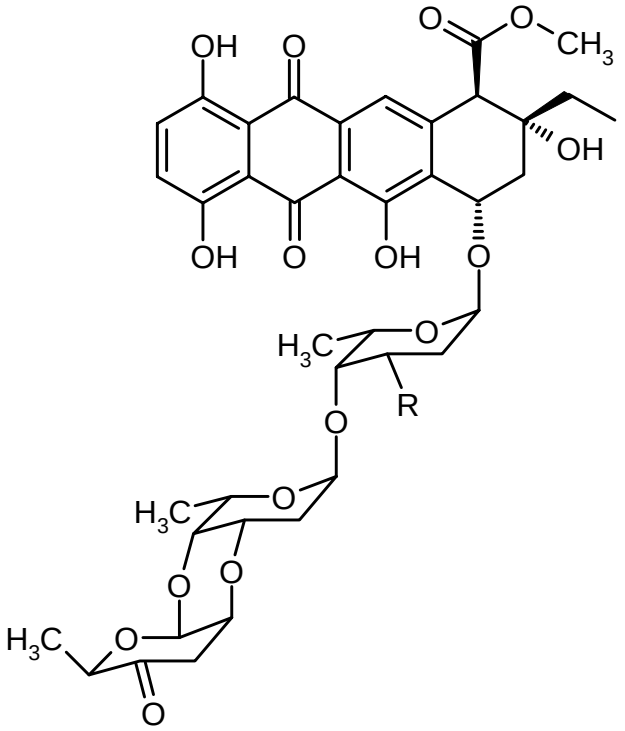


243

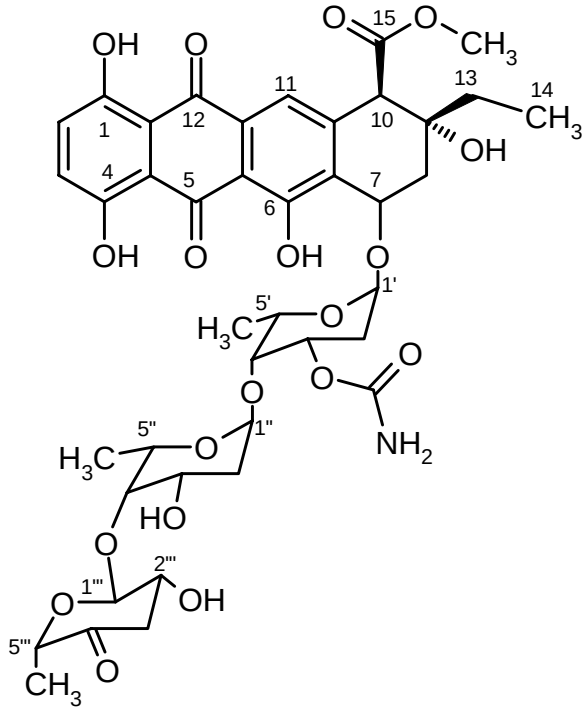


246

In cinerubin M (**254**), the 3'-N$(CH_3)_2$ residue of cinerubin B was replaced by a $NHCH_3$ group. Cinerubins are characterised by their activity against Gram-positive bacteria, viruses and tumours, as well as their cytotoxic and antifungal activity.

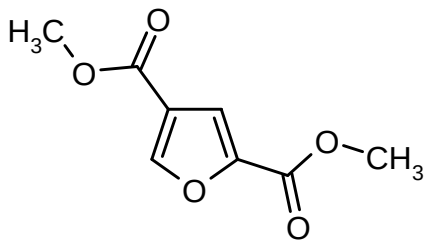


248: R = $N(CH_3)_2$, **254**: R = $NHCH_3$, **257**: R = $OCONH_2$

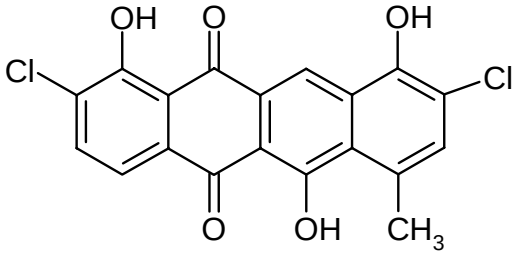


258

The pronounced bioactivity of the terrestrial *Streptomyces* sp. GW10/1828 against Gram-negative bacteria and fungi attracted the attention, and led to identify the two new natural compounds, furan-2,4-dicarboxylic acid dimethyl ester (**261**), and the chloroquinone, 2,9-dichloro-1,6,10-trihydroxy-7-methyl-naphathacene-5,12dion (**269**).

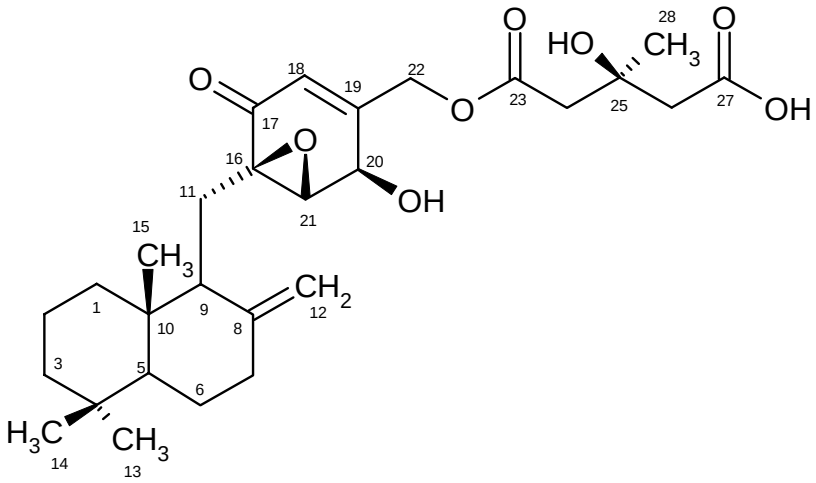


261

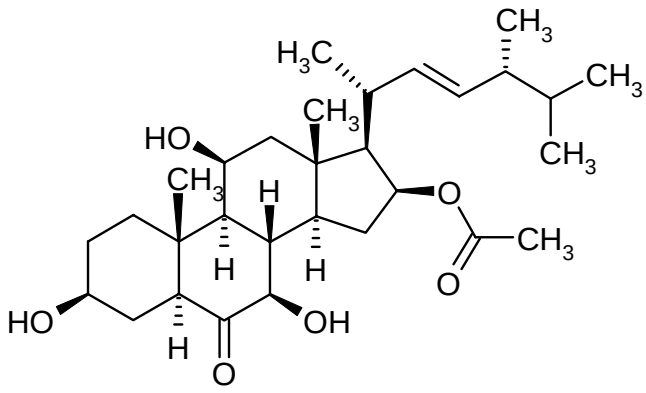


269

An additional cultivation of the strain as shaker culture led to the unusual steroids, cholic acid (**273**) and chenodeoxycholic (**274**), however, it was found out that both compounds were already present in the used Bacto-Pepton. A further fermentation on M_2 medium led to the isolation of macrophorin D (**275**) and anicequol (**278**). Anicquol (**278**) is an inhibitor for anchorage-independent growth of tumor cells and showed neuritogenic activity. Macrophorin D (**275**) is an antifungal against *Macrophoma*, which infects apple. The macrophorin analogues showed immunomodulatory activity.



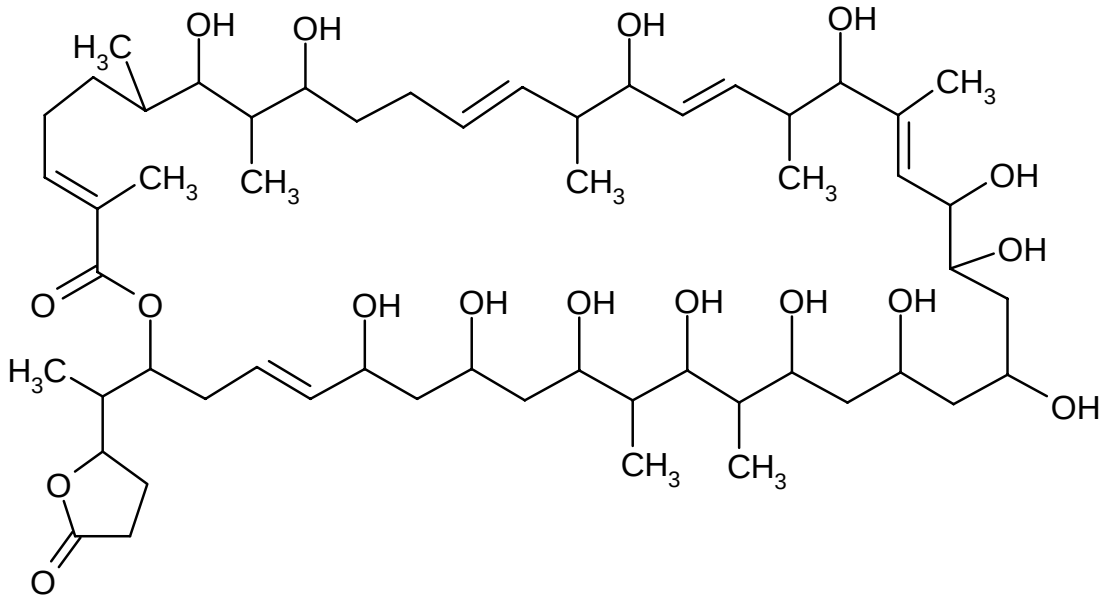
275



278

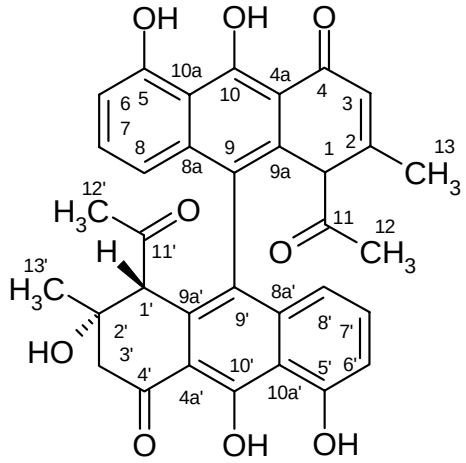
As fourth trial to enhance the concentration of the new chloroquinone **269** afforded only the antitumor antibiotic resistomycin (**279**).

Fermentation of the terrestrial *Streptomyces* sp. GW50/1568, as jar fermenter, led only to uninteresting metabolites, in addition to minor unidentified yellow compound. However, cultivation using a shaker culture for a long period led to the 42-membered macrolactone, oasomycin A (**281**), a member of the desertomycin family, which shows inhibitory effects on *de novo* cholesterol biosynthesis and the muscarine receptor.



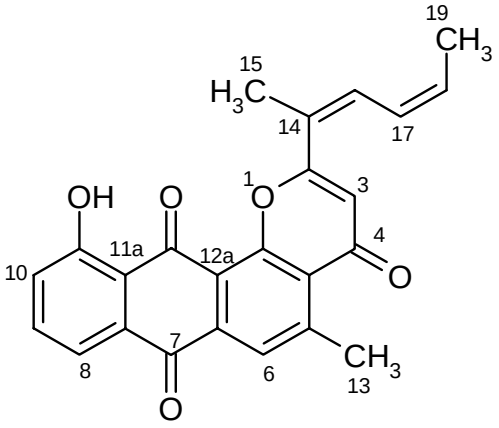
281

The high antibacterial activity and TLC screening results attracted the attention to the terrestrial *Streptomyces* sp. isolate GW44/1492. The interesting reddish-orange setomimycin (**282**) was isolated which exhibits a pronounced antibacterial and antitumor activity.

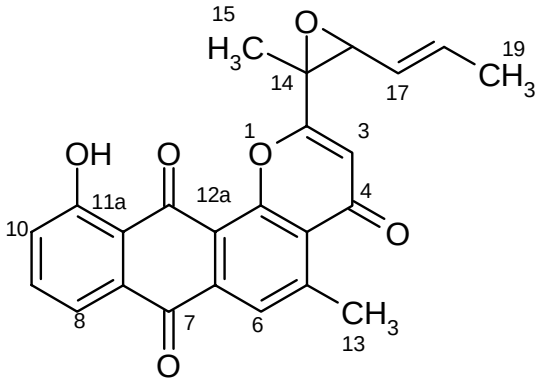


282

The terrestrial *Streptomyces* strain GW3/1130 was selected on the basis of its high antifungal and antibacterial activity. Cultivation in a jar fermenter led to an extract without bioactivity, and only 1-acetyl-*β*-carboline (**81**) was obtained. Cultivation, however, as shaker culture delivered rubiflavinon C-1 (**286**), saptomycin F (**289**), ε-indomycinone (**291**), *β*-indomycinone (**295**), saptomycin A (**297**) and γ-indomycinone (**298**). The ε-indomycinone (**291**) is a new antibiotic.



286



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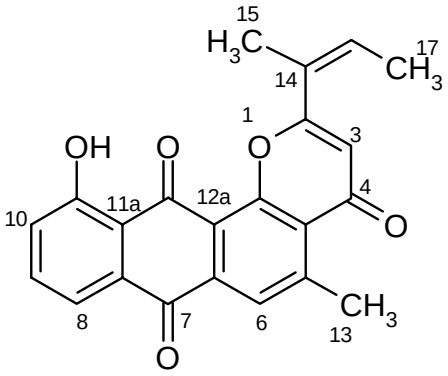

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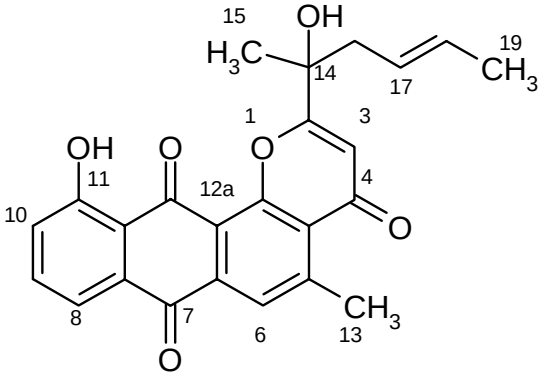

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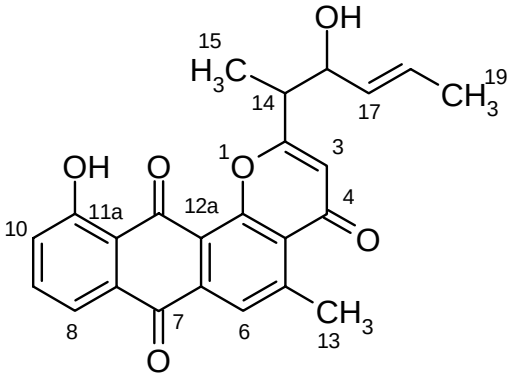
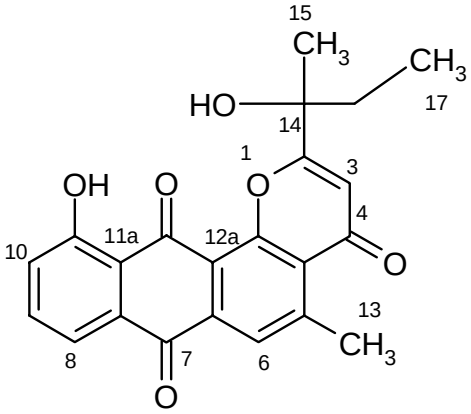
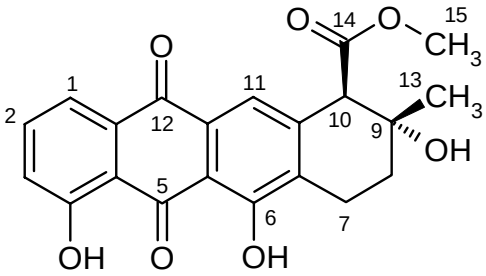


297

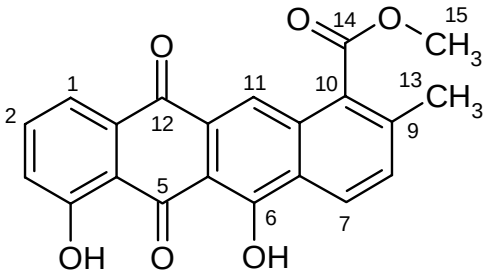


298

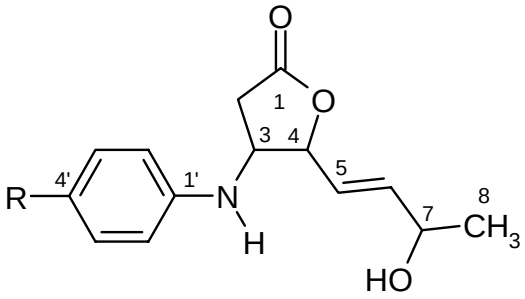
Cultivation of the terrestrial *Streptomyces* sp. GW22/3234 in a 50-liter fermenter delivered resomycin A (**299**) and resomycin D (**302**), in addition to the strongly green-fluorescent glycosidic coumarin antibiotic chartreusin (**308**).



299

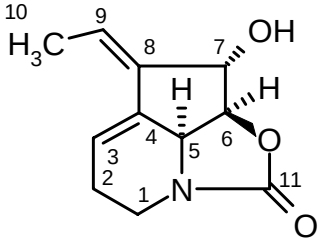


302

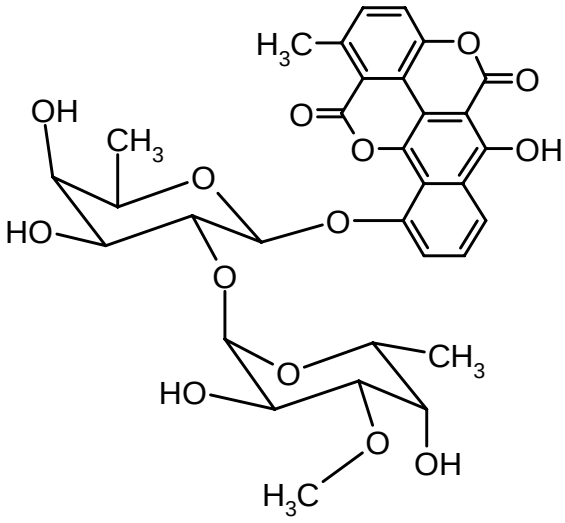


305: R = COOH, **306**: R = CHO, **307**: R = CH_2OH

In addition, obscurolides A1-A3 (**305, 306** and **307**), butyrolactone derivatives characterised by their phosphodiesterase inhibitory activity, were obtained, together with the antibacterial and antifungal streptazolin (**303**).



303



308

In this thesis, 17 *Streptomycetes* and 10 North Sea bacteria (among them 5 *Roseobacter* strains) were studied. From the North Sea strains, 47 compounds were obtained, from which eleven compounds can be considered as new secondary metabolites from bacteria. The 11 terrestrial *Streptomycetes* were found to produce 45 compounds, out of which 10 natural products are reported here for the first time. The marine *Streptomyces* were found to produce 25 compounds, out of which 5 were new natural products. The altogether 118 metabolites, including, 6 macrolides, one peptide, 13 quinones, and 3 polyenes (Table 13).

Table 13: Total number of isolated compounds from bacteria in this thesis.

Strains	No. of strains	No. of compounds	No. of new compounds
Terrestrial *Streptomycetes*	11	45	10
Marine *Streptomycetes*	6	25	5
North Sea Bacteria	5	47	11
Roseobacter sp.	5	1	-

Microorganisms provided a high variety of potentially useful natural products, however, rapid development of resistance is forcing the chemistry to explore new sources of potent bioactive natural products. Marine bacteria could be one of such sources.

The metabolic potency of the terrestrial and marine *Streptomycetes*, measured as the number of isolated compounds, did not differ at all in this investigation. However, the productivity of novel bio-active compounds from marine *Streptomycetes* is higher than that of the terrestrial *Streptomycetes*. Terrestrial and marine *Streptomycetes*, are an ideal target for discovery of novel potential bioactive compounds. Their investigation should be continued to extend the ability of discovering new compounds.

Strong biological activities are obviously also widespread amongst bacteria from the North Sea, and these microorganisms are at least as talented as the *Streptomycetes*. The structures of their metabolites are, however, less complex and seem to be derived from the amino acid pathways. They could nevertheless serve as an additional prolific source for more diversity and bioactive metabolic constituents.

6 Materials and Methods

6.1 General

Melting point: All melting- and decompose points were adjusted with the melting point apparatus of Fa. Electrothermal and were not corrected**. – IR spectra:** Perkin-Elmer 1600 Series FT–IR; Perkin-Elmer 297 infrared spectro-photometer; Beckman DU-640; Shimadzu FT-IR; (KBr tablet and film). **– UV/VIS spectra:** Perkin-Elmer Lambda 15 UV/VIS spectrometer. **– Optical rotations:** Polarimeter (Fa. Perkin-Elmer, model 241). – **^{1}H NMR spectra:** Varian Unity 300 (300 MHz), Bruker AMX 300 (300 MHz), Varian Inova 500 (499.8 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet doublet, t = triplet, q = quartet, m = multiplet, br = broad. – **^{13}C NMR spectra:** Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical shifts were measured relatively to tetramethylsilane as internal standard. **Abbreviations:** APT (Attached Proton Test): CH/CH_3 up and C_q/CH_2 down. **– 2D-NMR spectra:** H,H COSY (^{1}H,^{1}H-Correlated Spectroscopy), HMBC (Heteronuclear Multiple Bond Connectivity), HMQC (Heteronuclear Multiple Quantum Coherence) and NOESY (Nuclear Overhauser Effect Spectroscopy). - **Mass spectra**: EI MS with Varian MAT 95 Finigan (70 eV), high resolution with perflurokerosine as standard, ESI MS LCQ (Finnigan), DCI with Varian MAT 95 and NH_3 as reactand gas. – **High performance liquid chromatography (HPLC): Instrument I**: **Analytical**: Jasco multiwavelength detector MD-910, two pumps type Jasco Intelligent Prep. Pump PU-987 with mixing chamber, injection valve (type Rheodyne) with sample loop 20 µl, Borwin-HPLC-software. **Preparative**: sample loop 500 µl. **Analytical column:** 1) Eurochrom 4.6 × 125 mm without pre-column: stationary phase: Hypersil, ODS 120 × 5 µm; 2) Vertex 4.6 × 250 mm, stationary phase: Nucleosil NP 100-C-18, particle size 5 µm; **Preparative column**: 1) Vertex 16 × 250 mm with 16 × 30 mm pre-column, stationary phase: Eurospher C-18 RP 100 × 5 µm; 2) Vertex 16 × 250 mm with 16 × 30 mm pre-column, stationary phase: Nucleosil NP 100-C-18, particle size 5 µm, pore diameter 100 Å (Macherey–Nagel & Co.). **Instrument I**: Knauer HPLC-equipment containing: spectral-digital-photometer A0293, two pumps type 64 A0307, HPLC-software

V2.22, mixing chamber A0285, injection valve 6/1 A0263 (type Rheodyne) and sample loop 20 µl. **HPLC-solvents:** Acetonitrile / water-azeotrop (83.7% acetonitrile / 16.3% water, bp. 78.5 °C). The azeotrop was redistilled, filtered through a membrane filter (pore Ø: 0.45 µm, regenerated cellulose, Sartorius, Göttingen) and then degassed for 15 min by ultrasonic. – **Filter press:** Schenk Niro 212 B40. - **Photo reactor for algal growth:** Cylindrical photo reactor (Ø: 45 cm) with two vertical neon tubes Philips TLD 15 W/25. – **Liphiolizer drying:** a)Liomatic 3, Lioma GmbH, Göttingen, b) Christ Alpha 1-4, Christ Loc-1 m, W. krannich GmbH CO. K. G., Göttingen.

6.2 Materials

Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). – **Glass plates for chemical screening:** Merck silica gel 60 F254, (10 × 20 cm). - **Preparative thin layer chromatography (PTLC):** Silica gel P/UV_{254} (Macherey-Nagel & Co.) (55 g) is added to 120 ml of demineralised water with continuous stirring for 15 minutes. 60 ml of the homogenous suspension is poured on a horizontal held (20 × 20 cm) glass plate and the unfilled spaces are covered by distributing the suspension. The plates are air dried for 24 hours and activated by heating for 3 hours at 130 °C. – **Column chromatography (CC):** MN Silica gel 60: 0.05-0.2 mm, 70-270 inch (Macherey-Nagel & Co.); Silica gel for flash-chromatography: 30-60 µm (J. T. Backer). – **Size exclusion chromatography:** Sephadex LH-20 (Pharmacia) is used for size exclusion chromatography. – **Solid phase extraction**: Amberlite® XAD-7, 20-50 inch (FLUKA); Amberlite XAD-16, 13-80 inch (Serva); XAD-2, 300-1000 µm, (Serva, Heidelberg).

6.3 Spray reagents

Anisaldehyd/Sulphuric acid: 1 ml anisaldehyde is added to 100 ml solution containing 85 ml methanol, 14 ml acetic acid and 1 ml sulphuric acid. – **Ehrlich's Reagent:** 1 g of 4-dimethylaminobenzaldehyde was dissolved in a mixture of 25 ml hydrochloric acid (37%) and 75 ml methanol. - **Ninhydrin:** 0.3 g ninhydrin (2,2-dihydroxyindan-1,3-dion) was dissolved in 95 ml *iso*-propanol. The mixture was

added to 5 ml collidin (2,4,6-trimethylpyridin) and 5 ml acetic acid (96%). This reagent gave a blue to a violet colouration with amino acids, peptides and polypeptides with free amino groups, and gave yellow with cyclic or N-acylated peptides. **– Chlorine *o*-dianisidin reaction**: The reagent was prepared from 100 ml (0.032 %).*o*-dainisidin in 1 N acetic acid, 1.5 g Na_2WO_4. 2 H_2O in 10 ml Water, 115 ml acetone and 450 mg KI. The wettened TLC-plate was kept ca. 30 min in chlorine atmosphere (0.5 g $KClO_3$ + 2 ml conc. HCl) and then subjected to drying for ca 1 h, till the excess of chlorine was evaporated and then dipped into the reagent. The reagent is specific for peptides as universal spraying reagent. -**Palladium(II)-chloride.** 0.5 g of Palladium(II)-chloride are dissolved in 100 ml water in presence of few drops of HCl (25 %.). It is used to identify sulphur compounds, which give brown/grey colouration spots on the TLC after heating. - **NaOH or KOH** solutions are used to identify *peri*-hydroxyquinones by deepening of the colour from orange to violet.

6.4 Microbiologic materials

Fermenter: 20, 25-liter fermenter (Fa. Meredos GmbH, Göttingen) consisted of culture container, magnet-coupled propeller stirrer, cooler with thermostat, control unit with pH and antifoam regulation, Fa. Meredos GmbH Göttingen. 50-liter fermenter type Biostat U consisted of 70-liter metallic container (50-liter working volume), propeller stirrer, culture container covered with thermostat for autoclaving, refrigeration and thermostating, Fa. Braun Melsungen. – **Control unit**: pH regulator, antifoam regulator and air regulator, Fa. Meredos GmbH Göttingen. – **Storage of strains:** deep-freeze storage in a Dewar vessel, Fa. 1'Air liquid type BT 37 A. - **Capillaries for deep-freeze storage:** diameter 1.75 mm, length 80 mm, Fa. Hirschmann Laborgeräte Eberstadt. – Soil for soil culture**:** Luvos Heilerde LU-VOS JUST GmbH & Co. Friedrichshof (from the health shop). - **Ultraturrax:** Janke & Munkel KG. - **Shaker:** Infos AG (CH 4103 Einbach) type ITE. - **Laboratory shaker:** IKA-shaker type S50 (max. 6000 Upm). - **Autoclave:** Albert Dargatz Autoclave, volume 119 l, working temperature 121 °C, working pressure 1.2 kg/cm^2. - **Antibiotic assay disc:** 9 mm diameter, Schleicher & Schüll No. 321 261. - **Culture media:** glucose, bacto peptone, bacto agar, dextrose, soybean, mannit, yeast extract and malt extract were purchased from Fa. Merck. - **Antifoam solution:** Niax PPG 2025; Union Car-

bide Belgium N. V. (Zwijndrecht). - **Petri-dish:** 94 mm diameter, 16 mm hight with cam, Fa. Greiner Labortechnik, Nürtingen. – **Celite:** Celite France S. A., Rueil-Malmaison Cedex. - **Sterile filter:** Midisart 2000, 0.2 µm, PTFE-Filter, Sartorios, Göttingen. - **Laminar-Flow-Box:** Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1. - **Brine shrimp eggs** (***Artemia salina***)**:** SERA Artemia Salinenkrebseier, SERA Heinsberg. - Salinenkrebsfutter: micro cell DOHSE Aquaristik KG Bonn (brine shrimp eggs and food can be obtained from aquaristic shops).

6.5 Recipies

All cultures were autoclaved at 1.2 bar and 120 °C. Sterilisation time for 1-liter shaker culture: 33 min, 2-liter concentrated medium for fermenter: 50 min and fermenter containing 16 liters water: 82 min

6.5.1 Artificial sea water

Iron citrate	2 g (powder)
NaCl	389 g
$MgCl_2.6H_2O$	176 g
Na_2SO_4	68.8 g
$CaCl_2$	36.0 g
Na_2HPO_4	0.16 g
SiO_2	0.30 g
Trace element stock soln.	20 ml
Stock soln.	200 ml

All ingredients were dissolved in tap water and made to 20 liters.

Trace element stock solution

H_3BO_3	0.611 g
$MnCl_2$	0.389 g
$CuSO_4$	0.056 g
$ZnSO_4 \cdot 7H2O$	0.056 g
$Al_2 (SO_4)_3 \cdot 18H_2O$	0.056 g
$NiSO_4 \cdot 6H_2O$	0.056 g
$CO (NO_3)_3 \cdot 6 H_2O$	0.056 g
TiO_2	0.056 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.056 g
LiCl	0.028 g
$SnCl_2$	0.028 g
KI	0.028 g

All ingredients were dissolved in 1 liter of tap water.

Stock solution

KCl	110 g
$NaHCO_3$	32 g
KBr	16 g
$SrCl_2$. $6H_2O$	6.8 g (dissolved separately)
H_3BO_3	4.4 g
NaF	0.48 g
NH_4NO_3	0.32 g

All ingredients were dissolved in 2 liters of tap water.

6.5.2 Nutrients

M_2 without sea water

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of bacto agar.

M_2^+ with sea water

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Artificial sea water	500 ml
Tap water	500 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of bacto agar.

C with sea water

Meat extract	1 g
Glucose	10 g
Yeast extract	1 g
Pepton	2 g
Artificial sea water	500 ml
Tap water	500 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of bacto agar

Luria-Bertani-Medium (LB)

Trypton	10 g
Yeast extract	5 g
NaCl	10 g
Tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of bacto agar.

Soja-Mannit Medium

Soybean meal (defatted)	20 g
D (-)-Mannit	20 g
Tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of bacto agar.

M Test Agar (for test organisms *Escherichia coli*, *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus, Mucor miehei* (Tü284):

Malt extract	10 g
Yeast extract	4 g
Glucose	4 g
Bacto agar	20 g
Demineralised water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH.

Sabouraud-Agar

(for test organism *Candida albicans*)

Glucose	40 g
Bacto peptone	10 g
Bacto agar	20 g
Demineralised water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH.

Nutritional solution A

Soybean meal (defatted)	30 g
Glycerol	30 g
$CaCO_3$	2 g
Artificial sea water	750 ml
Demineralised water	250 ml

Nutritional solution B

Starch	10 g
NZ-Amine	5 g
Soybean meal	2g
Yeast extract	5 g
KNO_3	3 g
Algal extract	2.5 ml
Artificial sea water	750 ml
Demineralised water	250 ml

6.6 Stock solutions and media for cultivation of algae

Fe-EDTA

0.7 g of $FeSO_4 \cdot 7\ H_2O$ and 0.93 g EDTA (Titriplex III) are dissolved in 80 ml of demineralised water at 60 °C and then diluted to 100 ml.

Trace element Solution II:

Solution A:

$MnSO_4 \cdot H_2O$	16.9 mg
$Na_2MoO_4 \cdot 2H_2O$	13.0 mg
$Co\ (NO_3)_2 \cdot 6H_2O$	10.0 mg

Salts are dissolved in 10 ml of demineralised water.

Solution B:

$CuSO_4 \cdot 5H_2O$	5.0 mg
H_3BO_3	10.0 mg
$ZnSO_4 \cdot 7H_2O$	10.0 mg

Salts are dissolved each in 10 ml of demineralised water. Solutions A is added to B and diluted to 100 ml with demineralised water.

Bold's Basal medium (BBM): (for algae *Chlorella vulgaris, Chlorella sorokiniana* and *Scenedesmus subspicatus*.

$NaNO_3$	0.250 g
KH_2PO_4	0.175 g
K_2HPO_4	0.075 g
$MgSO_4 \cdot 7H_2O$	0.075 g
NaCl	0.025 g
$CaCl_2 \cdot 2\ H_2O$	0.025 g
Fe-EDTA	1.0 ml
Trace element solution II	0.1 ml

Salts are dissolved in 10 ml of demineralised water and added to Fe-EDTA and trace element solution II. The mixture made to one litre with demineralised water. Solid medium was prepared by adding 18 g of bacto agar.

6.7 Microbiological and analytical methods

6.7.1 Storage of Strains

All North Sea strains were stored in a liquid nitrogen for long time. The strains were used to inoculate agar plates with the suitable media at room temperature.

6.7.2 Pre-screening

The microbial isolates (obtained from culture collections) were cultured in a 1 liter scale in 1 liter-Erlenmeyer flasks each containing 200~250 ml of M_2 or (for marine strains) M_2^+ medium. The flasks were shaken for 3-5 days at 28 °C after which the entire fermentation broth was freeze-dried and the residue extracted with ethyl acetate. The extracts were evaporated to dryness and used for the antimicrobial tests in a concentration of 50 mg/ml.

6.7.3 Biological screening

The crude extract was dissolved in $CHCl_3$/10%MeOH (concentration 50 mg/ml) in which the paper disks impregnated, dried under sterile flow box and put on an agar plates inoculated with *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57), *Escherichia coli*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Candida albicans* and *Mucor miehei* (Tü284).

The plates were incubated at 37 °C for bacteria (12 hours), 27 °C for fungus (24 hours), and 24-26 °C under day-light for micro-algae (96 hours). The diameter of the inhibition zones were measured by ruler.

6.7.4 Chemical and pharmacological screening

Samples of the extracts were separated on silica gel glass plates (10 × 20 cm) with two solvent systems $CHCl_3$/5%MeOH and $CHCl_3$/10%MeOH. After drying, the plates were photographed under UV light and marked at 366 and 254 nm, and subsequently stained by anisaldehyde and Ehrlich's reagent. Finally, the plates were scanned for documentation. For the pharmacological investigations, approximately 25 mg of the crude extract was sent to industrial partners.

6.7.5 Brine shrimp microwell cytotoxicity assay [348]

To a 500 ml separating funnel, filled with 400 ml of artificial sea water, 1g of dried eggs of *Artemia salina L*. and 1 g food were added. The suspension was aerated by bubbling air into the funnel and kept for 24 to 48 hours at room temperature. After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining eggs dropped. In order to use only the active larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. 30 to 40 shrimp larvae were transferred to a deep-well microtiter plate (wells diameter 1.8 cm, depth 2 cm) filled with 0.2 ml of salt water. A solution of 20 µg of the crude extract in 5 to 10 µl DMSO was added and the plate kept at r.t. in the dark. After 24 h, the dead larvae were determined by counting the number of the dead animals in each well under microscope. The living larvae were killed by addition of *ca*. 0.5 ml methanol so that subsequently the total number of the animals could be determined. To each test row, blind sample was accompanied by adding DMSO. The mortality rate MS was calculated using the following formula:

$$M = \left[\frac{A\text{-}B\text{-}N}{G\text{-}B\text{-}N} \right] . 100$$

Where:

M	percent of the dead larvae after 24 h.
A	Number of the dead larvae after 24 h.
B	Average number of the dead larvae in the blind samples after 24 h
N	Number of the dead larvae before starting of the test.
G	Total number of larvae

6.7.6 Fermentation in 20 or 25-liter fermenter

The 20-liter glass fermenter was filled with 16 liters of water and closed with the metal lid. The aeration, acid/base and antifoam systems were connected to the fermenter where the inlet and outlet openings and tubes were closed with aluminium-foil and clamps. The pH electrode port was closed with a glass stopper. The fermenter was autoclaved for 90 minutes at 120 °C, after that it was taken out of the autoclave where the air supply, stirring motor and water circulation pumps were

switched on. The acid (2N HCl), base (2N NaOH) and antifoam (1% Niax/70% EtOH) were connected and filled. The pH electrode was sterilised for 30 minutes with 70% EtOH and then connected with the lid. Parallel to the preparation of the fermenter itself, two litres of concentrated medium containing suitable nutrients were prepared and autoclaved for 50 minutes at 120 °C. After cooling, the medium was added to the fermenter and the pre-culture was used to inoculate the fermenter.

6.8 Primary screening results

Bases of evaluation:

Antibiotic screening (disk diffusion test): The test is performed using paper discs with a diameter of 8-mm under standardized conditions (see above). If the inhibition zone is ranging from 11 to 20 mm, the compound is considered to be weakly active (+), from 21 to 30 mm designated as active (++) and over 30 mm is highly active (+++). - **Chemical screening:** evaluation of the separated bands by the number, intensity and colour reactions with different staining reagents by TLC. - **Toxicity test:** By counting survivors after 24 hrs, the mortality of the extracts was calculated. The extract, fraction or isolated compounds were considered inactive fewer than 10% (-), from 10 to 59 % weakly active (+), from 60 to 95 % active (++) and over 95% as strongly active (+++).

Cytotoxic activity

Nematode Test (Cytotoxic activity):

In sample tube, prepare a concentration of 7.5 mg/ml of the crude extract in methanol. In a tissue culture test plate put 40 µl of the tested solution by micropippet leaving two holes free one for the reference standard (postive control) and the second for the blank preparation (negative control = blind sample). In the first hole, put 3 mg of ivermectin in 40 µl methanol and in the second, put 40 µl methanol. Leave the solvent for 15 min to be evaporated. Take nematode from the agar plate by rinsing with M 9-buffer over a funnel in a centrifugal tube. In a nematode counting box, put 100 µl of Nematode solution and cover it, then calculate the nematode number with microscope (X = 50). Adjust the concentration of nematode to be 100 nematodes/100 µl with buffer. If the concentration is too low, centrifuge and take some of the super-

natant buffer and count again. After shaking, put 400 µl of the suspension with the micropipette in the hole of the tissue culture test plate. After 20 and 48 hours of incubation at room temperature, count the number of dead Nematodes, the test would be good if the postive control gives 100 % mortality. The percentage of cyctotoxcity was determined by the formula written down.

$$M = \left[\frac{A-B-N}{G-B-N} \right] . 100$$

Where:

M	percent of dead Nematodes after 20 and 48 h.
A	Number of dead Nematodes after 20 and 48 h.
B	Average number of dead Nematodes in the blind sample after 20 and 48 h
N	Number of dead Nematodes before starting of the test.
G	Total number of Nematodes

M9 Buffer for the Nematode test:

KH_2PO_4	3g
Na_2HPO_4	6 g
NaCl	5g
$MgSO_4$	1g
Tap water	1000 ml

Before autoclaving, the pH should be kept at 6.9

Cyctotoxcity test

The crude extracts are classified according to the number of dead larvae after 24 h. Under 10 % as uneffective (-), from 10 to 59 % as weak effective (+), from 60 to 95 % as effective (++) and over 95 % as strong effective (+++)

7 Metabolites from selected strains

North Sea bacteria were isolated from the free water column at the island of Helgoland from the German Wadden Sea by I. Wagner-Döbler (Braunschweig) and M. Meiners (Emden) in the Department of Microbiology at the National Research Institute for Biotechnology (GBF). The marine *Streptomyces* strains were obtained from Alfred-Wegener-Institutes für Polar- und Meeresforschung in Bremerhaven. All terrestrial *Streptomycetes* were obtained from the strain collection of bioLeads in Heidelberg.

7.1 *Cytophaga marinoflava* sp. Am13.1

The strain was inoculated on LB medium + artificial Sea water agar plates from soil culture and incubated for 4 days at 28 °C. It showed faint yellow gel colonies. One plate served to inoculate 12 of 1 liter-Erlenmeyer flasks each containing 200 ml of LB medium. The flasks were shaken at 95 rpm for 4 days at 28 °C. After two days, the colour of the medium changed to faint yellow. The culture was filtered over celite, and the water phase was extracted three times with ethyl acetate. The mycelial cake was kept in ethyl acetate, and dispersed by using ultrasound for 15 minutes to break the cells. The ethyl acetate extracts were combined and evaporated to obtain 114 mg of crude extract.

7.1.1 Pre-screening

Chemical screening by TLC showed one remarkable yellow zone that gave no colour reaction with 2N NaOH. In addition, numerous UV absorbing bands were observed, most of them coloured to violet-blue by spraying with anisaldehyde/sulphuric acid, pink with Ehrlich's reagent, in addition to some blue bands, were detected by chlorine/o-anisidine reaction. In the biological screening, the crude extract exhibited the activities shown in Table 14.

Table 14: Antimicrobial activity of the crude extract from strain Hel AM13,1 (1mg/ml, diameter of inhibition zones in mm)

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	12
Escherichia coli	12
Staphylococcus aureus	21
Streptomyces viridochromogenes (Tü57)	18
Mucor miehei (Tü284)	20
Chlorella vulgaris	20
Chlorella sorokiniana	20

7.1.2 Fermentation, isolation and identification of metabolites

The work-up procedure and the isolation of the new compounds, oligo-(ß-hydroxybutyric acid (cPHB)[343] (**318**), pharacine[89] **(73)** and 2,5-bis(3-methenylindolyl)-pyrazine[89] (**55**) have already been described.

The already known compounds, N$^{\beta}$-acetyltryptamin (**34**), mixture of 2-methylbutyramide (**35a**) and 3-methylbutyramide (**35b**), diketopiperazine mixture (probably *cyclo*(isoleucyl-valyl) **(37**)+ *cyclo*(isoleucyl-prolyl), *cis*-*cyclo*(tyrosyl-prolyl) (**39**), *cis*-*cyclo*(phenylalanyl-prolyl) (**40**), indolyl-3-ethylisovaleramid; madugin (**42**), N- (2-phenylethyl)-acetamide (**43**), tryptophol (**44**), indolyl-3-carboxylic acid (**45**), N-phenylethyl-isovaleramide (**58**), tryptanthrine (**63**), indolyl-3-acetic acid (**46a**), indolyl-3-acetic acid methyl ester (**46b**), 2-aminobenzamide (**47**), anthranilic acid (**48**), *o*-acetylaminobenzamide (**67**), phenyl acetic acid (**49a**), 2-phenylacetamid (**49b**) 2,2-dimethyl-2,3-dihydro-(1*H*)-quinazoline-4-on (**68**), *p*-hydroxyphenyl acetamide (**78**), 2-phenyllactic acid (**51a**), uracil (**52**) and thymine (**53),** are briefly characterized as follows:

N$^{\beta}$-Acetyltryptamin (34): $C_{12}H_{14}N_2O$ (202.11), was afforded from sub-fraction F_2a by applying to Sephadex LH 20 ($CHCl_3$/MeOH 3:2), PTLC ($CHCl_3$/5%MeOH), and finally by Sephadex LH-20 ($CHCl_3$/MeOH 3:2), as an UV absorbing colourless solid (80 mg), stained to pink/violet colour by anisaldehyde/sulphuric acid, and pink (later to blue) by Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.5 ($CHCl_3$ / 5 % MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 8.30 (s, br, 1 H, 1-NH), 7.60 (d, ^3J = 8.0 Hz, 1 H, 4-H), 7.38 (d, ^3J = 7.8 Hz, 1 H, 7-H), 7.26 (t, 7.8 Hz, 1 H, 6-H), 7.08 (t, 8.0, 1 H, 5-H) , 7.03 (d, 1.1 Hz, 1 H, 2-H), 5.70 (s, br, 1 H, NH), 3.58 (q, ^3J = 6.3 Hz, 2 H, 2'-CH_2), 2.98 (t, ^3J = 6.6 Hz, 2 H, 1'-CH_2), 1.93 (s, 3 H, 5'-CH_3). – **^{13}C NMR** ($CDCl_3$, 50 MHz): δ =

170.5 (C=O, C_q-4'), 136.4 (C_q-7a), 127.2 (C_q-3a), 122.3 (CH-6), 121.7 (CH-2), 119.0 (CH-5), 118.5 (CH-4), 112.3 (C_q-3), 111.5 (CH-7), 40.0 (CH_2-2'), 25.2 (CH_2-1'), 23.1 (CH_3-5'). – **CI MS (NH_3)**: *m/z* (%) = 422.3 ($[2\ M + NH_4]^+$, 6), 405.3 ($[2\ M+H]^+$ 5), 220.1 ($[M + NH_4]^+$, 100), 203.1 ($[M + H]^+$, 10).

Mixture of 2-Methylbutyramide (35a) and 3-Methylbutyramide (35b): $C_5H_{11}NO$ (101.148), applying sub-fraction F2a to HPLC (STM2, R_t = 81.20 min) led to a colourless solid (2 mg), turned to a faint pink colour with Ehrlich's reagent. - $\boldsymbol{R}_f$ = 0.52 ($CHCl_3$/MeOH 5 %). – **^{1}H NMR** ($CDCl_3$, 300 MHz) of **35a**: δ = 5.45 (s, br, 2 H, NH_2), 2.19 (m, 1 H, 2-H), 1.65 (m, 1 H, 3-H_A), 1.43 (m, 1 H, 3-H_B), 1.17 (d, ^3J = 6.8 Hz, 3 H, 5-CH_3), 0.92 (t, ^3J = 7.4 Hz, 3 H, 4-CH_3). **^{1}H NMR (**$CDCl_3$, 300 MHz) of **35b**: δ = 5.45 (s, br, 2 H, NH_2), 2.07 (m, 2 H, CH_2-2), 1.65 (m, 1 H, H-3), 0.98 (d, ^3J = 7.4 Hz, 6 H, CH_3-4,5). **– CI MS (NH_3)**: *m/z* **(%)** = 220.2 ($[2\ M + NH_4]^+$, 5.5), 203.2 ($[2\ M + H]^+$, 13.5), 136.1 ($[M + NH_4]^+$, 14), 119.1 ($[M + NH_4]$, 100), 102.1 ([M + H]$^+$, 21). – **EI MS** (70 eV): *m/z* (%) = 101.1 ($[M]^{+\cdot}$, 6), 86.1 ($[M – Me]^{+\cdot}$, 58), 73.0 ($[M- C=O]^{+\cdot}$, 100), 59.0 ($[H_2N\text{-}C=O\text{-}CH_3]^{+\cdot}$, 62), 57.1 ($[HN\text{-}C=O\text{-}CH_2\ ]^{+\cdot}$, 34), 44.0 ($[M - CONH_2]^{+\cdot}$, 67), 41.0 (58).

Diketopiperazine mixture (probably *cis-Cyclo*(isoleucyl-valyl) (37) + *cis-Cyclo*(leucyl-prolyl): $C_{11}H_{20}N_2O_2$ **(**212.289), colourless solid (12 mg), was isolated from sub-fraction F2a by Sephadex LH 20 ($CHCl_3$/MeOH 6:4) and HPLC (R_t = 20.20 min), as UV absorbing zone, coloured to pink, violet and blue by Ehrlich's reagent, anisaldehyde/sulphuric acid and chlorine/anisidine reaction, respectively. - $\mathbf{R}_f$ = 0.55 ($CHCl_3$ /5%MeOH). – **^{1}H NMR** ($CDCl_3$, 200 MHz) of the mixture: δ = 6.26, 6.02 (2 s br, 2 H, 2 NH), 4.11 (m, 1 H), 3.98 (s br, 1 H), 3.60 (m, 1 H), 2.41 (m, 1 H), 2.18-2.05 (m, 3 H), 1.55 (m, 2 H), 1.11-0.85 (m, 12 H, 4 CH_3). **– CI MS (NH_3)**: *m/z* (%) = 425 ($[2\ M + H]^+$, 2), 247.2 ($[M +NH_3 + NH_4]^+$, 10), 230.2 ($[M + NH_4]^+$, 100), 213.2 ($[M + H]^+$, 17).

***cis-Cyclo*(tyrosyl-prolyl) (39)**: $C_{14}H_{16}N_2O_3$ (260), colourless solid (15 mg), was isolated from the sub-fraction F2a by Sephadex LH20 followed by purification on HPLC (R_t = 13.80 min). It was detected as UV absorbing zone, violet by anisaldehyd /sulphuric acid and pink with Ehrlich's reagent, as well as blue with chlorine/anisidine reaction. – $\boldsymbol{R}_f$ = 0.82 ($CHCl_3$ 10 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.15 (d, ^3J = 8.6 Hz, 2 H, 3',5'-H), 6.72 (d, ^3J = 8.6 Hz, 2 H, 2',6'-H), 6.08 (s br, 1 H, NH), 4.21 (dd, ^3J = 7.8, J = 2.1 Hz, 1 H, 3-H), 4.08 (t, ^3J = 5.1 Hz, 1 H, 6-

H), 3.65-3.50 (m, 2 H, 9-CH_2), 3.42 (dd, ^2J = 14.2 Hz, ^3J = 5.1 Hz, 1 H, 10-H_a), 2.79 (dd, ^2J = 9.2 Hz, ^3J = 5.1 Hz, 1 H, 10-H_b), 2.30, 2.00-1.78 (2m, 1 + 3 H, 7-, 8-CH_2). **– EI MS** (70 eV): *m/z* (%) = 260 ($[M]^{+\cdot}$, 21), 154 (100), 126 (25), 107 (41), 70 (14).

***cis-Cyclo*(phenylalanyl-prolyl) (40)**: $C_{14}H_{16}N_2O_2$ (244.1), isolated from sub-fraction F2a by applying to Sephadex LH20 and HPLC (R_t = 14.90 min), as an UV absorbing colourless solid (10 mg), coloured to violet by anisaldehyde/sulphuric acid and pink by Ehrlich's reagent, respectively. – $\boldsymbol{R}_f$ = 0.45 ($CHCl_3$/5% MeOH), 0.67 ($CHCl_3$/10% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.29 (m, 5 H, Ar-H), 5.82 (s, br, 1 H, NH), 4.30 (dd, ^3J = 8.3 Hz, ^3J = 3.0 Hz, 1 H, 3-H), 4.08 (m, 1 H, 6-H), 3.62 (m, 3 H, 9-CH_2, 10-H_a), 2.80 (dd, ^2J = 14.1 Hz, ^3J = 8.3 Hz, 1 H, 10-H_b), 2.36, 2.20-1.81 (2 m, 1 + 3 H, 7,8-CH_2). – **^{13}C/APT NMR (**$CDCl_3$, 75 MHz): δ = 169.3 (C=O, C_q-2), 164.9 (C=O, C_q-5), 135.8 (C_q-1'), 129.1 (2 CH, C-3',5'), 128.9 (2 CH, C-2',6'), 127.2 (CH-4'), 60.0 (CH-6), 56.1 (CH-3), 45.2 (CH_2-9), 36.7 (CH_2-10), 28.2 (CH_2-7), 22.2 (CH_2-8). **– EI MS** (70 eV): *m/z* (%) = 244 ($[M^+]$, 100), 154 (44).

Indolyl-3-ethylisovaleramid; Madugin (42): $C_{15}H_{20}N_2O$ (244.3), was afforded from sub-fraction F_2b using Sephadex LH-20 followed by HPLC (R_t = 21.10 min), as colourless solid (5 mg). It showed UV-absorption, violet by spraying with anisaldehyde/sulphuric acid and pink (changing to blue) with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.45 ($CHCl_3$/ MeOH 5%). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.08 (s, br, 1 H, 1-NH), 7.62 (dd, ^3J = 7.9 Hz, ^4J = 1.1 Hz, 1 H, 4-H), 7.39 (dd, ^3J = 8.1 Hz, ^4J = 1.1 Hz, 1 H, 7-H), 7.21 (td, ^3J = 7.1 Hz, 1 H, 6-H), 7.13 (td, ^3J = 7.2 Hz, 1 H, 5-H), 7.05 (d, ^3J = 2.2 Hz, 1 H, 2-H), 5.55 (s, br, 1 H, 3' -NH), 3.64 (q, ^3J = 6.2, 2 H, 2'-H_2), 3.0 (t, ^3J = 6.1 Hz, 2 H, 1'-CH_2), 2.13 (m, 1 H, 6'-H), 2.00 (dd, ^3J = 7.0 Hz, 2 H, 5'-H), 0.90 (d, ^3J = 6.9 Hz, 6 H, 7',8'-CH_3). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 10.00 (s, br, 1 H, 1-NH), 7.60 (dd, ^3J = 7.9 Hz, ^4J = 1.1 Hz, 1 H, 4-H), 7.39 (dd, ^3J = 8.1 Hz, ^4J = 1.1 Hz, 1 H, 7-H), 7.18 (d, ^3J = 2.2 Hz, 1 H, 2-H) 7.11 (td, ^3J = 7.1 Hz, 1 H, 6-H), 7.00 (td, ^3J = 7.2 Hz, 1 H, 5-H), 3.50 (q, ^3J = 6.18, 2 H, 2'-CH_2), 2.92 (t, ^3J = 6.11 Hz, 2 H, 1'-CH_2), 2.05* (m, 3 H, 6'-CH, 5'-CH_2), 0.87 (d, ^3J = 6.95 Hz, 6 H, 7',8'-CH_3); * under solvent signal. – **^{13}C/APT NMR (**$CDCl_3$, 150 MHz): δ = 172.5 (C_q, HN-C=O), 136.4 (C_q-8), 127.3 (C_q-9), 122.2 (CH-6), 122.0 (CH-2), 119.5 (CH-5), 118.7 (CH-4), 113.1 (C_q-3), 111.2 (CH-7), 46.2 (CH_2-5'), 39.5 (CH_2-2'), 26.1 (CH-6'), 25.45 (CH_2-1'), 22.4 (CH_3-7'/8'). – **DCI (NH_3)**: *m/z* (%) = 506.4 ($[2\ M + NH_4]^+$, 4), 489.4 ($[2\ M + H]^+$, 6), 262.2 ($[M + NH_4]$, 100), 245.2 ($[M + H]^+$, 35). **– EI MS**

(70 eV): *m/z* (%) = 244.2 ($[M]^{+\cdot}$, 14), 143.1 ($[M- (H_2N-C=O-CH_2-CH (CH_3)2)]^{+\cdot}$, 100), 130.1 (42). **HREI MS**: *m/z* = 244.1576 (calcd. 244.15756 for $C_{15}H_{20}N_2O$).

N-(2-Phenylethyl)-acetamide (43): $C_{10}H_{13}NO$ (163.2), isolated from sub-fraction F_2b using 2 × Sephadex LH 20 and 1 × PTLC ($CHCl_3$/7%MeOH), as an UV absorbing colourless solid (4 mg), coloured to pink by Ehrlich's reagent and violet by anisaldehyde/sulphuric acid. - $\boldsymbol{R}_f$ = 0.50 ($CHCl_3$/ CH_3OH 90:10). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.38-7.16 (m, 5 H, Ar-H), 5.85 (s, br, 1 H, NH), 3.52 (q, ^3J = 5.1 Hz, 2 H, 2' -CH_2), 2.83 (t, ^3J = 5.1 Hz, 2 H, 1'-CH_2), 1.97 (s, 3 H, 5'-CH_3). **– EI MS (**70 eV): *m/z* (%) = 163.2 ($[M]^+$, 30), 104.1 (100), 91.1 (22), 72.1 (16), 43.1 (30).

Tryptophol; 3-Indolylethanol (44): $C_{10}H_{11}NO$ (161.2), afforded from sub-fraction F2c by applying to PTLC (20×20 cm, $CHCl_3$/8% MeOH) and Sephadex LH 20 ($CHCl_3$/MeOH 6:4), as an UV absorbing colourless solid (20 mg), turned to intense violet by anisaldehyde/sulphuric acid and pink with Ehrlich's reagent. **-** $\boldsymbol{R}_f$ = 0.28 ($CHCl_3$/5 % MeOH). **– ^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.05 (s, br, 1 H, NH), 7.63 (d, ^3J = 8.2 Hz; 1 H, 4-H), 7.39 (d, ^3J = 8.2 Hz, 1 H, 7-H), 7.21 (dd, ^3J = 8.2, ^4J = 1.3 Hz, 1 H, 5-H), 7.19 (dd, ^3J = 8.2 Hz, ^4J = 1.3 Hz, 1 H, 6-H), 7.10 (s, 1 H, 2-H), 3.93 (t, ^3J = 6.6 Hz, 2 H, 9-CH_2), 3.05 (t, ^3J = 6.6 Hz, 2 H, 8-CH_2), the OH is invisible. **– ^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 136.4 (C_q-7a), 127.4 (C_q-3a), 122.5 (CH-6), 122.2 (CH-2), 119.5 (CH-5), 118.8 (C_q-4), 112.2 (C_q-3), 111.2 (CH-7), 62.6 (CH_2-2'), 28.7 (CH_2-1'). **– EI MS** (70 eV): *m/z* (%) = 161 ($[M]^{+\cdot}$, 29), 130 (100), 103 (11), 77 (16).

Indolyl-3-carboxylic acid (45): $C_9H_7NO_2$ (161.16), isolated from sub-fraction F2c using PTLC (20 × 20 cm, $CHCl_3$/10% MeOH), followed by Sephadex LH 20, as an UV absorbing colourless solid (10 mg), coloured to orange (changed later to violet) by anisaldehyde/sulphuric acid and pink with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.43 ($CHCl_3$/MeOH 9:1). **– ^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 10.96 (s, br, 1 H, N-H), 8.15 (m, 1 H, 4-H), 8.06 (d, ^3J = 3.1, 1 H, 2-H), 7.52 (m, 1 H, 7-H), 7.21 (m, 2 H, 5,6-H). **– EI MS**: *m/z* (%) = 161 ($[M]^{+\cdot}$, 100), 144 ($[M-OH]^{+\cdot}$, 73), 117 ($[M-COO]^{+\cdot}$, 23), 89 (16).

Indolyl-3-acetic acid (46a): $C_{10}H_9NO_2$ (175.18), applying sub-fraction F3a to Sephadex LH 20 led to UV absorbing colourless needles (500 mg), stained to intense orange (later to violet) by anisaldehyde/sulphuric acid, and to pink with Ehrlich's

reagent. – $\boldsymbol{R}_f$ = 0.30 ($CHCl_3$ /5 % MeOH). – **^{1}H NMR (**[D_6]DMSO, 200 MHz): δ = 10.85 (s, br, 1 H, NH), 7.55 (dd, ^3J = 7.5 Hz, ^4J = 1.5 Hz, 1 H, 4-H), 7.48 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz 1 H, 7-H), 7.25 (d, ^3J = 2.1 Hz, 1 H, 2-H), 7.18 (t, ^3J = 8.3 Hz, 1 H, 6-H), 7.05 (t, ^3J = 7.6 Hz, 1 H, 5-H), 3.68 (s, 2 H, 8-CH_2). – **CI MS** (NH_3): *m/z* (%) = 176.1 ($[M + H]^+$, 5), 193.1 ($[M + NH_4]^+$, 100), 210.2 ($[M + NH_3 + NH_4]^+$, 368.2 ($[2 M + NH_4]^+$, 2). – **EI MS** (70 eV): *m/z* (%) = 175.0 ($[M]^{+\cdot}$, 40), 131.0 ([M-COO]$^{+\cdot}$, 10), 130.1 ([M-COOH]$^{+\cdot}$, 100), 103.0 (7), 77.0 (8).

Indolyl-3-acetic acid methyl ester (46b): $C_{11}H_{11}NO_2$ (189.2), To 10 mg of the indolyl-3-acetic acid dissolved in dry dichloromethane (5 ml) at 0 °C, 0.3 ml of ethereal diazomethane solution was added. After 1 min, the reaction mixture was evaporated to dryness to give a colourless solid. – $\boldsymbol{R}_f$ = 0.8 ($CHCl_3$ /5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 8.09 (s, br, 1 H, NH), 7.63 (dd, ^3J = 7.5 Hz, ^4J = 1.5 Hz, 1 H, 4-H), 7.38 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 7-H), 7.20 (td, ^3J = 8.3 Hz, 1.1 Hz, 1 H, 6-H), 7.18 (s, br, 1 H, 2-H), 7.14 (td, ^3J = 7.6 Hz, 1.2 Hz, 1 H, 5-H), 3.80 (s, 2 H, 8-CH_2), 3.71 (s, 3 H, 10-CH_3). – **EI MS** (70 eV): *m/z* (%) = 189.2 ($[M]^{+\cdot}$, 42), 130.1 ([M-$COOCH_3$]$^{+\cdot}$, 100), 103.0 (7), 77.0 (8).

2-Aminobenzamide (47): $C_7H_8N_2O$ (136.151), UV blue fluorescent colourless solid (50 mg), was obtained from sub-fraction F3a using PTLC (20×20 cm, $CHCl_3$/7%MeOH) followed by Sephadex LH-20 ($CHCl_3$/MeOH 6:4)]. It showed a colour staining to yellow, by either anisaldehyde/sulphuric acid or Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.25 ($CHCl_3$ /5% MeOH). – **^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 7.63 (s, br, 1 H, NH), 7.50 (dd, ^3J = 8.3 Hz, ^4J = 1.3 Hz, 1 H, 6-H), 7.13 (td, ^3J = 8.3 Hz, ^4J = 1.3 Hz, 1 H, 4-H), 7.00 (s, br, 1 H, NH), 6.68 (dd, ^3J = 8.3 Hz, ^4J = 1.3 Hz, 1 H, 3-H), 6.52 (s, br, 2 H, NH_2), 6.48 (t, ^3J = 8.3 Hz, 1 H, Ar-H). – **EI MS (**70 eV): *m/z* (%) = 136 ($[M]^{+\cdot}$, 94), 119 ([M-NH_3]$^{+\cdot}$, 100), 92 (62), 65 (27). – **CI MS (**NH_3): *m/z* (%) = 273 ($[2M + H]^+$, 4), 154 ($[M + NH_4]^+$,100), 137 ($[M + H]^+$, 82).

Anthranilic acid (**48)**: $C_7H_8N_2O_2$ (137.136), obtained from sub-fraction F3b by PTLC (20 × 20 cm, $CHCl_3$/MeOH 7 %) and Sephadex LH-20 ($CHCl_3$/MeOH 6:4), as yellow solid (22 mg), as an UV blue fluorescent substance, coloured to yellow by anisaldehyde/sulphuric acid and/or Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.22 ($CHCl_3$ /5% MeOH). – **^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 7.68 (dd, ^3J = 8.0 Hz, ^4J = 1.0, 1 H, 6-H), 7.18 (td, ^3J = 8.0 Hz, ^4J = 1.0, 1 H, 4-H), 6.68 (dd, ^3J = 8.0 Hz, ^4J = 1.1 Hz, 1

H, 3-H), 6.56 (td, ^3J = 8.2 Hz, ^4J = 1.1 Hz 1 H, 5-H). – **EI MS (**70 eV): *m/z* (%) = 137 ($[M]^{+\cdot}$, 72), 119 ($[M\text{-}NH_2]^{+\cdot}$, 100), 92 (52), 65 (17).

Phenyl acetic acid (49a): $C_8H_8O_2$ (136.148), delivered from sub-fraction F3b by applying to PTLC and Sephadex LH-20 ($CHCl_3$/MeOH 6.4), as an UV blue fluorescent colourless solid (12 mg). – $\boldsymbol{R}_f$ = 0.4 ($CHCl_3$ / MeOH 5%). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.32 (m, 5 H, Ph), 3.63 (s, 2 H, CH_2). – **EI MS** (70 eV): *m/z* (%) = 136 ($[M]^{+\cdot}$, 58), 91 (100), 65 (10).

2-Phenylacetamid (**49b)**: C_8H_9NO (135.1), purification of sub-fraction F3b, using PTLC followed by Sephadex LH-20 ($CHCl_3$/MeOH 6.4), led to an UV blue flourescent colourless solid (4 mg). It exhibited a faint pink colouration by spraying with Ehrlich's reagent and heating. – $\boldsymbol{R}_f$ = 0.52 ($CHCl_3$ / 5 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.39-7.22 (m, 5 H, Ph), 5.61 (s, br, 1 H, NH) 5.40 (s, br, 1 H, NH), 3.59 (s, 2 H, CH_2). – **EI MS** (70 eV): *m/z* (%) = 135 ($[M]^{+\cdot}$, 44), 92 (100), 91 (100), 65 (18), 44 (10).

***p* –Hydroxyphenyl-2-ethanol (50)**: $C_8H_{10}O_2$ (138.1), applying sub-fraction F3b (PTLC) to purification by HPLC (R_t = 12.79 min) led to an UV absorbing colourless solid (8 mg). – $\boldsymbol{R}_f$ = 0.77 ($CHCl_3$/MeOH 10%). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.08 (d, ^3J = 8.8 Hz, 2 H, 2',6'-H), 6.80 (d, ^3J = 8.8 Hz, 2 H, 3',5'-H), 3.82 (t, ^3J = 7.29, 2 H, 1-CH_2), 2.80 (t, ^3J = 7.3 Hz, 2 H, 2-CH_2). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 154.2 (C_q-4'), 130.5 (C_q-1'), 130.2 (CH-2',6'), 115.4 (CH-3',5'), 63.8 (CH_2-1), 38.2 (CH_2-2). – **CI MS** (NH_3): *m/z* (%) = 294.2 ([M + NH_4], 2), 173.1 ([M + NH_4 + NH_3], 51), 156.1 ($[M + NH_4]^+$, 100). – **EI MS (**70 eV): *m/z* (%) = 138.0 ($[M]^{+\cdot}$, 32), 107.0 ($[M\text{- }(CH_2\text{-}OH)]^{+\cdot}$, 100), 85.9 (26), 83.9 (38).

2-Phenyllactic acid (51a): $C_9H_{10}O_3$ (166.14), delivered from fraction 4, by PTLC and purified on HPLC (R_t = 12.10 min) as an UV absorbing colourless solid (4 mg). – $\boldsymbol{R}_f$ = 0.40 ($CHCl_3$ /MeOH 10%). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 7.35-7.18 (m, 5 H, Ph), 4.39 (dd, ^3J = 8.3, 3.4 Hz, 1 H, 2-H), 3.11 (ABX, J_{AB} = 14.4, J_{AX} = 3.4, J_{BX} = 3.4 Hz, 1 H, 3-H_A), 2.92 (ABX, J_{AB} = 14.4, J_{AX} = 7.1, J_{BX} = 7.2, 1 H, 3-H_B). – **EI MS (**70 eV): *m/z* (%) = 166.1 ($[M]^{+\cdot}$, 13), 148.1 ($[M\text{-}H_2O]^{+\cdot}$, 26), 91.0 (100).

Uracil (52): $C_4H_4N_2O_2$ (112.0)**,** was obtained from fraction 5 by applying to HPLC (R_t = 3.67 min), as an UV absorbing colourless solid (100 mg), coloured to

faint brown by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.23 ($CHCl_3$, MeOH 9:1). – **^{1}H NMR (**[D_6]DMSO, 200 MHz): δ = 10.85 (s, br, 2 H, 2 NH), 7.38 (d, ^3J = 8.2 Hz, 1 H, 6-H), 5.43 (d, ^3J = 7.5 Hz, 1 H, 5-H). – **CI MS (**NH_3): *m/z* (%) = 242 ([2 M + NH_4]$^+$, 44), 147.1 ([M + NH_4 + NH_3]$^+$, 86), 130.1 ([M + NH_4]$^+$, 100), 113.1 ([M + H]$^+$ 38).

Thymine; 5-Methyluracil (53): $C_5H_6N_2O_2$ (126), isolated from fraction 5, using HPLC (R_t = 5.02 min), as an UV absorbing colourless solid (32 mg), coloured to faint brown by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.30 ($CHCl_3$,/ 10% MeOH). – **^{1}H NMR (**[D_6] DMSO, 200 MHz): δ = 10.92 (s, br, 1 H, NH), 10.52 (s, br, 1 H, NH), 7.22 (d, ^3J = 5.2 Hz, 1 H, 6-H), 1.72 (s, 3 H, 5-CH_3). – **EI MS (**70 eV): *m/z* (%) = 126 ([M]$^{+\cdot}$, 100), 112 (62), 69 (30), 55 (40).

N-Phenylethyl-isovaleramide (58): $C_{13}H_{19}NO$ (205.29), delivered from sub-fraction F2d by Sephadex LH 20 followed by PTLC ($CHCl_3$/8% MeOH), and purified again on Sephadex LH 20 ($CHCl_3$/MeOH 6:4), to give an UV absorbing colourless solid (5 mg) which became orange by anisaldehyde/sulphuric acid, and pink (blue after few min) with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.53 ($CHCl_3$ /5% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.37-7.18 (m, 5 H, Ar-H), 5.58 (s, br, 1 H, NH), 3.53 (q, ^3J = 6.8 Hz, 2 H, 2'-CH_2), 2.81 (t, ^3J = 7.1 Hz, 2 H, 1'-CH_2), 2.09 (m, 1 H, 6'-H), 1.98 (d, ^3J = 6.8 Hz, 2 H, 5'-CH_2), 0.92 (d, ^3J = 6.8 Hz, 6 H, 7',8'-CH_3). – **EI MS**: *m/z* (%) = 205 ([M]$^{+\cdot}$, 52), 114 (18), 105.1 (26), 104.1 (100), 91 (18), 85.1 (46), 57.1 (34), 46.0 (22).

Tryptanthrine (63): $C_{15}H_8N_2O_2$ (248.2), applying sub-fraction F2d to purification using Sephadex LH 20 (2 times), followed by PTLC (20 × 20 cm; $CHCl_3$/MeOH 5%) led to deep yellow solid (12 mg). It exhibits no colour change with either 2 N NaOH or sulphuric acid. – $\boldsymbol{R}_f$ = 0.95 ($CHCl_3$ / MeOH 9:1). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.62 (d, ^3J = 7.9 Hz, 1 H, 11-H), 8.42 (d, ^3J = 7.9 Hz, 1 H, 5-H), 8.05 (d, ^3J = 8.1 Hz, 1 H, 2-H), 7.95- 7.75 (m, 3 H, 3,4,8-H), 7.65 (t, ^3J = 7.9 Hz, 1 H, 10-H), 7.43 (t, ^3J = 7.9 Hz, 1 H, 9-H). – **^{13}C/APT NMR (**125 MHz): δ = 182.6 (C_q-6), 158.1 (C_q-12), 146.6 (C_q-6_a), 146.3 (C_q-2_a), 144.3 (C_q-7_a), 138.3 (CH-3), 135.1 (CH-9), 130.7 (CH-4), 130.3 (CH-1), 127.6 (CH-5) 127.2 (CH-8), 125.4 (CH-11), 123.7 (C_q-5_a), 121.9 (C_q-11_a), 118.0 (CH-2). – **CI MS** (NH_3): *m/z* (%) = 266.1 ([M + NH_4]$^+$, 30), 249 ([M + H]$^+$, 58), 235 (100).

***o*-Acetylaminobenzamid (67)**: $C_9H_{10}N_2O_2$ (178.18), isolated from sub-fraction F3b by PTLC (20×20 cm; $CHCl_3$/MeOH 10%) followed by HPLC (R_t = 13.04 min), as UV blue fluorescent colourless solid (4 mg), coloured to grey-brown by anisaldehyde/sulphuric acid, and yellow by Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.19 ($CHCl_3$ /5 % MeOH). – **^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 11.55 (s, br, 1 H, NH), 8.41 (d, ^3J = 8.0 Hz, 1 H, 6-H), 8.20 (s, br, 1 H, NH), 7.79 (dd, ^3J = 8.0 Hz, ^4J = 1.6 Hz, 1 H, 3-H), 7.63 (s, br, NH), 7.43 (td, ^3J = 8.0 Hz, ^4J = 1.6 Hz, 1 H, 4-H), 7.10 (td, ^3J = 8.0 Hz, ^3J = 1.6 Hz, 1 H, 5-H), 2.08 (s, 3 H, 8-CH_3). – **^{13}C/APT NMR (**[D_6]DMSO, 125 MHz): δ = 170.6 (C=O, C_q-7), 168.9 (C=O, C_q-8), 139.5 (C_q-2), 132.0 (CH-4), 128.4 (CH-6), 122.2 (CH-5), 120.1 (CH-3), 119.7 (C_q-1), 24.8 (CH_3-9). – **EI MS** (70 eV): *m/z* (%) = 178.1 ([M]$^{+\cdot}$, 47), 146.1 (9), 136 ([M-C=O-CH_2]$^{+\cdot}$, 71), 119 (100), 92 (16), 66 (16), 43 (18). – **HREI MS**: *m/z* = 178.0742 (calcd. 178.0742 for $C_9H_{10}N_2O_2$).

2,2-Dimethyl-2,3-dihydro-(1*H*)-quinazoline-4-on (68): $C_{10}H_{12}N_2O$ (176.1), purification of the sub-fraction F3b by PTLC (20 × 20 cm, $CHCl_3$/10 MeOH) and followed by HPLC (R_t = 15.00 min) led to an UV blue flourecent colourless solid (6 mg). – $\boldsymbol{R}_f$ = 0.37 ($CHCl_3$ /MeOH 7%). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.83 (dd, ^3J = 8.1 Hz, ^4J = 2.1 Hz, 1 H, 5-H), 7.27 (td, ^3J = 8.1 Hz, ^4J = 1.8 Hz, 1 H, 7-H), 6.81 (t, ^3J = 8.1 Hz, 1 H, 6-H) 6.63 (s br, 2 H, 2 NH), 6.59 (dd, ^3J = 8.1 Hz, ^4J = 2.1 Hz, 1 H, 8-H), 1.56 (s, 6 H, 2-$(CH_3)_2$). – **EI MS** (70 eV): *m/z* (%) = 176.1 ([M]$^{+\cdot}$, 18), 161.1 ([M- Me]$^{+\cdot}$, 100), 120.0 (26), 92.1 (16). **HREI MS**: *m/z* = 176.0949 (calcd. 176.0950 for $C_{10}H_{12}N_2O$).

***p*–Hydroxyphenyl acetamide (78)**: $C_8H_9NO_2$ (151.16), applying fraction 4 to PTLC (20 × 20 cm, $CHCl_3$/MeOH 13 %) and HPLC (R_t = 2.40 min) led to an UV absorbing colourless solid (5 mg), which turned to faint pink by Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.4 ($CHCl_3$ / MeOH 10%). – **IR (**KBr) ν_{max} = 3394, 2926, 1660, 1616, 1543, 1515, 1421, 1383, 1314, 1280, 1255, 1232, 1191, 1027, 800, 688 cm^{-1}. – **^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 9.12 (s br 1 H, NH) 7.25 (s, br, 1 H, OH), 7.05 (d, ^3J = 8.8 Hz, 2 H, 2',6'-H), 6.70 (s, br, 1 H, NH), 6.65 (d, ^3J = 8.8 Hz, 2 H, 3',5'-H), 3.22 (s, 2 H, CH_2). – **^{13}C/APT NMR (**[D_6]DMSO, 125MHz): δ = 172.7 (C=O, C_q-1), 155.7 (C_q-4'), 129.8 (CH-2'/6'), 126.5 (C_q-1'), 114.8 (CH-3'/5'), 40.1 (CH_2). – **EI MS (**70 eV): *m/z* (%) = 151.1 ([M]$^{+\cdot}$, 46), 108.0 (22), 107.0 ([M- (CO-NH_2)]$^{+\cdot}$, 100), 90.0 ([M- (CO-NH_2 + OH)]$^{+\cdot}$, 4), 77.0 (8). – **HREI MS**: *m/z* = 151.0633 (calcd. 151.0663 for $C_8H_9NO_2$).

7.2 Marine *Streptomyces* sp. B1848

The marine *Streptomyces* sp. B1848 was growing with a white aerial mycelium on agar plates using M_2^+ medium at 28 °C for 3 days.

7.2.1 Pre-screening

A well grown agar plate of the marine *Streptomyces* isolate B1848, was inoculated to four 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2^+ medium on circular shaker culture (95 rpm) for 72 hours at 28 °C, and gave a white aerial mycelium with a yellowish fermentation broth. One litre of the culture broth was filtered and extracted with ethyl acetate to yield 120 mg of a brown residue.

The TLC of the resulting ethyl acetate extract showed various UV absorbing bands, stained mostly to blue by anisaldehyde/sulphuric acid. One fluorescence zone was shown, which stained to yellow by spraying with anisaldehyde/sulphuric acid, two red zones, and one orange band. The latter three coloured zones showed no colour change by treatment with NaOH or sulphuric acid.

Table 15: Antimicrobial activity of the crude extract from the strain B 1848

Tested microorganism	Inhibition zone ∅ [mm]
Staphylococcus aureus	10
Streptomyces viridochromogenes (Tü57)	13
Mucor miehei (Tü284)	30
Candida albicans	20
Chlorella vulgaris	16
Chlorella sorokiniana	12

7.2.2 Fermentation and working up

Twelve 1-liter Erlenmeyer flasks, each containing 250 ml of M_2^+ medium were adjusted to pH 7.8 before sterilisation and inoculated with the strain B1848 and shaken (95 rpm) for 72 h at 28 °C. The seed culture was inoculated into 25-liter jar fermenter, containing 21 liters of the culture medium M_2^+. The fermentation process was carried out for additional 3 days at 29 °C with agitation at 120 rpm. The resulting culture broth was mixed with diatomaceous earth (celite, ca. 1 kg), and filtered under pressure. Both of filtrate and mycelial cake were extracted four times with ethyl acetate. The mycelial cake was further extracted two times with acetone, where the extract was concentrated under reduced pressure. The resultant aqueous solution was extracted three times with ethyl acetate. The combined organic phases were con-

centrated under reduced pressure. The residue was dried to give 10.53 g of crude extract.

7.2.2.1 Isolation and identification of metabolites

The extract was defatted with cyclohexane (2.50 g), and the afforded methanol extract (7.0 g) was applied to flash silica gel column chromatography (30 × 600 mm), using gradient of $CHCl_3$-MeOH. After monitoring by TLC ($CHCl_3$/5; 10% MeOH), five fractions were obtained. Purification of the fractions led to 1-acetyl-*β*-carboline (**81**, 10 mg), 2'–deoxyadenosine (**87**, 8 mg), 6-hydroxy isatine (**93**, 1.5 mg), 2'-deoxythymidine (**95**, 25 mg), 2'-deoxyuridine (**96**, 12 mg), 4-methylamino-7,8-dimethyl-isoquinoline-3,6-dione (**97**, 1.7 mg), and 7-methylamino-3*H*-pyrrolo[2,3-c]-isoquinoline-6,9-dione **(102**, 1.5 mg).

7.2.3 Optimization of the marine *Streptomyces* sp. B1848

The producing strain was optimized using 6 different media and conditions of pH (6.5, 7.8), temperature (28, 35 °C), and shaking speed (110, 95 rpm) for 4 days (Table 16). TLC analysis and microbial assays (using the agar diffusion method) of the obtained extracts (1 liter-shaker culture) indicated the C medium as the optimum one for the desired components.

Table 16: Optimization of the marine *Streptomyces* sp. B1848

Medium	PH	Ingredients	Temp.	Cult. Period/day
A, M_2^+-medium	7.8, 6.5	10 g malt ext., 4 g yeast, 4 g glucose	28, 35 °C	4
B, SM-Medium	7.8, 6.5	20 g soja fatt frei, 20 g mannite	28, 35 °C	4
C, Meat extract medium	7.8, 6.5	10 g glucose, 2 g pepton, 1 yeast, 1 g meat ext.	28, 35 °C	4
D,$CaCl_2$-Medium	7.8, 6.5	40 g yeast, 5 g glucose, 45 g $CaCl_2$	28, 35 °C	4
E, LB-Medium	7.8, 6.5	10 g trypton, 5 g, yeast, 10 NaCl, 5 g glucose	28, 35 °C	4
F, Fish powder Medium	7.8, 6.5	21 g glucose, 5 g fish powder, 10 g flour (typ 405), 0.5 g $MgSO_4$, 1 g NaCl, 0.5 g $CaCl_2$, 10 ml trace element solution	28, 35 °C	4

Twelve 1-liter Erlenmeyer flasks, each containing 250 ml of C medium, were adjusted to pH 7.8 before sterilisation and inoculated with the strain B1848, and shaked (95 rpm) for 96 h at 28 °C. The seed culture was inoculated into a 50-liter jar fermenter, containing the same nutrient medium. The fermentation process was car-

ried out for additional 4 days at 28 °C with agitation at 120 rpm. The resulting culture broth (pale yellow) was mixed with diatomaceous earth (celite, ca. 1.8 kg), and filtered under pressure. The mycelial cake was extracted with ethyl acetate (three times), and two times with acetone. The acetone extract was concentrated under reduced pressure, and the aqueous solution was extracted once more with ethyl acetate. The combined organic phases were concentrated in *vacuo*, yielding 4.8 g of dried reddish-orange residue.

The culture filtrate was subjected to a Amberlite XAD-2 column, and the adsorbed metabolites were eluted with a methanol-water gradient. The methanol water solutions were concentrated, and the water residue was extracted with ethyl acetate. The resulting ethyl acetate extract was evaporated in *vacuo* to dryness, yielding 10.2 g of a dark crude extract.

TLC analysis of the water phase extract exhibited solely uninteresting components, and was ignored. The mycelial cake extract was applied to flash silica gel column chromatography (30 × 600 mm) using a DCM-MeOH gradient. After monitoring by TLC ($CHCl_3$/5; 10% MeOH), four fractions were obtained. Purification of the fractions II-IV, using PTLC and Sephadex LH 20, lead to isolation of five dark red compounds, among them compounds **97** (3 mg) and **102** (6 mg). The other three closely related compounds B14f (4.2 mg), B14g (8 mg) and B14r (4.1 mg) are still under investigation.

1-Acetyl-β-carboline (81): $C_{13}H_{10}N_2O$ (210.0), was obtained by applying fraction II (1.50 g) to PTLC (20 × 20 cm; $CHCl_3$/ 5% MeOH), followed by Sephadex LH-20 ($CHCl_3$/MeOH 6:4) to give an UV blue fluorescent, as faint yellow solid (10 mg). By TLC, it turned to yellow by anisaldehyde/sulphuric acid or Ehrlich's reagent. – R_f = 0.90 ($CHCl_3$/10% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 10.31 (s, br, 1 H), 8.58 (d, ^3J = 6.1 Hz, 1 H, 3-H), 8.19-8.16 (m, 2 H, 4,5-H), 7.62 (m, 2 H, 7,8-H), 7.33 (m, 1 H, 6-H), 2.91 (s, 3 H, 11-CH_3). – **^{1}H NMR** ([D_6]acetone, 300 MHz): δ = 11.22 (s, 1 H, 9-NH), 8.52 (d, ^3J = 5 Hz, 1 H, 3-H), 8.36 (d, ^3J = 5.1 Hz, 1 H, H-4), 8.28 (dd, ^3J = 8.1 Hz, ^4J = 1.3 Hz, 1 H, H-5), 7.84 (dd, ^3J = 8.1 Hz, ^4J = 1.3 Hz, 1 H, H-8), 7.61 (td, ^3J = 8.1 Hz, ^4J = 1.3 Hz, 7-H), 7.33 (td, ^3J = 8.1 Hz, ^4J = 1.3 Hz, 1 H, 6-H), 2.78 (s, 3 H, 11-CH_3). – **^{13}C NMR (**[D_6]acetone, 150 MHz): δ = 202.5 (C=O, C_q-9), 142.7 (C_q-8a), 138.6 (CH-3), 137.2 (C_q-1), 135.6 (C_q-1a), 132.1 (C_q-4a), 129.8 (CH-7), 122.5 (CH-5), 121.4 (C_q-5a), 121.2 (CH-6), 119.8 (CH-4), 25.7

(CH_3, 10-CH_3). – **(+)-ESI MS**: *m/z* (%) = 211 ($[M + H]^+$). – **EI MS** (70 eV): *m/z* (%) = 210.0 ($[M]^{+\cdot}$, 100), 182.0 ($[M\text{-}CO]^{+\cdot}$, 34), 168.0 ($[M\text{-} (COCH_2)]^{+\cdot}$, 92), 140.0 (22). – **CI MS** (NH_3): *m/z* = 211 ($[M + H]^+$, 100).

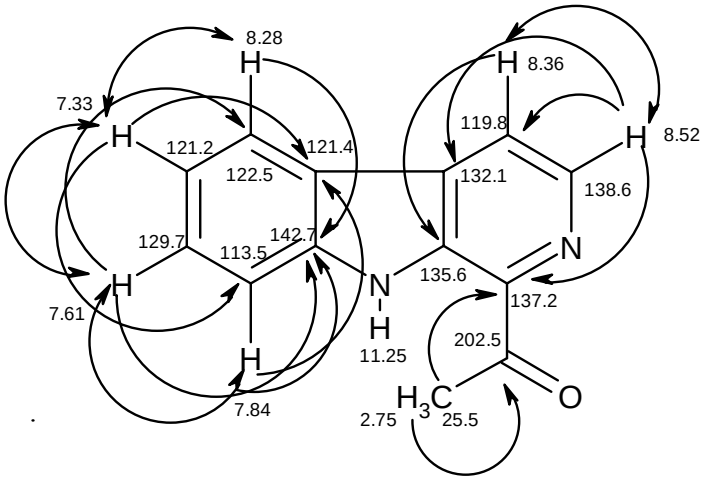


Figure 167: HMBC and H,H COSY correlations of 1-acetyl-*β*-carboline (**81**)

2'–Deoxyadenosine (87): $C_{10}H_{13}N_5O_3$ (251.2)**,** a colourless solid (8 mg), was found as intensive UV absorbing band in fraction III (2.21 g). It turned to green-blue by anisaldehyde/sulphuric acid. It was isolated by applying the fraction to Sephadex LH-20 (MeOH), PTLC (20×20 cm, $CHCl_3$/12% MeOH), and finally purified with the aid of Sephadex LH-20 ($CHCl_3$/MeOH 6:4). – R_f = 0.15 ($CHCl_3$/10%MeOH). – **^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 8.35 (s, 1 H, 2-H), 8.14 (s, 1 H, 6-H), 7.23 (s, br, 2 H, NH_2), 6.35 (td, ^3J = 6.1 Hz, ^4J = 1.2 Hz, 1 H, 1'-H) 5.24 (d, ^3J = 4.2, 1 H, OH), 5.16 (t, ^3J = 4.8 Hz, 1 H, OH), 4.40 (d, ^3J = 5.2 Hz, 1 H, 3'-H), 3.86 (m, 1 H, 4'-H), 3.65-3.49 (ABX, ^3J = 16.2 Hz, 8.1 Hz, 5.2 Hz, H, 5'-CH_2), 2.68 (dd, ^3J = 8.1 Hz, 3.1 Hz, 1 H, 2'-H_A), 2.24 (m, 1 H, 2'-H_B). – **^{13}C/APT NMR (**[D_6] DMSO, 75 MHz): δ = 156.1 (C_q-6), 152.3 (CH-2), 151.5 (C_q-4), 140.5 (C_q-5), 139.5 (CH-8), 88.0 (CH-1'), 83.9 (CH-4'), 70.9 (CH-3'), 61.9 (CH_2-5'), 40.8* (CH_2-2'). *CH_2-2' signal, hidden by solvent. – **EI MS** (70 eV): *m/z* (%) = 251 ($[M]^{+\cdot}$, 6), 162 (44), 136.0 (56), 135.0 (100), 108 (16). – **CI MS** (NH_3): *m/z* (%) = 252.1 ($[M + H]^{+\cdot}$, 26), 153 (20), 136 (100), 132 (18), 102 (66). – **(+)-ESI MS**: *m/z* (%) = 525 ($[2M + Na]^+$, 7), 274 ($[M + Na]^+$, 100).

6-Hydroxy isatine (93): $C_8H_5NO_3$ (163.0), an intensive orange powder (1.5 mg), was obtained from fractions III (1.21 g) and IV (1.51 g) by PTLC (20×20 cm, $CHCl_3$/10% MeOH) and purified on Sephadex LH-20 ($CHCl_3$/MeOH 6:4). It showed

no colour change by sulphuric acid or NaOH. – $\boldsymbol{R}_f$ = 0.71 ($CHCl_3$/10 % MeOH). – **UV (**MeOH): λ_{max} = 261, 268, 280 (sh), 348.9 (br), 398 (sh br), 449 (br) nm. – **^{1}H NMR (**[CD_3OD, 300 MHz): δ = 7.42 (d, ^3J = 8.3 Hz, 1H, 4-H), 6.38 (dd, ^3J = 8.3 Hz, ^4J = 2.4 Hz, 1H, 5-H), 6.26 (d, ^4J = 2.4 Hz, 1H, 7-H). – **^{13}C NMR (**[CD_3OD, 150 MHz): δ = 178.0 (CO, C_q-3), 166.8 (C_q-6), 155.9 (C_q-2), 129.7 (CH-4), 116.8 (CH-5), 102.8 (CH-7). Two quaternary signals are missing due to the small amount. – **(+)-ESI MS**: *m/z* (%) = 186 ($[M + Na]^+$, 16). – **(-)-ESI MS**: *m/z* (%) = 162 ($[M-H]^-$, 100). – **EI MS** (70 eV): *m/z* (%) = 163 ($[M]^+$, 100), 147 (40), 135 (77), 119 (19), 108 (14). – **HREI MS**: *m/z* = 163.0269 (calcd. 163.02694 for $C_8H_5NO_3$).

2'-Deoxythymidine (95): $C_{10}H_{14}N_2O_5$ (242.23), delivered from fraction IV (1.51 g) by applying to PTLC (20 × 20 cm, $CHCl_3$/13% MeOH), followed by Sephadex LH 20 (MeOH), as an UV absorbing colourless solid (25 mg) which turned to blue by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.25 ($CHCl_3$ / 10 % MeOH). – **^{1}H NMR** ([D_6] DMSO, 300 MHz): δ = 11.20 (s, br, NH), 7.68 (s, 1 H, 6-H), 6.16 (t, ^3J = 7.2 Hz, 1 H, 1'-H), 5.20 (s, br, 1 H, OH), 4.98 (s, br, 1 H, OH), 4.24 (m, 3'-H), 3.76 (dd, ^3J = 7.2 Hz, 1.1 Hz, 1 H, 4'-H), 3.58 (ABX, ^2J = 9.1 Hz, ^3J = 2.2 Hz, 2 H, 5'-CH_2), 2.08 (m, 2 H, 2'-CH_2), 1.78 (s, 3 H, 7-CH_3). – **^{13}C/APT NMR** ([D_6]DMSO, 75 MHz): δ = 163.8 (C_q-4), 150.5 (C_q-2), 136.2 (CH-6), 109.4 (C_q-5), 87.3 (CH-4'), 83.8 (CH-1'), 70.5 (CH-3'), 61.4 (5'-CH_2), 39.5 (2'-CH_2) under solvent signal, 12.3 (5-CH_3). – **EI MS** (70 eV): *m/z* (%) = 242 ($[M]^{+\cdot}$, 17), 153 (8), 127 (26), 126 (33), 117 (100), 99 (23), 73 (36).

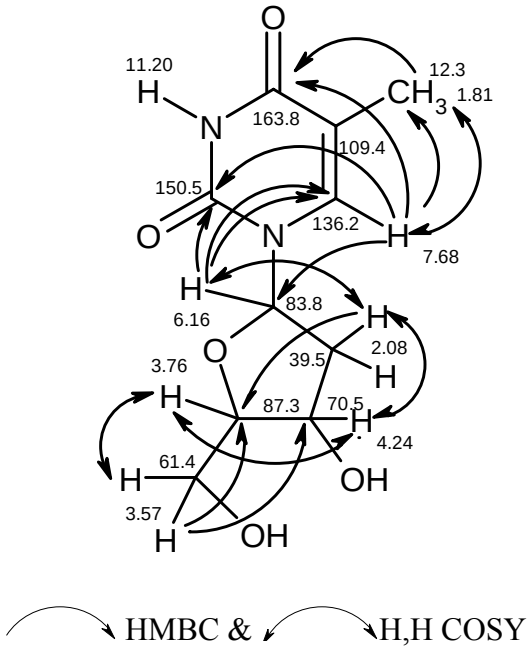


Figure 168: H,H COSY and HMBC correlations of 2'-deoxythymidine (**95**)

2'-Deoxyuridine (96): $C_9H_{12}N_2O_5$ (228.2), was purified from fraction IV (1.51 g) by PTLC (20 × 20 cm, $CHCl_3$/13% MeOH), followed by Sephadex LH-20 (MeOH), to give an UV absorbing colourless solid (12 mg), which turned to blue by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.2 ($CHCl_3$ /10 % MeOH). – **^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 11.20 (s, br, 1 H, NH), 7.82 (d, ^3J = 8.1 Hz, 1 H, 6-H), 6.15 (t, ^3J = 7.1 Hz, 1 H, 1'-H), 5.62 (d, ^3J = 8.2 Hz, 1 H, 5-H), 5.20 (s, br, 1 H, OH), 4.95 (s, br, 1 H, OH), 4.23 (br, m, 1 H, 3'-H), 3.79 (dd, ^3J = 7.1 Hz, 1 H, 4'-H), 3.78 (s, br, 2 H, 5'-CH_2), 2.08 (m, 2 H, 2'-CH_2). – **^{1}H NMR (**CD_3OD, 300 MHz): δ = 7.98 (d, ^3J = 8.1 Hz, 1 H, 6-H), 6.23 (t, ^3J = 7.1 Hz, 1 H, 1'-H), 5.65 (d, ^3J = 8.1 Hz, 1 H, 5-H), 4.38 (m, 1 H, 3'-H), 3.92 (m, 1 H, 4'-H), 3.73, 3.69 (ABX, ^2J = 14.2 Hz, ^3J = 5.1 Hz, 2 H, 5'-CH_2), 2.21 (m, 2 H, 2'-CH_2). – **EI MS** (70 eV): *m/z* (%) = 228 ($[M]^{+\cdot}$, 2), 137 (14), 127 (16), 118 (94), 113 (100), 99 (26), 73 (24), 70 (30), 69 (62), 55 (18), 46 (20), 45 (28), 43 (34).

4-Methylamino-7,8-dimethyl-isoquinoline-3,6-dione (97): $C_{12}H_{12}N_2O_2$ (216), applying of fraction IV (1.51 g) by PTLC ($CHCl_3$/ 5% MeOH) and Sephadex LH-20 (MeOH) led to a dark red solid (1.7 mg). It showed no colour change by treatment with sodium hydroxide or sulphuric acid. – $\boldsymbol{R}_f$ = 0.22 ($CHCl_3$/5 %MeOH). – **UV** (MeOH): λ_{max} = 232, 273, 339, 447 nm. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 9.02 (s, 1 H, 2-H), 5.82 (s, br, 1 H, 4-NH), 5.72 (s, 1 H, 5-H), 2.93 (d, *J* = 4.6 Hz, 3 H, 4-$NHCH_3$), 2.76 (s, 3 H, 7-CH_3), 2.68 (s, 3 H, 8-CH_3). – **^{13}C NMR** ($CDCl_3$, 125 MHz):

δ = 185.0 (CO, C_q-6), 181.9 (CO, C_q-3), 167.4 (C_q-8), 147.7 (C_q-4), 145.1 (CH-2), 135.2 (C_q-6a), 131.9 (C_q-7), 123.5 (C_q-2a), 103.3 (CH-5), 29.0 (CH_3, 4-NHCH$_3$), 24.7 (CH_3-8), 16.0 (CH_3-7). – **CI MS** (NH_3): *m/z* (%) = 234.1 ($[M + NH4]^+$, 12), 217.1 ($[M + H]^+$, 100). – **EI MS** (70 eV): *m/z* (%) = 216.1 ($[M]^{+\cdot}$, 100), 188.1 ($[M-CO]^{+\cdot}$, 6). – **HREI MS**: *m/z* = 216.0899 (calcd. 216.0899 for $C_{12}H_{12}N_2O_2$).

7-Methylamino-3*H*-pyrrolo[2,3-c]-isoquinoline-6,9-dione (102): $C_{12}H_9N_3O_2$ (227.0), isolated from fraction IV (1.51 g) using PTLC ($CHCl_3$/ 5% MeOH), and followed by Sephadex LH-20 (MeOH), as a dark red solid (1.5 mg). It exhibited no colour change by treatment with sodium hydroxide or sulphuric acid. – $\boldsymbol{R}_f$ = 0.15 ($CHCl_3$/5 %MeOH). – **UV** (MeOH): λ_{max} = 233, 287, 295 (sh), 372, 422 (sh), 481 (br) nm. – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 10.25 (s, br, 1 H, NH), 9.12 (s, 1 H, 5-H), 7.79 (t, ^3J = 2.6 Hz, 2-H), 6.81 (t, ^3J = 2.9 Hz, 1-H), 6.25 (s, br, 1 H, 7-NH), 5.69 (s, 1 H, 8-H), 2.98 (d, *J* = 5.1 Hz, 3 H, 7-NHCH$_3$). – **^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 11.88 (s, br, 1 H, NH), 8.98 (s, 1 H, 5-H), 7.92 (t, ^3J = 2.6 Hz, 2-H), 7.82 (q br, ^3J = 4.9 Hz, 1 H, 7-NH), 6.69 (t, ^3J = 2.9 Hz, 1-H), 5.61 (s, 1 H, 8-H), 2.83 (d, *J* = 5.0 Hz, 3 H, 7-NHCH$_3$). – ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 182.8 (CO, C_q-9), 181.2 (CO, C_q-6), 153.5 (C_q-1a), 149.9 (C_q-7), 140.7 (CH-5), 137.6 (CH-2), 122.4 (C_q-3a), 121.9 (C_q-9a), 102.7 (CH-3), 99.0 (CH-8), 29.3 (CH_3, 7-NHCH$_3$). – **CI MS** (NH_3): *m/z* (%) = 245.0 ($[M + NH_4]^+$, 5), 228.0 ($[M + H]^+$, 100). – **EI MS** (70 eV): *m/z* (%) = 227.0 ($[M]^{+\cdot}$, 100), 199.0 ($[M-CO]^{+\cdot}$, 8), 186.0 (16), 145 (9), 116.0 (8), 59 (12), 43.0 (8). – **HRESI MS**: *m/z* = 228.0695 ($[M + H]^+$, 100) (calcd. 227.0694 for $C_{12}H_9N_3O_2$).

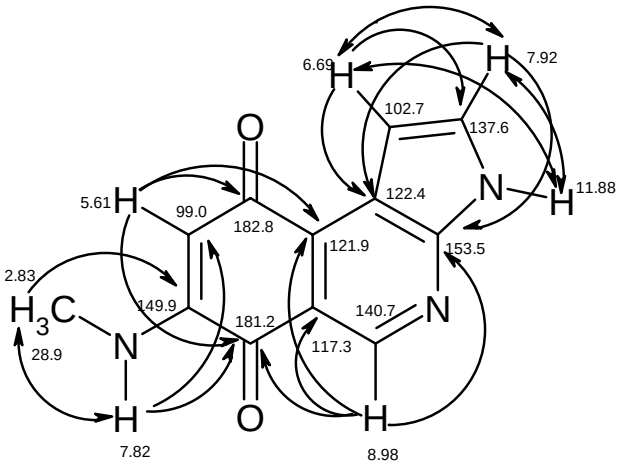


Figure 169: H,H COSY and HMBC correlations of 7-methylamino-3*H*-pyrrolo[2,3-c]-isoquinoline-6,9-dione (**102**).

***p*-Hydroxy benzoic acid (105a)**: $C_7H_6O_3$ (138.12), was delivered from fraction V (0.31 g) by PTLC (20 × 20 cm, $CHCl_3$/13%MeOH) and Sephadex LH-20 (MeOH), as an UV absorbing colourless solid (8 mg). – $\boldsymbol{R}_f$ = 0.36 ($CHCl_3$/MeOH 9:1). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 7.91 (d, ^3J = 8.5 Hz, 2 H, 2,6-H), 6.92 (d, ^3J = 8.6 Hz, 2 H, 3,5-H). – **^{13}C-NMR** [D_6]acetone, 125 MHz): δ = 162.6 (C=O, 1-COOH), 132.5 (CH, C-2,6), 115.86 (CH-3,5). – **EI MS** (70 eV): *m/z* (%) = 138 ($[M]^{+\cdot}$, 95), 121 ($[M\text{-}OH]^{+\cdot}$, 100), 93 ($[M\text{-}COOH]^{+\cdot}$, 20), 65 (15).

***p*-Methoxy benzoic acid methyl ester (105b)**: $C_9H_{10}O_3$ (166.18), an ethereal diazomethane (0.3 ml) was poured for 1 min to 2 mg (0.014 mmol) of *p*-hydroxybenzoic acid in dry dichloromethane (5 ml) at 0 °C. The reaction mixture was then evaporated under vacuum to dryness, giving an UV absorbing colourless solid (2.2 mg). – $\boldsymbol{R}_f$ = 0.8 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.98 (d, ^3J = 8.5 Hz, 2 H, 2,6-H), 6.84 (d, ^3J = 8.6 Hz, 2 H, 3,5-H), 3.91 (s, 3 H, OCH_3), 3.75 (s, 3 H, O=C-OCH_3).

7.3 Alteromonas distincta sp. Hel69

Faint yellow coloured gel colonies were obtained of the marine bacteria *Alteromonas distincta* Hel69 on agar plate, when incubated on LB-medium + 50% Sea water at 28 °C for 3 days.

7.3.1 Pre-screening

Small pieces of a well grown agar plate of the strain Hel69 were used to inoculate 12 × 1 liter- Erlenmeyer flasks each containing 200 ml of LB-medium. Fermentation was carried out at 28 ºC for 3 days (pH = 7.8, and 95 rpm). One litre of the culture broth was filtered and extracted with ethyl acetate to yield 210 mg of faint yellow residue. TLC of the extract exhibited several UV absorbing bands of low, and middle polar components (R_f = 0.2-0.95, $CHCl_3$/10 MeOH), most of them showed a colour staining to violet-blue, when sprayed with anisaldehyde/sulphuric acid, Ehrlich's reagent, or chlorine/*o*-anisidine.

Table 17: Antimicrobial activity of the crude extract produced by Hel 69 strain.

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	14
Escherichia coli	17
Streptomyces viridochromogenes (Tü57)	20
Staphylococcus aureus	14

7.3.2 Fermentation and working up

One agar plate of the marine strain *Alteromonas distincta* Hel69 served to inoculate 12 of 1 liter- Erlenmeyer flasks, each containing 250 ml of LB-medium. The fermentation was carried out on circular shaker 3 days at 28 °C. The resulting preculture was inseeded into 20-liter jar fermenter. The pH value was in permanent increasing during the fermentation process (pH>8). Both of filtrate and mycelial cake were extracted with ethyl acetate. The mycelial cake was further extracted with acetone where the extract was concentrated under reduced pressure. The resultant aqueous solution was extracted again with ethyl acetate. The ethyl acetate extracts were combined and concentrated under reduced pressure. The residue was dried to give a faint yellowish-brown crude extract (2.3 g).

7.3.3 Isolation and identification of metabolites

The crude extract was dissolved in methanol and subjected to defatting by cyclohexane. The resulting cyclohexane extract (200 mg) was treated with acetonitrile, affording an insoluble solid substance (80 mg). After filtration, it was further purified on Sephadex LH-20 (CH_2Cl_2/MeOH 6:4) to give a colourless solid, identified as poly-3-hydroxybutyric acid; sPHB, n > 50,000 (**317**, 56 mg**)**. The acetonitrile and methanol extracts were combined (2.05 g). An application of the resulting extract to Sephadex LH-20 column chromatography (MeOH) led to five fractions. Further training and purification of the obtained fraction gave the metabolites described below.

Indole (106): C_8H_7N (117.148), a subjection of the low polar fraction I (1.11 g) to Sephadex LH-20 (MeOH) two times led to an UV absorbing faint yellow semi-solid (150 mg), which exhibited an orange colour by spraying with anisaldehyde/sulphuric acid, and pink by Ehrlich's reagent and heating. – $\boldsymbol{R}_f$ = 0.96 ($CHCl_3$ /10 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.12 (s br, 1 H, NH), 7.65 (dd, ^3J = 7.5 Hz, ^4J = 0.9 Hz, 1 H, 4-H), 7.39 (dd, ^3J = 7.5 Hz, ^4J = 0.9, 1 H, 7-H), 7.24-7.06

(m, 3 H, 2,5,6-H), 6.56 (m, 1 H, 3-H). – **^{13}C/APT NMR (**$CDCl_3$, 50 MHz): δ = 135.7 (C_q-7a), 127.8 (C_q-3a), 124.1 (CH-2), 122.0 (CH-5), 120.7 (CH-4), 119.8 (CH-6), 111.0 (CH-7), 102.6 (CH-3). – **EI MS (**70 eV): *m/z* (%) = 117 ($[M]^{+\cdot}$, 100), 90 ([M-HCN]$^{+\cdot}$, 37), 63 (15), 59 (12).

Brevianamide F (107): $C_{16}H_{17}N_3O_2$ (283.3)**,** isolated from fraction III (0.320 g) by applying to PTLC (20×20 cm, $CHCl_3$/10 % MeOH), Sephadex LH-20 (MeOH) and HPLC (R_t = 16.10), as an UV absorbing colourless solid (12 mg), turned to violet by anisaldehyde/sulphuric acid, and pink with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.47 ($CHCl_3$/5% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.14 (s br, 1 H, NH), 7.59 (d, ^3J = 7.5 Hz, 4-H), 7.40 (d, ^3J = 7.5 Hz, 7-H), 7.24 (t, ^3J = 7.5 Hz, 1 H, 6-H), 7.14 (m, 2 H, 2,5-H), 5.72 (s br, 1 H, NH), 4.39 (dd, *J* = 11, 3.5 Hz, 9-H), 4.08 (t, ^3J = 7.5 Hz, 12-H), 3.76 (dd, *J* = 15, 3.5 Hz, 8-Hb), 3.64 (m, 2 H, 15-CH_2), 2.97 (dd, ^3J = 10.9 Hz, ^2J = 15.1 Hz, 1 H, 8-Ha), 2.36 (m, 1 H, 17-Ha), 2.10-1.90 (m, 3 H, 17-Hb, 16-CH_2). – **EI MS (**70 eV): *m/z* (%) = 283 ($[M]^{+\cdot}$, 17), 154 (10), 130 (100).

7.4 Terrestrial *Streptomyces* sp. GW3/1538

The terrestrial *Streptomyces strain* GW3/1538 exhibited white aerial mycelia when sub-cultured in M_2 medium with agar and incubated for 3 days at 28 °C.

7.4.1 Pre-screening

One agar plate was used to inoculate 12 of 1 liter-Erlenmeyer flasks, each containing 200 ml of M_2 medium. The flasks were kept at 28 ºC for 3 days and 95 rpm shaker. The culture was filtered and extracted with ethyl acetate to give 100 mg of crude extract. TLC of the extract showed several UV absorbing bands of middle polar components, changed to violet-orange when sprayed with anisaldehyde/sulphuric acid and heating, and to pink with Ehrlich's reagent. In the biological screening, the crude extract showed bio-activity as shown in Table 18.

Table 18: Antimicrobial activity of the crude extract produced by strain GW3/1538.

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	11
Escherichia coli	14
Streptomyces viridochromogenes (Tü57)	11
Staphylococcus aureus	14
Chlorella vulgaris	11
Chlorella sorokiniana	11

7.4.2 Fermentation and working up

The strain was subjected first to cultivation as 3 liters shaker (95 rpm) for 3 days at 28 °C. The faint yellow broth was inseeded to 20-liter jar fermenter for additional 3 days at 28 °C. After harvesting, the culture broth was separated from the mycelium by filtration on celite. The water and mycelial cake phases were extracted repeatedly with ethyl acetate and acetone, respectively. The combined extracts were evaporated at 40 °C under vacuum to yield 9.71 g of faint brown crude extract.

7.4.3 Isolation and identification of metabolites

The resulting extract was adsorbed on silica gel, and submitted to flash column chromatography (30 × 1000 mm) on silica gel, and eluted with $CHCl_3$/MeOH-gradient. After controlling by TLC ($CHCl_3$/5%MeOH), five fractions were obtained.

The crude extract and the isolated fractions were tested antimicrobially, and fractions I-IV exhibited activities against *Staphylococcus Aureus, E. coli, Streptomyces viridochromogenes* (Tü57), *Bacillus subtilis, Candida albicans* and *Mucor miehei* (Tü284). Further isolation and purification of the afforded fractions led to the metabolites shown below.

6-Ethyl-4-hydroxy-3,5-dimethyl-2-pyrone (113): $C_9H_{12}O_3$ (168.1)**,** applying fraction II (2.12 g) to PTLC (20 × 20 $CHCl_3$/5%MeOH) followed by Sephadex LH-20 ($CHCl_3$/MeOH 3:2) led to 6.5 mg of an UV absorbing as colourless solid. – $\boldsymbol{R}_f$ = 0.55 ($CHCl_3$/MeOH, 5 %). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 2.56 (q, ^3J = 7.4 Hz, 2 H, 7'-CH_2), 1.99 (s, 3 H, CH_3-9), 1.96 (s, 3 H, 10-CH_3), 1.22 (t, ^3J = 7.4 Hz, 3 H, 8-CH_3). – **^{13}C NMR (**$CDCl_3$, 125 MHz): δ = 166.0 (C_q-2), 164.6 (C_q-4), 160.1 (C_q-6), 105.8 (C_q-3), 98.1 (C_q-5), 24.2 (CH_2-7), 11.7 (CH_3-9), 9.5 (CH_3-10), 8.4 (CH_3-8). – **CI MS (**NH_3): *m/z* (%): = 337.2 ($[2\ M + H]^+$, 4), 186.1 ($[M + NH_4]^+$, 100), 169.1 ($[M + H]^+$, 40).

2,5-Furandimethanol (114): $C_6H_8O_3$ (128.1), was afforded as a colourless semi-solid (8 mg) from fraction II (2.12 g) by PTLC (20 × 20 cm, $CHCl_3$/7%MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 3:2). It is weakly UV absorbing substance, which turned to brown by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.39 ($CHCl_3$/10% MeOH). – **^{1}H NMR - (**[D_6]DMSO, 300 MHz): δ = 6.17 (s, 2 H, 3,4-H), 5.09 (t, ^3J = 6.1 Hz, OH), 4.35 (d, ^3J = 6.1 Hz, 2,5-CH_2). – **^{1}H NMR (**$CDCl_3$**,** 200 MHz): δ =

6.23 (s, 2 H, 2 H, 3,4-H), 4.59 (s, 4 H, 2,5-CH_2). – **^{13}C/APT NMR** ($CDCl_3$, 50 MHz): δ = 154.0 (C_q-2,5), 108.4 (CH-3,4), 56.8 (CH_2-2,5). – **EI MS** (70 eV): *m/z* (%) = 128 ($[M]^{+\cdot}$, 67), 111 ($[M\text{-}OH]^{+\cdot}$, 20), 101 (28), 97 ($[M\text{-}(CH_2\text{-}OH)]^{+\cdot}$, 100), 85 (24), 74 (68), 69 (22), 60 (16), 55 (28), 45 (72), 43 (35), 41 (22).

3-Hydroxy-2-methyl-γ-pyrone; Maltol (116): A colourless solid (6 mg), $C_6H_6O_3$ (126.1), was obtained from fraction III (0.411 g) by PTLC (20×20 cm, $CHCl_3$/7% MeOH), and purified on Sephadex LH-20 ($CHCl_3$/MeOH 3:2). It was a middle polar UV absorbing substance, with no colour reaction by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.32 ($CHCl_3$ 5% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.73 (d, ^3J = 6.2 Hz, 1 H, 6-H), 6.42 (d, ^3J = 6.1 Hz, 1 H, 5-H), 2.38 (s, 3 H, 7-CH_3). – **EI MS** (70 eV): *m/z* (%) = 126 ($[M]^{+\cdot}$, 100), 83 (10), 55 (48), 54 (18), 43 (4).

***cis*-*Cyclo*(leucyl-prolyl) (119)**: $C_{11}H_{18}N_2O_2$ (210.27)**,** delivered from from fraction III (0.411 g) by PTLC (20×20 cm, $CHCl_3$/7% MeOH), and purified on Sephadex LH-20 ($CHCl_3$/MeOH 3:2) to give an UV absorbing colourless solid (6 mg), as a middle polar substance, turned to violet, pink, blue by anisaldehyde/sulphuric acid, Ehrlich's reagent and chlorine/*o*-anisidine reaction, respectively. – $\boldsymbol{R}_f$ = 0.58 ($CHCl_3$/5 CH_3OH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 6.00 (s br, 1 H, NH), 4.15 (t, ^3J = 8.1 Hz, 1 H, 3-H), 4.04 (dd, ^3J = 10.2 Hz, ^3J = 6.2 Hz, 1 H, 6-H), 3.64-3.54 (m, 2 H, 9-CH_2), 2.37 (m, 1 H, 10-H_A), 2.22-1.98 (m, 3 H, 10-H_B, 7,8-H_A), 1.96 (m, 1 H, 8-H_B), 1.75 (m, 2 H, 7-CH_2), 1.55 (m, 1 H, 11-H), 1.03 (d, ^3J = 8.1 Hz, 3 H, 12-CH_3), 0.96 (d, ^3J = 8.1 Hz, 3 H, 13-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 75 MHZ): δ = 170.3 (C_q-2), 166.1 (C_q-5), 59.0 (CH-6), 53.4 (CH-3), 45.5 (CH_2-9), 38.6 (CH_2-7), 28.1 (CH_2-10), 24.7 (CH-11), 23.3 (CH_3-12), 22.7 (CH_2-8), 21.2 (CH_3-13). – **CI MS** (NH_3): *m/z* (%) = 438 ($2\,M + NH_4]^+$, 7), 421 ($[2M + H]^+$,4), 245 ($[M + NH_3 + NH_4]^+$, 64), 228 ($[M + NH_4]^+$, 100), 211 ($[M + H]^+$, 40).

(S)-Dihydro-4-hydroxy-2(3*H*)-furanone (122): A colourless oil (7 mg), $C_4H_6O_3$ (102.1), was obtained from fraction III (0.411 g) by PTLC (20 × 20 cm, $CHCl_3$/7% MeOH) and Sephadex LH-20, as weakly UV absorbing substance, which turned to brown by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.23 ($CHCl_3$ /5% MeOH). – $[\alpha]^{20}_D$ = -19.6° (EtOH, *c* 0.665 mg/ml). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 4.73 (m, 1 H, 4-H), 4.41 (dd, ^2J = 14, ^3J = 7.1 Hz, 5-H_A), 4.27 (dd, ^2J = 14, ^4J = 1.3 Hz, 1 H, 5-H_B), 2.78 (dd, ^2J = 16.1, ^3J = 6.8 Hz, 3-H_A), 2.52 (dd, ^2J = 16.1, J = 1.2 Hz, 3-H_B).

– ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 175.5 (CO, C_q-2), 75.7 (CH-4), 67.6 (CH_2-5), 37.8 (CH_2-3). **– CI MS (**NH_3): *m/z* (%) = 222.1 ($[2\ M + NH_4]^+$, 1), 137.1 ($[M+ NH_4 + NH_3]^+$, 20), 120.1 ($[M + NH_4]$, 60).

7.5 Strain Bio134

The marine bacteria strain Bio134 exhibited white gelatinous colonies when sub-cultured in LB medium (with 50% artificial sea water) on agar plates at 28 °C for 3 days.

7.5.1 Pre-screening

One agar plate was used to inoculate 12 of 1 liter-Erlenmeyer flasks, each containing 200 ml of LB medium. The flasks were kept at 28 ºC for 3 days and 95 rpm, affording a white aerial mycelium with a faint yellow broth. The culture was filtered and extracted with ethyl acetate to give 300 mg of yellowish-brown crude extract. TLC screening of the extract exhibited several UV absorbing zones, one of them as middle polar, turned to blue by spraying with anisaldehyde/sulphuric acid.

Table 19: Antimicrobial activity of the crude extract produced by strain Bio 134

Tested microorganism	Inhibition zone ∅ [mm]
Streptomyces viridochromogenes (Tü57)	11
Staphylococcus aureus	14
Mucor miehei (Tü284)	12
Candida albicans	12
Chlorella sorokiniana	12

7.5.2 Fermentation and working up

Fermentation was carried out as follows: Well-grown agar subcultures of the marine strain Bio 134 served to inoculate 12 × 1 liter-Erlenmeyer flasks, each containing 250 ml of LB medium + 50% Sea water. The flasks were incubated with 110 rpm at 28 °C for 3 days. The faint yellow broth was used to inseed a 25-liter jar fermenter (containing 22 liters LB-medium), which was stirred for 3 days. The cultures were separated by filtration with the aid of celite into mycelial cake and filtrate. Both filtrate and residue were extracted repeatedly with ethyl acetate and acetone, respectively. The extracts (filtrate and mycelia) were evaporated separately at 40 °C under vacuum to give the fractions: Bio134A (biomass, 2.1 g) and Bio134B (filtrate, 8.41

g). TLC monitoring of the fraction Bio134A (biomass) exhibited non interesting metabolites which were neglected.

7.5.3 Isolation and identification of metabolites

The extract Bio134 (8.41 g) was defatted by cyclohexane. The resulting methanolic extract (6.1 g) was further fractionated using flash silica gel column chromatography (30 × 1000 mm), and elution was performed using $CHCl_3$-MeOH-gradient to give 4 fractions. Working up of the obtained fractions led the metabolites given below.

13-Methyltetradecanoic acid (123): A colourless oil (45 mg), $C_{15}H_{30}O_2$ (242.4), was isolated from the fast moving fraction I (0.810 g), which stained to violet by anisaldehyde/sulphuric acid after heating. It was isolated by PTLC (20 × 20 cm, $CHCl_3$/5%MeOH) and then purified on Sephadex LH-20 (MeOH). – $\boldsymbol{R}_f$ = 0.83 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**$CDCl_3$, 200 MHz): δ = 10.38 (s br, 1 H), 2.28 (m, 2 H, CH_2), 1.60-1.42 (m, 3 H), 1.38-1.10 (m, 18 H), 0.83 (d, ^3J = 7.7 Hz, 6 H, 14,15-CH_3). – **EI MS** (70 eV): *m/z* (%) = 242 ($[M]^{+\cdot}$, 36), 228 (4), 214 (10), 185 (20), 171 (18), 154 (18), 129 (52), 115 (20), 97 (36), 83 (46), 73 (100), 57 (84), 43 (88).

3'-Acetoxy-2'-deoxy-thymidine (124): $C_{12}H_{16}N_2O_6$ (284.27), isolated by applying fraction III (0.33 g) to Sephadex LH-20 (MeOH), PTLC (20 × 20 cm, $CHCl_3$/10% MeOH) and HPLC (R_t = 11.20), obtained as colourless middle polar, UV absorbing substance (9 mg), blue-green by spraying with anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.43 ($CHCl_3$/ MeOH 90:10). – **IR** (KBr, cm^{-1}): ν = 3475, 3077, 2926, 2855, 1711, 1666, 1477, 1408, 1375, 1306, 1291, 1257, 1206, 1130, 1100, 1069 and 1027. – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.43 (s br, 1 H, NH), 7.48 (s, 1 H, H-6), 6.22 (t, ^3J = 7.2 Hz, 1'-H), 5.33 (m, 1 H, 3'-H), 4.08 (m, 1 H, 4'-H), 3.90 (m, 2 H, 6'-CH_2), 2.37 (m, 2 H, 2'-CH_2), 2.10 (s, 3 H, 9'-CH_3), 1.90 (s, 3 H, 5-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 170.7 (C_q-8'), 163.6 (C_q-4), 150.4 (C_q-2), 136.3 (CH-6), 111.4 (C_q-5), 86.0 (CH-1'), 85.0 (CH-4'), 74.7 (CH-3'), 62.6 (CH_2-6'), 37.2 (CH_2-2'), 21.0 (CH_3-9'), 12.6 (CH_3-5). – **EI MS** (70 eV): *m/z* (%) = 284.1 ($[M]^{+\cdot}$, 6), 193.0 (8), 159.0 (35), 154.1 (13), 150 (12), 126.0 (24), 110.1 (7), 99.0 (100), 81.0 (6), 69.0 (80), 43.0 (36). – **CI MS** (NH_3): *m/z* (%) = 586.3 ($[2\ M + NH4]^+$, 2), 302.2 ($[\ M + NH_4]^+$, 52), 285 ($[M + H]^+$, 12), 202 (100), 134 (40). – **HREI MS**: *m/z* = 284.1008 (calcd. 284.1008 for $C_{12}H_{16}N_2O_6$).

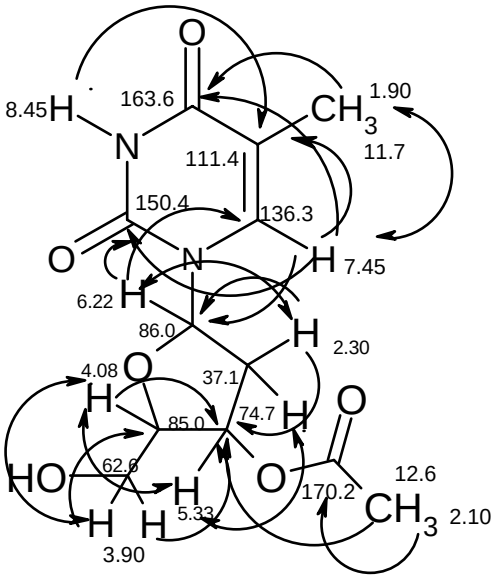


Figure 170: H,H COSY and HMBC correlations of 3-acetoxy-2-deoxy-thymidine (**124**)

Pyrrole-2-carboxylic acid (125): $C_5H_5NO_2$ (111.10), applying fraction III (0.33 g) to Sephadex LH-20 (6:4 $CHCl_3$/MeOH) led to an UV absorbing colourless powder (4 mg), turned to brown-orange by spraying with anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.55 ($CHCl_3$/10 % MeOH). – **^{1}H NMR (**[CD_3OD, 300 MHz): δ = 6.93 (dd, ^3J = 2.6, ^4J = 1.5 Hz, 1H, 3-H), 6.82 (dd, ^3J = 3.8, ^4J = 1.5 Hz, 1 H, 5-H), 6.15 (dd ^3J = 2.6, ^3J = 2.7 Hz, 1 H, 4-H). – **^{13}C NMR (**[CD_3OD, 150 MHz): δ = 165.1 (C_q-COOH), 124.2 (CH-5), 123.2 (C_q-2)*, 116.4 (CH-3), 110.5 (CH-4). *from internet computer program. – **EI MS** (70 eV): *m/z* (%) = 111.1 ([M]$^{+\cdot}$, 100), 93.0 ([M-CO], 80), 65 ([M-COO]$^{+\cdot}$, 28).

7.6 Marine *Streptomyces* sp. B8876

Marine *Streptomycete* B8876 was grown on M_2^+ medium (with 50% Sea water) agar plates for 3 days at 28 ºC. After 72 hours, the plates showed a dark red colour. The strain was cultivated in 12 × 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. The flasks were shaken at 28 ºC for 3 days on a rotary shaker at 95 rpm. The fermentation broth was lyophilised and extracted with ethyl acetate. After evaporation, a dark red residue was obtained (85 mg).

7.6.1 Pre-screening

TLC of the strain extract exhibited a non polar pink coloured band, which turned to yellow by treatment with 2 N NaOH. In addition, two UV absorbing bands were observed, which stained violet by anisaldehyde/sulphuric acid.

In the biological screening, the crude extract exhibited activity against the following test organisms (Table 20).

Table 20: Antimicrobial activity of the crude extract produced by strain B 8876

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	17
Escherichia coli	20
Streptomyces viridochromogenes (Tü57)	12

7.6.2 Fermentation, isolation and identification of metabolites

The marine *Streptomyces* strain B8876 was cultivated in 12 of 1L-Erlenmeyer flasks, each containing 250 ml of M_2^+ medium for 72 h at 28 °C on rotary shaker. The seed culture was used to inoculated a 20-liter jar fermenter, containing 17-liter of the nutrient M_2^+ medium. The fermentation was carried out for additional 3-days at 28 °C (with agitation at 120 rpm). The red culture broth was filtered with the aid of celite, and both the filtrate and mycelial cake were extracted four times with ethyl acetate. The biomass was additionally extracted with acetone. The ethyl acetate extracts were evaporated to dryness, to give a dark pink crude extract (*ca* 17.1 g). The extract was chromatographed on a flash silica gel (30 × 1000 mm, $CHCl_3$-MeOH gradient) to afford four fractions by monitoring with TLC analysis. The low polar fraction II (10.12 g), containing the pink-red zone, was sub-fractionated to IIa (3.21 g). The last sub-fraction was applied to Sephadex LH-20 ($CHCl_3$/MeOH 6:4), giving a pink component, which was absorbed on basic alumina column chromatography (type III), and eluted with chloroform. The obtained yellow zone on the column was dissolved in acidic chloroform ($CHCl_3$ + 2 N HCl), and dried over Na_2SO_4. The isolated component was defatted by washing with *n*-pentane at 0 °C, giving a dark-pink solid of undecylprodigiosin (**128**)

Undecylprodigiosin (128): $C_{25}H_{35}N_3O$ (393.5), a dark pink solid (5 mg). Unpolar on silica gel, changed to yellow by 2 N NaOH, while blue when sprayed with anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.5 ($CHCl_3$ 100 %). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 12.93 (s br, 1 H, 1-H_B), 12.75 (s br, 1 H, 1-H_A), 12.70 (s br, 1 H, 1-H_C),

7.25* (ddd, ^3J = 3.1 Hz, ^4J = 1.5, ^3J = 1.7 Hz, 5-H_A), 7.05 (s, 1 H, 1''-H), 6.94 (m, 1 H, 3_A-H), 6.85 (ddd, ^3J = 3.5 Hz, ^4J = 1.5, ^4J = 2.1 Hz, 1 H, 4_C-H), 6.39 (m, 1 H, 4_A-H), 6.22 (m, 1 H, 3_C-H), 6.08 (s, 1 H, 4_B-H), 4.04 (s, 3 H, 3_B-OCH_3), 2.95 (t, ^3J = 7.2 Hz, ^4J = 1.0 Hz, 2 H, 1'-CH_2), 1.78 (m, 2 H, 2'-CH_2), 1.45–1.30 (m, 16 H, 3'-10'-CH_2), 0.88 (t, ^3J = 6.7 Hz, 3 H, 11'-CH_3); * hidden by $CDCl_3$. – **EI MS (**70 eV): *m/z* (%) = 393.4 ($[M]^{+\cdot}$, 100), 252.2 ($[M-(CH_2)_9CH_3]^{+\cdot}$, 23). – **(+)-ESI MS**: *m/z* (%) = 394.6 ($[M + H]^+$).

7.7 Marine *Streptomyces* sp. B7936

Agar plates of the marine *Streptomycete* B7936 were incubated at 28 °C for 3 days, where white aerial mycelia was growing on the agar using M_2^+ medium. The strain was fermented at 28 °C for 72 hours in M_2^+ medium with 50% synthetic sea water using a rotary shaker (95 rpm). Extraction of the culture broth (1 liter) with ethyl acetate afforded 105 mg of crude extract.

7.7.1 Pre-screening

The chemical screening by TLC showed two major coloured bands. The first of them was orange, and turned to red by anisaldehyde/sulphuric acid, while the second one was yellow and changed to brown by treatment with sulphuric acid. A third polar yellow band was green fluorescent under UV, and turned to blue by spraying with anisaldehyde/sulphuric acid and heating. Additionally, the crude extract showed a high bio-activity against the test microorganisms (Table 21)

Table 21: Antimicrobial activity of the crude extract produced by strain B 7936

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	24
Escherichia coli	25
Streptomyces viridochromogenes (Tü57)	20
Staphylococcus aureus	17
Mucor miehei (Tü284)	27
Candida albicans	18
Chlorella vulgaris	16
Chlorella sorokiniana	18
Scenedesmus subspicatus	11

7.7.2 Fermentation and working up

The marine *Streptomyces* strain B B7936 was cultivated in 12 1 liter-Erlenmeyer flasks of M_2^+ medium, each containing 250 ml (at pH 7.8) for 72 h at 28 °C with the

aide of rotary shaker (95 rpm). The seed culture was inoculated to 25-liter jar fermenter containing 22-liter of the nutrient culture M_2^+ medium, and fermented for additional 3 days at 28 °C (with agitation at 120 rpm). After harvesting, the formed yellowish/orange culture broth was mixed with celite, and filtered with the aide of filter press. The filtrate and biomass were extracted repeatedly with ethyl acetate. The combined organic phases were evaporated at 40 °C under vacuum. The residue was dried to afford an orange crude extract (5.21 g).

7.7.3 Isolation and identification of metabolites

The extract was dissolved in 200 ml methanol and defatted with cyclohexane (3 × 100 ml). The methanolic layer was evaporated and the residue (4.1g) was subjected to column chromatography on flash silica gel (30 × 600 mm), and eluted by chloroform-methanol-gradient, revealing four fractions. Further separation of the fractions led to the metabolites listed below.

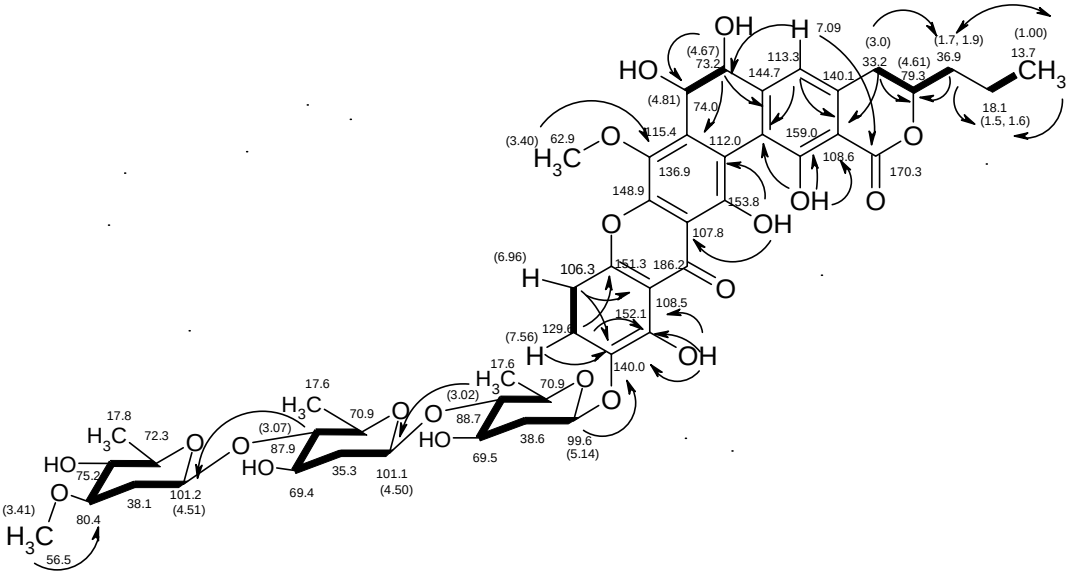
Actinomycin D (130): $C_{62}H_{86}N_{12}O_{16}$ (1255.4), isolated reddish-orange powder (300 mg) from fraction II (1.51g) by PTLC (20 × 40 cm, $CHCl_3$/5%MeOH) and Sephadex LH-20 (two times MeOH). On TLC, it turned to red by anisaldehyde/sulphuric acid but no colour change by treatment with 2 N NaOH. – $\boldsymbol{R}_{f=}$ 0.37 ($CHCl_3$/MeOH 95:5). – **^{1}H NMR** ($CDCl_3$, 300 MH_Z): δ = 8.13 (d br, ^3J = 6.4 Hz, 1 H, NH-Val$_\alpha$), 7.97 (d br,^3J = 6.4 Hz, 1 H, NH-Val$_\beta$), 7.81 (d br, ^3J = 6.4 Hz, 1 H, NH-Thr$_\beta$), 7.67 (d, ^3J = 7.9 Hz, 1 H, 8-H), 7.39 (d, ^3J = 7.9 Hz, 1 H, 7-H), 7.21 (d br, ^3J = 6.4 Hz, 1 H, NH-Thr$_\alpha$), 6.03 (d, ^3J = 9.1 Hz, 1 H, 2-H Pro$_\alpha$), 5.96 (d, ^3J = 9.1 Hz, 1 H, 2-H Pro$_\beta$), 5.25-5.15 (m, 2 H, 3-H, Thr$_{\alpha,\beta}$), 4.81 (d, ^3J = 18.1 Hz, 1 H, 2-Ha, Sar$_\alpha$), 4.73 (d, ^3J = 18.8 Hz, 1 H, 2-Ha, Sar$_\beta$), 4.61 (dd, ^3J = 6.4 Hz, ^3J = 2.3 Hz, 1 H, 2-H Thr$_\alpha$), 4.49 (dd, ^3J = 6.4, ^3J = 2.3 Hz, 1 H, 2-Thr$_\beta$), 4.03-3.40 (m, 8 H, 2-H$_b$ Sar$_{\alpha,\beta}$, 2-H, Val$_{\alpha,\beta}$, 5-H$_2$, Pro$_{\alpha,\beta}$), 2.93, 2.90, 2.88 (3 s, 3 H + 3 H + 6 H, NCH_3 MeVal$_{\alpha,\beta}$, NCH_3, Sar$_{\alpha,\beta}$), 2.67, 2.57 (m + s, 6 + 3, 3-Ha Pro$_{\alpha,\beta}$, 2,3-H, MeVal$_{\alpha,\beta}$, 6-CH_3), 2.38-1.76 + 2.23 (m + s, 8 + 3 H, 3-H$_b$-, 4-H$_2$ Pro$_{\alpha,\beta}$, 3-H Val$_{\alpha,\beta}$, 4-CH_3), 1.28 (m, 6 H, CH_3, Thr$_{\alpha,\beta}$), 1.17-1.07 (m, 6 H, CH_3 Val$_{\alpha,\beta}$), 0.99-0.82 (m, 12 H, CH_3 Val$_{\alpha,\beta}$, CH_3 Me Val$_{\alpha,\beta}$), 0.75 (d, ^3J = 4.9 Hz, 6 H, CH_3 MeVal$_{\alpha,\beta}$). – **^{13}C/APT NMR (**$CDCl_3$, 50 MHz): δ = 179.0 (C_q), 173.6 (C_q), 173.3 (C_q), 173.2 (C_q), 173.1 (C_q), 168.9 (C_q), 168.4 (C_q), 167.7 (C_q), 167.6 (C_q), 166.6 (C_q), 166.5 (C_q), 166.4 (C_q), 166.2 (C_q), 147.5 (C_q), 145.7 (C_q), 145.5 (C_q), 140.3 (C_q), 132.2 (C_q), 130.2 (CH), 129.0 (C_q),

127.7 (C_q), 125.5 (CH), 113.4 (C_q), 101.5 (C_q), 75.0 (CH), 74.8 (CH), 71.2 (CH), 71.0 (CH), 58.7 (CH), 58.5 (CH), 57.9 (CH_2), 56.5 (CH), 56.3 (CH), 55.0 (CH), 54.6 (CH), 51.3 (CH_2), 50.2 (CH), 47.5 (CH_2), 47.3 (CH_2), 39.1 (CH), 39.0 (CH), 34.9 (CH), 34.8 (CH_3), 31.6 (CH_3), 31.4 (CH_3), 31.1 (CH_2), 30.8 (CH_2), 26.8 (CH_3), 25.0 (CH_3), 22.8 (CH_2), 22.7 (CH_2), 21.5 (CH_3), 21.4 (CH_3), 19.1 (CH_3), 19.0 (CH_3), 18.9 (CH_3), 18.8 (CH_3), 18.1 (CH_3), 17.6 (CH_3), 17.1 (CH_3), 14.9 (CH_3), 7.6 (CH_3). – **(+)-ESI MS**: *m/z* (%) = 1277 ($[M + Na]^+$, 100), 1255 ($[M + H]^+$, 6). – **(-)-ESI MS**: *m/z* (%) = 1254 ($[M]^-$)

Actinomycin D analogue (131): $C_{61}H_{84}N_{12}O_{16}$ (1240.43), was obtained as reddish-orange powder (2 mg) from fraction II (1.51g) along with (**130**). It showed a reddish-orange spot during the TLC analysis, which coloured to red by spraying with anisaldehyde/sulphuric acid, while no colour change with 2 N NaOH. – $\boldsymbol{R}_f$ = 0.35 ($CHCl_3$/ MeOH, 5%). – **(+)-ES MS**: *m/z* (%) = 1263 ($M + Na]^+$, 100). – **(-)-ESI MS**: *m/z* (%) = 1239 ($[M-H]^-$). – (+)-**ESI MS^2**: *m/z* (%) = 1263 ($[M+Na]^{+,}$), 1150.4, 1037.4, 966.4, 740.2, 669.4, 572.2, 473.2, 455.2.

FD-594 (135): $C_{47}H_{56}O_{20}$ (940.9), isolated by applying fraction III (0.32 g) to PTLC (20 × 20 cm, $CHCl_3$/7% MeOH), followed by Sephadex LH-20 (two times, $CHCl_3$/MeOH 3:2) as yellow solid (12 mg) , middle polar yellow substance, which turned to brown by sulphuric acid, yellowish-green with Ehrlich's reagent, while no colour change occurred with 2 N NaOH. – $\boldsymbol{R}_f$ = 0.5 ($CHCl_3$ /10% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.36 (s br, 1H, 15-OH), 11.98 (s br, 1 H, 16-OH), 11.88 (s br, 1 H, 13-OH), 7.57 (d, ^3J = 9.1 Hz, 1 H, 11-H), 7.09 (s, 1 H, 5-H), 6.97 (d, ^3J = 9.1 Hz, 1 H, 10-H), 5.68 (s br, 1 H, OH), 5.15 (dd, *J* = 9.7, 2.0 Hz, 1 H, 1'-CH), 4.67 (s br, 1 H, OH), 4.76 (s, 1 H, 3''-OH), 4.67 (d, br, ^3J = 9.3, 2 H, 6,7-H), 4.65 (m, 1 H, 3-H), 4.57 (td, ^3J = 9.8 Hz, ^4J = 1.8, 2 H, 1''/1'''-CH), 4.08 (s, 3 H, 8-OCH_3), 3.77-3.59 (m, 2 H, 3,5''-CH), 3.43 (s, 3 H, 3'''-OCH_3), 3.40 (m, 1 H, 5'-CH), 3.25-3.16 (ddm, *J* = 12.4, 8.6, Hz, 2 H, 3'',3'''-CH), 3.14 (t, ^3J = 8.6 Hz, 1 H, 4'''-CH), 3.07 (m, 1 H, 1 H, 4''-CH), 3.05 (t, ^3J = 8.6 Hz, 1 H, 4'-CH), 2.99 (m, 1 H, 4-CH), 2.62 (ddd, *J* = 12.6, 5.4, 2.1 Hz, 2 H, 2'-CH_2), 2.41 (d, br, *J* = 3.1 Hz, 4'''-OH), 2.35 (ddd, *J* = 12.5, 4.5, 2.1, 2 H, 2'''-CH_2), 2.29 (ddd, *J* = 13.0, 5.3, 1.8 Hz, 2 H, 2''-CH_2), 1.97-1.83 (m, 4 H, 2',17-CH_2), 1.79-1.45 (m, 6 H, 2'',2''',18-CH_2), 1.38 (d, ^3J = 6.1 Hz, 3 H, 6'''-CH_3), 1.37 (d, ^3J = 6.3 Hz, 3 H, 6''-CH_3), 1.32 (d, ^3J = 6.1 Hz, 3 H, 6'-CH_3), 1.01 (t, ^3J = 7.3 Hz, 3 H, 19-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ =

186.0 (C_q-14), 170.4 (C_q-1), 158.7 (C_q-16), 153.8 (C_q-15), 151.6 (C_q-13), 151.0 (C_q-9a), 148.7 (C_q-8a), 144.8 (C_q-5a), 140.0 (C_q-4a), 139.9 (C_q-12), 138.9 (C_q-7a), 136.8 (C_q-8), 129.0 (CH-11), 115.1 (C_q-15b), 112.8 (CH-5)*, 111.8 (C_q-15a), 108.6 (C_q-16a), 108.5 (C_q-13a), 107.8 (C_q-14a), 106.3 (CH-10), 101.2 (CH-1'''), 101.1 (CH-1''), 99.6 (CH-1'), 88.1 (CH-4'), 87.7 (CH-4''), 80.3 (CH-3'''), 79.3 (CH-3), 75.0 (CH-4''')**, 74.95 (CH-7), 73.1 (CH-6), 72.1 (CH5'''), 70.7 (CH-5'), 70.7 (CH-5''), 69.3 (CH_2-3'), 69.2 (CH_2-3''), 62.9 (8-OCH_3), 56.6 (3'''-OCH_3), 38.6 (CH_2-2'), 38.1 (CH_2-2''), 36.8 (CH_2-17), 35.1 (CH_2-2'''), 33.2 (CH_2-4), 18.1 (CH_2-18), 17.7 (CH_3-6'''), 17.6 (CH_3-6''), 17.6 (CH_3-6'), 13.8 (CH_3-19). – **(+)-ESI MS** (Na): *m/z* (%) = 963 ([M + Na]$^+$, 100), 941 ([M + H]$^+$, 10). – **(-)-ESI MS**: *m/z* (%) = 939 ([M - H]$^-$).



Fungichromin (14-Hydroxyfilipin III) (138a): $C_{35}H_{58}O_{12}$ (670.82): A greenish-yellow solid (26 mg), was obtained from fraction IV (0.22 g) by PTLC (20 × 20 cm, $CHCl_3$/15%MeOH), Sephadex LH-20 (MeOH) and HPLC (R_t = 5.32), as polar substance. It is green fluorescent under UV, and turned to blue by spraying with anisaldehyde/sulphuric acid and heating. – ***R***$_f$ = 0.18 ($CHCl_3$/10% MeOH). – **UV (**MeOH): λ_{max} = 290, 303, 318, 337, 356 nm. – **^{1}H NMR (**[D_6] DMSO, 300 MHz): δ = 6.50-6.20 (m, 7 H, 6.05 (dd, ^3J = 14.1, 6.2 Hz, 1 H), 5.92 (d, ^3J = 10.1 Hz, 1 H), 5.18 (d br, ^3J = 6.1 Hz, 1 H, OH), 5.12 (s br, 2 H, OH), 4.98 (s br, 1 H, OH), 4.88 (s br, 1H, OH), 4.76 (s br, 2 H, OH), 4.68 (d, br, ^3J = 5.2 Hz, 1 H, OH), 4.62 (t, ^3J = 6.5 Hz, 1 H, CH-O), 4.43 (d, br, ^3J = 5.1 Hz, 1 H, OH), 4.22 (d, br, ^3J = 7.0 Hz, 1 H, OH), 3.99 (m, 2 H), 3.92 (m, 3 H), 3.78 (m, 1 H), 3.71 (dd, ^3J = 8.1, 4.2 Hz, 2 H), 3.48 (d, br, ^3J = 7.2 Hz, 1 H), 3.15 (m, 1 H), 2.43 (dd, ^3J = 8.1 Hz, 7.1 Hz, 1 H, 2-H),

1.68 (s, 3 H, 29-CH_3), 1.42-1.22 (m, 18 H, 9 CH_2), 1.19 (d, ^3J = 7.0 Hz, 3 H, 28-CH_3), 0.83 (m, 3 H, 35-CH_3). – **^{13}C/APT NMR (**[D_6] DMSO, 125 MHz): δ = 171.0 (C_q-1), 138.7 (C_q-16), 135.0 (CH-19), 133.2 (CH-21/25), 133.0 (CH-23), 132.2 (CH-20), 131.2 (CH-22), 129.1 (CH-24), 127.8 (CH-17), 127.1 (CH-18), 78.1 (CH-15), 76.5 (CH-14), 73.1 (CH-27) 71.2 (CH-9), 71.1 (CH-5), 70.4 (CH-7), 70.3 (CH-3), 70.1 (CH-26), 69.6 (CH-1'), 69.2 (CH-11), 68.3 (CH-13), 58.6 (CH-2), 44.0 (CH_2-8), 43.8 (CH_2-6), 42.9 (CH_2-10), 40.3 (CH_2-4), 38.7 (CH_2-12)*, 34.2 (CH_2-2'), 31.3 (CH_2-4'), 24.5 (CH_2-3'), 22.0 (CH_2-5'), 17.7 (CH_3-29), 13.9 (CH_3-6'), 11.6 (CH_3-28), * hidden by solvent. – **(+)-ESI MS**: *m/z* (%) = 1363 ($[M + Na]^+$, 46), ($[M + Na + H]^+$, 100). – **(-)-ESI MS**: *m/z* (%) = 1341 ($[2M-H]^-$, 78), 670 ($[M]^-$, 50).

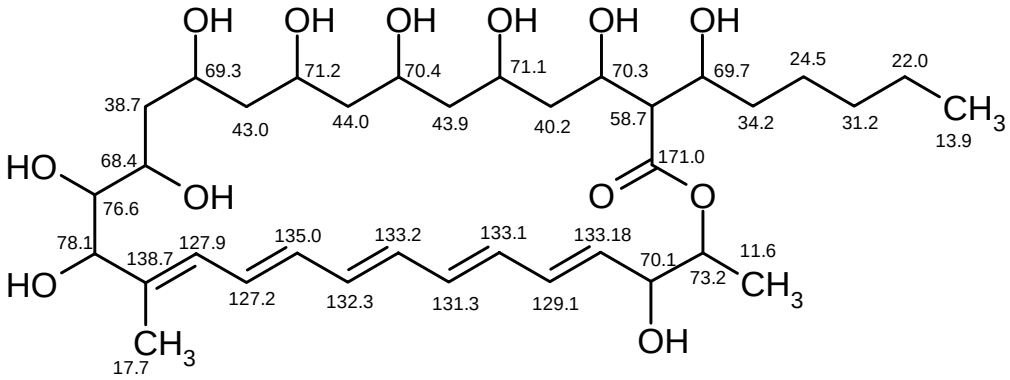


Figure 171: ^{13}C NMR values of fungichromin (**138a**)

7.8 Terrestrial *Streptomyces* sp. GW2/577

The terrestrial *Streptomyces* strain GW2/577 displayed white aerial mycelia when incubated on M_2 agar for 3 days at 28 °C.

7.8.1 Pre-screening

Well grown agar plates of the terrestrial *Streptomyces* strain GW2/577 served to inculcate 1 liter of M_2 medium using a rotary shaker for 3 days at 28 °C. The TLC of the resulting faint yellow extract (220 mg) showed numerous middle polar UV absorbing bands, most of them turned to violet-pink by spraying with anisaldehyde/sulphuric acid. Additional UV absorbing bands were explored, but showed no colour staining with the spray agents. Moreover, the crude extract was tested for antimicrobial activity; the results are presented in Table 22

Table 22: Antimicrobial activity of the crude extract produced by strain GW2/577

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	15
Escherichia coli	20
Streptomyces viridochromogenes (Tü57)	13
Staphylococcus aureus	11
Chlorella vulgaris	12
Chlorella sorokiniana	12
Scenedesmus subspicatus	11

7.8.2 Fermentation and working-up

Twelve of 1 litre-Erlenmeyer flasks, each containing 250 ml of M_2 medium were inoculated with well grown agar plates of the producing strain GW2/577, and leaved to grow for 72 h at 28 °C on circular shaker (95 rpm). The obtained well grown broth (2.5 liters) served to inoculate a 27-liter jar fermenter, using the same M_2 medium and kept to fermentation for further 3 days at 28 °C. The fermenter broth was harvested, mixed with celite (*ca*. 1.5 kg), and filtered by a press filter. Both the filtrate and mycelial cake were extracted repeatedly with ethyl acetate. The organic phases were evaporated at 40 °C under vacuum to dryness. As a result, a faint brown crude extract (10.13 g) was obtained.

7.8.3 Isolation and identification of metabolites

The extract was dissolved in 150 ml methanol, and defatted with cyclohexane (2 × 100 ml). The methanolic layer was evaporated and the extract (6.1 g) was subjected to column chromatography on Sephadex LH-20 (30 × 700 mm, MeOH). Eluates were monitored by TLC and pooled into four fractions. In addition, a methanol insoluble white solid was obtained, which was dissolved in chloroform and filtered. The filtrate was evaporated and the resulting white residue was purified on Sephadex LH-20 ($CHCl_3$/MeOH, 6:4) to a give a colourless solid powder of oligo-(*ß*-hydroxybutyric acid (cPHB [n = 8 – 30], **318**). The isolated compounds were: 3-Hydroxy-N-phenethyl-butyramide[343] (**145**) (colourless solid, 2.5 mg, R_f = 0.45[CH_3/MeOH 5%]), 5-methyl-1*H*-quinazoline-2,4-dione[193] (**148**) (colourless solid, 1.5 mg, R_f = 0.5[CH_3/MeOH 10%], R_t = 13.3 min). The new compounds crotonic acid-*ß*-phenylethyl amide[343] (**147**) (colourless solid, 60 mg; yield = 16 %), R_f = 0.61[CH_3/MeOH 5%]), 8-methyl-1*H*-quinazoline-2,4-dione[193] (**149**) (colourless solid, 500 mg; yield = 71 %), R_f = 0.52[CH_3/MeOH 10%]), and 4,12-dimethyl-5*H*-quinazolino[4,3-b]quinazoline-6,8-dione[193] (**151**) (yellow solid, 10 mg [1.5 %], R_f =

0.65[CH_3/MeOH 5%]) have already been described[193,343]. *p*-Hydroxyphenethyl propionamide (**144**) and the already known compounds, N-(2-phenylethyl)-propionamide (**141)** and *p*-hydroxyphenethylacetamide (**144**), are described now.

N-(2-Phenylethyl)-propionamide (141): $C_{11}H_{15}NO$ (177.2), applying fraction II to Sephadex LH-20 ($CHCl_3$/MeOH 3:2), PTLC ($CHCl_3$/5%MeOH) and finally with HPLC (R_t = 16.21 min) delivered a middle polar UV absorbing colourless solid (3 mg). It was stained to violet by anisaldehyde/sulphuric acid, and pink by Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.56 ($CHCl_3$/ MeOH, 5%). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.34-7.18 (m, 5 H, Ar-H), 5.48 (s br, 1 H, NH), 3.57 (q ^3J = 8.0 Hz, 2 H, 2'-CH_2), 2.83 (t, ^3J = 7.5 Hz, 2 H, 1'-CH_2), 2.18 (q, ^3J = 7.0 Hz, 2 H, 5'-CH_2), 1.13 (t, ^3J = 7.0 Hz, 3 H, 6' -CH_3). – **CI MS** (NH_3): *m/z* (%) = 372.2 ($[2\ M + NH_4]^{+\cdot}$, 42), 355.1 ($[2\ M + H]^{+\cdot}$, 100), 195.1 ($[M + NH_4]^{+\cdot}$, 68), 178.1 ($[M + H]^{+\cdot}$, 29). – **EI MS** (70 eV): *m/z* (%) = 177.0 ($M^{]+\cdot}$, 68), 104.0 ($[M - (H_2N\text{-}COCH_2CH_3)]^{+\cdot}$, 100).

***p*-Hydroxyphenethylacetamide; N-Acetyl-tyramine (142)**: $C_{10}H_{13}NO_2$ (179.21), delivered from fraction II by Sephadex LH-20 ($CHCl_3$/MeOH 3:2), PTLC ($CHCl_3$/5%MeOH), and finally purified with HPLC (R_t = 16.82 min), giving a middle polar UV absorbing colourless solid (4.5 mg), which turned to violet by anisaldehyde/sulphuric acid, and pink with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.45 ($CHCl_3$ / MeOH 5%). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 8.73 (s br, 1 H, OH), 7.51 (s br, 1 H, NH), 7.02 (d, ^3J = 8.8 Hz, 2 H, 2,6-H), 6.73 (d, ^3J = 8.9 Hz, 2 H, 3,5-H), 3.37 (q, ^3J = 6.2 Hz, 2 H, 2'-CH_2), 2.67 (t, ^3J = 6.1 Hz, 2 H, 1'-CH_2), 1.88 (s, 3 H, 5'-CH_3). – **EI MS** (70 eV) **(%)**: *m/z* (%) = 179.2 ($[M]^{+\cdot}$, 7), 120.1 ($[M- (H_2N\text{-}C{=}O\text{-}CH_3)]^{+\cdot}$, 100), 107.1 (30), 91 (11), 77.0 (8).

***p*-Hydroxyphenethyl propionamide (144)**: $C_{11}H_{15}NO_2$ (193.2), was obtained from fraction II by Sephadex LH-20 ($CHCl_3$/MeOH 3:2), PTLC ($CHCl_3$/5%MeOH), and finally with HPLC (R_t = 13.32 min), as colourless solid (6.4 mg), as UV absorbing substance, which turned to violet by anisaldehyde/sulphuric acid, and pink with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.4 ($CHCl_3$/5% MeOH). – **IR** (KBr, cm^{-1}): ν = 3309, 2991, 2878, 1651, 1554-1453, 1453, 1379, 1236 and 1201. – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 7.05 (d, ^3J = 8.8 Hz, 2 H, 2/6-H), 6.80 (d, ^3J = 8.9 Hz, 2 H, 3/5-H), 5.57 (s br, 1 H, NH), 3.48 (q, ^3J = 8.1 Hz, 2 H, 2'-CH_2), 2.77 (t, ^3J = 7.5 Hz, 2 H, 1'-CH_2), 2.17 (q, ^3J = 7.0 Hz, 2 H, 5'-CH_2), 1.12 (t, ^3J = 7.0 Hz, 3 H, 6'-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 150 MHz): δ = 174.4 (C_q-4'), 155.0 (C_q-4), 130.0 (C_q-1), 129.7 (CH-2/6),

115.6 (CH-3/5), 40.8 (CH_2-2'), 34.6 (CH_2-1'), 29.7 (CH_2-5'), 9.9 (CH_3-6'). **– EI MS (**70 eV): *m/z* (%) = 193.1 ($[M]^{+\cdot}$, 9), 154.1 (64), 135.1 (36), 120.1 (100), 107.1 (16), 92.1 (69). **– HREI MS**: *m/z* = 193.1103 (calcd. 193.11027 for $C_{11}H_{15}NO_2$).

7.9 Strain Bio215

The strain Bio215 was grown on LB-medium (with 50% Sea water) agar plates for 3 days at 28 °C, and developed intensive yellowish-green mycelium colonies.

7.9.1 Pre-screening

Small pieces of well grown sub-cultured agar plats of Bio 215 were used to inoculated 4 of 1 liter-Erlenmeyer flasks, each containing 250 ml of LB-medium using a rotary shaker for 3 days at 28 °C. The obtained yellow-green broth was filtered and extracted by ethyl acetate. After evaporation, a yellowish-green residue was obtained (250 mg). TLC analysis of the resulting extract showed two middle polar pale yellow bands and one with yellowish-green UV florescence, which coloured to yellow when sprayed with anisaldehyde/sulphuric acid. Additionally, other middle polar UV absorbing active bands were detected, most of them turned to violet-orange by spraying with anisaldehyde/sulphuric acid and pink-blue with Ehrlich's reagent. Moreover, the extracted was biologically tested as shown in Table 23.

Table 23: Antimicrobial activity of the crude extract produced by strain Bio 215

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	19
Escherichia coli	18
Staphylococcus aureus	15
Streptomyces viridochromogenes (Tü57)	12
Mucor miehei (Tü284)	12
Chlorella vulgaris	22
Chlorella sorokiniana	15
Scenedesmus subspicatus	16

7.9.2 Fermentation and working up

The said strain was sub-cultured in LB medium in 50% synthetic Sea water on 4 agar plates at 28 ºC for 3 days. Pieces of agar (1 cm^2) from the Bio 215 culture were used to inoculate 12 of 1 liter-Erlenmeyer flasks, each containing 250 ml of LB medium. The flasks were kept at 28 ºC for 5 days and 95 rpm. After 5 days, two different colours were obtained: the first as faint yellow broth, and the second an intensive yellow (as corresponding to agar plate colouration). Therefore, the different coloured

broths were fermented separately as 20 (for 3 days) and 27-liter jar fermenters (for 5 days), respectively. Both broths were filtered and extracted with ethyl acetate repeatedly (separately) giving, a) 8.4 g of yellowish-brown and b) 4.1 g of intensive yellowish green of the two extracts, respectively.

7.9.3 Isolation and identification of metabolites

TLC analysis ($CHCl_3$/7% MeOH) of both extracts gave identical results. Therefore, they were combined to give 12.5 g of crude extract, which was chromatographed on Sephadex LH-20 column ((30 × 1200 mm, MeOH) to give five fractions. Purification of fraction IV (0.81 g) on Sephadex LH-20 (MeOH), followed by PTLC (20 × 20 cm, 10% MeOH/$CHCl_3$), and again on Sephadex LH-20 ($CHCl_3$/MeOH 6:4) led to *p*-hydroxy phenyl acetic acid **(174)**, as colourless solid (4 mg; $\boldsymbol{R}_f$ = 0.72 ($CHCl_3$/ MeOH 10%)). By applying fraction V (0.311 g) to Sephadex LH-20 (MeOH) and HPLC (R_t = 5.82), a colourless solid (30 mg) of adenine (**175)** was obtained (R_f = 0.45 ($CHCl_3$ /MeOH 10 %)). Working up of fractions II and IV, led to the compounds shown below.

***cis-Cyclo*(valyl-prolyl) (164)**: $C_{10}H_{15}N_2O_2$ (196.2), was purified from fraction II (2.12 g) by applying fraction II to Sephadex LH-20 ($CHCl_3$/MeOH 3:2), PTLC (20 × 20 cm, $CHCl_3$/10%MeOH), and at the end to HPLC (R_t = 11.80 min), as middle polar UV absorbing colourless solid (6 mg), which turned to violet by anisaldehyde/sulphuric acid, and to pink with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.66 ($CHCl_3$ /10 % MeOH). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 4.21 (td, 1 H, J = 6.2 Hz, 0.8 Hz, 6-H), 3.94 (d, 1 H, 0.8 Hz, 3-H), 3.57-3.38 (m, 2 H, 9-CH_2), 2.49 (m, 1 H, 10-H), 2.21 (m, 1 H, 8-H_A), 1.90 (m, 3 H, 8-H_B, 7-CH_2), 1.10 (d, ^3J = 7.1 Hz, 3 H, 11-CH_3), 0.94 (d, ^3J = 6.8 Hz, 3 H, 12-CH_3). – **CI MS** (NH_3): *m/z* (%) = 393 ($[M + H]^+$, 6), 231 ($[M + NH_3 + NH_4]^+$, 10), 214 ($[M + NH_4]^+$, 100), 197 ($[M + H]^+$, 48).

Flazin (166): $C_{17}H_{12}N_2O_4$ (308.293), was obtained as yellowish-green solid (200 mg) from fraction IV (0.81 g) by applying to Sephadex LH-20 (MeOH), PTLC (20 × 20 cm, 10% MeOH/$CHCl_3$), and finally with an additional Sephadex LH-20 ($CHCl_3$/MeOH 6:4), which was delivered as UV green fluorescence, turned to yellow by anisaldehyde/sulphuric acid after heating. – $\boldsymbol{R}_f$ = 0.23 ($CHCl_3$ /10 % MeOH). – **^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 11.58 (s br, 1 H, NH), 8.83 (s, 1 H, 4-H), 8.41 (d, ^3J = 8.2 Hz, 1 H, 5-H), 7.82 (d, ^3J = 8.1 Hz, 1 H, 8-H), 7.62 (t, ^3J = 8.1 Hz, 1 H, 7-

H), 7.41 (d, ^3J = 3.5 Hz, 1 H, 4'-H), 7.32 (t, ^3J = 8.2 Hz, 1 H, 6-H), 6.62 (d, ^3J = 3.5 Hz, 1 H, 3'-H), 5.43 (s br, 1 H, OH), 4.64 (s, 2 H, 2'-CH_2). – **^{13}C/APT NMR (**[D_6] DMSO): δ = 166.6 (C=O, 3-COOH), 157.2 (C_q-2'), 151.4 (C_q-5'), 141.5 (C_q-8_a), 132.5 (C_q-1), 132.0 (C_q-4_b), 130.0 (C_q-3), 129.0 (CH-7), 122.0 (CH-5), 121.0 (2 C_q-$4a/8_b$), 120.7 (CH-6), 115.7 (CH-4), 112.9 (CH-8), 111.2 (CH-4'), 109.4 (CH-3'), 56.0 (2'-CH_2). – **(+)-ESI MS**: *m/z* (%) = 353.8 ([M – H + 2 Na]$^+$, 100), 331.8 ([M + Na]$^+$, 83), 309.7 ([M+H]$^+$, 10). – **(-)-ESI MS**: *m/z* (%) = 307.8 ([M - H]$^-$). – **DCI** (NH_3): *m/z* (%) = 309.2 ([M + H]$^+$). – **EI MS** (70 eV): *m/z* (%) = 308.3 ([M]$^{+\cdot}$, 60), 264.3 ([M - COO]$^{+\cdot}$, 100), 247.3 (28), 205.2 (34), 177.2 (12), 91.1 (54), 44.1 (42), 41 (40). – **HREI MS**: *m/z* = 308.0803 (calcd. 308.0797 for $C_{17}H_{12}N_2O_4$).

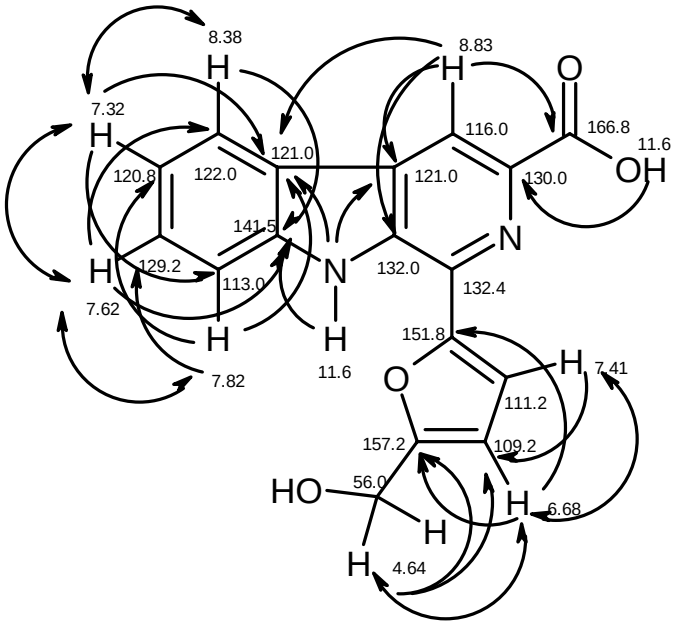


Figure 172: H,H COSY and HMBC correlations of flazin (**166**)

1-(9H-*β*-carbolin-1-yl)-3-hydroxy-propan-1-one (171): $C_{14}H_{12}N_2O_2$ (240.25), was isolated from fraction IV (0.81 g), by applying to Sephadex LH-20 (MeOH), PTLC (20 × 20 cm, 10% MeOH/$CHCl_3$), and finally by Sephadex LH-20 ($CHCl_3$/MeOH 6:4), as yellowish-green solid (2 mg). – R_f = 0.29 ($CHCl_3$ /10 % MeOH). – **^{1}H NMR** ([D_6]acetone, 300 MHz): δ = 8.49 (d, ^3J = 4.8 Hz, 1H, H-3), 8.38 (d, ^3J = 4.8 Hz, 1H, 4-H), 8.29 (dd, ^3J = 8.1 Hz, ^4J = 1.2 Hz, 1 H, 5-H), 7.86 (dd, ^3J = 8.1 Hz, ^4J = 1.1 Hz, 1 H, 8-H), 7.61 (td, ^3J = 8.1 Hz, ^4J = 1.2 Hz, 1 H, 7-H), 7.33 (t, ^3J = 8.1 Hz, 1 H, 6-H), 4.05 (t, ^3J = 6.2 Hz, 3'-CH_2), 3.55 (t, ^3J = 6.2 Hz, 4'-CH_2). – **EI MS** (70 eV): *m/z* (%) = 240.0 ([M]$^{+\cdot}$, 36), 211.0 ([M-CHO]$^{+\cdot}$, 100), 193.0

(8), 168.0 ([M - (CH_2CH_2-COO)]$^{+\cdot}$, 76), 140.0 (21), 130.0 (22), 107 (20), 72.0 (16), 60.0 (52), 42 (24). – **HREI MS**: *m/z* = 240.0899 (calcd. 240.08987 for $C_{14}H_{12}N_2O2$).

7.10 Strain Pic009

The strain Pic009 was grown on LB-medium + glucose (with 50% Sea water) agar plates for 3 days at 28 °C, developing faint yellow mycelial colonies.

7.10.1 Pre-screening

The strain was cultivated as 1 liter-shaker culture at 28 °C for 120 h with 95 rpm. After 5 days, the mycelium and nutritional medium were faint yellow coloured. The obtained broth was filtered and extracted by ethyl acetate, giving a residue of 280 mg. The crude extract showed several UV absorbing bands on TLC, most of them turned to violet-blue, when sprayed with anisaldehyde/sulphuric acid or Ehrlich's reagent. Additional polar UV absorbing bands were detected, which showed no colour staining when exposed to the spray reagents. Additionally, the residue was tested antimicrobially as shown in Table 24

Table 24: Antimicrobial activity of the crude extract produced by strain Pic009

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	23
Escherichia coli	12
Streptomyces viridochromogenes (Tü57)	11
Mucor miehei (Tü284)	11

7.10.2 Fermentation and working up

Fermentation was carried out in shaking flasks. The said strain was sub-cultured in LB medium + glucose in 50% synthetic sea water on 4 agar plates at 28 ºC for 3 days. Pieces of agar from the Pic009 culture were used to inoculate 100 of 1 liter-Erlenmeyer flasks, each containing 250 ml. The flasks were kept at 28 ºC for 96 hours and 95 rpm. After harvesting, celite (~ 1 kg) was added to the culture broth, and the mixture was homogenized by shaking for 1~3 min. By filtration using a filter press, the liquid phase was separated and extracted four times with ethyl acetate. On the other hand, the mycelial cake was extracted (3 times) with acetone (5-liter), and the acetone was removed in *vacuo*. After evaporation, the aqueous layer was extracted (3 times) with ethyl acetate, and the extract was evaporated to give crude ma-

terial. For the isolation of metabolites, the combined organic layers were dried at the oil pump to give a faint brown crude extract (2.91 g).

7.10.3 Isolation and identification of metabolites

The resulting crude extract was dissolved in methanol, and subjected to column chromatography on Sephadex LH-20 (30× 1200 mm; MeOH). With the aid of TLC monitoring, four fractions were obtained. Further working up, and purification of the fractions delivered the compounds mentioned below.

***o*-Hydroxy phenyl acetic acid (177)**: $C_8H_8O_3$ (152.15), was isolated from fraction III (1.21 g), by Sephadex LH-20 ($CHCl_3$/MeOH 3:2), and followed by HPLC (R_t = 2.32 min), as an UV absorbing colourless solid (9 mg). – $\boldsymbol{R}_f$ = 0.80 ($CHCl_3$/ MeOH, 10 %). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 7.18 (dd, ^3J = 8.8 Hz, ^4J = 1.1 Hz, 1 H, 6'-H), 7.08 (td, ^3J = 8.8 Hz, ^4J = 1.0 Hz, 1 H, 4'-H), 6.85 (d, ^3J = 8.8 Hz, 1 H, 5'-H), 6.78 (td, ^3J = 8.8 Hz, ^4J = 1.0 Hz, 1 H, 3'-H), 3.62 (s, 2 H, 2-CH_2). – **EI MS** (70 eV): *m/z* (%) = 152.1 ($[M]^{+\cdot}$, 98), 134.1 ($[M- H_2O]^{+\cdot}$, 75), 106.1 ($[M-HCOOH]^{+\cdot}$, 100), 78.0 (49), 43.1 (23).

Isoxanthohumol; 4',7-Dihydroxy-5-methoxy-8-prenylflavonone (180): $C_{21}H_{22}O_5$ (354.39), was isolated from fraction III (1.21 g), by subjecting to Sephadex LH-20 ($CHCl_3$/MeOH 3:2), and the remaining impure substance, was purified on HPLC (R_t = 4.83 min), delivering an UV blue fluorescent colourless solid (1.5 mg), which turned to faint yellow by exposing to ammonia. – $\boldsymbol{R}_f$ = 0.11 ($CHCl_3$ / MeOH 10 %). – **^{1}H NMR** ([D_6]acetone, 500 MHz): δ = 9.38 (s br, 1 H, 7-OH), 8.51 (s br, 1 H, 4'-OH), 7.39 (d, ^3J = 8.5 Hz, 2 H, 2',6'-H), 6.88 (d, ^3J = 8.3 Hz, 2 H, 3',5'-H), 6.26 (s, 1 H, 6-H), 5.35 (dd, $J_{2,3a}$ = 12.5, $J_{2,3b}$ = 2.5 Hz, 1 H, 2-H), 5.19 (t, ^3J = 7.2 Hz, 1 H-*β*, 10-H), 3.73 (s, 3 H, 5-OCH_3), 3.30-3.22 (ABX, J_{AB} = 17.5 Hz, J_{AX} = 7.2 Hz, J_{BX} = 2.5 Hz, 2 H, 9-CH_2), 2.93 (ABX, J_{AB} = 16.92, J_{AX} = 8.25, J_{BX} = 6.23, 1 H, 3-H_A), 2.61 (ABX, J_{AB} = 16.7 Hz, J_{AX} = 8.2 Hz, J_{BX} = 6.1 Hz, 1 H, 3-H_B), 1.60 (s, 3 H, 12-CH_3), 1.58 (s, 3 H, 13-CH_3). – **(+)-ESI MS**: *m/z* (%) = 731.1 ($[2 M + Na]^+$, 100), 377.5 ($[M + Na]^+$, 10). – **(-)-ESI MS**: *m/z* (%) = 353.5 ($[M-H]^+$). – **EI MS (**70 eV): *m/z* (%) = 354.0 ($[M]^{+\cdot}$, 100), 339.0 ($[M- Me]^{+\cdot}$, 12), 311.0 ($[M-CH=C(Me)_2)]^{+\cdot}$, 42), 299.0 ($[M-CH_2CH = C(Me)_2]^{+\cdot}$, 15), 247.0 (6), 234.0 (22), 219.0 (24), 191.0 (20), 179.0 (52), 147.0 (7), 120.0 (11), 91.0 (6), 45.0 (5). – **HREI MS**: *m/z* = 354.1464 (calcd. 354.1467 for $C_{21}H_{22}O_5$).

Uridine* (187): $C_9H_{12}N_2O_6$ (244.2), was afforded from fraction III (1.21 g) by subjecting to Sephadex LH-20 ($CHCl_3$/MeOH 3:2) followed by HPLC (R_t = 3.22 min), as an UV absorbing colourless solid (12 mg), which turned to blue when sprayed by anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R}_f$ = 0.32 ($CHCl_3$/MeOH, 10 %). – **^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 11.22 (s br, 1 H, NH), 7.84 (d, ^3J = 8.1 Hz, 1 H, 6-H), 5.78 (d, ^3J = 6.1 Hz; 1H, 1'-H), 5.62 (d, ^3J = 8.1 Hz, 1 H, 5-H), 5.29 (d, ^3J = 6.1 Hz, 1 H, OH), 5.02 (m, 2 H, 2 OH), 4.08 (m, 2 H, 2',4'-H), 3.83 (q, ^3J = 8.1 Hz, 1 H, 3'-H), 3.56 (m, 2 H, 6'-CH_2). – **(+)-ESI MS (Na)**: *m/z* (%) = 1023.2 ([4 M + 2 Na + H]$^+$, 15), 777.1 ([3 M + 2 Na + H]$^+$, 60), 534.3 ([2 M + 2 Na]$^+$, 35), 533.4 ([2 M + 2 Na – H]$^+$, 100), 511.3 ([2 M + Na]$^+$, 21), 267.5 ([M + Na]$^+$, 52). – **CI MS** (NH_3): *m/z* (%) = 262.0 ([M + NH_4]$^+$). *uridine was obtained as a mixture with uracil.

7.11 Terrestrial *Streptomyces* sp. GW10/580

The terrestrial *Streptomyces* strain GW10/80 exhibited white mycelial colonies when incubated on M_2 agar for 3 days at 28 °C.

7.11.1 Pre-screening

Fermentation was carried out in 4 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium at 28 °C for 3 days with vigorous shaking at 95 rpm. The culture broth (1litre) was extracted with ethyl acetate to afford 53 mg of a faint yellow culture broth. Chemical screening (TLC, $CHCl_3$/5~10% MeOH) of the crude extract showed a low polar yellowish-green band. One middle polar brown band was detected by spraying with anisaldehyde/sulphuric acid, in addition to numerous UV absorbing bands. Furthermore, the residue was subjected to antimicrobial activity, and the results were displayed in Table 25.

Table 25: Antimicrobial activity of the crude extract produced by the strain W10/580

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	30
Escherichia coli	23
Staphylococcus aureus	30
Streptomyces viridochromogenes (Tü57)	30
Mucor miehei (Tü284)	21
Candida albicans	20
Chlorella vulgaris	20
Chlorella sorokiniana	30
Scenedesmus subspicatus	24

7.11.2 Fermentation, and Isolation of metabolites (shaker culture)

A big shaker culture was carried out in 96 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. The inoculated flasks were incubated for 3 days at 28 °C on a linear shaker (110 rpm). The culture broth (faint yellow, 22-liter) was mixed with *ca*. 1 kg celite and filtered through a press filter to separate the mycelium, and the aqueous phase. The mycelium and aqueous phase were extracted repeatedly with ethyl acetate and the organic layer was evaporated to dryness. The residual solids from both organic phases, were combined to yield 4.52 g of brown crude extract.

The crude extract was subjected to silica gel column chromatography (30 × 600 mm), with a stepwise elution, using $CHCl_3$/MeOH. During TLC and spraying agents control ($CHCl_3$ /5-10%MeOH) four fractions were collected. On further isolation and purification of the fractions, the metabolites shown below were obtained.

Phenazine-1-carboxylic acid; Tubermycin B (188): $C_{13}H_8N_2O_2$ (224.2), was obtained from the fast mobile fraction I (0.66 g) by PTLC (20 × 20 cm, $CHCl_3$/2%MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH, 3:2), as an amorphous yellow solid (7 mg). It showed a colour change from yellowish-green to reddish-brown by anisaldehyde/sulphuric acid after heating, but no colour change occurred with 2 N NaOH. – $\boldsymbol{R}_f$ = 0.67 ($CHCl_3$ /10% MeOH). – **^{1}H NMR** ([D_6]acetone, 300 MHz): δ = 8.92 (dd, ^3J = 7.2 Hz, ^4J = 1.5 Hz, 6-H), 8.60 (dd, ^3J = 8.8 Hz, ^4J = 1.5 Hz, 1 H, 9-H), 8.49 (dd, ^3J = 8.4 Hz, ^4J = 1.3 Hz, 2-H), 8.38 (dd, ^3J = 8.3 Hz, ^4J = 1.5 Hz, 4-H), 8.22-8.08 (m, 3 H, 3,7,8-H). – **EI MS** (70 eV): *m/z* = 224 ($[M]^{+\cdot}$, 6), 180 ($[M\text{-}CO_2]^{+\cdot}$, 100), 153 (5), 130 (16), 71 (12), 57 (14), 43 (8).

Surfactin C (189): $C_{53}H_{93}N_7O_{13}$ (1036.3), an application of faction II (1.21 g) to silica gel column chromatography (30 × 400 mm, $CHCl_3$/MeOH), and then Sephadex LH-20 ($CHCl_3$/MeOH 3:2), led to a colourless solid (300 mg) of homologues. It showed a colour change to brown by anisaldehyde/sulphuric acid, was blue with chlorine/anisidine, and yellow with Ninhydrin. – $\boldsymbol{R}_f$ = 0.55 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.4-7.30 (s br, 6 H, 6 NH), 5.25-4.06 (m, 8 H), 2.88-1.15 (m, 36 H), 1.05 – 0.85 (m, 30 H, 10 CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 75 MHz): δ = 178-169 (10 C_q), 75 – 48 (8 CH), 42-28 (17 CH_2), 25 – 12 (10 CH_3). – **(+)-ESI MS**: *m/z* = 1058.9 $[M + Na]^+$. – **(-)-ESI MS**: *m/z* (%) = 1035.7 $[M - H]^-$. – (+)-**ESI MS^2**: *m/z* = 1036.70 $[M + H]^+$, 1018 $[M - (H_2O) + H]^+$, 923.3 [M – (Leu) + H]$^+$,

810.2 [M - (Leu + Leu) +H]$^+$, 695.2 [M - (Asp + Leu + Leu) + H]$^+$, 596.2 [M- (Val + Asp + Leu + Leu) + H]$^+$, 483.1 [M - (Leu + Val + Asp + Leu + Leu) + H]$^+$, 370.0 (M- (Leu + Leu + Val + Asp + Leu + Leu) +H]$^+$. – (+)-**ESI MS^3**: *m/z* = 667.2 [Leu + Leu + Val + Asp +Leu + Leu + H]$^+$, 554.1 [Leu + Leu + Val + Asp + Leu + H]$^+$, 441.1 [Leu + Leu + Val + Asp + H]$^+$.

3-Pyridine carboxylic acid; Nicotinic acid (190): $C_6H_5NO_2$ (123.1), was delivered from fraction III (0.512 g) by Sephadex LH-20 (MeOH), PTLC (20×20 cm, $CHCl_3$/7%MeOH), and then Sephadex LH-20 ($CHCl_3$/MeOH 3:2), as an UV absorbing colourless solid (3.5 mg), turned to faint pink by Ehrlich's reagent after heating. – R_f = 0.45 ($CHCl_3$/10%MeOH). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 9.80 (s br, 1 H, COOH), 9.16 (dd, ^4J = 0.9 Hz, ^4J = 0.9 Hz, 1 H, 2-H), 8.78 (dd, ^3J = 8.3 Hz, ^4J = 1.5 Hz, 1 H, 6-H), 8.31 (dt, ^3J = 8.1 Hz, ^4J = 1.5 H, 1 H, 4-H), 7.52 (dd, ^3J = 6.0 Hz, 3.10 Hz, 1 H, 5-H). – **EI MS** (70 eV): *m/z* (%) = 123.1 ([M]$^{+\cdot}$), 78.1 ([M-COOH]$^{+\cdot}$, 31), 45.1 (35). – **CI MS** (NH_3): *m/z* (%) = 158.1 ([M + NH_3 + NH_4]$^+$, 7), 141.1 ([M + NH_4]$^+$, 100), 124.1 ([M + H]$^+$, 75). – **(+)-ESI MS (**Na): *m/z* (%) = 124.5 ([M + H]$^+$, 100). – **(-) ESI MS**: *m/z* (%) = 124.5 ([M + H]$^+$). – **(-) ESI MS**: *m/z* (%) = 245.2 ([2M - H]$^+$, 39), 122.5 ([M - H]$^+$, 100). – **HREI MS**: *m/z* = 123.0320 (calcd. 123.0320, for $C_6H_5NO_2$).

2-Acetamidophenol; 2-Hydroxyacetanilide (192): $C_8H_9NO_2$ (151.16), a purification of fraction III (0.512 g), using Sephadex LH-20 (MeOH), PTLC (20×20 cm, $CHCl_3$/7%MeOH), and followed by Sephadex LH-20 ($CHCl_3$/MeOH 3:2), led a colourless solid (5 mg) of middle polar UV absorbing component, which turned to pink by Ehrlich's reagent, and to violet with anisaldehyde/sulphuric acid. – R_f = 0.25 ($CHCl_3$/MeOH 5%). – **^{1}H NMR** ([D_6]acetone, 300 MHz): δ = 9.29 (s br, 2 H, NH, OH), 7.39 (dd, ^3J = 8.3 Hz, ^4J = 0.7 Hz, 1 H, 3-H), 7.02 (td, ^3J = 8.2 Hz, ^4J = 0.7 Hz, 1 H, 5-H), 6.88 (dd, ^3J = 8.25, ^4J = 0.8 Hz, 1 H, 6-H), 6.80 (td, ^3J = 8.3 Hz, ^4J = 0.8 Hz, 1 H, 4-H), 2.20 (s, 3 H, O=C-CH_3). – **EI MS (**70 eV): *m/z* (%) = 151.1 ([M]$^{+\cdot}$, 34), 135.1 (7), 109.1 ([M-O=C-CH_2]$^+$, 100), 92.1 (10), 91.1 (14), 80.1 (17), 43.0 (17).

3-Indolylcarbaldehyde (194): C_9H_7NO (145.16), an application of fraction IV (0.312 g) to PTLC (20 × 20 cm, $CHCl_3$/10%MeOH), followed by Sephadex LH-20 (MeOH) led to a colourless solid (11 mg), as middle polar UV absorbing substance coloured to orange by anisaldehyde/sulphuric acid and to pink with Ehrlich's re-

agent. – $\boldsymbol{R}_f$ = 0.35 ($CHCl_3$/10 MeOH). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 11.19 (s br, 1 H, NH), 10.01 (s, 1 H, CHO), 8.22 (m, 2 H, 2,4-H), 7.52 (m, 1 H, 7-H), 7.23 (m, 2 H, 5,6-H). – **EI MS (**70 eV): *m/z* (%) = 145 ($[M]^{+\cdot}$, 84), 144 ($[M-H]^{+\cdot}$, 100), 130 (20), 116 (30), 84 (96), 66 (94), 46 (16).

Cytoxazone (195): $C_{11}H_{13}NO_4$ (223.22), colourless solid (2 mg), was afforded from fraction IV (0.312 g), during the application of PTLC (20 × 20 cm, $CHCl_3$/10%MeOH) and Sephadex LH-20 (MeOH). It delivered an UV absorbing polar band, which coloured to violet by anisaldehyde/sulphuric acid, and to pink with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.50 ($CHCl_3$/10% MeOH). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 7.25 (d, ^3J = 8.1 Hz, 2 H, 2',6'-H), 6.95 (d, ^3J = 8.1 Hz, 2 H, 3',5'-H), 5.01 (d, ^3J = 8.3 Hz, 1 H, 4-H), 4.81 (m, 1 H, 5-H), 3.78 (s, 3 H, 4'-OCH_3), 3.21-3.18 (m, 2 H, 6-CH_2). – **CI MS** ($[NH_3]^+$): *m/z* (%) = 464.2 ($[2M + NH_4]^+$, 36), 241.1 ($[M + NH_4]^+$, 100). – **EI MS (**70 eV): *m/z* (%) = 223.1 ($[M]^{+\cdot}$, 50), 192.1 ($[M - OCH_3]^{+\cdot}$, 2), 180.1 ($[M - HNCO]^{+\cdot}$, 4), 163.1 ($[M - H_2NCOO]^{+\cdot}$, 49), 135.1 (100), 134.1 (58), 120.1 (20), 107.1 (12), 91.1 (18), 77.0 (8). – **HREI MS**: *m/z* = 223.0853 (calcd. 223.0844 for $C_{11}H_{13}NO_4$).

7.11.3 Fermentation and isolation of metabolites (fermenter)

For cultivation, small pieces of an agar culture of the terrestrial *Streptomyces* GW10/580 grown on M_2 medium were used to inoculate 12 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. The shaker flasks were incubated for 4 days at 28 °C with 95 rpm. This culture (faint yellow) was used to inoculate a 25-liter jar fermenter containing 22 liters of fermentation M_2 medium. The fermentation was carried out at 28 °C for 4 days. The culture broth was divided into culture filtrate and mycelial cake by filtration with the aid of celite. The mycelial cake was stirred with acetone (3 times). After filtration, the acetone was evaporated under reduced pressure, and the aqueous layer extracted with ethyl acetate. The water phase was also extracted four times with ethyl acetate. The combined ethyl acetate extract was filtered and evaporated to dryness *in vacuo*, yielding 5.34 g of brown crude extract

The TLC analysis of the extract showed a low polar yellow band of phenazine-1-carboxylic acid (**188)**. In addition, three intensive overlapped middle polar brown-violet bands were detected after spraying with anisaldehyde/sulphuric acid and heating. The extract was subjected to a flash silica gel chromatography, with chloroform-

methanol gradient, to furnish four fractions. Purification of the main fraction III (2.12 g) using PTLC ((CHCl$_3$/MeOH 7-10 %), followed by Sephadex LH-20 (CH$_2$Cl$_2$/MeOH 3:2) two times, led to isolation of three colourless oily compounds: Feigrisolide B (**198**), feigrisolide A (**202**) and feigrisolide C (**206**).

Feigrisolide B (198): $C_{11}H_{21}O_4$ (216), a colourless oil (12 mg), turned to violet by spraying with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R}_f$ = 0.42 (CHCl$_3$/ 10% MeOH). – **^{1}H NMR** (CDCl$_3$, 300 MHz): δ = 6.90 (s br, 2 H, 2 OH), 4.23 (m, 1H, 6-H), 4.05 (q br, ^3J = 8.3 Hz, 1 H, 3-H), 3.78 (m, 1H, 8-H), 2.51 (dq, ^3J = 8.3 Hz, 7.0 Hz, 1 H, 2-H), 2.03 (m, 2 H, 5-H$_B$, 4-H$_A$), 1.67 (m, 4 H, 4-H$_B$, 5-H$_A$, 7-CH$_2$), 1.49 (m, 2 H, 9-CH$_2$), 1.16 (d, ^3J = 7.1 Hz, 3 H, 11-CH$_3$), 0.94 (t, ^3J = 7.6 Hz, 3 H, 10-CH$_3$). – **^{13}C NMR** (CDCl$_3$, 75.476 MHz): δ = 178.1 (C$_q$-1), 80.9 (CH-3), 77.4 (CH-6), 70.2 (CH-8), 45.3 (CH-2), 40.7 (CH$_2$-7), 30.6 (CH$_2$-5), 29.7 (CH$_2$-9), 28.8 (CH$_2$-4), 13.5 (CH$_3$-11), 10.0 (CH$_3$-10). – **(+)-ESI MS**: *m/z* (%) = 455.4 ([2 M + Na]$^+$, 32), 239.4 ([M + Na]$^+$, 38). – **(-)-ESI MS**: *m/z* (%) = 453.8 ([2 M – 2 H + Na]$^+$, 100), 215.5 ([M - H]$^-$, 93).

Feigrisolide A (202): $C_{10}H_{18}O_4$ (202), colourless oil (6 mg), turned to violet by anisaldehyde/sulphuric acid. – $\boldsymbol{R}_f$ = 0.32 (CHCl$_3$/ 10% MeOH). – **^{1}H NMR** (CDCl$_3$, 300 MHz): δ = 6.08 (s br, 2 H, 2 OH), 4.22 (m, 1 H, 6-H), 4.09 (m, 1 H, 8-H), 4.00 (q, ^3J = 8.3 Hz, 1 H, 3-H), 2.48 (dq, ^3J = 8.3 Hz, 7.2 Hz, 1 H, 2-H), 2.05 (m, 2 H, 4,5-H$_A$), 1.68 (m, 4 H, 7-CH$_2$, 4,5-H$_B$), 1.21 (d, ^3J = 6.4 Hz, 3 H, 9-CH$_3$), 1.17 (d, ^3J = 7.2 Hz, 3 H, 11-CH$_3$). – **(+)-ESI MS**: *m/z* (%) = 427.0 ([2 M + Na]$^+$, 74), 225.2 ([M + Na]$^+$, 26). – **(-)-ESI MS**: *m/z* (%) = 201.1 ([M-H]$^-$)

Feigrisolide C (206): $C_{21}H_{36}O_7$ (400), colourless oil (13 mg), coloured to violet by anisaldehyde/sulphuric acid after heating. – $\boldsymbol{R}_f$ = 0.27 (CHCl$_3$/ 10% MeOH). – **^{1}H NMR** (CDCl$_3$, 300 MHz): δ = 6.45 (s br, 3 H, 3 OH), 5.08-4.87 (m, 1 H, 12-H), 4.18 (m, 1 H, 5-H), 3.98 (m, 3 H, 1-H, 8-H, 14-H), 3.75 (m, 1H, 20-H), 2.50 (m, 2 H, 2-H, 9-H), 2.08-1.93 (m, 4 H, 15,16-CH$_2$), 1.86-1.42 (m, 10 H, 6,7,13,19,21-CH$_2$), 1.24 (d, ^3J = 6.4 Hz, 3 H, 24-CH$_3$), 1.17 (d, ^3J = 7.2 Hz, 3H, 18-CH$_3$), 1.13 (d, ^3J = 7.2 Hz, 3 H, 23-CH$_3$), 0.96 (t, ^3J = 6.2 Hz, 3 H, 22-CH$_3$). – **^{13}C/APT NMR (**CDCl$_3$, 75 MHz): δ = 178.5 (C$_q$-3), 174.2 (C$_q$-10), 80.6 (CH-1), 80.5 (CH-8), 77.2 (CH-5), 76.4 (CH-14), 69.9 (CH-20), 69.0 (CH-12), 45.4 (CH-9), 45.3 (CH-2), 42.2 (CH$_2$-13), 41.0 (CH$_2$-19), 31.0 (CH$_2$-15), 30.5 (CH$_2$-7), 29.8 (CH$_2$-21), 28.4 (CH$_2$-6), 28.2 (CH$_2$-16), 20.2 (CH$_3$-25), 13.3 (CH$_3$-18), 13.1 (CH$_3$-24), 9.1 (CH$_3$-22). – **(+)-ESI**

MS: *m/z* (%) = 845.3 ([2 M + 2 Na - H]$^+$, 68), 823.3 ([2 M + Na]$^+$, 24), 423.5 ([M + Na]$^+$, 100). **– (-)-ESI MS**: *m/z* (%) = 399.3 ([M - H]$^-$).

7.12 Marine *Streptomyces* sp. B8335

The marine *Streptomyces* sp. B8335 was cultured on agar with M_2^+ medium (50% synthetic sea water) and incubated at 28 °C for 3 days, exhibiting white aerial mycelial colonies.

7.12.1 Pre-screening

The fermentation was carried out in 5 of 1 liter-Erlenmeyer flasks, each containing 200 ml of M_2^+ medium with 50% artificial sea water. Each flask was inoculated with 1 cm^2 area of a 3 days agar culture. After 3 days of cultivation on a shaker (95 rpm) at 28 °C, the culture broth (~l liter) was harvested and extracted with ethyl acetate to give 82 mg of reddish-orange crude extract. The extract was applied to chemical screening using TLC. During the TLC analysis, two orange middle polar bands were detected, which turned to red by anisaldehyde/sulphuric acid, in addition, a yellow, greenish UV fluorescent band, which changed to grey by anisaldehyde/sulphuric acid. An additional four low polar UV absorbing bands were detected, which turned to violet-blue by anisaldehyde/sulphuric acid after heating. The microbial screening of the extract was performed, giving the results shown below (Table 26)

Table 26: Antimicrobial activity of the crude extract produced by the strain B 83335

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	28
Escherichia coli	20
Streptomyces viridochromogenes (Tü57)	16
Mucor miehei (Tü284)	13

7.12.2 Fermentation and working up

For cultivation, small pieces of agar culture of the marine *Streptomyces* B8335 grown on M_2^+ medium with 50% artificial sea water were used to inoculate 96 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2^+ medium. The shake flasks were incubated for 3 days at 28 °C on a linear shaker culture (110 rpm). The culture broth (25-liter) was divided into culture filtrate and mycelial cake by filtration with the aid of celite. The mycelial cake was stirred with acetone. After filtration, the ace-

tone was evaporated and the aqueous layer extracted with ethyl acetate. The water phase was also extracted repeatedly with ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness *in vacuo*, yielding 3.78 g of orange crude extract**.**

7.12.3 Isolation and identification of metabolites

The crude extract was applied to Sephadex LH-20 (30 × 1200 mm, MeOH) to give six fractions by monitoring with TLC, and the spraying reagents. The main fraction II (1.51 g), after purification by Sephadex LH-20 (MeOH), led to obtain Actinomycin D (**130**). Further purification of the fractions II~IV the metabolites shown below were obtained.

Actinomycin HKI 0155 (208): $C_{62}H_{86}N_{12}O_{17}$ (1272), was obtained as red powder (4.5 mg) from fraction II (1.51 g), by Sephadex (MeOH), PTLC ($CHCl_3$/5% MeOH), and finally on Sephadex LH-20 ($CHCl_3$/MeOH 3:2), as middle polar red-orange substance, which turned to red by anisaldehyde/sulphuric acid but gave no colour change with 2 N NaOH. **–** $\boldsymbol{R}_f$ = 0.35 ($CHCl_3$/5MeOH). **– (+)-ESI MS**: *m/z* = 1295.9 ($[M + Na]^+$). **– (-)-ESI MS**: *m/z* (%) = 1271 ($[M-H]^-$).

N-Phenyl-1-naphthylamine (209a): $C_{16}H_{13}N$ (219.28), was delivered from fraction IV by applying to PTLC ($CHCl_3$/5%MeOH), and Sephadex LH-20 ($CHCl_3$/MeOH 3:2), as UV absorbing colourless solid (8 mg), turned to violet by anisaldehyde/sulphuric acid. **–** $\boldsymbol{R}_f$ = 0.90 ($CHCl_3$ /MeOH 5 %). **– ^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.02 (m, 1 H, Ar-H), 7.86 (m, 1 H, Ar-H), 7.57 (m, 1 H, Ar-H), 7.52-7.46 (m, 2 H, 2 Ar-H), 7.38 (m, 1 H, 2-H), 7.22 (dt, ^3J = 8.5 Hz, ^4J = 1.3 Hz, 1 H, Ar-H), 7.01, 6.99 (dd, *J* = 8.1, 1.2 Hz, 2 H, 2Ar-H), 6.88 (t, ^3J = 7.9 Hz, 1 H, Ar-H). **– ^{13}C/APT NMR (**$CDCl_3$, 50 MHz): δ = 144.7 (C_q-1), 138.7 (C_q-1'), 134.6 (C_q-4a), 129.3 (CH-3'/5'), 128.5 (CH-5), 127.7(C_q-8a), 126.1 (CH-3), 126.0 (CH-6), 125.6 (CH-7), 122.9 (CH-8), 121.8 (CH-4), 120.4 (CH-4'), 117.4 (CH-2'/6'), 115.8 (CH-2). **– EI MS** (70 eV): *m/z* (%) = 219.1 ($[M]^{+\cdot}$, 100), 218.1 (42), 165.1 (9), 108.6 (17), 85.1 (6), 71.1 (7), 57.0 (12), 43.1 (8).

7.13 Strain Hel59b

The North Sea strain Hel59b was sub-cultured in LB + glucose medium with 50% synthetic sea water on agar plates for three days at 28 °C. After 3 days, faint yellow gel colonies were grown, and the surrounding agar showed a yellow colour.

7.13.1 Pre-screening

The strain was cultivated as 1 liter-shaker culture at 28 °C for 72 h with 95 rpm. After 3 days, the mycelium and nutritional medium were harvested and extracted with ethyl acetate to give 200 mg of brown extract. Chemical screening by TLC of the extract showed numerous overlapping middle, and polar absorbing bands, most of them exhibited a colour staining to violet-orange or pink by spraying with anisaldehyde/sulphuric acid or Ehrlich's reagent. Furthermore, in the preliminary screening using the agar diffusion method, the extract exhibited the bio-activity shown below (Table 27).

Table 27: Antimicrobial activity of the crude extract produced by the strain Hel 59b

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	15
Escherichia coli	15
Staphylococcus aureus	13
Streptomyces viridochromogenes (Tü57)	15
Mucor miehei (Tü284)	12
Candida albicans	16
Chlorella vulgaris	15
Chlorella sorokiniana	15
Scenedesmus subspicatus	11

7.13.2 Fermentation and working up

Well-grown agar sub-cultures of the marine bacterium Hel 59b served to inoculate 12 of l liter-Erlenmeyer flasks, each containing 250 ml of LB-medium + glucose. The flasks were kept on a circular shaker (95 rpm) for 3 days at 28 °C. The broth was used to inseed a 40-liter jar fermenter held at 28 °C for 72 hours (pH 6.5~ 7.8, 250 rpm), to afford a white aerial mycelium with a faint yellow broth. After harvesting, the broth was mixed with celite and filtered. The mycelial cake was extracted with ethyl acetate followed by acetone. After evaporation of the acetone, the water residue was extracted again by ethyl acetate. The filtrate was extracted by ethyl acetate repeatedly. The ethyl acetate layers were combined and evaporated to dryness under reduced pressure at 40 °C yielding 11.17 g of a brown crude extract.

7.13.3 Isolation and identification of metabolites

The ethyl acetate extract residue of the entire culture was dissolved in methanol. The methanolic solution (50 ml) was applied to Sephadex LH-20 (30×1200 mm; MeOH). With the aid of TLC monitoring, five fractions were yielded. On further

purification of the fractions, the metabolites discussed below were isolated. The fast mobile fraction I (3.12 g) yielded benzoic acid (**211**) (15 mg, colourless solid; R_f = 0.55 ($CHCl_3$ / MeOH 5 %)) using Sephadex LH-20, followed by HPLC (R_t = 20.11 min). Further purification of the fractions led to isolation the metabolites discussed below.

3-(Methylthio) propanoic acid (210): $C_4H_8O_2S$ (120.0), was afforded from fraction I (3.12 g), by applying to Sephadex LH-20 ($CHCl_3$/MeOH 3:2), and the resulting impure component (25 mg), was purified on HPLC (R_t = 19.22 min) to a pale yellow oil (12 mg). It changed to brown by spraying with $PdCl_2$ reagent and heating. – $\boldsymbol{R}_f$ = 0.30 ($CHCl_3$). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 9.25 (s br, 1 H, COOH), 2.77 (A_2B_2, ^3J = 6.1 Hz, 2 H, 3-CH_2), 2.68 (A_2B_2, ^3J = 6.1 Hz, 2 H, 2-CH_2), 2.15 (s, 3 H, 5-CH_3). – **EI MS** (70 eV): *m/z* (%) = 122.0 ($S^{34}[M]^{+\cdot}$, 5), 120.0 ($[M]^{+\cdot}$, 100), 107 ($S^{34}[M\text{-}CH_3]^{+\cdot}$, 0.8), 105 ($[M\text{-}CH_3]^{+\cdot}$, 8), 89.0 ($S^{34}$, 3), 87.0 (16), 85.0 (35), 83.9 (56), 82.9 (42), 74.0 (45), 73.0 (16), 63.0 ($[M\text{-}(CH_2\text{-}S^{34}\text{-}CH_3)]^+$, 6) 61.0 ($[M\text{-}(CH_2\text{-}S\text{-}CH_3)]^{+\cdot}$, 98), 59.0 ($[HO\text{-}C{=}O\text{-}CH_2]^{+\cdot}$, 11), 48.9 (11), 46.9 (27), 44.9 ($[COOH]^{+\cdot}$, 26), 41.0 (9). – **HREI MS**: *m/z* = 120.0245 (calcd. 120.0245 for $C_4H_8O_2S$).

Quinoline-2-one-4-carboxylic acid methyl ester (212): $C_{11}H_9NO_3$ (203.19), was isolated as colourless solid (2 mg) from fraction II (1.12 g), by applying to Sephadex LH-20 ($CHCl_3$/MeOH; 3:2), PTLC ($CHCl_3$/5% MeOH), and further followed by HPLC (R_t = 10.22 min). It is a middle polar UV absorbing substance, which turned to a violet by anisaldehyde/sulphuric acid, and to pink with Ehrlich's reagent. – $\boldsymbol{R}_f$ = 0.75 ($CHCl_3$ /MeOH 10 %). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 11.42 (s br, 1 H, NH), 8.45 (d, ^4J = 1.2 Hz, 1 H, 3-H), 8.30 (m, 1 H, 5-H), 7.58 (m, 1 H, 8-H), 7.31 (m, 2 H, 6,7-H), 3.92 (s, 3 H, 4-OCH_3). – **EI MS (**70 eV): *m/z* (%) = 203.0 ($[M]^{+\cdot}$, 26), 114.0 ([M-$OOCH_3$), 100), 116.0 ([M- ($COOCH_3$, C=O)]$^{+\cdot}$, 16), 89.0 (15).

3-(Hydroxy acetyl)-indole (214): $C_{10}H_9NO_2$ (175.18), purified from fraction III (0.413 g), with aid of PTLC ($CHCl_3$/10%MeOH), and followed by HPLC (R_t = 13.12 min), as UV absorbing colourless solid (8 mg). It exhibited a colour change to orange and pink by anisaldehyde/sulphuric acid and Ehrlich's reagent, respectively. – $\boldsymbol{R}_f$ = 0.55 ($CHCl_3$/ MeOH 5%). – **^{1}H NMR (**[D_6]acetone, 300 MHz): δ = 11.18 (s br, 1 H, NH), 8.31 (d, ^3J = 2.1 Hz, 1 H, 2-H), 8.27 (m, 1 H, 4-H), 7.54 (m, 1 H, 7-H), 7.24 (m, 2 H, 5,6-H), 4.69 (s, 2 H, 11-CH_2), 3.84 (s br, 1 H, OH). – **EI MS (**70 eV): *m/z*

(%) = 175.0 ($[M]^{+\cdot}$, 20), 144.2 ($[M- (CH_2\text{-}OH)]^{+\cdot}$, 100), 116.0 (18), 89.0 (11), 84.0 (2).

3-Pyridinecarboxamide, Nicotinamide (215): $C_6H_6N_2O$ (122.12)**,** isolated as UV absorbing colourless solid (5 mg) from fraction V (0.215 g) by HPLC (R_t = 18.12), which showed pink colour by anisaldehyde/sulphuric acid and/or Ehrlich's reagent. **–** $\boldsymbol{R}_f$ = 0.30 ($CHCl_3$ /MeOH 5 %). **– ^{1}H NMR** ([D_6]acetone, 300 MHz): δ = 9.11 (d, ^4J = 1.2 Hz, 1 H, 2-H), 8.70 (dd, ^3J = 8.4 Hz, ^4J = 1.2 Hz, 1 H, 6-H), 8.25 (dd, ^3J = 8.5 Hz, ^4J = 1.3 Hz, 1 H, 3-H), 7.68 (s br, 1 H, NH_A), 7.50 (dd, ^3J = 8.5 Hz, ^4J = 1.3 Hz, 1 H, 5-H), 6.90 (s br, 1 H, NH). **– EI MS (**70 eV): *m/z* (%): = 122 ($[M]^{+\cdot}$, 98), 105 (92), 91 (100), 78.0 ([M - $CONH_2$), 10), 77.0 (58).

7.14 Terrestrial *Streptomyces* sp. GW3/1786

The terrestrial *Streptomyces* strain GW3/1786 was cultured on agar plates (M_2medium) for 3 days at 28 °C. As a result, white aerial mycelial colonies were exhibited.

7.14.1 Pre-screening

The strain was inoculated into 4 of 1 liter-Erlenmeyer flasks each containing 250 ml of M_2 -medium. The fermentation was carried out at 28 °C for 72 hours on a rotary shaker at 95 rpm. After 3 days, the broth (intensive yellow) was filtered and extracted with ethyl acetate. TLC analysis of the residue (104 mg) exhibited numerous overlapping low polar yellow bands, which changed to red-violet by treatment with NaOH. Two additional yellowish-green bands were detected, which turned to red by spraying with anisaldehyde/sulphuric acid, but not with NaOH. Additionally, the extract was tested antimicrobially using the agar diffusion method, and the results are shown in Table 28.

Table 28: Antimicrobial activity of the crude extract produced by the strain GW3/1786

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	30
Escherichia coli	23
Staphylococcus aureus	30
Streptomyces viridochromogenes (Tü57)	30
Mucor miehei (Tü284)	21
Candida albicans	20
Chlorella vulgaris	20

Chlorella sorokiniana	30
Scenedesmus subspicatus	24

7.14.2 Isolation and identification of metabolites (shaker)

The terrestrial *Streptomyces* sp. isolate GW 3/1786 grew well on agar with M_2 medium in about 72 hours with faint yellowish-white aerial mycelium. 96 of 1-litre Erlenmeyer shaking flasks, each containing 250 ml of M_2 medium, were inoculated with pieces of well grown agar plates, and kept for 5 days at 28 °C and 95 rpm. The entire culture broth was mixed with *ca*. 1 kg of diatom earth, and pressed through a pressure filter. The mycelia cake was stirred with acetone repeatedly and filtered. After evaporation of the acetone, the water residue was extracted with ethyl acetate. The filtrate was subjected to column chromatography on Amberlite XAD-2, and the adsorbed metabolites were eluted with methanol-water gradient. The methanol water solutions were evaporated. The rest water residue was extracted with ethyl acetate. All the ethyl acetate layers were combined, and evaporated at 40 °C under vacuum to dryness, yielding 2.71 g of a yellowish-orange crude extract.

This was subjected to flash silica gel column chromatography (30 × 600 mm) using 1000 ml DCM and then a DCM-MeOH gradient. After monitoring by TLC analysis, four fractions were obtained. Purification of fractions II~IV (1.22 g), using PTLC (DCM/2%MeOH) several times, and then Sephadex LH-20 (DCM/MeOH, 3:2) resulted in the four yellow solid compounds: *α*-Indomycinone (**286**), *β*-indomycinone (**295**), saptomycin A (**297**) and *ε*-indomycinone (**291**) (see strain GW3/1130, 362). Working up of the fast mobile fraction I (0.312 g) with Sephadex LH-20 (2 × MeOH) resulted in the green-yellow phencomycin methyl ester (**217**), as shown below.

Phencomycin methyl ester (217): $C_{16}H_{12}N_2O_4$ (296.3), greenish-yellow powder (4 mg), showed a colour change to red by anisaldehyde/sulphuric acid, but not with NaOH. – $\boldsymbol{R}_f$ = 0.91 ($CHCl_3$/3%MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 8.49 (dd, ^3J = 8.2, ^4J = 1.2 Hz, 4,9-H), 8.29 (dd, ^3J = 7.2, ^4J = 1.2 Hz, 2,7-H), 7.88 (dd, ^3J = 8.2 Hz, ^3J = 7.2 Hz, 3,8-H), 4.15 (s, 3 H, 1,6-OCH_3). – **^{13}C/APT** NMR ($CDCl_3$, 150 MHz): δ = 166.8 (2 CO, C_q-1,6), 142.9 (C_q-4a,9a), 140.9 (C_q-5a,10a), 134.3 (CH-2,7), 132.9 (CH-4,9), 131.1 (C-1,6), 129.5 (CH-3,8), 52.7 (OCH_3-1,6). – **EI MS** (70 eV): *m/z* (%) = 296 $[M]^{+\cdot}$, 40), 265 ($[M\text{-}CH_3O]^{+\cdot}$, 22), 238 ($[M - COOCH_2]^{+\cdot}$, 100), 222 (15), 117 (12), 103 (6).

7.14.3 Isolation and identification of metabolites (fermenter)

The strain was cultured at 28 °C for 3 days on a rotary shaker (95 rpm) in 12 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. 2.5 Litres of the broth were used to inoculate a 25-liter jar fermenter, which was kept at 28 °C for 96 hours. The harvested broth (faint yellow) was filtered with celite, and the filtrate was extracted four times with EtOAc. The mycelial cake was stirred two times with acetone and filtered. After distillation, the aqueous residue was extracted with EtOAc. Also, the water phase was extracted with ethyl acetate. The resulting combined organic phases were evaporated under vacuum to afford a yellowish-brown crude extract (6.41 g).

TLC analysis of the residue showed one low polar yellow band. It exhibited a red colour when sprayed with anisaldehyde/sulphuric acid and heating. Additionally, other middle polar UV absorbing bands were detected, which predominantly coloured to violet-brown by anisaldehyde/sulphuric acid after heating.

The extract was dissolved in methanol (150 ml), and defatted by cyclohexane (3 × 100 mL). The methanol extract (4.2 g) was then applied to Sephadex LH-20 column (30×100 mm, MeOH), yielding five fractions. Applying the fraction II (1.55 g) containing a greenish-yellow metabolite to silica gel column, and elution with C_6H_{12}-CH_2Cl_2 gradient gave a yellow solid. The yellow compound was further purified on Sephadex LH-20 (CH_2Cl_2/MeOH, 3:2) to give a greenish-yellow solid of 1-phenazinecarboxylic acid methyl ester (**223**).

1-Phenazinecarboxylic acid methyl ester (223): $C_{14}H_{10}N_2O_2$ (238.25), yellowish-green powder (2 mg). – R_f = 0.8 ($CHCl_3$ / 5 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ 8.42 = (dd, ^3J = 8.1 Hz, ^4J = 1.2 Hz, Ar-H), 8.34 (m, 1 H, Ar-H), 8.24 (dd, ^3J = 7.1 Hz, ^4J = 1.2 Hz, 2 H, 2 Ar-H), δ 7.95-7.82 (m, 3 H, 3 Ar-H), 4.15 (s, 3 H, 1-OCH_3). – **EI MS** (70 eV): *m/z* (%) = 238 ($[M]^{+\cdot}$, 100), 222 (16), 179 ($[M\text{-}CO_2]^{+\cdot}$, 8), 117 (10), 60 (36), 43 (16). – **(+)-ESI MS**: *m/z* (%) = 499 ($[2\ M + Na]^+$), 100), 261 ($[M + Na]^+$, 58) and 239 ($[M+H]^+$, 4).

7.15 Marine *Streptomyces* sp. B2150

The marine *Streptomyces* strain B2150 was exhibited as white aerial mycelous colonies when cultivated on M_2^+ medium agar plat for 3 days at 28 °C.

7.15.1 Pre-screening

Well grown agar plates of the marine *Streptomyces* sp. B2150, were transferred into 4 of 1 liter-Erlenmeyer flasks, each loaded with 250 ml M_2^+ medium, and cultured on a shaker for 72 h at 28 °C. The white aerial mycelium, and the brown broth were filtered and extracted with EtOAc. After evaporation under vacuum at 40 °C, 120 mg of brown extract was obtained. TLC analysis of extract showed numerous UV absorbing bands. Most of the bands turned to violet-orange by anisaldehyde/sulphuric acid. An additional faint yellow greenish UV-fluorescent spot was detected, which coloured to yellow by anisaldehyde/sulphuric acid after heating. Bioactivity of the strain extract was determined on basis of agar diffusion method as shown in Table 29.

Table 29: Antimicrobial activity of the crude extract produced by the strain B 2150

Tested microorganism	Inhibition zone ∅ [mm]
Escherichia coli	11
Streptomyces viridochromogenes (Tü57)	12
Mucor miehei (Tü284)	30
Candida albicans	20
Chlorella vulgaris	16

7.15.2 Fermentation and working up

3 Liters culture broth of the marine *Streptomyces* sp. B2150 was used to inseed a 25-liter jar fermenter for 3 days at 28 °C. The resulting brown broth was divided into filtrate and mycelial cake, after adding celite. Both biomass and filtrate were extracted separately with acetone and ethyl acetate, respectively. The acetone extract was evaporated, and the yielded aqueous layer was extracted once more by ethyl acetate. The ethyl acetate extracts were combined and evaporated under vacuum at 38 °C giving 1.75 g of a brown crude extract.

7.15.3 Isolation and identification of metabolites

The extract was applied to Sephadex LH-20 (MeOH). With the aid of TLC monitoring, five fractions were delivered. TLC of fraction III (0.21 g), exhibited a yellowish-green UV fluorescent band. Purification of the fraction by PTLC ($CHCl_3$/13%MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 3:2) led to isolation of flazin (**166**). Purification of the polar fraction IV (0.312 g), using PTLC

($CHCl_3$/10%MeOH), followed by Sephadex LH-20 ($CHCl_3$/MeOH 3:2) led to indolyl-3-lactic acid (**225**).

Indolyl-3-lactic acid (225): $C_{11}H_{11}NO_3$ (205.2), middle polar UV absorbing colourless solid (7 mg), turned to violet by anisaldehyde/sulphuric acid and heating. **– R_f** = 0.35 ($CHCl_3$ /5%MeOH). **– ^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 10.75 (s br, 1 H, NH), 7.52 (dd, ^3J = 7.9 Hz, ^4J = 1.1 Hz, 1 H, 4-H), 7.35 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 1 H, 7-H), 7.11 (d, ^3J = 0.8 Hz, 1 H, 2-H), 7.02 (td, ^3J = 7.2 Hz, ^4J = 1.2 Hz, 1 H, 5-H), 6.98 (td, ^3J = 7.2 Hz, ^4J = 1.1 Hz, 1 H, 6-H), 4.21 (m, 1 H, 1'-H), 3.03 (dd, $^2J_{AB}$ = 11.1 Hz, $^3J_{AX}$ = 4.2 Hz, 1 H, 2'-CH_A), 2.92 (dd, ^3J = 6.1 Hz, 1.1, 1 H, 2'-CH_B). **– EI MS** (70 eV): *m/z* (%) = 205.2 ([M]$^{+\cdot}$, 24), 130.1 ([M- (HO-CH-COOH)]$^{+\cdot}$, 100).

7.16 Terrestrial *Streptomyces* sp. GW12/3995

The terrestrial *Streptomyces* strain GW12/3995 was cultivated on agar M_2 medium for 3 days at 28 °C. After 72 hours, the agar plate showed white aerial mycelia.

7.16.1 Pre-screening

One agar plate was used to inoculate four of 1L-Erlenmeyer flasks, each containing 250 ml of M_2 medium. The fermentation was carried out at 28 °C for 3 days with 95 rpm. The culture broth (1 L) was lyophilised and extracted with ethyl acetate yielding 65 mg of yellowish-brown crude extract. TLC analysis of the extract showed two polar UV absorbing spots, one of which coloured to a faint brown by anisaldehyde/sulphuric acid after heating. An additional middle polar band was detected, which turned strongly to brown by anisaldehyde/sulphuric acid and heating.

Table 30: Antimicrobial activity of the crude extract produced by the strain GW 12/3995

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	11
Escherichia coli	10.5
Staphylococcus aureus	13
Streptomyces viridochromogenes (Tü57)	19

7.16.2 Fermentation and working up

The terrestrial *Streptomyces* sp. GW 12/3995 grew strongly on agar with M_2 medium in about 72 hours. 12 of 1-litre Erlenmeyer flasks, each containing 250 ml of M_2 medium, were inoculated with pieces of well grown agar plates, and kept for 3

days at 28 °C and 95 rpm. The entire culture broth was inseeded to a 20-liter jar fermenter, which was grown for additional 3 days at 28 °C. The obtained broth (pale yellow) was mixed with *ca*. 1 kg of diatomaceous earth, pressed through a pressure filter, and both filtrate and residue were extracted separately with ethyl acetate. Since both extracts showed the same components by TLC, they were combined and evaporated to dryness to yield 3.1g of an organic crude extract.

7.16.3 Isolation and identification of metabolites

The extract was subjected to flash silica gel column chromatography (30 × 600 mm), using 500 ml DCM and then a DCM-MeOH gradient, yielding four fractions. TLC of fraction III (0.32 g) showed a middle polar UV absorbing band. After purification, using PTLC ($CHCl_3$/ 10% MeOH) and Sephadex LH-20 (MeOH), the entire component was isolated as colourless solid (13 mg) of 3,4-dihydroxy benzoic acid (**226**).

3,4-Dihydroxy benzoic acid (226): $C_7H_6O_4$ (154.12), colourless solid (13 mg). It exhibited no colour with spraying reagents. – $\boldsymbol{R}_f$ = 0.32 ($CHCl_3$/10%MeOH). – **^{1}H NMR (**[CD_3OD, 300 MHz): δ = 7.42 (d, ^4J = 1.4 Hz, 2-H), 7.37 (dd, ^3J = 8.3 Hz, ^4J = 1.4 Hz, 1 H, 6-H), 6.77 (d, ^3J = 8.3 Hz, 1 H, 5-H). – **^{13}C/APT NMR (**[CD_3OD, 300 MHz): δ = 174.2 (C=O, C_q-COOH), 149.6 (C_q-4), 145.5 (C_q-3), 128.4 (C_q-1), 123.2 (CH-6), 117.8 (CH-2), 115.3 (CH-5). – **EI MS** (70 eV): *m/z* (%) = 154 $[M]^{+\cdot}$, 100), 137 ($[M\text{-}OH]^{+\cdot}$, 96), 109 (16), 60 (30), 43 (18).

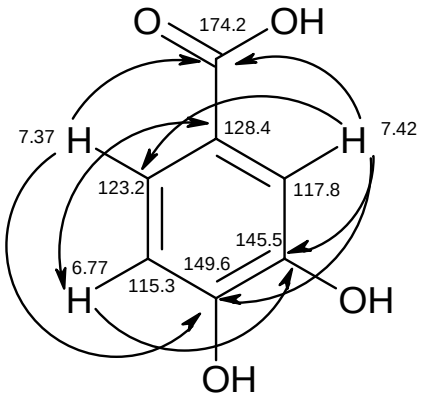


Figure 173: HMBC and HMQC correlation of 3,4-dihydroxybenzoic acid (**226**)

7.17 Roseobacter Strains (DFL12, DFL38, DFL16, DFL30, DFL27)

The marine *Roseobacter* sp. (DFL12, DFL38, DFL16, DFL30, and DFL27) were obtained with unidentified genetic taxonomy by Wagner Döbler, institute of Gesellschaft für Biotechnologische Forschung, Braunschweig. They were cultivated on agar (LB medium with 50 % artificial Sea water) in Petri dishes for 3 days at 28 °C. After 72 h, the agar showed a red colour with the formation of dark-red colonies.

7.17.1 Pre-screening

TLC and HPLC-MS analysis of the strains extracts, showed the presence of iso-forms metabolic components. During TLC, they exhibited a highly low polar dark red zone, with equal R_f values (0.95~90; $CHCl_3$), which turned to blue by treatment with sulphuric acid. Additionally, middle polar UV absorbing bands were detected, which turned to violet by anisaldehyde/sulphuric acid and heating. The crude extracts showed very weak biological activities against bacteria, fungus and algae which are shown in Table 31.

Table 31: Antimicrobial activities of the crude extracts produced by the strains DFL 12,16,38, 27,30

Strain ext. / Test.M.	*B. sub.*	*St. aur.*	*Strept.* (Tü57)	*E. coli*	*Cand alb*	*Mucor miehei*	*Clorevul ga*	*Clore soroki*	*Scened. subspic*
DFL 12	0	0	0	0	0	0	0	0	0
DFL 16	0	0	0	17	0	0	0	12	12
DFL 27	0	0	0	0	0	0	0	0	0
DFL 30	0	0	0	0	0	0	0	0	0
DFL 38	11	13	0	0	0	0	0	0	0

7.17.2 Fermentation and working up

For cultivation, small pieces of agar culture of the marine *Roseobacter* sp. strains DFL12 and 38 grown on LB medium with 50% artificial sea water were used to inoculate 40 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2^+ medium. The shaker flasks were incubated for 3 days at 28 °C with 95 rpm. The culture broth (dark red) of both stains were filtered off with the aid of celite. The bacterial cake was stirred with acetone. After filtration, the acetone was evaporated under reduced pressure, and the aqueous layer extracted with ethyl acetate. The water phase was also extracted four times with ethyl acetate. The combined ethyl acetate extract was

filtered and evaporated to dryness *in vacuo*, yielding of 3.10 g (of DFL12) and 2.51 (of DFL38) dark red crude extracts, respectively.

7.17.3 Isolation and identification of metabolites

The two strains extracts (of DFL12 and 38) were investigated by TLC and were found alike especially for their obtained red zones. This was established by HPLC-ESI MS. As a result, the two extracts were combined. The crude extract (5.61 g) was then subjected to column chromatography on flash silica gel, and eluted by CH_2Cl_2-MeOH gradient. On the basis of TLC monitoring, two fractions were obtained. The fraction II exhibited several middle polar UV absorbing bands, most of them turned to pink-violet by anisaldehyde/sulphuric acid. Purification of the fraction using PTLC and Sephadex LH-20 (DCM/MeOH 3:2), led to *cyclo*(leucyl-prolyl), *cyclo*(phenylalanyl-prolyl) and linoleic acid (**239**), as three colourless solids. Purification of the main fraction I (3.21 g), containing the dark red carotenoid, led to spheroidenone (**230**), as described below.

Spheroidenone (230): $C_{41}H_{58}O_2$ (582.92). The fraction I (3.12 g) was dissolved in 100 ml of 5 % methanolic potassium hydroxide and stirred for 3 hours. The reaction mixture was poured into cold water (150 ml), and extracted by diethyl ether. The ethereal layer (containing the red pigment) was washed with water (3 times), and then dried over sodium sulphate and filtered. The filtrate was evaporated under vacuum. The resulting fat-free red pigment was further purified using inactivated aluminium oxide column chromatography, and additionally purified on Sephadex LH-20 ($CHCl_3$/MeOH, 3:2). At the end, the concentrated $CHCl_3$-solution was precipitated with 10 ml of n-pentane at 0°C, giving a dark red powder (15 mg). The red compound turned to blue by treatment with sulphuric acid, but gave no colour change with 2 N NaOH. – $\boldsymbol{R}_f$ = 0.90 ($CHCl_3$), 0.48 (cyclohexane/30%EtOAc). – **UV** ($CHCl_3$): λ_{max} = 261 (sh), 304 (sh), 382 (sh), 493. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 7.47 (d, ^3J = 15.3 Hz, 1 H), 6.76 (d, ^3J = 15.3 Hz, 1 H), 6.68-6.18 (m, 12 H), 5.95 (d, ^3J = 15.2 Hz, 1 H), 5.17-5.09 (m, 2 H), 3.22 (s, 3 H, OCH_3), 2.18-2.04 (m, 4 H, 2 CH_2), 1.97 (m, 12 H, 4 CH_3), 1.68 (s, 3 H, CH_3), 1.60 (s, 9 H, 3 CH_3), 1.32 (s, 6 H, 2 CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 125 MHz): δ = 203.0 (CO, C_q), 148.4 (CH), 142.4 (CH), 141.3 (CH), 140.0 (C_q), 139.8 (CH), 137.2 (C_q), 135.9 (C_q), 135.9 (C_q), 135.6 (C_q), 135.4 (CH), 135.2 (CH), 134.1 (CH), 133.8 (C_q), 131.3 (CH), 131.0 (CH), 129.3 (CH), 125.7 (CH), 125.4 (CH), 124.5 (C_q), 124.3 (CH), 124.3 (CH), 123.8

(CH), 123.8 (CH), 118.0 (CH), 81.7 (C_q), 52.2 (OCH_3), 26.8 (CH_2), 26.7 (CH_2), 26.7 (CH_2), 26.6 (CH_2), 25.7 (CH_3), 22.9 (CH_3), 22.9 (CH_3), 17.7 (CH_3), 17.7 (CH_3), 16.0 (CH_3), 12.85 (CH_3), 12.85 (CH_3), 12.8 (CH_3), 12.7 (CH_3). – **(+)-ESI MS**: *m/z* (%) = 583 ($[M+H]^+$). – **EI MS (**70 eV): *m/z* (%) = 582 ($[M]^{+\cdot}$, 56), 476 (18), 277 (28), 73 ($[-C(OCH_3)(CH_3)_2]^{+\cdot}$, 100), 69 ($[-C_5H_9]^{+\cdot}$, 20). **HREI MS**: *m/z* = 582.4437 (calcd. 582.4436 for $C_{41}H_{58}O_2$).

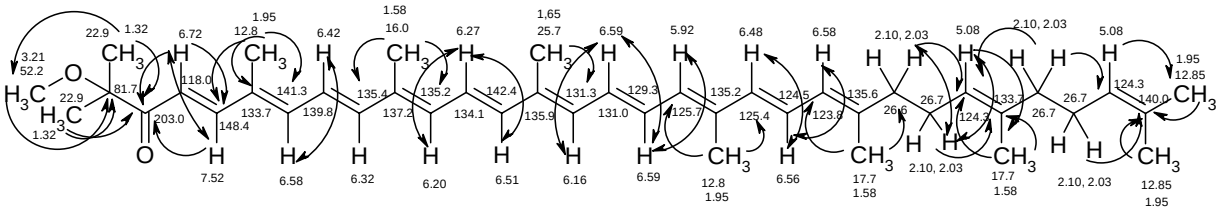


Figure 174: H,H COSY (H↔H) and HMBC (H → C) correlations of spheroidenone (**230**)

7.18 Terrestrial *Streptomyces* sp. GW10/1818

The terrestrial *Streptomyces* strain GW10/1818 formed a white aerial mycelium and the surrounding agar was grey by incubation on M_2 -medium for 3 days at 28 °C.

7.18.1 Pre-screening

The agar from one plate was cut into small pieces, and used to inoculate 4 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. Fermentation was carried out on a shaker at 95 rpm for 3-days at 28 °C. The obtained reddish grey culture broth was filtered on celite and extracted with ethyl acetate. Chemical screening with the aide of TLC analysis of the extract (180 mg) showed a low polar UV absorbing zone, which stained to brown by anisaldehyde/sulphuric acid after heating. Furthermore, the extract was tested biologically on the basis of agar diffusion method, as shown below in Table 32

Table 32: Antimicrobial activity of the crude extract produced by the strain GW 10/1818

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	11
Escherichia coli	0
Staphylococcus aureus	0
Streptomyces viridochromogenes (Tü57)	12
Mucor miehei (Tü284)	34
Candida albicans	0
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

7.18.2 Fermentation and working up

Well grown agar plats of the terrestrial *Streptomyces* sp. GW10/1818 were used to inoculate 96 × 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium, which were cultivated on a linear shaker (110 rpm) for 5 days at 28 °C. After harvesting, the broth (grey) was filtered giving mycelial cake and water phase, which were extracted repeatedly with ethyl acetate. The organic phases were collected and evaporated under vacuum giving 1.21 g of a brown crude extract.

7.18.3 Isolation and identification of metabolites

The crude extract was fractionated based on TLC (DCM/3%MeOH), using flash column chromatography with a stepwise gradient system of increasing polarity. As a result, two fractions were obtained. The main fraction II (1.11 g) exhibited the wanted compound. Purification of the fraction was carried out using PTLC ($CHCl_3$/5%MeOH), and the obtained pale-yellow oily crude component was further purified on Sephadex LH-20 (CH_2Cl_2/MeOH, 3:2), resulting in a pale yellow oil of hexahydromenaquinone MK-9 (II,III,VIII-H6) (**235**). The compound exhibited an UV absorbance and turned to brown by anisaldehyde/sulphuric acid after heating.

Hexahydromenaquinone MK-9 (II,III,VIII-H6) (235): $C_{56}H_{86}O_2$ (791.31), Pale yellow oil (32 mg). – R_f = 0.89 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.08 (m, 2 H, 5,8-H), 7.69 (m, 2 H, 6,7-H), 5.18-5.08 (m, 6 H, CH=C), 3.38 (d, ^3J = 6.2 Hz, 2 H, 1'-CH_2), 2.20 (s, 3 H, 3-CH_3), 2.13-1.90 (m, 20 H), 1.78 (s, 3 H, CH_3), 1.69 (s, 3 H, CH_3), 1.61 (s, 15 H, 5 CH_3), 1.42-1.18 (m, 21 H), 0.88-0.78 (m, 9 H, 3 CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 75 MHz): δ = 185.3 (CO, C_q), 184.5 (CO, C_q), 146.1 (C_q), 143.3 (C_q), 137.9 (C_q), 135.2 (C_q), 134.9 (2 C_q), 134.6 (C_q)

134.5 (CH), 133.3 (CH), 133.2 (C_q), 132.1 (C_q), 131.2 (C_q), 126.3 (CH), 126.1 (CH), 124.9 (CH), 124.4 (CH), 124.2 (2 CH), 124.0 (CH), 118.7 (CH), 40.0 (CH_2), 39.9 (CH_2), 39.7 (4 CH_2), 38.6 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 37.0 (CH_2), 36.6 (CH_2), 32.7 (CH), 32.6 (CH), 32.4 (CH), 29.7 (2 CH_2), 29.6 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 27.8 (CH_3), 26.7 (2 CH_2), 26.6 (CH_2), 26.0 (CH_2), 25.7 (CH_3), 25.7 (CH_3), 25.5 (CH_2), 22.6 (CH_3), 19.7 (CH_3), 17.7 (CH_3), 16.3 (CH_3), 16.0 (2 CH_3), 15.9 (CH_3), 12.7 (CH_3). – **EI MS (**70 eV): *m/z* (%) = 791 ($[M]^{+\cdot}$, 12), 225 ([fragment **234**]$^{+\cdot}$, 100), 69 ($[-C_5H_9]^+$, 58). – (+)- and **(-)-ESI MS** gave wrong results.

7.19 Terrestrial *Streptomyces* sp. GW5/1749

The terrestrial *Streptomyces* strain GW5/1749 showed a white aerial mycelium with a yellow-orange colouration on agar by incubation on M_2 medium for 3 days at 28 °C.

7.19.1 Pre-screening

The agar from one plate was served to inoculate 4 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. Fermentation was carried out on shaker at 95 rpm for 3-days at 28 °C. After three days, the colour of the broth in the flasks changed to reddish-orange. The broth was harvested after 3 days, and both the filtrate and the mycelial cake were extracted three times with ethyl acetate. TLC of the EtOAc extract showed the presence of one unpolar UV absorbing band, which turned to brown by anisaldehyde/sulphuric acid after heating. An additional intensive unpolar yellow band was detected, which turned to blue when treated with sulphuric acid. The biological activity of the metabolites is shown in Table 33.

Table 33: Antimicrobial activity of the crude extract produced by the strain GW5 /1749

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	25
Escherichia coli	24
Staphylococcus aureus	25
Chlorella vulgaris	14
Chlorella sorokiniana	12

7.19.2 Fermentation and working up

The strain was grown on agar plates with M_2 medium without artificial sea water at 28 °C for three days. These agar plates were used to inoculate 96 of 1 liter-

Erlenmeyer flasks, each containing 200 ml of M_2 medium. The fermentation conditions were applied, as described in the pre-screening step. The culture broth (20-liter, intensive yellow) was filtered. The mycelial cake was stirred with acetone (2 × 2.5 liters) and filtered. After evaporation, the aqueous residue was extracted with ethyl acetate. The water phase was extracted, using the Amberlite XAD-2, and the adsorbed metabolites were extracted by MeOH-H_2O (80:20). After evaporation, the water residue was extracted also by ethyl acetate. TLC analysis of the two phases showed that, the main metabolite was concentrated in biomass fraction. This was separately concentrated in *vacuo* to dryness to yield 2.1 g extract. The filtrate fraction was neglected.

7.19.3 Isolation and identification of metabolites

The reddish-orange biomass fraction (2.1 g) was subjected to a fractionation using middle pressure column chromatography on flash silica gel (30×600 mm), with a CH_2Cl_2-MeOH-gradient. As a result, it was divided into two fractions, with the aid of TLC monitoring (CH_2Cl_2/5%MeOH). Purification of the main fraction II (1.11 g) using PTLC ($CHCl_3$/5%MeOH), and then Sephadex LH-20 ($CHCl_3$/MeOH 3:2) led to isolation of menaquinone MK-9 (II, III-H4) (**237**) as pale yellow oil (23 mg).

Menaquinone MK-9 (II, III-H4) (237): $C_{56}H_{84}O_2$ (789.29), pale yellow UV absorbing oil (23 mg), brown by anisaldehyde/sulphuric acid after heating. – $\boldsymbol{R}_f$ = 0.41 (C_6H_{12}/10%EtOAc). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.08 (m, 2 H, 5,8-H), 7.68 (m, 2 H, 6,7-H), 5.17-5.07 (m, 7 H, CH =CCH_3), 3.38 (d, ^3J = 7.2 Hz, 2 H, 1'-CH_2), 2.19 (s, 3 H, 9-CH_3), 2.13-1.88 (m, 24 H, CH_2CH_2), 1.78 (s, 3 H, CH_3), 1.67 (s, 3 H, CH_3), 1.61 (s br, 18 H, 6 CH=CH_3), 1.42-1.21 (m, 14 H), 0.91-0.82 (m, 6 H, 2 CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 75 MHz): δ = 185.3 (CO, C_q), 184.4 (CO, C_q), 146.1 (C_q), 143.3 (C_q), 137.8 (C_q), 134.8 (4C_q), 134.5 (C_q), 133.2 (CH), 133.2 (CH), 132.1 (C_q), 132.1 (C_q), 131.1 (C_q), 126.2 (CH), 126.1 (CH), 124.9 (CH), 124.4 (CH), 124.2 (4 CH), 118.8 (CH), 40.0 (CH_2), 39.7 (3 CH_2), 37.3 (CH_2), 37.2 (CH_2), 37.1 (CH_2), 36.6 (CH_2), 34.3 (CH), 32.6 (CH), 32.4 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 26.7 (CH_2), 26.6 (CH_2), 25.9 (CH_2), 25.6 (CH_2), 25.4 (CH_3), 25.2 (CH_3), 24.9 (CH_2), 19.7 (CH_3), 19.6 (CH_3), 17.6 (CH_3), 16.3 (CH_3), 16.0 (3 CH_3), 15.9 (CH_3), 12.6 (CH_3). – **EI MS** (70 eV): *m/z* (%) = 788.9 ($[M]^{+\cdot}$, 6), 648

(4), 606 (8), 225 ([fragment **234**]$^{+\cdot}$, 22), 69 ([M-C_5H_9]$^{+\cdot}$, 100). – **CI MS**: *m/z* (%) = 807 ([M+NH_4]$^+$, 100), 624.9 (9), 347 (13), 333 (36), 302 (27) .

7.20 Marine *Streptomyces* sp. B8904

The marine *Streptomyces* strain B8904 exhibited a dark red colouration on the agar plate with white aerial mycelium, when cultivated on M_2^+ medium at 28 °C for 4 days.

7.20.1 Pre-screening

The strain was fermented at 28 °C for 7 days in M_2^+ medium with 50% synthetic sea water as shaker culture (95 rpm). Extraction of the culture broth (1 liter) with ethyl acetate afforded 37 mg of dark red crude extract. TLC of the extract exhibited numerous coloured zones: a pink one turned to yellow with 2 N NaOH, numerous bands were orange-red at different R_f values (0.21~0.94; $CHCl_3$/10MeOH). The treatment of these bands with dilute sodium hydroxide solution, gave a blue~violet colouration. As shown in Table 34 the crude extract had biological activity against all of our test organisms.

Table 34: Antimicrobial activity of the crude extract produced by the strain B 8904

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	19
Escherichia coli	21
Staphylococcus aureus	19
Streptomyces viridochromogenes (Tü57)	26
Mucor miehei (Tü284)	15
Chlorella vulgaris	11
Chlorella sorokiniana	14

7.20.2 Fermentation and isolation of metabolites (a: fermenter)

12 of 1 liter Erlenmeyer flasks, each containing 250 ml of M_2^+ medium were adjusted to pH 7.8 before sterilisation. The media were inoculated with small pieces of well grown agar plats and cultivated for 7 days at 28 °C on rotary shaker (95 rpm). The obtained broth (2.7 liters, dark red), were inseeded into 23-liter jar fermenter at 28 °C and cultivated for additional 5 days. After harvesting, celite (~ 1 kg) was added to the culture broth, and the mixture was homogenized by shaking for 1~3 min. By filtration using a filter press, the liquid phase and mycelial cake were separated, and extracted with ethyl acetate repeatedly. The obtained organic phases were

evaporated in *vaccuo* separately, giving 3.21 g (pink-red) of mycelial cake, and 10.61g (dark red) of filtrate.

On the basis of TLC analysis, the two extracts exhibited similar components, and were combined. The extract was applied to flash silica gel column chromatography (30 × 1000 mm) using a $CHCl_3$/MeOH gradient. As a result, five fractions were obtained after monitoring with TLC.

Linoleic acid; (9Z,12Z)-9, 12-octadecanoic acid (239): $C_{18}H_{32}O_2$ (280.45), was delivered from fractions I and II by applying to PTLC ($CHCl_3$/5%MeOH), and then Sephadex LH-20 ($CHCl_3$/MeOH 3:2), as an UV absorbing colourless oil (45 mg), which turned to blue by anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R}_f$ = 0.90 ($CHCl_3$/MeOH, 10%). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.98 (s, br, 1 H, COOH), 5.43-5.28 (m, 4 H, 9,10,12,13-CH), 2.78 (t, ^3J = 6.0 Hz, 2 H, 11-CH_2), 2.38 (t, ^3J = 7.2 Hz, 2 H, 2-CH_2), 2.08 (m, 4 H, 8,14-CH_2), 1.63 (m, 2 H, 3-CH_2), 1.42-1.23 (m, 14 H, 4,5,6,7,16,17-CH_2), 0.85 (m, 3 H, 18-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 50 MHz): δ = 180.1 (CO, C_q), 130.1 (CH-13), 129.9 (CH-9), 128.0 (CH-10), 127.8 (CH-12), 31.5 (CH_2-2), 29.6 (CH_2-16), 29.6 (CH_2-11), 29.5 (CH_2-14), 29.3 (CH_2-8), 29.1 (CH_2-7), 29.0 (CH_2-6), 29.0 (CH_2-5), 27.1 (CH_2-4), 25.6 (CH_2-3), 24.7 (CH_2-15), 22.5 (CH_2-17) 14.0 (CH_2-18). – **EI MS** (70 eV): *m/z* (%) = 280.4 (80), 264 (28), 137 (10), 124 (15), 110 (28), 95 (60), 81 (84), 67 (100), 55 (92), 41 (92).

Adenosine (242): $C_{10}H_{13}N_5O_4$ (267.24), was afforded as a colourless solid (13 mg) from fraction V by PTLC ($CHCl_3$/13%MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 3:2). It showed an UV absorbance, and was coloured to greenish-blue by treatment with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R}_f$ = 0.12 ($CHCl_3$/ 10 % MeOH). – **^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 8.34 (s, 1 H, 2-H), 8.12 (s, 1 H, 6-H), 7.28 (s, 2 H, NH_2), 5.87 (d, ^3J = 6.1 Hz, 1 H, 1' -H), 5.50-5.20 (s, br, 2 H, 2 OH), 4.59 (t, ^3J = 7.1 Hz, 1 H, 2'-H), 4.17 (m, 1 H, 3'-H), 3.95 (m, 1 H, 4'-H), 3.65, 3.55 (ABX, ^3J = 12.0, 4.1 Hz, 1 H, 5'$_a$,5'$_b$-H). – **EI MS** (70 eV): *m/z* (%) = 267 ([M]$^{+\cdot}$, 8), 237 (12), 178 (36), 164 (86), 136 (64), 135 (100), 108 (24).

7.20.3 Fermentation and isolation of metabolites (shaker culture)

Well-grown agar sub-cultures of the marine *Streptomyces* B8904 served to inoculate 100 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2^+ medium. They were kept on a linear shaker (110 rpm) for 7 days at 28 °C. The obtained red

broth was harvested and mixed with diatomaceous earth (celite, ca. 1 kg), and filtered with the aid of a filter press. The mycelial cake was extracted with ethyl acetate, and acetone. After evaporation of the acetone, the water residue was extracted again by ethyl acetate. Also the filtrate was repeatedly extracted with ethyl acetate. The EtOAc layers were combined and evaporated to dryness under reduced pressure at 40 °C, yielding 3.20 g of a dark red extract. The extract was then chromatographed on flash silica gel (30×600 mm), and eluted with a $CHCl_3$-MeOH-gradient. With the aid of TLC monitoring, five fractions were collected. Purification of the orange-red metabolites present in the fractions II~IV, led to the compounds discussed below.

ζ-Pyrromycinone (243): $C_{22}H_{20}O_8$ (412.4). An orange-red solid (100 mg) was isolated from fraction II (0.812 g) by PTLC ($CHCl_3$/4% MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 3:2), from a low polar orange band which turned to violet with NaOH. – $\boldsymbol{R}_f$ = 0.57 $CHCl_3$/5% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 13.02 (s, 1 H, OH), 12.61 (s, 1 H, OH), 12.28 (s, 1 H, OH), 7.69 (s, 1 H, 11-H), 7.29, 7.25 (AB, ^3J = 9.4 Hz, 2 H, 2,3-H), 3.96 (s, 1 H, 10-H), 3.74 (s, 3 H, 15-OCH_3), 3.07, 2.86 (ABX$_2$, J_{AB} = 14.1 J_{AX} = 2.2 Hz, J_{AX} = 2.4 Hz, 2 H, 7_a,7_b-H), 2.33 (m, 1 H, 8-H_A), 1.95 (m, 1 H, 8-H_B), 1.72, 1.62 (m, 2 H, 12-CH_2), 1.09 (t, ^3J = 7.6 Hz, 3 H, 13-CH_3). **- (-)-ESI MS)**: *m/z* (%) = 845 ([2 M+Na-2H]$^-$, 100), 411 ([M-H]$^-$, 76). – **CI MS** (NH_3): *m/z* (%) = 430 ([M+NH_4]$^+$).

η-Pyrromycinone (244): $C_{22}H_{16}O_7$ (392.37) was obtained from fraction II (0.812 g) by PTLC ($CHCl_3$/4% MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 3:2), as low polar reddish-orange, on TLC under UV orange fluorescent solid (25 mg), which turned to violet with NaOH. – $\boldsymbol{R}_f$ = 0.65 $CHCl_3$/5% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 13.84 (s, 1 H, OH), 13.11 (s, 1 H, OH), 12.32 (s, 1 H, OH), 8.53 (d, ^3J = 8.6 Hz, 7-H), 8.27 (s, 1 H, 11-H), 7.61 (d, ^3J = 8.6 Hz, 8-H), 7.29 (s, 2 H, 2,3-H), 4.13 (s, 3 H, 15-OCH_3), 2.85 (q, ^3J = 5.3 Hz, 2 H, 13-CH_2), 1.35 (t, ^3J = 5.3 Hz, 3 H, 14-CH_3). – **EI MS** (70 eV): *m/z* (%) = 392 ([M]$^{+\cdot}$), 361 ([M-OCH_3]$^+$, 18).

Musettamycin (246): $C_{36}H_{45}NO_{14}$ (715.75), was purified from fraction III (0.51 g) by PTLC ($CHCl_3$/10%MeOH) and Sephadex LH-20 (2 × MeOH), and obtained as red powder (7 mg), as a middle polar substance with an orange UV fluorescence, turned to violet by treatment with dilute sodium hydroxide. – $\boldsymbol{R}_f$ = 0.65 ($CHCl_3$/10% MeOH). – **^{1}H NMR (**$CDCl_3$, 499 MHz): δ = 12.93 (s, br, 1 H, OH), 12.80 (s, br, 1 H,

OH), 12.22 (s, br, 1 H, OH), 7.69 (s, 1 H, 11-H), 7.30, 7.27 (AB, ^3J = 9.4 Hz 2 H, 2,3-H), 5.51 (d, ^3J = 2.4 Hz, 1 H, 1'-H), 5.25 (d, ^3J = 2.2 Hz 1 H, 7-H), 5.07 (d, ^3J = 2.3 Hz, 1 H, 1"-H), 4.46 (q, ^3J = 5.4 Hz, 1 H, 3"-H), 4.13 (s, 1 H, 10-H), 3.99 (m, 1 H, 5"-H,), 3.79 (s, 1 H, OH), 3.72-3.63 (m, 1 H, 4'-H), 3.68 (s, 3 H, OCH_3), 3.62 (s, b, 1 H, OH), 2.51 (dd, J = 14.2, 2.2 Hz, 1 H, 8-H_{eq}), 2.35-2.17 (m+s, 7 H, 8-H_{ax}, NMe_2), 2.09-1.94 (m, 2 H, 2"-H_2), 1.88-1.78 (m, 2 H, 2'-CH_2), 1.72 (m, 1 H, 13-H_A), 1.51 (m, 1 H, 13-H_B), 1.27 (d, ^3J = 7.3 Hz, 3 H, 6"-CH_3), 1.18 (d, ^3J = 7.3 Hz, 3 H, 6'-CH_3), 1.08 (t, ^3J = 7.4 Hz, 3 H, 14-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 190.6 (CO, C_q-5), 185.8 (CO, C_q-12), 171.3 (CO, C_q-15), 162.2 (C_q-6), 158.4 (C_q-4), 157.8 (C_q-1), 142.4 (C_q-10a), 132.8 (C_q-11a), 131.3 (C_q-6a), 130.1 (CH-3), 129.7 (CH-2), 120.4 (CH-11), 114.8 (C_q-5a), 112.5 (C_q-12a), 112.3 (C_q-4a), 101.3 (CH-1'), 99.0 (CH-1"), 73.8 (CH-4'), 71.7 (C_q-9), 71.4 (CH-5'), 70.7 (CH-4"), 68.2 (CH-3"), 66.3 (CH-5"), 65.7 (CH-7), 61.5 (CH-3'), 57.0 (CH-10), 52.5 (15-OCH_3), 42.8 (3'-$N(CH_3)_2$), 33.8 (CH_2-8), 32.7 (CH_2-13), 32.1 (CH_2-2'), 28.8 (CH_2-2"), 18.0 (CH_3-6'), 16.7 (CH_3-6"), 6.7 (CH_3-14). – **(+)-ESI MS**: *m/z* (%) = 716 ($[M + 1]^+$). – **(+)-ESI MS^2**: *m/z* (%) = 716.1 ($[M + H]^+$, 12), 586 (M - (2-Deoxyfucoe + H)]$^+$, 100), 393.1 ([M - (2-deoxyfucose + rhodosamin + H)]$^+$, 5). – **(+)-ESI MS^3**: *m/z* (%) = 586.0 (M - (2-deoxyfucoe + H)]$^+$, 28), 392.9 ([M - (2-deoxyfucose + rhodosamin + H)]$^+$, 100 %).

Cinerubin B (248): $C_{42}H_{51}NO_{16}$ (825.87), red amorphous (30 mg), yielded from fraction III (0.51g) with the aide of PTLC ($CHCl_3$/10MeOH), and Sephadex LH-20 (MeOH), middle polar red substance with an orange UV fluorescence, turned to blue-violet with 2 N NaOH. – R_f = 0.60 ($CHCl_3$/10% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.97 (s, br, 1 H, OH), 12.80 (s, br, 1 H, OH), 12.21 (s, br, 1 H, OH), 7.73 (s, 1 H, 11-H), 7.32, 7.28 (AB, ^3J = 9.4 Hz, 2 H, 2,3-H), 5.51 (d, ^3J = 2.4 Hz, 1'-H), 5.28 (d, ^3J = 2.3 Hz, 1 H, 7-H), 5.20 (d, ^3J = 2.4 Hz, 1 H, 1"-H), 5.17 (d, ^3J = 2.1 Hz, 1'"-H), 4.81 (q, ^3J = 6.4 Hz, 1 H, 5'- H), 4.68 (q, ^3J = 5.2 Hz, 1 H, 5"-H), 4.38 (m, 3 H, 3',4",5'"-H), 4.13 (s, 1 H, 10-H), 4.04 (m, 2 H, 5",2'"-H), 3.83 (s, 1 H, 4'-H), 3.72 (s, 15-OCH_3), 2.60 (d, ^3J = 3.4 Hz, 2 H, 3'"-CH_2), 2.48 (m, 2 H, 8-H_{eq}, 2'-H_{eq}), 2.38-2.17(m+s, 7 H, 8-H_{ax}, NMe_2), 1.95 (m, 3 H, 2"-CH_2, 2'-H_{eq}), 1.75 (m, 1H, 13-H_A), 1.53 (m, 1 H, 13-H_B), 1.37 (d, ^3J = 7.2, 3 H, 6'"-CH_3), 1.30 (d, ^3J = 7.2 Hz, 3 H, 6'-CH_3), 1.23 (d, ^3J = 7.3 Hz, 3 H, 6"-H_3), 1.09 (t, ^3J = 7.4 Hz, 3 H, 14-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 208.3 (CO, C_q-4'"), 190.5 (CO, C_q-5), 185.6

(CO, C_q-12), 171.3 (CO, C_q-15), 162.2 (C_q-6), 158.3 (C_q-4), 157.7 (C_q-1), 142.3 (C_q-10a), 132.7 (C_q-11a), 131.4 (C_q-6), 130.0 (CH-3), 129.7 (CH-2), 120.3 (CH-11), 114.7 (C_q-5a), 112.4 (C_q-12a), 112.2 (C_q-4a), 101.5 (CH-1'), 99.0 (CH-1''), 91.5 (CH-1'''), 77.9 (CH-5'''), 74.0 (CH-4'), 71.6 (C_q-9), 70.6 (CH-5'), 68.2 (CH-4''), 67.2 (CH-3''), 66.8 (CH-5''), 65.2 (CH-7), 62.9 (CH-2'''), 61.5 (CH-3'), 57.1 (CH-10), 52.5 (15-OCH_3), 43.2 (3'-$N(CH_3)_2$), 39.7 (CH_2-3'''), 33.7 (CH_2-8), 32.1 (CH_2-13), 29.2 (CH_2-2'), 26.9 (CH_2-2''), 17.8 (CH_3-6'), 16.1 (CH_3-6'''), 16.0 (CH_3-6''), 6.7 (CH_3-14). – **(+)-ESI MS**: *m/z* (%) = 1650.9 ($[2M+H]^+$, 1), 826 ($[M+1]^+$, 100), 586 (M-[Cinerulose B+2-Deoxyfucoe)+H]$^+$, 36), 393 ([M-(Cinerulose B+2-Deoxyfucose+Rhodosamin)+H]$^+$, 6). – **(-)-ESI MS**: *m/z* (%) = 1671 ($[2M+Na-2H]^+$, 100), 824 ($[M-H]^+$, 48).

Cinerubin M (254): $C_{41}H_{49}NO_{16}$ (811.84), red powder (2.5 mg), was afforded from fraction III (0.51g) using PTLC ($CHCl_3$/10 MeOH), followed by Sephadex LH-20 (MeOH), turned to blue-violet when exposed to 2 N NaOH. – $\boldsymbol{R}_f$ = 0.57 ($CHCl_3$/10% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.94 (s br, 1 H, OH), 12.80 (s br, 1 H, OH), 12.22 (s br, 1 H, OH), 7.71 (s, 1 H, 11-H), 7.33, 7.30 (AB, ^3J = 9.4 Hz, 2 H, 2,3-H), 5.45 (d, ^3J = 2.4 Hz, 1'-H), 5.24 (d, ^3J = 2.3 Hz, 1 H, 7-H), 5.18 (d, ^3J = 2.1 Hz, 1''-H), 5.08 (d, ^3J = 1.2 Hz, 1 H, 1'''-H), 4.76 (q, ^3J = 6.4 Hz, 1 H, 5'- H), 4.38-4.26 (m, 4 H, 3',3'',4'',5'''-H), 4.13 (s, 1 H, 10-H), 4.08 (q, ^3J = 6.4 Hz, 1 H, 5''-H), 4.03 (d, br, *J* = 1.2 Hz, 1 H, 2'''-H), 3.72 (m, br, 1 H, 4'-H), 3.69 (s, 3 H, 15-OCH_3), 2.58 (d, ^3J = 3.4 Hz, 2 H, 3'''-CH_2), 2.48 (m, 2 H, 8, 2'-H_{eq}), 2.36 (s, 3 H, NHMe), 2.29 (m, 1 H, 8-H_{ax}), 2.09 (br, m, 2 H, 2''-CH_2), 1.92 (dd, *J* = 10.1, 5.2 Hz, 1 H, 2'-H_{ax}), 1.75 (m, 1H, 13-H_A), 1.48 (m, 1 H, 13-H_B), 1.37 (d, ^3J = 7.2, 3 H, 6'''-H_3), 1.29 (d, ^3J = 7.2 Hz, 3 H, 6'-CH_3), 1.27 (d, ^3J = 7.3 Hz, 3 H, 6''-CH_3), 1.08 (t, ^3J = 7.4 Hz, 3 H, 14-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 171.3 (CO, C_q-15), 162.2 (C_q-6), 158.4 (C_q-4), 157.9 (C_q-1), 142.3 (C_q-10a), 132.9 (C_q-11a), 130.1 (CH-3), 129.7 (CH-2), 120.4 (CH-11), 114.9 (C_q-5a), 112.4 (C_q-4a, 12a), 100.5 (CH-1'), 91.4 (CH-1'''), 78.0 (CH-5'''), 71.6 (C_q-9), 68.2 (CH-4''), 67.9 (CH-3''), 66.7 (CH-5''), 66.5 (CH-7), 63.0 (CH-2''), 57.2 (CH-10), 54.7 (CH-3'), 52.6 (15-OCH_3), 40.8 (3'-$NHCH_3$), 39.6 (CH_2-3'''), 32.6 (CH_2-8), 32.1 (CH_2-13), 29.7 (CH_2-2'), 27.0 (CH_2-2''), 18.1 (CH_3-6'), 16.2 (CH_3-6'''), 16.2 (CH_3-6''), 6.7 (CH_3-14). – **(+)-ESI MS**: *m/z* (%) = 1624 ($[2M+2H]^+$, 18), 812 ($[M + H]^+$, 100). – **(+)-ESI MS^2**: *m/z* (%) = 571 ([M-cinerulose + 2-deoxyfucose) + H]$^+$), 393 ([M-cinerulose + 2-deoxyfucose + N-

methyl-daunosamine + 2 H_2O) + H]$^+$). – **(-)-ESI MS**: *m/z* (%) = 1644 ([2 M + Na - H]$^-$, 64), 810 ([M - H]$^-$, 100). – **(+)-ESIHR MS**: *m/z* = 812.31241 (M + H) (calcd. 812.3115 for $C_{41}H_{50}NO_{16}$); 572.21264 (572.2126 for $C_{29}H_{34}NO_{11}$; [M-(cinerulose + 2-deoxyfucose) + 2 H]$^+$)

Islamomycin A (257): $C_{41}H_{47}NO_{18}$ (841.83), was delivered from fraction IV (0.31 g) by applying to PTLC ($CHCl_3$/13% MeOH) and Sephadex LH-20 (MeOH), as a dark red powder (10 mg), which showed a colour changing to violet-blue by treatment with NaOH. – $\boldsymbol{R}_f$ = 0.23 ($CHCl_3$/10% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.90 (s br, 2 H, OH), 12.22 (s br, 1 H, OH), 7.66 (s, 1 H, 11-H), 7.25, 7.23 (AB, ^3J = 9.4 Hz, 2 H, 2,3-H), 5.63 (s br, 1' -H), 5.42 (s, br, 1"-H), 5.22 (q, ^3J = 6.3 Hz, 1 H, 3'-H), 5.19 (d, ^3J = 2.2 Hz, 1 H, 1'"-H), 5.12 (d, ^3J = 2.2 Hz , 1 H, 7-H), 5.08 (s br, 1H, OH), 4.72 (q, ^3J = 6.4 Hz, 1 H, 5'"-H), 4.42 (s, 1 H, 4'-H), 4.34 (m, 1 H, 2'"-H), 4.30-4.19 (m, br, 3 H, 5',3",4"-H), 4.08 (s, 1 H, 10-H), 4.02 (s, br, 1H, 5"-H), 3.68 (s, 3 H, 15-OCH_3), 2.58 (m, 2 H, 3'"-CH_2), 2.46-2.40 (m, 2 H, 2"-H_{eq}, 2'-CH_2), 2.28 (m, 1 H, 8-CH_2), 1.94 (m, 1 H, 2"-H_{ax},), 1.72 (m, 1 H, 13-H_A), 1.56 (m, 1H, 13-H_B), 1.37 (d, ^3J = 7.2, 3 H, 6'"-CH_3), 1.27 (d, ^3J = 7.2 Hz, 3 H, 6'-CH_3), 1.21 (d, ^3J = 7.3 Hz, 3 H, 6"-CH_3), 1.10 (t, ^3J = 7.4 Hz, 3 H, 14-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 207.8 (CO, C_q-4'"), 190.5 (CO, C_q-5), 185.6 (CO, C_q-12), 171.2 (CO, C_q-15), 169.1 (CO, C_q-7'), 162.3 (C_q-6), 158.4 (C_q-4), 157.8 (C_q-1), 142.9 (C_q-10a), 132.5 (C_q-11a), 131.5 (C_q-6), 130.2 (CH-3), 129.7 (CH-2), 120.4 (CH-11), 114.6 (C_q-5a), 112.4 (C_q-12a), 112.2 (C_q-4a), 100.0 (CH-1'), 99.1 (CH-1"), 91.3 (CH-1'"), 77.9 (CH-5'"), 72.8 (CH-4'), 70.8 (C_q-9), 70.4 (CH-7), 69.2 (CH-5'), 67.6 (CH-3'), 66.6 (CH-3",4"), 66.1 (CH-5"), 63.1 (CH-2'"), 56.3 (CH-10), 52.5 (15-OCH_3), 40.7 (CH_2-2'), 39.6 (CH_2-3'"), 32.4 (CH_2-8), 31.9 (CH_2-13), 26.5 (CH_2-2"), 18.3 (CH_3-6'), 16.2 (CH_3-6'"), 16.0 (CH_3-6"), 6.7 (CH_3, C-14). – **(+)-ESI MS**: *m/z* (%) =1683 ([2M + H]$^+$, 100), 842 ([M + H]$^+$, 50). – **(+)-ESI MS^2**: *m/z* (%) = 842 (M + H], 100), 798 ([M- $CONH_2$]$^+$, 2), 602 (M-[cinerulose B + 2-deoxyfucoe] + H]$^+$, 45), 393 ([M- (cinerulose B + 2-deoxyfucose)-fragment]-rhodosamine + H]$^+$, 94). – **(-)-ESI MS**: *m/z* (%) = 840 (M-H]$^-$, 50). – **(+)-ESI HRMS**: *m/z* = 842.2871 (M + H) (calcd. 842.2871 for $C_{41}H_{48}NO_{18}$).

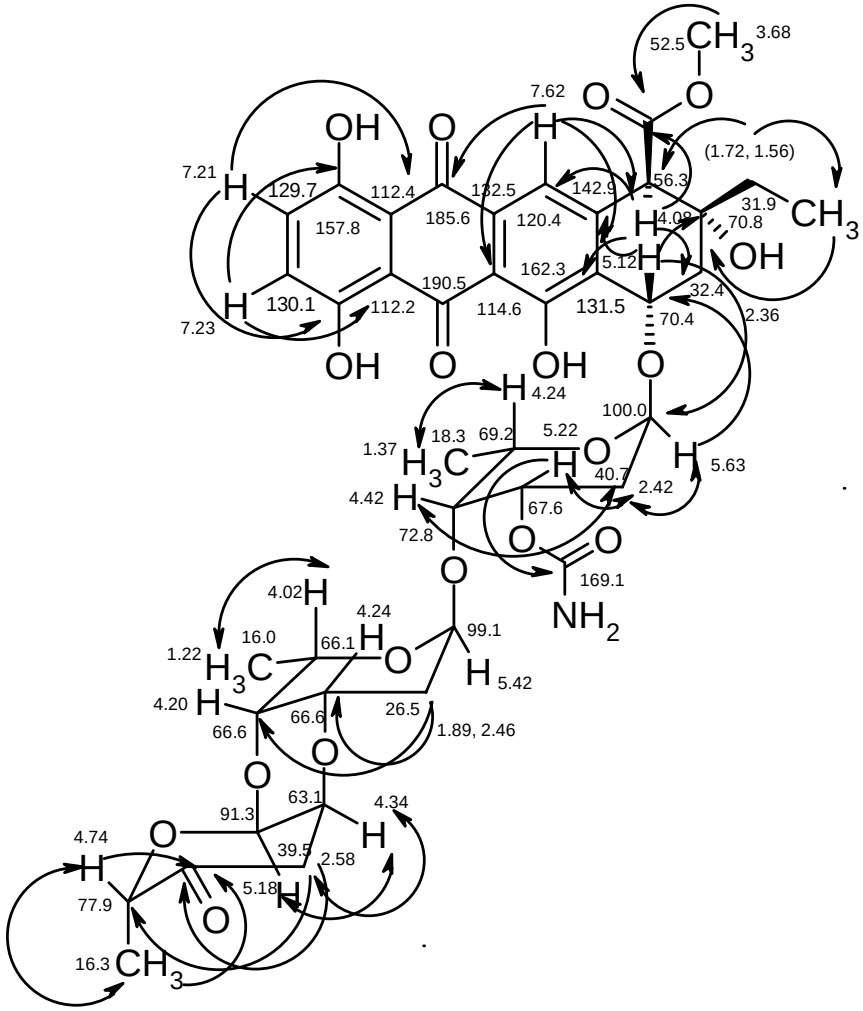


Figure 175: H,H COSY and HMBC correlations of islamomycin A (**257**).

Islamomycin B (258): $C_{41}H_{49}NO_{19}$ (859.84), purification of the fraction IV (0.31 g), using PTLC ($CHCl_3$/13%MeOH) and Sephadex LH-20 (MeOH), led to 2.1 mg of a dark red powder, turned to violet-blue by treatment with NaOH. – $\boldsymbol{R}_f$ = 0.20 ($CHCl_3$/10%MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.97 (s br, 1 H, OH), 12.83 (s br, 1 H, OH), 12.22 (s br, 1 H, OH), 7.73 (s, 1 H, 11-H), 7.30, 7.28 (AB, ^3J = 9.4 Hz 2 H, 2,3-H), 5.63 (d, ^3J = 1.2 Hz, 1 H, 1' -H), 5.44 (d, ^3J = 3.2 Hz, 1 H, 1''-H), 5.28 (m, 1 H, 1'''-H), 5.18 (d, ^3J = 3.1 Hz, 1 H, 7-H), 4.84 (t, ^3J = 4.1 Hz 1 H, 5'-H), 4.50 (s, 1 H, 3''-H), 4.43 (s, 1 H, 4''-H), 4.30 (t, ^3J = 4.8 Hz, 1 H, 5'''-H), 4.15 (m, 1H, 2'''-H), 4.08 (s, 1 H, 10-H), 3.78 (s, 1 H, 4'-H), 3.70 (s, 3 H, 15-OCH_3), 2.60-2.44 (m, 3 H, 8-H_{eq}, 2''-H_{eq}, 3'''-H_{eq}), 2.41 (t, ^3J = 5.1 Hz, 2 H, 2'-CH_2), 2.04-1.96 (m, 2 H, 8-,3'''-H_{ax}), 1.89 (m, 1 H, 2''-H_{ax},), 1.72 (m, 1 H, 13-H_A), 1.52 (m, 1H, 13-H_B), 1.36 (d, ^3J = 7.2, 3 H, 6'''-CH_3), 1.26 (d, ^3J = 7.2 Hz, 3 H, 6' -CH_3), 1.17 (d, ^3J = 7.3 Hz, 3 H, 6''-CH_3), 1.08 (t, ^3J = 7.4 Hz, 3 H, 14-CH_3) – **(+)-ESI MS**: *m/z* (%) = 860 ([M +

$H]^+$). **- (+)-ESI MS^2:** *m/z* (%) = 860 ($[M + H]^+$, 17), 602 (M-(cinerulose B + 2-deoxyfucoe) + $H]^+$, 100). **– (+)-ESIHR MS**: *m/z* = 860.33354 (M + H) (calcd. 860.3335 for $C_{41}H_{50}NO_{19}$).

7.21 Terrestrial *Streptomyces* sp. GW10/1828

The strain GW10/1828 was inoculated on M_2 medium agar plates, and incubated for 3 days at 28 °C. The colour of the aerial mycelium was white, while the agar was stained to black.

7.21.1 Pre-screening

The fermentation process was carried out in 4 of 1liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium at 28 °C for 3 days, with vigorous shaking at 95 rpm. The culture broth (1litre) was extracted with ethyl acetate, to afford 257 mg of greenish-black crude extract.

Chemical screening (TLC, $CHCl_3$/5-10% MeOH) of the crude extract showed a number of middle polar yellowish-green and orange coloured bands. These bands turned to red by treatment with sodium hydroxide. Additional UV absorbing bands changed to pink, violet and brown by anisaldehyde/sulphuric acid after heating. The crude extract had high biological activity against several of our test organisms, as shown in Table 35

Table 35: Antimicrobial activity of the crude extract produced by the strain GW10/1828

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	26
Escherichia coli	20
Staphylococcus aureus	17
Streptomyces viridochromogenes (Tü57)	20
Mucor miehei (Tü284)	24
Chlorella vulgaris	14

7.21.2 Fermentation and working up

7.21.3 Fermentation and isolation of metabolites (shaker)

A shaker culture was carried out in 96 of 1 liter-Erlenmeyer flasks, each containing 250 ml of nutrient medium M_2. The flasks were incubated with small species of well-grown agar plates of the terrestrial *Streptomyces* sp. GW10/1828. The inocu-

lated flasks were incubated for 7 days at 28 °C on a linear shaker (110 rpm). The culture broth (black, 25-liter) was mixed with *ca*. 1 kg celite, and filtered through a press filter, to separate the mycelium and the aqueous phase. The mycelium was extracted with acetone. After evaporation, the resultant aqueous solution was extracted with ethyl acetate and concentrated *in vacuo*. The aqueous phase was then extracted repeatedly with ethyl acetate, and the organic layer was evaporated to dryness. The residual solids from both organic phases were combined to yield 2.51 g of crude greenish-black extract.

The extract was dissolved in methanol (150 ml) and defatted with cyclohexane (3 × 100 ml). The two phases, methanol (1.81 g) and cyclohexane (0.7 g) were worked up separately. The methanol extract was chromatographed on flash silica gel column (30 × 600 mm) and eluted with a CH_2Cl_2-MeOH-gradient. On the basis of TLC monitoring, five fractions were yielded. Also the cyclohexane extract was subjected to a flash silica gel column and eluted by CH_2Cl_2. The obtained main fraction II (0.21 g), containing an UV absorbing low polar component (brown by anisaldehyde/sulphuric acid and heating), was purified by Sephadex LH-20 (DCM/MeOH 3:2), giving a colourless solid of furan-2,4-dicarboxylic acid dimethyl ester (**274**). The main fraction II (0.312 g) from the methanolic extract contained several greenish-yellow bands, and was further purified by PTLC ($CHCl_3$/6%MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 6:4), affording 1.4 mg of the yellowish-green 2,9-dichloro-1,6,10-trihydroxy-7-methyl-naphthacene-5,12-dion (**261**).

Furan-2,4-dicarboxylic acid dimethyl ester (261): $C_8H_8O_5$ (184.14), colourless UV absorbing solid (5 mg), which turned to brown with anisaldehyde/sulphuric acid. – R_f = 0.90 ($CHCl_3$/ 5%MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.12 (s, 1 H, 5-H), 7.44 (s, 1 H, 3-H), 3.94 (s, 3 H, 7-OCH_3), 3.86 (s, 3 H, 9-OCH_3). – **^{13}C/APT NMR (**$CDCl_3$, 125 MHz): δ = 162.4 (C_q-8), 158.5 (C_q-6), 150.1 (CH-5), 145.5 (C_q-2), 121.0 (C_q-4), 117.0 (CH-3), 52.3 (CH, 7-OCH_3), 52.0 (CH, 9-OCH_3). – **EI MS** (70 eV): *m/z* (%) = 184.1 ($[M]^{+\cdot}$, 53), 153.0 ($[M\text{-}OCH_3]^{+\cdot}$, 100), 149.0 (5), 121.0 ($[M\text{-}(2OCH_3 + H)]^{+\cdot}$, 15), 93.0 ($[M\text{-}(2OCH_3 + CHO)]^{+\cdot}$, 8).

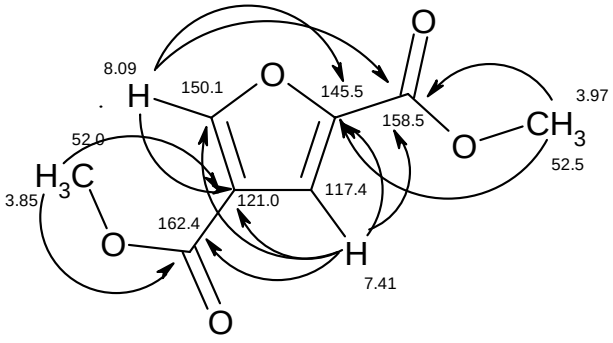


Figure 176: HMBC-correlations of furan-2,4-dicarboxylic acid methyl ester (**261**)

2,9-Dichloro-1,6,10-trihydroxy-7-methyl-naphthacene-5,12-dione (269): $C_{19}H_{10}Cl_2O_5$ (389.2), Greenish-yellow solid (1.4 mg), turned to red with 2 N NaOH. – R_f = 0.69 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**$CDCl_3$, 500 MHz): δ = 12.21 (s br, 1 H, OH), 11.94 (s, 1 H, OH), 9.86 (s br, 1 H, OH), 8.35 (s, 1 H, 12-H), 7.60 (AB, ^3J = 8.3 Hz, 2 H, 7,8-H), 6.98 (s, 1 H, 3-H), 2.52 (s, 3 H, 4-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 150 MHz): δ = 141.0 (C_q-1), 137.7 (CH-8), 121.7 (CH-3), 121.6 (CH-7), 119.4 (CH-12), 22.7 (CH_3). – **(+)-ESI MS**: *m/z* (%) = 803 ($[2M + Na]^+$, 100) 413 ($[M + Na + H]^{+,}$ 46). – **(-) ESI MS**: *m/z* (%) = 799 ($[2M + Na - H]^-$, 38), 388 ($[M-H]^-$, 100). - **EI MS** (70 eV): *m/z* (%) = 388 ($[M]^{+\cdot}$, 100), 354 ($[M - Cl]^{+\cdot}$, 43), 325 ($[M- (CO + Cl)]^{+\cdot}$, 24), 297 ($[M- (2CO + Cl)]^{+\cdot}$, 7). – **HREI MS**: *m/z* = 387.9900 (calcd. 387.9905 for $C_{19}H_{10}Cl_2O_5$).

7.21.4 Optimisation of strain GW10/1828

The strain was optimized, using the conditions outlined below (Table 36), to get optimal yields.

Table 36: Optimization of the terrestrial *Streptomyces* sp. GW10/1828

Medium	PH	Ingredients	Temp.	Cult. Period/day
Bacto-peptone-Medium	8.0	5g/l Bacto-peptone, 1g/l yeast extract	28 °C	5
Bennett's-medium	7.8	10g/l glucose, 2g/l peptone, 1g/l yeast extract	28 °C	5
Bennett's-medium	6.0	10g/l glucose, 2g/l peptone, 1g/l yeast extract	28 °C	5
M_1- medium	8.0	5g/l Peptone, 1g/L yeast extract, 1g/l KBr	28 °C	5
M_2- medium	6.0	10g/l malt extract, 4g/l yeast, 4g/l glucose,	28 °C	5
M_2- medium	7.8	10g/l malt extract, 4g/l yeast, 4g/l glucose,	28 °C	5

Bennett's and M_1 medium yielded only uninteresting metabolites. Bacto-peptone and M_2 medium exhibited similar results, but M_2 medium was more affluent with the desired metabolites. Therefore the strain was cultivated on a big scale, a) as 25-liter

shaker culture Bacto-peptone medium, and b) in a 50-liter jar fermenter using M_2 medium.

7.21.5 Fermentation and isolation (Bacto-peptone medium, shaker)

The strain was cultivated on 25-liter Bacto-peptone medium for 5 days at 28 °C, as linear shaking culture (110 rpm). The greenish-black broth was filtered on celite with the aid of a filter press. Both biomass and water phase were extracted with ethyl acetate separately. The biomass extract (0.512 g) was ignored because of its insufficient composition. TLC of the water extract (2.11 g) showed two blue bands after spraying with anisaldehyde/sulphuric acid and heating. The extract was dissolved in methanol (100 ml) and defatted by cyclohexane (2 × 75 ml). The methanolic extract was chromatographed on silica gel, followed by PTLC (DCM/7%MeOH) and Sephadex LH-20 (MeOH). As a result, two colourless solid compounds were obtained: Cholic acid (**273**) and chenodeoxycholic acid (**274**).

7.21.6 Fermentation and isolation (M_2 medium; fermenter)

Fermentation was carried out primarily in shaking flasks. The strain was subcultured in M_2 medium on 3 agar plates at 28 ºC for 4 days. Pieces of agar (1 cm^2) from the strain GW10/1828 were used to inoculate 20 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. The flasks were kept at 28 ºC for 7 days and 95 rpm. The broth served to inoculate a 50-liter jar fermenter which was kept for additional 7 days. After harvesting, celite (~ 2 kg) was added to the culture broth (50 liters, black-grey), and the mixture was homogenized with the aid of ultraturrax for 1~3 min. After filtration using a filter press, both of the liquid phase and biomass were extracted repeatedly with ethyl acetate. The extract was evaporated to give 20.1 g of greenish-black crude material.

TLC analysis of the extract exhibited numerous bands, which were stained to brown by anisaldehyde/sulphuric, however, showed no UV absorption. The extract was chromatographed on flash silica gel (column 30×1200 mm), and eluted with CH_2Cl_2-MeOH. As a result, five fractions were isolated, of which the most two interesting compounds were concentrated in fractions III-V (1.13, 1.11, 0.51 g). They are observed as two blue bands after spraying with anisaldehyde/sulphuric acid and heating with no UV absorption. Purification of the fractions (III-V) led to the two colourless solid compounds, macrophorin D (**275**) and anicequol (**278**).

Macrophorin D (275): $C_{28}H_{40}O_8$ (504.63). was obtained by applying of fractions III-IV by PTLC ($CHCl_3$/7% MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 6:4), as colourless solid (25 mg), not UV absorbing. – $\boldsymbol{R}_f$ = 0.5 ($CHCl_3$ / 10 % MeOH). – **UV/VIS** (MeOH): λ_{max} = end absorption. – **IR** (KBr): ν = 3426, 3000, 2922, 1736-1637, 1560-1511, 1442-1382, 1100-1033 cm^{-1}. – **^{1}H NMR (**$CDCl_3$ 300 MHz): δ = 5.90 (s, 1 H, 18-H), 4.89 (d, *J* = 17.1 Hz, 1 H, 22-H_A), 4.81 (s, 1 H, 12-H_A), 4.75 (d, *J* = 17.1 Hz, 1 H, 22-H_B), 4.63 (s, 1 H, 20-H), 4.54 (s, 1 H, 12-H_B), 3.79 (d, ^3J = 2.1 Hz, 1 H, 21-H), 2.81-2.55 (m, 4 H, 24,26-CH_2), 2.41-2.26 (m, 2 H, 3,11-H_A), 2.03-1.46 (m, 8 H, 3,11-H_B,7,6-H_A, 2,9-CH_2), 1.42 (m, 1 H, 1-H_A), 1.38 (s, 3 H, 28-CH_3), 1.35-1.07 (m, 3H, 1,6-H_B, 5-H), 0.87 (s, 3 H, 13-CH_3), 0.80 (s, 3 H, 14-CH_3), 0.70 (s, 3 H, 15-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 75 MHz): δ = 193.1 (C_q-17), 174.9 (C_q-27), 170.8 (C_q-23), 151.8 (C_q-19), 149.0 (C_q-8), 122.4 (CH-18), 106.7 (CH_2-12), 70.0 (C_q-16), 64.9 (CH-20), 63.3 (CH_2-22), 60.8 (C_q-25), 60.5 (CH-21), 55.4 (CH-5), 51.3 (CH-9), 44.9 (CH_2-24), 44.8 (CH_2-26), 41.9 (CH_2-1), 39.6 (C_q-10), 38.7 (CH_2-7), 38.0 (CH_2-3), 33.5 C_q-4), 33.5 (CH_3-13), 27.4 (CH_3-28), 24.3 (CH_2-6), 21.6 (CH_3-14), 20.8 (CH_2-11), 19.3 (CH_2-2), 14.4 (CH_3-15). – **(+)-ESI MS**: *m/z* (%) = 1031.3 ($[2M + Na]^+$, 100), 527.6 ($[M + Na]^+$, 55). – **(-)- ESI MS**: *m/z* (%) = 1007.7 ([2 M-H]$^-$, 100), 503.8 ([M-H]$^-$, 45). – **ESIHR MS**: *m/z* = 504.2723 (calcd. 504.2723 for $C_{28}H_{40}O_8$).

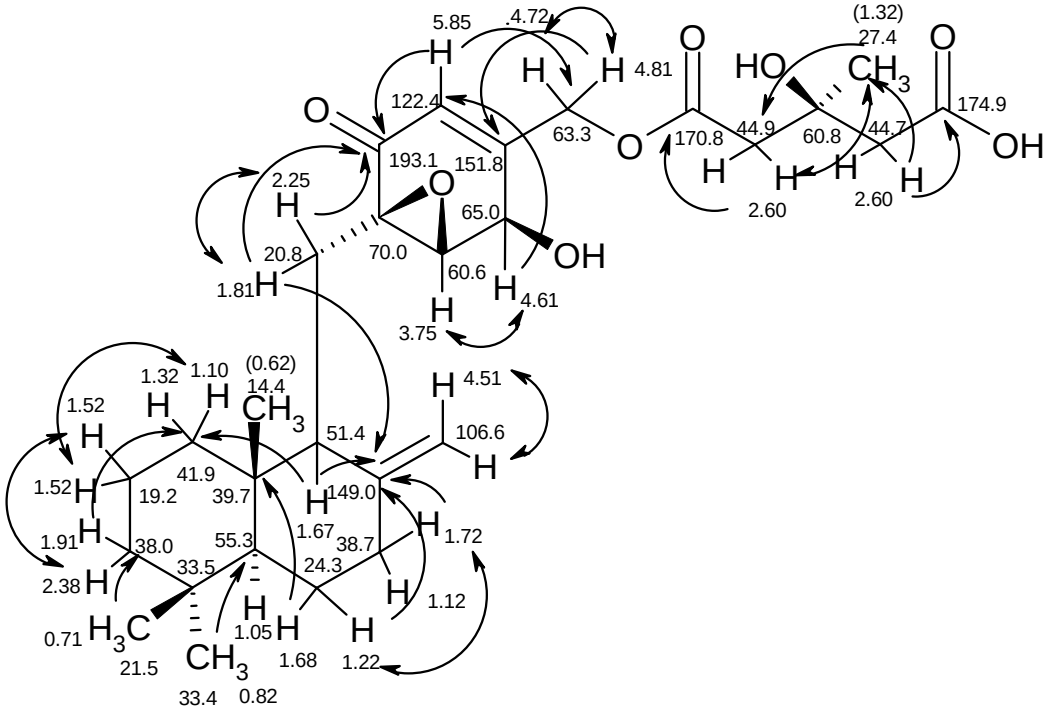


Figure 177: HMBC and H,H COSY correlations of macrophorin D (**275**)

Anicequol (278): $C_{30}H_{48}O_6$ (504.71), was purified from fractions IV-V by PTLC ($CHCl_3$/12%MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 6:4).) and obtained as colourless solid (40 mg). – R_f = 0.32 ($CHCl_3$ / 10 % MeOH). – **^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 5.19, 5.17 (AB, *J* = 15.3, 3.9 Hz, 2 H, 22,23-H), 4.85 (m, 1 H, 16-H), 4.53 (br, 1 H, 3-OH), 4.24 (s br, 11-OH), 4.18 (m, br, 1 H, 11-H), 3.67 (dd, ^3J = 12.6, 5.0 Hz, 1 H, 7-H), 3.35 (m, 1 H, 3-H), 2.51-2.38 (m, 2 H, 20,15a H), 2.28 (dd, *J* = 17.1, 3.2 Hz, 1 H, 5-H), 2.14 (dd, *J* = 17.1, 3.2 Hz, 1 H, 12-H), 1.93 (s, 3 H, 30-CH_3), 1.91 (m, 1 H, 8-H), 1.83 (dd, *J* = 14.0 Hz, 2.7 Hz, 1 H, 1-Ha), 1.75 (m, 1 H, 24-H), 1.65-52 (m, 2 H, 2,4-Ha), 1.42-1.17 (m, 8 H, 1b,2b,4b,9,14,15b,17,25-H), 1.05 (s, 3 H, 18-CH_3), 1.01 (d, ^3J = 7.3 Hz, 3 H, 21-CH_3), 0.81 (d, ^3J = 6.7 Hz, 3 H, 28-CH_3), 0.79 (s, 3 H, 19-CH_3), 0.78 (d, ^3J = 6.1 Hz, 3 H, 26-CH_3), 0.76 (d, ^3J = 6.1 Hz, 3 H, 27-CH_3). – **^{13}C/APT NMR (**[D_6]DMSO, 75 MHz): δ = 210.2 (C_q-6), 169.3 (C_q-29), 135.2 (CH-23), 131.8 (CH-22), 78.4 (CH-7), 74.6 (CH-16), 68.4 (CH-3), 66.3 (CH-11), 59.0 (CH-17), 55.2 (CH-14), 54.6 (CH-9), 53.8 (CH-5), 47.7 (CH_2-12), 42.7 (C_q-13), 42.6 (CH-8), 41.7 (CH-24), 40.3 (C_q-10), 36.8 (CH_2-15), 35.2 (CH_2-1), 33.9 (CH-20), 32.4 (CH-25), 30.3 (CH_2-4), 29.5 (CH_2-2), 21.1 (CH_3-30), 20.7 (CH_3-21), 19.8 (CH_3-26), 19.4 (CH_3-27), 17.6 (CH_3-28), 15.8 (CH_3-19), 15.1 (CH_3-18). – **(+)-ESI MS**: *m/z* (%) = 1535 ([3 M + Na]$^+$, 100), 1031 ([2 M + Na]$^+$, 83), 527 ([M + Na]$^+$, 10). – **(+)-ESI MS^2**: *m/z* (%) = 527 ([M + Na]$^+$, 48), 468 ([M +Na + H - ($HOOCCH_3$)]$^+$, 9). – **(-)-ESI MS**: *m/z* (%) = 1007.7 ([2 M - H]$^-$, 100), 503.9 ([M - H]$^-$, 40).

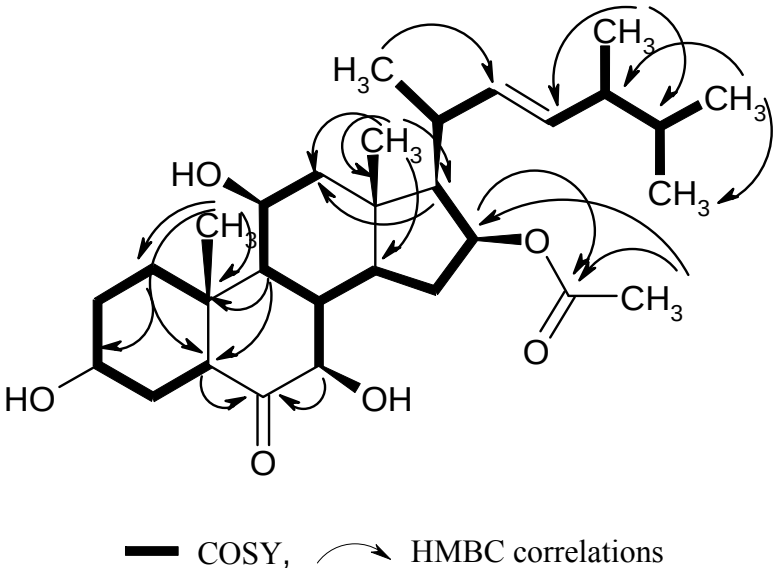


Figure 178: HMBC and H,H COSY correlations of anicequol (**278**)

7.21.7 Fermentation, isolation of metabolites (M_2 medium; shaker)

In a fourth trial, the terrestrial *Streptomyces* GW10/1828, was re-cultivated two times, each 25-liter shaker culture (110 rpm) for 5 days at 28 °C. After harvesting, TLC of both broths (greenish-black), indicated similar metabolites. They were combined, giving 30 liters. After mixing with celite (ca. 1.2 kg), the broth was filtered. The water and mycelial cake were extracted repeatedly by ethyl acetate. The combined organic phases were evaporated to dryness, yielding 3.21 g of greenish-black crude extract. TLC analysis of the extract exhibited a main yellow middle polar band with red UV fluorescence, which turned to reddish-brown by sulphuric acid. A pre-fractionation of the extract on a middle pressure silica gel column led to reveal five fractions. The main yellow band, present in fraction III (1.22 g) was further isolated with the aid of PTLC (2 × DCM/5%MeOH) followed by Sephadex LH-20 (DCM/MeOH 3:2). As a result, 17 mg of the yellow solid resistomycin (**279**) were obtained.

Resistomycin (**279**): $C_{22}H_{16}O$ (376.1), yellow solid, – R_f = 0.64 ($CHCl_3$/10%MeOH). – **^{1}H NMR** ([D_6]acetone, 300 MHz): δ = 14.73 (s, 1H, OH), 14.47 (s, 1 H, OH), 14.16 (s, 1 H, OH), 7.44 (s, 1 H, 11-H), 7.09 (s, 1 H, 8-H), 6.36 (s, 1 H, 4-H), 3.03 (s, 3 H, CH_3), 1.64 (s, 6 H, 2 CH_3). – **(-)-ESI MS**: *m/z* (%) = 750.9 ($[2M-H]^+$, 12), 375.0 ($[M-H]^-$, 100).

7.22 Terrestrial *Streptomyces* sp. GW50/1568

The terrestrial *Streptomyces* strain GW50/1568 delivered a white aerial mycelium by incubation on M_2 agar for 3 days at 28 °C.

7.22.1 Pre-screening

The extract was obtained by cultivating well grown agar plates of strain GW50/1568 on 3-liter M_2-medim for 3 days shaker culture (95 rpm), which gave a faint yellow broth. TLC exhibited unpolar yellow band, which showed no colour change with 2 N NaOH or sulphuric acid. Additionally, a highly polar zone gave a dark violet colouration with anisaldehyde/sulphuric acid, but showed no UV absorbance. The bio-activity of the extract was determined, on the basis of agar diffusion method, and gave the results shown below in Table 37.

Table 37: Antimicrobial activity of the crude extract produced by the strain GW50/1568

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	13
Escherichia coli	13
Streptomyces viridochromogenes (Tü57)	13
Mucor miehei (Tü284)	13

7.22.2 Fermentation and isolation of metabolites (fermenter)

12 of 1-litre-Erlenmeyer flasks, each containing 250 ml of M_2 medium (pH 7.8) were inoculated with the producing strain GW50/1568, and kept for 96 h at 28 °C on rotary shaker (95 rpm). The culture broth (faint yellow) was used to inseed a 20-liter jar fermenter, which was kept for further 3 days at 29 °C. The broth (pale yellow) was mixed with (*ca*. 1 kg), and filtered off. Both the water phase and biomass were extracted separately with ethyl acetate (for filtrate) and acetone (for biomass). After evaporation of acetone, the water residue was extracted again with ethyl acetate. The combined organic phases were evaporated to dryness, resulting in 5.52 g of a pale yellow crude extract.

The crude extract was dissolved in a little amount of methanol (45 ml) and then subjected to Sephadex LH-20 (30 × 1200; MeOH). On the basis of TLC analysis, four factions were obtained. The fast mobile fraction I (1.11 g) was purified by PTLC and Sephadex (DCM/MeOH 3:2), yielding 1.3 mg of yellow solid unidentified substance (*m/z* = 386; R_f = 0.95, $CHCl_3$/5%MeOH). It showed no colour change with either NaOH or sulphuric acid. Work up of fraction III (0.87 g), using PTLC (DCM/10% MeOH) followed by Sephadex LH-20 (MeOH), led to a colourless solid (20 mg) of *p*-hydroxybenzaldehyde (**280**) (R_f = 0.44 ($CHCl_3$/10%MeOH).

7.22.3 Fermentation and isolation of metabolites (shaker)

By the same way, the terrestrial *Streptomyces* sp. GW 50/1568 was re-cultivated as 25-liter shaker culture for 7 days at 28 °C. The reddish-brown broth (18-liter) was mixed with celite (ca. 1 kg), and filtered off with the aide of a filter press. The water fraction and mycelial cake were extracted repeatedly with ethyl acetate. The combined organic phases were evaporated to dryness, yielding 1.31 g of brown crude extract.

TLC analysis of the extract exhibited a highly polar zone, which showed no UV absorption, and turned dark violet with anisaldehyde/sulphuric acid (after heating), red-pink with vanillin/sulphuric acid (without heating), brown/grey with Ehrlich's reagent. A pre-fractionation of the extract using middle pressure silica gel, gave six fractions, after monitoring by TLC. Purification of the main fraction IV (100 mg) by Sephadex LH-20 (3 × MeOH), led to oasomycin A (**281**).

Oasomycin A (281): $C_{55}H_{94}O_{17}$ (1027.4), colourless solid (35 mg), highly polar dark violet band after spraying with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R}_f$ = 0.2 (CH_2Cl_2/MeOH: 6:4). – **^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 6.69 (t, ^{3}J = 7.1 Hz, 1 H, 3-H), 5.51-5.22 (m, 7 H, 12,13,16,17,21,38,39-H), 4.98 (m, 1 H, H-41), 4.76 (d, ^3J = 4.2 Hz, OH), 4.64-4.01 (m, 11 H, 22,33,37,43-H, 7 OH), 3.95-3.57 (m, 8 H, 9,15,23,25,27,29,31,35-H), 3.50 (m, H-19), 3.25 (m, H-7), 2.56-2.24 (m, 4 H, 40,45-H_2), 2.20-2.00 (m, br, 7 H, 11_A,14,18,42,44_A-H, 4-CH_2), 1.90 (m, 11,44-H_B), 1.88 (s br, 3 H, 47-CH_3), 1.58-1.11 (m, 21 H, 6,8,30,32-H, 5,10,24,26,28,34,36-CH_2, 52-H_3), 0.99 (d, ^3J = 6.2 Hz, 3 H, 50-CH_3), 0.92-0.72 (m, 9 H, 53,48,55-CH_3), 0.64 (m, 6 H, 54,49-CH_3). – ^{13}C/**APT NMR (**[D_6]DMSO, 75 MHz): δ = 176.7 (CO, C_q-46), 166.4 (CO, C_q-1), 142.5 (CH-3), 138.2 (CH-38), 138.1 (C_q-20), 132.7 (CH-17), 132.5 (CH-13), 130.9 (CH-16), 129.5 (CH-12), 126.9 (CH-21), 126.8 (C_q-2), 122.4 (CH-39), 81.0 (CH-19), 80.4 (CH-43), 74.7 (CH-7), 74.3 (CH-15), 73.0 (CH-41), 72.9 (CH-29), 72.0 (CH-9), 70.9 (CH-22), 70.8 (CH-23), 70.8 (CH-31), 66.8 (CH-33), 66.8 (CH-27), 63.8 (CH-35), 63.7 (CH-25), 45.7 (CH_2-36), 45.6 (CH_2-26), 42.2 (CH-14), 42.2 (CH-34), 42.2 (CH_2-28), 41.4 (CH-8), 40.4 (CH_2-24), 40.1 (CH-32), 39.9 (CH-42)*, 39.5 (CH-30)*, 39.2 (CH-18)*, 34.3 (CH-6), 32.9 (CH_2-5), 32.4 (CH_2-40), 32.1 (CH_2-10), 28.9 (CH_2-11), 28.2 (CH_2-45), 26.1 (CH_2-4), 24.8 (CH_2-44), 16.8 (CH_3-51), 15.2 (CH_3-50), 12.2 (CH_3-48), 12.1 (CH_3-47), 11.5 (CH_3-52), 11.2 (CH_3-49), 10.5 (CH_3-54), 9.6 (CH_3-55), 9.5 (CH_3-53), *hidden by solvent. – **(+)-ESI MS**: *m/z* (%) = 1050.2 ($[M + Na]^+$). **–(-)-ESI MS**: *m/z* (%) = 1026.1 ($[M-H]^-$). – **(+)-ESI MS**: *m/2z (*%) = 536.5 ($[M + Na]^+$).

7.23 Terrestrial *Streptomyces* sp. GW44/1492

The terrestrial *Streptomyces* strain GW44/1492 was cultured on agar with M_2 medium, and incubated at 28 °C for 3 days exhibiting a white aerial mycelium.

7.23.1 Pre-screening

Well grown agar plates of GW44/1492 were used to inoculate 3 liters of M_2 medium. After 3 days on a shaker (95 rpm) at 28 °C, a strongly yellow broth was obtained. TLC of the ethyl acetate extract exhibited a middle polar yellow band, which showed a yellowish-red UV fluorescence, and turned to reddish-brown by treatment with sulphuric acid. The yellow band showed no colour change by NaOH. The biological activities of the crude extract are shown in Table 37.

Table 38: Antimicrobial activity of the crude extract produced by the strain GW44/1492

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	20
Escherichia coli	13
Staphylococcus aureus	22
Streptomyces viridochromogenes (Tü57)	20
Mucor miehei (Tü284)	12
Candida albicans	12

7.23.2 Fermentation and working up

For cultivation, small pieces of agar culture of the terrestrial *Streptomyces* GW44/1499 grown on M_2 medium, were used to inoculate 96 of sterilised 1 liter-Erlenmeyer flasks, each containing 200 ml of M_2 medium. The flasks were incubated for 4 days at 28 °C with 95 rpm. The obtained yellow broth was mixed with celite (ca. 1 kg), and filtered off using a filter press. The two fractions, water and biomass were extracted with ethyl acetate repeatedly. The combined organic phases were evaporated in *vacuo* to dryness, yielding 1.21 g of a brown crude extract.

7.23.3 Isolation and identification of metabolites

The crude extract delivered 3 fractions on flash silica gel (CH_2Cl_2/MeOH). The fraction II (300 mg), containing the yellow band, was purified by PTLC ($CHCl_3$/7%MeOH) and Sephadex LH-20 (MeOH). As a result, 18 mg of setomimycin (**282**), were obtained as a yellow solid.

Setomimycin (282): $C_{34}H_{28}O_9$ (580.1), yellow solid (18 mg). – $\boldsymbol{R}_f$ = 0.66 (CH_2Cl_2/7%MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 16.57 (s, 1 H, OH), 16.51 (s, 1 H, OH), 10.12 (s, 1 H, OH), 10.00 (s, 1 H, OH), 7.42 (t, ^3J = 8.2 Hz, 1 H, 7-H), 7.28 (t, ^3J = 8.1 Hz, 1 H, 7'-H), 7.02 (d, ^3J = 8.2 Hz, 1 H, 6-H), 6.92 (d, ^3J = 8.1 Hz, 1 H, 6'-H), 6.67 (d, ^3J = 8.2, 1 H, 8-H), 6.27 (s, 1 H, 3-H), 6.26 (d, ^3J = 8.1 Hz, 1H, 8'-

H), 4.03 (s, 1 H, 1-H), 3.72 (s, 1 H, 1'-H), 3.20 (d, ^2J = 18.0 Hz, 1 H, 3'-H_A), 2.64 (d, ^2J = 18 Hz, 1 H, 3'-H_B), 2.02 (s, 3 H, 12-CH_3), 1.71 (s, 3 H, 12'-CH_3), 1.42 (s, 3 H, 13-CH_3), 1.25 (s, 3 H, 13'-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 150 MHz): δ = 208.3 (C_q-11'), 203.2 (C_q-4'), 199.5 (C_q-11), 191.0 (C_q-4), 166.4 (C_q-10'), 164.8 (C_q-10), 158.8 (C_q-5'), 158.5 (C_q-5), 158.0 (C_q-2), 138.6 (C_q-8a'), 137.0 (C_q-8a), 134.3 (C_q-9a), 133.8 (C_q-9a'), 133.5 (CH-7'), 133.2 (CH-7), 125.8 (CH-3), 125.0 (CH_q-9), 124.3 (C_q-9'), 117.4 (CH-8'), 116.5 (CH-8), 113.4 (C_q-10a), 113.0 (C_q-10a'), 112.6 (CH-6), 112.5 (CH-6'), 109.2 (C_q-4a'), 108.6 (C_q-4a), 72.1 (C_q-2'), 60.8 (CH-1), 60.5 (CH-1'), 47.2 (CH_2-3'), 33.9 (CH_3-12'), 29.5 (CH_3-13'), 28.2 (CH_3-12), 23.0 (CH_3-13). – **(+)-ESI MS**: *m/z* (%) = 1182.8 ([2 M + Na]$^+$, 100), 603.3 ([M + Na]$^+$, 58). **–(-)-ESI (-)**: *m/z* (%) = 579.3 ([M - H]$^-$).

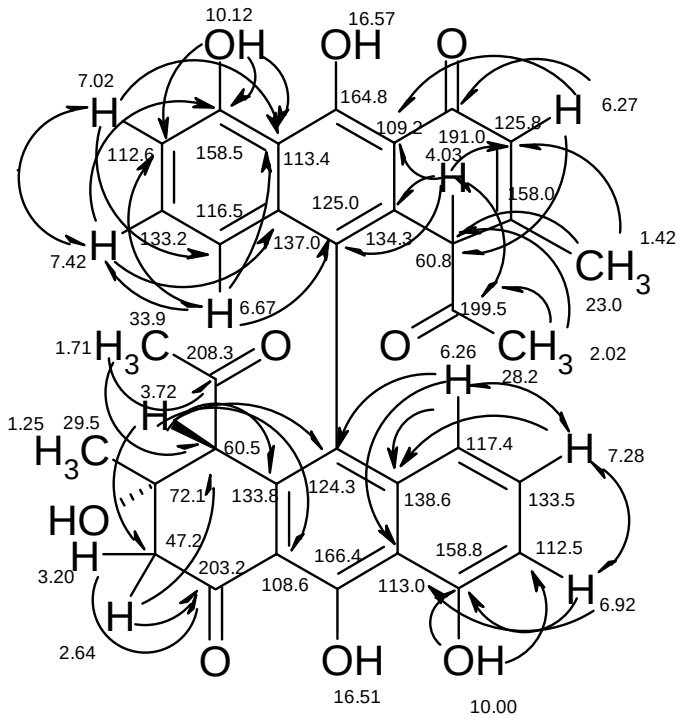


Figure 179: HMBC and H,H COSY correlations of setomimycin (**282**)

7.24 Terrestrial *Streptomyces* sp. GW3/1130

The terrestrial *Streptomyces* strain GW3/1130 was inoculated on agar plates with M_2 medium. After 3 days of incubation at 28 °C, faint yellowish-white mycelous colonies had grown, and the surrounding agar showed a dark yellow colour.

7.24.1 Pre-screening

The strain was cultivated as 1 liter-shaker culture at 28 °C for 5 days with 95 rpm. After 5 days, the mycelium and nutritional medium were orange-yellow coloured. After extraction with ethyl acetate and evaporation *in vacuo*, 200 mg of orange residue were obtained. TLC analysis of the extract exhibited numerous unpolar yellow bands, which turned to red by treatment with NaOH, and brown by sulphuric acid. They showed orange UV fluorescence.

Table 39: Antimicrobial activity of the crude extract produced by the strain GW3/1130

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	16
Staphylococcus aureus	20
Streptomyces viridochromogenes (Tü57)	30
Mucor miehei (Tü284)	25
Candida albicans	20
Chlorella vulgaris	11
Chlorella sorokiniana	11
Scenedesmus subspicatus	11

7.24.2 Fermentation and isolation of metabolites (fermenter)

The strain was cultured at 28 °C for 4 days on a rotary shaker (95 rpm) in 12 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. The pre-culture was used to inseed a 25liter fermenter, charged with 22 liters of M_2 medium, which was kept at 28 °C for 96 hours. The fermentation broth (pale yellow, 25 liters) was filtered with the aid of celite, and the resulting mycelial cake was extracted with acetone. After removal of the acetone, the aqueous residue was extracted with ethyl acetate. The filtered broth was extracted repeatedly using ethyl acetate. The combined organic phases were evaporated to dryness under vacuum at 40 °C, giving 6.0 g of pale yellow crude extract (6.0 g). The organic extract was applied to a flash column of silica gel (30 × 600 mm), and eluted with $CHCl_3$/MeOH-gradients. As a result, four fractions were obtained by TLC monitoring. TLC analysis of fraction III (1.12 g) exhibited a middle polar, blue UV fluorescence band. By purification of the fraction III by PTLC ($CHCl_3$/7%MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 6:4), a pale yellow solid (10 mg) of 1-acetyl-*β*-carboline (**81)** was obtained.

7.24.3 Fermentation and isolation of metabolites (shaker)

Pieces of agar (*ca*. 1 cm^2) from the GW3/1130 culture were used to inoculate 96 of 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2 medium. The flasks were kept at 28 ºC for 6 days and 95 rpm. After harvesting, celite (~ 1 kg) was added to the culture broth (25-liter), and the mixture was homogenized by mechanical stirring for 1~3 min. By filtration using a filter press, the liquid phase was separated and extracted with ethyl acetate. The mycelial cake was extracted three times with acetone (5 liters), and the acetone was removed in *vacuo*, and the aqueous layer was extracted with ethyl acetate. For the isolation of metabolites, the combined organic layers were evaporated in *vacuo* at 40 °C to give 4.02 g of an orange crude extract.

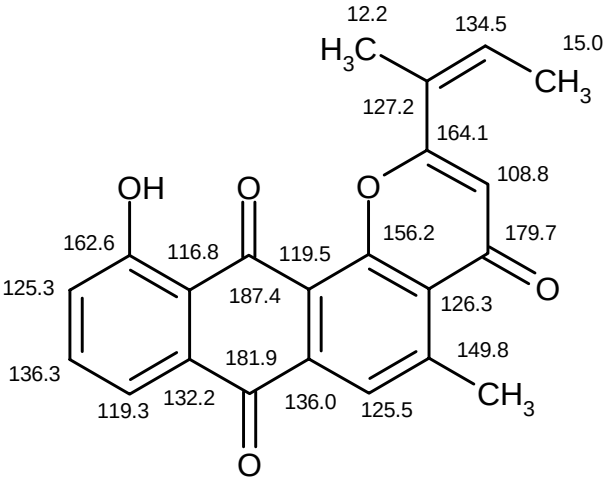
The crude residue was chromatographed on Sephadex LH 20 (30 × 1000 mm, MeOH) yielding four fractions. The main fraction II containing all the yellow components was subjected to further fractionation, using PTLC ($CHCl_3$/ 2 % MeOH), to give 6 yellow components. These were further purified with Sephadex LH-20 ($CHCl_3$/MeOH 6:4), and assigned as, α-indomycinone (**286**), saptomycin F (**289**), ε-indomycinone (**291**), *β*-indomycinone (**295**), saptomycin A (**297**) and γ-indomycinone (**298**).

Rubiflavinon C-1; α-Indomycinone (286): $C_{24}H_{18}O_5$ (386.11), yellow amorphous (20 mg), as orange UV fluorescent substance, turned to red by treatment with 2 N NaOH, and brown with sulphuric acid. – $\boldsymbol{R}_f$ = 0.8 ($CHCl_3$/5 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.93 (s br, 1H, OH), 8.40 (d, ^3J = 11.7 Hz, 1 H, 16-H), 8.03 (s, 1 H, 6-H), 7.83 (dd, ^3J = 7.6 Hz, ^4J = 1.3 Hz, 1 H, 8-H), 7.68 (t, ^3J = 8.0 Hz, 1 H, 9-H), 7.37 (dd, ^3J = 8.4 Hz, ^4J = 1.3 Hz, 10-H), 6.53 (ddq, ^3J = 11.7, ^3J = 10.7, ^4J = 1.7 Hz, 1 H, 17-H), 6.42 (s, 1 H, 3-H), 6.08 (dq, ^3J = 10.7, ^3J = 7.6 Hz, 1 H, 18-H), 2.98 (s, 3 H, 13-CH_3), 2.16 (dd, ^3J = 7.6, ^4J = 1.7 Hz, 3 H, 19-CH_3), 2.06 (s, 3 H, 15-CH_3). – **(+)-ESI MS**: *m/z* (%) = 795.2 ($[2\ M + Na]^+$). – **(-)-ESI MS**: *m/z* (%) = 793.5 ($[2\ M - 2\ H + Na]^-$, 100), 386.7 ($[M]^+$, 59). – **EI MS** (70 eV): *m/z* (%) = 386.3 ($[M]^{+\cdot}$, 100), 371.3 ($[M - Me]^{+\cdot}$, 95), 360.3 (20), 343.3 (16), 299.3 (9), 281.2 (80).

Saptomycin F (289): $C_{24}H_{18}O_6$ (402), yellow powder (2 mg), orange UV fluorescent compound, changed to red by 2 N NaOH, and brown by sulphuric acid. – $\boldsymbol{R}_f$ = 0.6 ($CHCl_3$/MeOH 2%). – **^{1}H NMR (**$CDCl_3$, 300.145 MH): δ = 12.85 (s br, 1 H,

OH), 8.08 (s, 1 H, 6-H), 7.82 (dd, ^3J = 7.4 Hz, ^4J = 1.2 Hz, 1 H, 8-H), 7.67 (t, ^3J = 7.4 Hz, 1 H, 9-H), 7.36 (dd, ^3J = 8.4 Hz, ^4J = 1.2 Hz, 10-H), 6.53 (s, 1 H, 3-H), 6.05 (dq, ^3J = 10.7, ^3J = 7.2 Hz, 1 H, 18-H), 5.42 (ddq, ^3J = 8.1 Hz, 11.1 Hz, ^4J = 1.7 Hz, 17-H), 4.24 (d, ^3J = 8.0 Hz, 16-H) 3.02 (s, 3 H, 13-CH_3), 1.89 (dd, ^3J = 7.6, ^4J = 1.7, 3 H Hz, 19-CH_3), 1.83 (s, 3 H, 15-CH_3). – **(-)-ESI MS**: *m/z* (%) = 825.2 ([2 M + Na – 2 H]$^-$, 100), 401.3 ([M-H]$^-$, 38).

ε-Indomycinone (291): $C_{22}H_{16}O_5$ (360), unpolar yellow powder (6 mg), orange UV fluorescence. It exhibited a colour change to red by 2 N NaOH, and brown with Sulphuric acid. – R_f = 0.65 ($CHCl_3$/2%MeOH). – **IR** (KBr): ν = 3440, 2924, 2366, 1736, 1720, 1640, 1638, 1582, 1512, 1457, 1380, 1315, 1266, 1219, 1163, 1097, 1079, 797 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300 MH): δ = 12.88 (s br, 1 H, OH), 798 (s, 1 H, 6-H), 7.79 (dd, ^3J = 7.4, ^4J = 1.2 Hz, 1 H, 8-H), 7.65 (t, ^3J = 7.4 Hz, 1 H, 9-H), 7.48 (dq, ^3J = 8.1, ^4J = 1.1 Hz, 16-H), 7.33 (dd, ^3J = 8.4, ^4J = 1.2 Hz, 10-H), 6.36 (s, 1 H, 3-H), 2.98 (s, 3 H, 13-CH_3), 2.00 (d, ^3J = 8.1 Hz, 3 H, 17-H_3), 1.97 (s, 3 H, 15-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 150 MHz): δ = 187.4 (C_q-12), 181.9 (C_q-7), 179.7 (C_q-4), 164.1 (C_q-2), 162.6 (C_q-11), 156.2 (C_q-12b), 149.8 (C_q-5), 136.3 (CH-9), 136.0 (C_q-6a), 134.5 (CH-16), 132.2 (C_q-7a), 127.2 (C_q-14), 126.3 (C_q-4a), 125.5 (CH-6), 125.3 (CH-10), 119.5 (C_q-12a), 119.3 (CH-8), 116.8 (C_q-11a), 108.8 (CH-3), 24.2 (CH_3-13), 15.0 (CH_3-17), 12.2 (CH_3-15). – **(-)-ESI MS**: *m/z* (%) = 741.0 ([2 M + Na – 2 H]$^-$, 100), 360 (60). – **EI MS** (70 eV): *m/z* (%) = 360 ([M]$^{+\cdot}$, 100), 281 ([M-C_6H_7]$^{+\cdot}$, 95), 252 ([M- (C_6H_7 + CHO)]$^{+\cdot}$, 5); – **HREI MS**: *m/z* = 360.0998 (calcd. 360.0998 for $C_{22}H_{16}O_5$).



***β*-Indomycinone (295)**: $C_{24}H_{20}O_6$ (404.4), yellowish-orange amorphous powder (15 mg), showed a colour change to red by treatment with NaOH, and brown with sulphuric acid. It is orange UV fluorescent. – $\boldsymbol{R}_f$ = 0.78 ($CHCl_3$/5 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.85 (s br, 1 H, OH), 8.08 (s, 1 H, 6-H), 7.83 (dd, ^3J = 7.6, ^4J = 1.3 Hz, 1 H, 8-H), 7.68 (t, ^3J = 8.0 Hz, 1 H, 9-H), 7.38 (dd, ^3J = 8.4, ^4J = 1.3 Hz, 10-H), 6.56 (s, 1 H, 3-H), 5.74 (m, 1 H, 18-H), 5.39 (m, 1 H, 17-H), 3.02 (s, 3 H, 13-CH_3), 2.91 (dd, ^3J = 14.4, ^3J = 8.5 Hz, 1 H, 16-H_A), 2.78 (dd, ^3J = 14.4, ^3J = 7.2 Hz 1 H, 16-H_B), 1.68 (s, 3 H, 15-CH_3), 1.64 (d, ^3J = 6.8 Hz, 19-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 75 MHz): δ = 187.3 (C_q-12), 181.6 (C_q-7), 179.2 (C_q-4), 171.9 (C_q-2), 162.6 (C_q-11), 156.0 (C_q-12b), 150.0 (C_q-5), 136.5 (CH-9), 136.0 (C_q-6a), 132.2 (C_q-7a), 129.5 (CH-18), 126.2 (C_q-4a), 125.7 (CH-6), 125.4 (CH-10), 123.1 (CH-17), 119.6 (C_q-12a), 119.4 (CH-8), 116.7 (C_q-11a), 109.6 (CH-3), 73.7 (C_q-14), 38.1 (CH_2-16), 26.1 (CH_3-15), 24.2 (CH_3-13), 13.0 (CH_3-19). – **CI MS (NH_3)**: *m/z* **(**%) = 422.4 ($[M + NH_4]^+$, 16), 405.3 ($[M + H]^+$, 100). – **EI MS** (70 eV): *m/z* (%) = 350 ([M - C_4H_6], 20), 349.2 ($[M - C4H_7]^{+\cdot}$, 100). – **(-)-ESI MS**: *m/z* (%) = 403 ($[M-H]^-$).

Saptomycin A (297): $C_{24}H_{20}O_6$ (404.4), yellow amorphous powder (5 mg), turned to red by treatment with NaOH, and to brown by sulphuric acid. Orange UV fluorescence on TLC. – $\boldsymbol{R}_f$ = 0.70 ($CHCl_3$/5 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.64 (s br, 1 H, OH), 8.09 (s, 1 H, 6-H), 7.83 (dd, ^3J = 7.6 Hz, ^4J = 1.3 Hz, 1 H, 8-H), 7.69 (t, ^3J = 8.0 Hz, 1 H, 9-H), 7.38 (dd, ^3J = 8.4, ^4J = 1.3 Hz, 10-H), 6.28 (s, 1 H, 3-H), 5.65 (m, 1 H, 18-H), 5.53 (td, ^3J = 10.5, ^3J = 6.8 Hz, 1 H, 17-H), 5.01 (dd, ^3J = 8.5, ^3J = 3.8 Hz, 1 H, 16-H), 3.02 (s, 3 H, 13-CH_3), 2.99 (dq, ^3J = 3.8, ^3J = 7.5 Hz, 1 H, 14-H), 1.71 (d, ^3J = 7.0 Hz, 3 H, 19-CH_3), 1.45 (d, ^3J = 7.0, Hz, 3 H, 15-CH_3). – ^{13}C/**APT NMR (**$CDCl_3$, 75 MHz): δ = 187.8 (C_q-12), 181.8 (C_q-7), 179.0 (C_q-4), 171.0 (C_q-2), 162.9 (C_q-11), 156.7 (C_q-12b), 150.4 (C_q-5), 136.9 (CH-9), 136.1 (C_q-6a), 132.3 (C_q-7a), 130.3 (CH-18), 128.2 (CH, C-17), 126.6 (C_q-4a), 126.1 (CH-10), 125.8 (CH-6), 119.8 (CH-8), 119.7 (C_q-12a), 116.8 (C_q-11a), 112.4 (CH-3), 69.0 (CH-16), 45.1 (CH-14), 24.5 (CH_3, C-13), 13.6 (CH_3-15), 12.9 (CH_3-19). – **CI MS** (NH_3): *m/z* **(**%) = 422.4 ($[M + NH_4]^+$, 5), 405.3 ($[M + H]^+$, 26).

γ-Indomycinone (298): $C_{22}H_{18}O_6$ (378.11), yellow amorphous powder (4 mg), turned to red when treated with NaOH and to brown by sulphuric acid. – $\boldsymbol{R}_f$ = 0.75 ($CHCl_3$/2 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.83 (s br, 1 H, OH), 8.09 (s, 1 H, 6-H), 7.83 (dd, ^3J = 7.5 Hz, ^4J = 1.2 Hz, 1 H, 8-H), 7.69 (t, ^3J = 7.9 Hz, 1 H,

9-H), 7.38 (dd, ^3J = 8.3 Hz, ^4J = 1.2 Hz, 10-H), 6.52 (s, 1 H, 3-H), 3.04 (s, 3 H, 13-CH_3), 2.10 (dq, ^3J = 14.2, ^3J = 7.5 Hz, 1 H, 16-H_A), 1.98 (dq, ^3J = 14.2, ^3J = 7.5 Hz, 1 H, 16-H_B), 1.67 (s, 3 H, 15-CH_3), 0.97 (t, ^3J = 7.5 Hz, 3 H, 17-CH_3). – **CI MS** (NH_3): *m/z* (%) = 396.4 ($[M + NH_4]^+$, 10), 379.3 ($[M + H]^+$, 100). **– EI MS** (70 eV): *m/z* (%) = 378.4 ($[M]^{+\cdot}$, 40), 350 ($[M\text{-}CO]^+$, 22), 349.2 ($[M\text{-}CHO]^{+\cdot}$, 100), 281 (30).

7.24.4 Optimisation of the strain GW3/1130

The terrestrial *Streptomyces* strain GW3/1130 was additionally, re-cultivated, as 25-liter shaker culture (95 rpm) for 7 days (longer period) on M_2 medium at 28 °C. After harvesting, the yellowish-orange broth was mixed with celite and filtered off. The fractions, mycelial cake and water phase, were extracted repeatedly with ethyl acetate. The combined organic phases were evaporated in *vacuo*, yielding 10.1 g of red crude extract. With the aid of flash silica gel column chromatography (CH_2Cl_2/MeOH), eight fractions were obtained. The fractions I-V showed an approximate equal R_f value (CH_2Cl_2) of the yellow components. After purification, using PTLC (C_6H_{12}/ 50 % EtOAc), and further Sephadex LH-20 ($CHCl_3$/MeOH 6:4), α-indomycinone (**286**, 10 mg), saptomycin F (**289**, 1.2 mg), ε-indomycinone (**291**, 10 mg), β-indomycinone (**295**, 30 mg), saptomycin A (**297**, 8 mg), and γ-indomycinone (**298**, 3.5 mg), were obtained.

7.25 Terrestrial *Streptomyces* sp. GW22/3234

The strain GW 22/3234 developed on M_2 agar for 3 days at 28 °C grey mycelial colonies.

7.25.1 Pre-screening

One agar plate served to inoculate 12 of 1 liter-Erlenmeyer flasks, each containing 200 ml of M_2 medium. Incubation was done at 28 °C for 3 days, giving a brown culture broth. On TLC, there were numerous UV absorbing bands visible. The antimicrobial activity of the extract was weak, as shown in Table 40

Table 40: Antimicrobial activity of the crude extract produced by the strain GW22/3234

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	13
Escherichia coli	11

Staphylococcus aureus	14
Mucor miehei (Tü284)	17

7.25.2 Fermentation and working up

Well-grown agar cultures of *Streptomyces* sp. GW22/3234, served to inoculate 12 of 1 liter-Erlenmeyer flasks, each filled with 250 ml of M_2 medium. The flasks were incubated on a shaker (95 rpm) for 3 days at 28 °C. The afforded culture broth was used to inseed a 50-liter jar fermenter, held at 28 °C for 72 hours (pH 6.5~ 7.8, 250 rpm). The fermentation broth was harvested and mixed with celite (~2 kg) and filtered off. The resulting water phase and mycelial cake were extracted with ethyl acetate and acetone. The extracts were combined together and concentrated under reduced pressure to give a brown crude extract (15.1 g). TLC analysis showed a metabolic pattern, completely different, than that in the pre-screening. It showed two low polar orange-yellow bands, which turned to red-violet by NaOH, and several middle polar UV absorbing bands, which turned to yellow by Ehrlich's reagent, and to blue by anisaldehyde/sulphuric acid. An additional polar greenish-yellow band with green UV florescence turned to blue by anisaldehyde/sulphuric acid, red by NaOH and brown with sulphuric acid, respectively. Finally, a weak UV absorbing band was detected which turned to yellow by Ehrlich's reagent. The extract pronounced higher biological activity, than that exhibited in pre-screening, as shown in Table 41.

Table 41: Antimicrobial activity of the crude extract produced by the strain GW22/3234 (fermenter)

Tested microorganism	Inhibition zone ∅ [mm]
Bacillus subtilis	25
Escherichia coli	22
Staphylococcus aureus	27
Streptomyces viridochromogenes (Tü57)	30
Mucor miehei (Tü284)	25

7.25.3 Isolation and identification of metabolites

The extract was subjected to flash silica gel column chromatography (30 × 1200 mm) using a CH_2Cl_2-MeOH gradient. On the basis of TLC analysis, seven fractions were given. Further fractionation and purification of the obtained fractions (II-VII), lead to the metabolites shown below.

Resomycin A (299): $C_{21}H_{18}O_7$ (382.37). Fraction II (0.5 g) delivered a yellowish orange solid (15 mg), from an unpolar yellow band, with an orange UV fluorescence, which turned to violet by NaOH. It was purified on a silica gel column (2 × 40 cm; CH_2Cl_2 /1-5% MeOH) followed by Sephadex LH-20 (CH_2Cl_2/MeOH 6:4). – $\boldsymbol{R}_f$ = 0.84 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 12.51 (s br, 1 H, OH), 12.10 (s br, 1 H, OH), 7.82 (dd, ^3J = 7.5, ^4J = 1.1 Hz, 1 H, 1-H), 7.67 (t, ^3J = 8.3 Hz, 1 H, 2-H), 7.62 (s, 1 H, 11-H), 7.29 (dd, ^3J = 8.3, ^4J = 1.1 Hz, 1 H, 3-H), 3.91 (s br, 1 H, 10-H), 3.75 (s, 3 H, 15-OCH_3), 3.06 (ddd, ^2J = 19.3, ^3J = 6.9, 3.1 Hz, 1 H, 7_a-H), 2.88 (ddd, J = 19.3, 6.9, 3.1 Hz, 1 H, 7_b-H), 2.34 (m, 1 H, 8_a-H), 1.92 (m, 1 H, 8_b-H), 1.42 (s, 3 H, 13-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 150 MHz): δ = 192.8 (C_q-5), 181.6 (C_q-12), 171.7 (C_q-14), 162.5 (C_q-4), 161.0 (C_q-6), 142.0 (C_q-10a), 137.1 (CH-2), 133.7 (C_q-12a), 133.6 (C_q-6a), 130.7 (C_q-11a), 124.5 (CH-3), 121.2 (CH-1), 119.9 (CH-11), 115.9 (C_q-4a), 113.6 (C_q-5a), 69.6 (C_q-9), 57.4 (CH-10), 52.5 (CH_3-15), 30.8 (CH_2-8), 27.5 (CH_3, C-13), 20.1 (CH_2-7). – **(-)-ESI MS**: *m/z* (%) = 1167.4 [3 M-2 H + Na]$^-$, 65), 785.4 [2 M + Na – 2 H]$^-$, 35), 381.5 ([M-H]$^-$, 100).

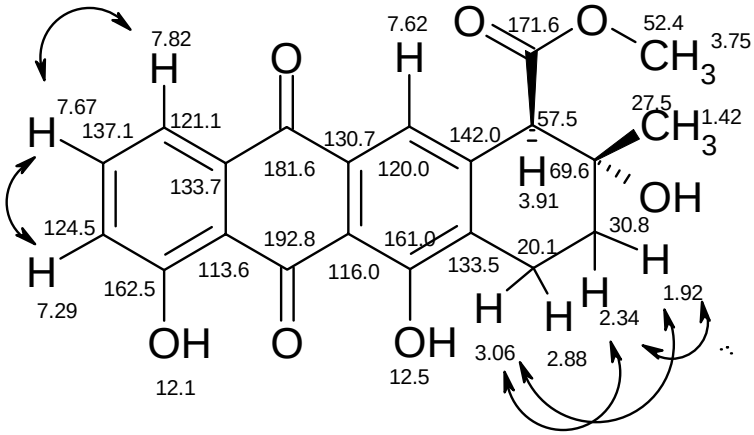


Figure 180: H,H COSY-correlations and ^{13}C-values of resomycin A (**299**)

Resomycin D (302): $C_{21}H_{14}O_6$ (362.34). Fraction III (1.91 g) delivered by Sephadex LH-20 (MeOH) sub-fraction IIIa (0.3 g). The yellow band was purified by PTLC (CH_2Cl_2/3%MeOH) and Sephadex LH-20 (CH_2Cl_2/MeOH, 6:4) to deliver a low polar yellow solid (2 mg), which showed an orange UV fluorescence, and turned to violet by NaOH. – $\boldsymbol{R}_f$ = 0.92 ($CHCl_3$/5% MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 13.76 (s br, 1 H, OH), 12.24 (s br, 1 H, OH), 8.50 (d, ^3J = 8.6 Hz, 1 H, 7-H), 8.24 (s, 1 H, 11-H), 7. 88 (dd, ^3J = 7.6, ^4J = 1.1 Hz, 1 H, 1-H), 7.70 (t, ^3J = 8.4 Hz, 1 H, 2-H), 7.56 (d, ^3J = 8.6 Hz, 1 H, 8-H), 7.30 (dd, ^3J = 8.4 Hz, ^4J = 1.1, 1 H, 3-H), 4.08 (s,

3 H, 15-OCH_3), 2.54 (s, 3 H, 13-CH_3). – **(-)-ESI MS**: *m/z* (%) = 1107.6 [3 M + Na – 2 H]$^-$, 40), 745.7 [2 M + Na – 2 H]$^-$, 100), 361.9 [M-H]$^-$, 78).

Streptazolin (303): $C_{11}H_{13}NO_3$ (207.23), was obtained from sub-fraction IIIa (1.21 g) by two times Sephadex LH-20 (MeOH), as colourless oil (300 mg). It is a middle polar UV absorbing substance, and coloured to orange by anisaldehyde/sulphuric acid, and to yellow by Ehrlich's reagent after heating. – $\boldsymbol{R}_f$ = 0.56 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 6.08 (q, ^3J = 7.1 Hz, 1 H, 9-H), 5.92 (m, 1 H, 3-H), 4.65 (s br, 1 H, 5-H), 4.60 (d, ^3J = 7.1 Hz, 1 H, 7-H), 4.18 (d, ^3J = 7.2 Hz, 1 H, 6-H). 3.28 (m, 2 H, 1-CH_2), 2.40, 2.10 (m, 2 H, 2-CH_2), 1.77 (d, ^3J = 7.1 Hz, 3 H, 10-CH_3). – **^{13}C/APT NMR (**$CDCl_3$, 75 MHz): δ = 159.5 (C_q-11), 142.8 (C_q-4), 137.9 (C_q-8), 123.4 (CH-9), 117.7 (CH-3), 82.0 (CH-6), 73.6 (CH-7), 58.8 (CH-5), 39.6 (CH_2-1), 22.4 (CH_2-2), 14.5 (CH_3-10). – **(+)-ESI MS**: *m/z* (%) = 851([4 M + Na]$^+$, 6), 644 ([3 M + Na]$^+$, 13), 437.4 ([2 M + Na]$^+$, 100), 230.4 ([M + Na]$^+$, 12).

Table 42: ^{1}H NMR data of streptazolin (**303**) in $CDCl_3$ and CD_3OD.

	δ_H ($CDCl_3$)	δ_H (CD_3OD)	δ_H ($CDCl_3$)[349]	δ_H ($CDCl_3$)[350]
1-H_2	3.25-3.32 (m)	3.31-3.43 (m)	3.36-3.43 (m)	3.35 (m)
2-H_2	2.38, 2.13 (m)	2.48, 2.24 (m)	2.48, 2.20 (m)	1.8-2.7 (m)
3-H	5.92 (m)	6.11 (m)	6.02 (m)	5.90 (m)
5-H	4.65 (s)	4.75 (s)	4.85 (s)	4.79 (s)
6-H	4.18 (d, *J* = 7)	4.35 (d, *J* = 7)	4.29 (d, *J* = 7)	4.25 (d,*J* = 7)
7-H	4.60 (d, *J* = 7)	4.68 (d, *J* = 7)	4.73 (d, *J* = 7)	4.67 (d, *J* = 7)
9-H	6.08 (q, *J* = 7)	6.20 (q, *J* = 7)	6.16 (q, *J* = 7)	6.25 (m)
10-H_3	1.74 (d, *J* = 7)	1.88 (d, *J* = 7)	1.89 (d, *J* = 7)	1.84 (d, *J* = 7)

Obscurolide A1 (305): $C_{15}H_{17}NO_5$ (291.31), was obtained as pale yellow solid (12 mg) from fraction IV (6.0 g), using firstly silica gel column (CH_2Cl_2-MeOH-gradient), followed by PTLC and Sephadex (MeOH). It is a middle polar UV absorbing substance, turned to blue by anisaldehyde/sulphuric acid, yellow with Ehrlich's reagent, and brown with vanillin-sulphuric acid. – $\boldsymbol{R}_f$ = 0.42 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**CD_3OD, 300 MHz): δ = 7.83 (d, ^3J = 8.1 Hz, 2 H, 2',6'-H), 6.65 (d, ^3J = 8.1 Hz, 2 H, 3',5'-H), 5.75-6.01 (m, 2 H, 5,6-H), 4.83 (m, 1 H, 4-H), 4.31 (m, 1 H, 7-H), 4.17 (m, 1 H, 3-H), 3.07 (dd, $J_{2a,\,2b}$ = 17.5 Hz, $J_{2a,3}$ = 8.5 Hz, 1 H, 2-H_A) and 2.42 (dd, $J_{2b,\,2a}$ = 17.5 Hz, $J_{2b,3}$ = 5.0 Hz, 1 H, 2-H_B), 1.15-1.22 (dd, ^3J = Hz, ^4J = Hz, 3 H, 7-CH_3). – **(-)- ESI MS**: *m/z* (%) = 603 ([2 M + Na – 2 H]$^-$, 38), 290 ([M - H]$^-$, 100).

Obscurolide A2 (306): $C_{15}H_{17}NO_4$ (275.3), pale yellow solid (10 mg), isolated from fraction IV (6.0 g) by the same method carried out for obscurolide A1 (**305**). – R_f = 0.48 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**CD_3OD, 300 MHz): δ = 9.60 (s, 1 H, CHO), 7.68 (d, ^3J = 8.1 Hz, 2 H, 2',6'-H), 6.71 (d, ^3J = 8.1 Hz, 2 H, 3',5'-H), 5.98-5.80 (m, 2 H, 5,6-H), 4.87 (m, 1 H, H-4), 4.36-4.19 (m, 2 H, 3,7-H), 3.10 (dd, $J_{2a,2b}$ = 17.5 Hz, $J_{2a,3}$ = 8.5 Hz, 1 H, 2-CH_A), 2.51 (dd, $J_{2b,2a}$ = 17.5 Hz, $J_{2b,3}$ = 5.0 Hz, 1 H, 2-CH_B), 1.20 (d, ^3J = 6.1 Hz, 3 H, 7-CH_3). – **EI MS** (70 eV): *m/z* (%) = 275 ($[M]^{+\cdot}$, 8), 257 [M - H_2O]$^{+\cdot}$ 4), 174 (52), 147.1 (100), 132.1 (26), 118.1 (42), 91.1 (28), 43.1 (24).

Obscurolide A3 (307): $C_{15}H_{19}NO_4$ (277.3), pale oil (50 mg), was obtained from fraction IV showing the same chromatographic properties, as obscurolides A1(**305**) and A2 (**306**). **-** R_f = 0.55 ($CHCl_3$/5%MeOH). – **^{1}H NMR (**CD_3OD, 300 MHz): δ = 7.17 (d, ^3J = 8.1 Hz, 2 H, 2',6'-H), 6.61 (d, ^3J = 8.1 Hz, 2 H, 3',5'-H), 5.95-5.78 (m, 2 H, 5,6-H), 4.80 (m, 1H, 4-H),4.45 (s, 2 H, 7'-CH_2), 4.28 (m, 1 H, 7-H), 4.08 (m, 1 H, 3-H), 3.07 ($J_{2a,2b}$ dd, $J_{2a,2b}$ = 17.5 Hz, $J_{2a,3}$ = 4.5 Hz, 2-H_B), 2.42 (dd, $J_{2b,2a}$ = 17.5 Hz, $J_{2b,3}$ = 8.0 Hz, 2-H_B), 1.25-1.19* (d, ^3J = 6.2 Hz, 3 H, 7-CH_3). *split. – **(+)-ESI MS**: *m/z* (%) = 300.5 ($[M + Na]^+$).

Chartreusin (308): $C_{32}H_{32}O_{14}$ (640.60), was isolated as a greenish-yellow solid (200 mg) from fraction VII (1.2 g) by Sephadex LH-20 (MeOH), which followed by a further Sephadex LH-20 column (CH_2Cl_2/MeOH 6:4). It showed a greenish-yellow UV fluorescence, turned blue by anisaldehyde/sulphuric acid and heating, and red-dish-brown with sulphuric acid, but gave no change with NaOH. – R_f = 0.22 ($CHCl_3$/5% MeOH). – **^{1}H NMR (**[D_6]DMSO, 300 MHz): δ = 11.40 (s br, 1 H, 6-OH), 8.12 (d, ^3J = 8.1 Hz, 1 H, 7-H), 7.75 (t, ^3J = 8.1 Hz, 2 H, 2,8-H), 7.65 (d, ^3J = 8.1 Hz, 1 H, 3-H), 7.54 (d, ^3J = 8.1 Hz, 1 H, 9-H), 5.44 (d, ^3J = 3.2 Hz, 1 H, 1''-H), 5.39 (d, ^3J = 8.1 Hz, 1 H, 1'-H), 4.83 (d, ^3J = 8.1 Hz, 1 H, 2'-H), 4.51 (d, ^3J = 6.1 Hz, 1 H, 5''-H), 4.22 (d, ^3J = 6.1 Hz, 1 H, 2''-H), 4.18 (d, ^3J = 4.2 Hz, 3'-H), 4.01-3.90 (m, 2 H, 4'',5'-H), 3.73 (m, br, 1 H, OH), 3.62 (m, br, 2 H, 4',3''-H), 3.18 (s, 3 H, 3''-OCH_3), 2.83 (s, 3 H, 1-CH_3), 1.23 (d, ^3J = 6.1 Hz, 3 H, 5'-CH_3), 0.97 (d, ^3J = 6.1 Hz, 3 H, 5''-CH_3). – **^{13}C/APT NMR (**[D_6]DMSO, 75 MHz): δ = 163.8 (C_q-5), 158.5 (C_q-12), 155.4 (C_q-6), 154.1 (C_q-10), 146.2 (C_q-3a), 138.5 (C_q-10b), 138.4 (C_q-1), 132.9 (CH-2), 128.4 (CH-8), 126.1 (C_q-6a), 120.8 (CH-3), 119.6 (C_q-12b), 117.9 (C_q-10a), 117.0 (C_q-12a), 116.3 (CH-7), 114.3 (CH-9), 108.4 (C_q-12c), 99.6 (CH-1'), 99.4

(CH-1''), 96.9 (C_q-5a), 79.7 (CH-2'), 77.9 (CH-3''), 72.0 (CH-3'), 71.2 (CH-4'), 70.2 (CH-5'), 67.4 (CH-5''), 66.9 (CH-4''), 65.8 (CH-4''), 55.8 (CH_3, C3''-OCH_3), 21.6 (CH_3, C-1-CH_3), 16.4 (CH_3-6'), 16.4 (CH_3-6"). **– (+)-ESI MS**: *m/z* (%) = 1303 [2 M + Na]$^+$, 100), 663.5 ([M + Na]$^+$, 32). **– (-)-ESI MS**: *m/z* (%) = 1301.1 ([2 M + Na – 2 H]$^-$, 40), 1279.1 ([2M - H], 50), 639.7 ([M - H]$^-$, 100). **– (+)-ESI MS^2**: *m/z* (%) = 663 ([M + Na]$^+$), 503 [M - (D-digitalose) + Na]$^+$), 329 [M - (D-fucose + D-digitalose) + Na]$^+$). **– (-)-ESI MS^2**: *m/z* (%) = 639 ([M - H]$^-$, 100), 479 ([M - (D-digitalose) - H]$^-$, 58), 333 ([M - (D-fucose + D-digitalose) - H]$^-$, 76), 332 ([M - (D-fucose + D-digitalose) – 2 H]$^-$, 35).

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Lebenslauf

Am 30.01.1969 wurde ich als erster Sohn der Eheleute Fatma Mohamed El-Awady und Attia Shaaban Mahmoud in Esbett-Karam, Tanah, El-Mansoura, Ägypten geboren.

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Am 21. Januar 1999 heiratete ich die Apothekerin, Zeinab Ali Shaaban Mahmoud. Am 02. Dezember 1999 kam unsere Tochter Israa zur Welt, am 26. November 2002 unser Sohn Islam.

Am 30. November 1999 kam ich mit einem ägyptischen Stipendium nach Deutschland, und lernte für 4 Monate Deutsch (bis zu Mittelstufe zwei) im Goethe-Institut in Göttingen gelernt. Von April 2000 bis September 2004 fertige ich die vorliegende Arbeit im Institut für Organische Chemie der Universität Göttingen unter der Leitung von Prof. Dr. H. Laatsch an.

Ich besitze die ägyptische Staatsbürgerschaft.