Khaled Attia Shaaban Mahmoud

Nafisamycin, Cyclisation Product of a New Enediyne Precursor, Highly Cytotoxic Mansouramycins, Karamomycins Possessing a Novel Heterocyclic Skeleton and Further Unusual Secondary Metabolites from Terrestrial and Marine Bacteria

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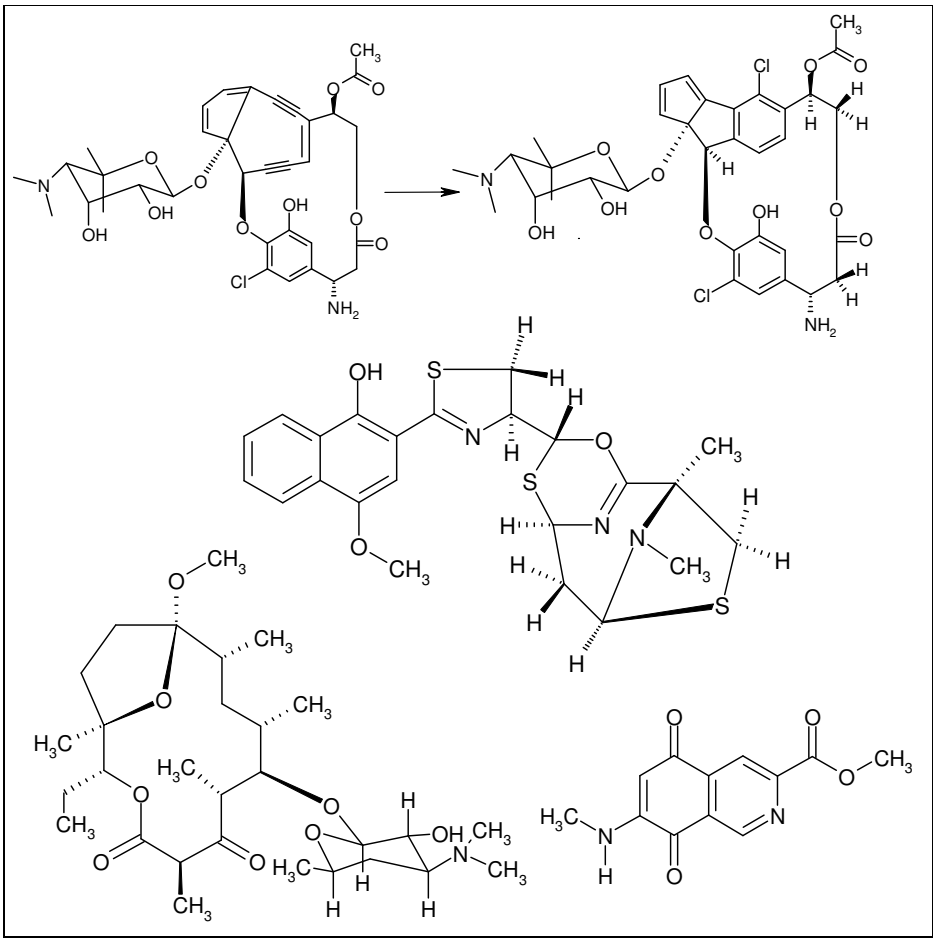


Dissertation

Nafisamycin, Cyclisation Product of a New Enediyne Precursor, Highly Cytotoxic Mansouramycins, Karamomycins Possessing a Novel Heterocyclic Skeleton and Further Unusual Secondary Metabolites from Terrestrial and Marine Bacteria

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultäten

der Georg-August-Universität zu Göttingen

vorgelegt von

Khaled Attia Shaaban Mahmoud

aus

El-Mansoura (Ägypten)

Göttingen 2008

D7

Referent: Prof. Dr. H. Laatsch

Korreferent: Prof. Dr. A. Zeeck

Tag der mündlichen Prüfung:

Die vorliegende Arbeit wurde in der Zeit von Januar 2005 bis Dezember 2008 im Institut für Organische Chemie der Georg-August-Universität zu Göttingen unter der Leitung von Herrn Prof. Dr. H. Laatsch angefertigt.

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 Tochter Rawan

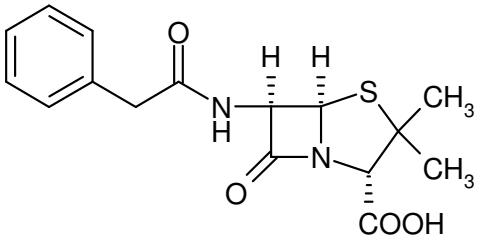
1 Introduction

Natural materials play an essential role in the application of bioactive materials with medicinally useful properties since thousands of years, however, the science of isolating and elucidating their active constituents is not older than 150 years. Natural products are defined as chemical compounds, isolated/derived from living organisms (e.g. plants, animals and microorganisms). Predominantly these compounds are secondary metabolites[1]. So, chemistry of natural products is principally related to the biosynthesis, isolation, and structure elucidation of new products obtained from nature.

Medicinal plants were and are still in use as folk medicines, especially in the developing countries, for treatment of different diseases. The World Health Organization (WHO) estimated that 80% of the earth inhabitants mainly depend on traditional medicines for their health care[2]. Plants have been the roots of the traditional medicine that has existed for thousands of years starting from the first records about 2600 BC. Some of these plants are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation[3].

According to WHO, more than 17 million lives are lost yearly due to the high number of infectious diseases (e.g. AIDS, hepatitis, Ebola, SARS), cancer and drug resistance phenomena[4-6]. Therefore, there is worldwide attention to search for new sources of bioactive constituents, as most medicinal plants (~97%) have been totally investigated. So, many other natural sources were being taken into consideration, especially those located in marine habitats e.g. soft corals, sponges, jellyfishes, algae, and microorganisms. Microorganisms are living symbiotically or located in sediments and represent a huge source of diverse bioactive secondary metabolites. Such diversity increases the possibility of structural novelty as well as bioactivity.

Penicillin (**1**), one of the first antibiotics from microorganisms, was discovered in 1928 by Fleming, showing a bactericidal activity against the *Staphylococcus* sp. For this great discovery, Fleming, Chian and Florey were honoured with the Nobel Prize in 1945 [7]. Since the discovery of penicillin (**1**) and mycophenolic acid (**2**) before[8], microorganisms, especially bacteria, played an essential role in the production of antibiotics and other drugs for the treatment of current diseases[9, 10].



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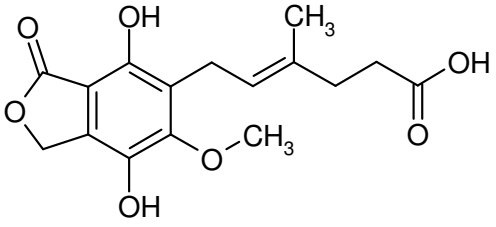

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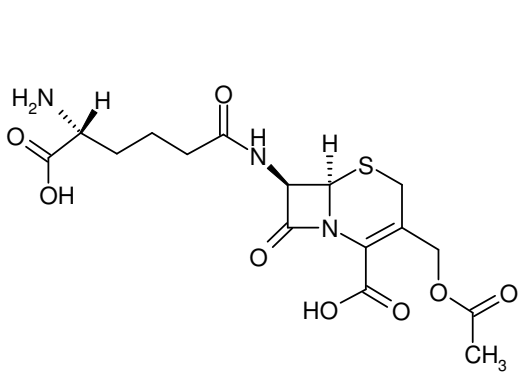
1.1 History of Antibiotics

The development of penicillin by Florey and his colleagues opened the door to the "Golden Age of Antibiotics" that has dominated medical practice for several decades. It is an active agent produced by *Penicillium notatum* and has inhibitory effect against Gram-positive bacteria, including the disease-causing species from the two genera *Streptococcus* and *Staphylococcus.* A post penicillin hunt for further antibiotics began in the 1940s and resulted eventually in the identification of thousands of microbial metabolites with a wide array of biological properties. Till the mid eighties, almost all groups of important antibiotic were discovered: the antibacterial cephalosporin C (**3**), streptomycin, tetracyclines, erythromycin A (**4**), vancomycin (**5**), the antifungal amphotericin B (**6**), imidazoles, griseofulvin, strobilurins, the antiviral acyclovir (**7**), vidarabine and many other compounds that play a role in therapeutics and agriculture[11].

The Greek-derived word *antibiose* had been coined to describe antagonistic effects between microorganisms. According to Waksman (1941), an antibiotic was defined as a "secondary metabolite, produced by microorganisms, which has the ability to inhibit the growth and even to destroy bacteria and other microorganisms in a very low concentration"[8]. Not all the isolated secondary metabolites act as antibiotics, many of them serve as plant growth factors and enzyme inhibitors[12] and as self regulating factors in some bacteria (e.g. A-Factor; γ-butylrolactone)[13]. The virginiae butanolides induce the production of virginamycin in *Streptomyces virginiae*, others promote pigment[14] or spore formation, and some are cytotoxic or enzyme inhibitors.

Zähner *et al.* proposed the existence of a "playground" of secondary metabolism, which is closely connected to five distinct primary cellular levels. These five levels are: intermediary metabolism, regulation, transport, differentiation and mor-

phogenesis[15]. As a result, the definition of secondary metabolites is not confined to antibiotics but should be extended to all those metabolites, which regulate all physiological and biochemical activities in the life cycle of organisms[1]. It is logically therefore to understand secondary metabolites as 'words' in a language, where chemo-ecology is the grammar[16].



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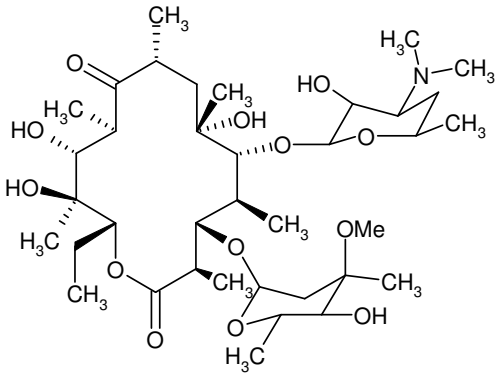

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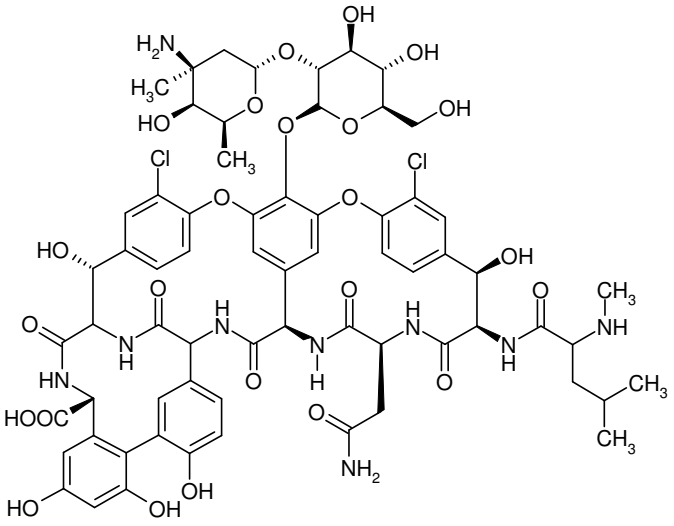

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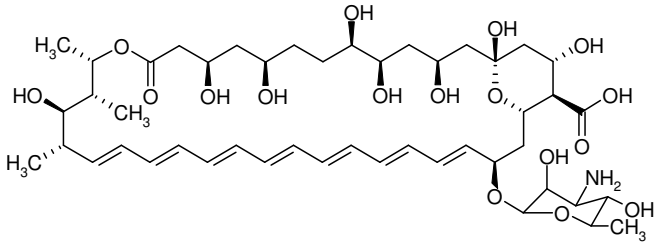

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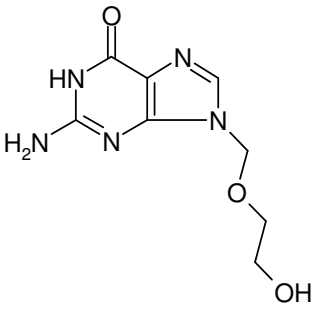

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1.2 A Search for New Therapeutically Useful Natural Products

The escalation of clinical resistance, multi-drug resistance (MDR), new pathogens, viral diseases *etc.,* represent serious problems, which cost millions of lives on earth. Moreover, β-lactam antibiotics had been used as the first defence line against the pathogenic bacteria until the emergence of β-lactamases. Consequently, a search for β-lactamase-resistant drugs became vital.

Throughout the years, extensive chemical programs were developed worldwide to synthesize bioactive compounds and to understand their mode of action. Such increasing need for drugs directed a number of scientists to search in the oceans for other natural sources.

1.3 Screening Methods for New Natural Products

In order to discover new bioactive compounds, crude extracts can be evaluated by chemical screening or by various biological or pharmaceutical screening approaches. The latter can be focused on bioactive substances of greater sensitivity using High-throughput-Screening (HTS)[17]. However, novel compounds, which may be active against other targets, are overlooked. To overcome this problem, Zähner and many other researchers began to investigate systematically chemical screening methods in the 1980s[18]. The chromatographic characteristics of metabolites using TLC plates, as well as their chemical reactivity towards staining reagents under defined reaction conditions, allows visualising an almost complete fingerprint of a secondary metabolite pattern[19]. Using this method led to the isolation of all metabolites firstly and then testing of these compounds later for biological activity. In such a way, known compounds were often re-isolated.

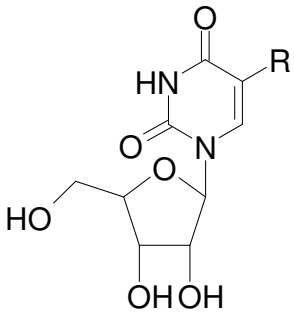
Recently, sensitivity of MS and NMR instruments was increased. Moreover, a considerable number of diverse natural products databases combined with HPLC/DAD, HPLC/CD, HPLC/MS, HPLC/NMR/MS or GC/MS systems and TLC scanners was designed. So, researchers are now able to distinguish the known from the new molecules directly in crude extracts. Accordingly, the avoidance of known compounds from isolation will be enhanced. Furthermore, microbiologists developed a PCR-based screening assay (polymerase chain reaction) for genes e.g. polyketide synthases, non-ribosomal polypeptide synthases (NRPSs), dNDP-glucose dehydra-

tase or halogenases[20]. So, the screening efficiency for secondary metabolites from bacteria has been strongly enhanced.

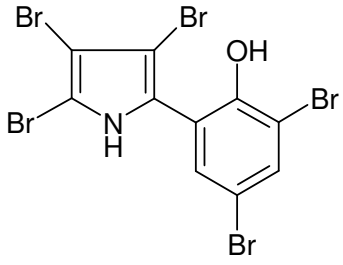
1.4 Marine Environment as Novel Source for Bioactive Metabolites

The Oceans, which cover almost 70% of the earth's surface and over 90% of volume of its crust[10,21], contain a variety of species belonging to 36 phyla, of which 34 were originally located in oceans. In contrast, only 17 phyla are present in the terrestrial environment[22].

It is only sixty years ago that the first marine antibiotic, cephalosporin C (**3**) (1948), was isolated by Giuseppe Brotzu from the fungus *Cephalosporium acremonium*. In the early 1950s, Bergmann and his coworkers isolated uridine (**8**) and thymidine (**9**) [23], the first naturally occurring nucleosides, from a marine sponge. Later, Burkholder and his cooperators had isolated pentabromopseudiline (**10**), as first marine bacterial metabolite from the bacterium *Pseudomonas bromoutilis*[24]. Pentabromopseudilin (**10**), a highly brominated pyrrole antibiotic, containing more than 70% of the molecular weight as bromine[24-26]. Because of its high toxicity, compound **10** could not used as medicament. In contrast to **10**, the chlorine analogue **11** was isolated in 1978 from the terrestrial *Actinoplanes* strain ATCC 33002[27]; it is a fascinating example of co-evolution.



8: R = H, **9:** R = CH_3



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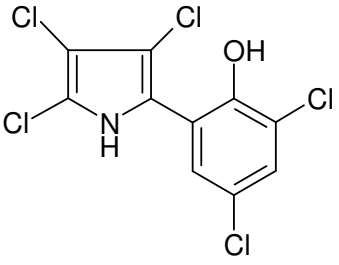


11

The systematic investigations of marine environment as sources of novel biologically active agents began intensively in the mid 1970s. Among the phyla in the oceans, bacteria, fungi, algae, sponges, coelenterates, sea hares, bryozoans, tunicates and nudibranchs have been studied[28,29]. There is, however, a continual increasing of the reported new compounds from marine sources since 1965 (Figure 1). Sponges followed by coelenterates were the most studied marine organisms. Since that time,

the share of marine sources has not lost its significance and stayed more or less in a constant level. In contrast, sponges have lost the interest of the natural products' scientists (Figure 2, A~C). The bioactivity profiles of marine metabolites include neurotoxic, antiviral, antitumor, antimicrobial or cytotoxic properties and are of considerable biotechnological interest.

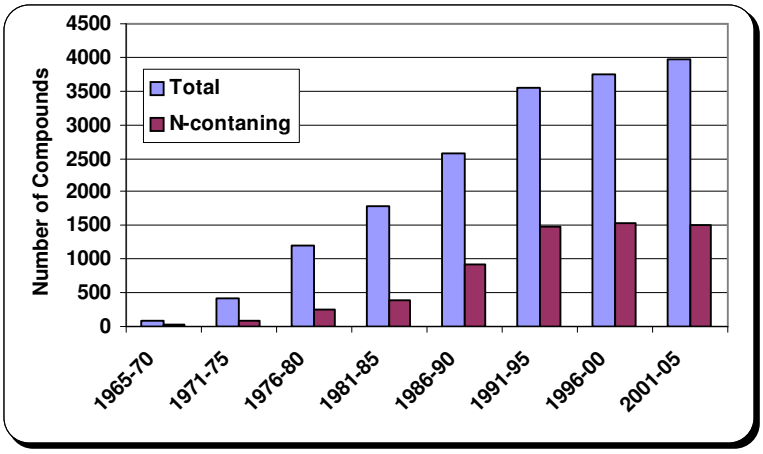


Figure 1: Numbers of marine natural products for the period 1965–2005.

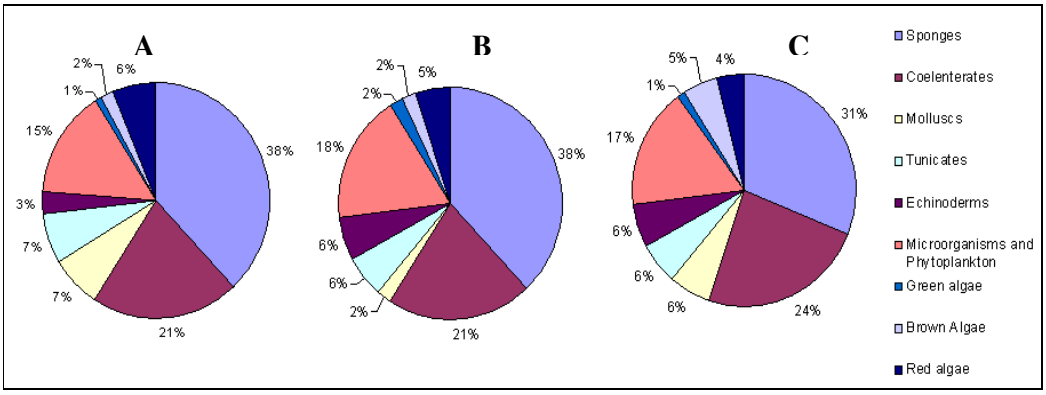
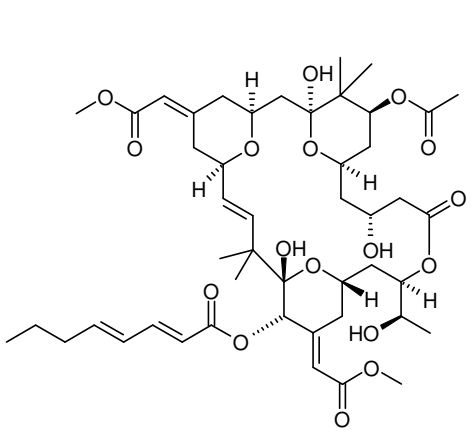


Figure 2: Percentage of isolated compounds from different phyla till 2001 (**A**), 2002 (**B**) and 2003 (**C**)[28], modified by Blunt *et al.* [30].

1.4.1 Marine Secondary Metabolites of Interesting Activities

Many of the marine derived anti-inflammatory, neurotoxic and antitumor compounds had been included in clinical research trials. *Conus* venoms are highly constrained peptides of 10-30 amino acids in length. This peptide groups target nicotinic acetylcholine receptors, voltage-sensitive calcium channels and sodium channels[31]. Bryostatins (*e.g.* bryostatin 1, **12**), macrocyclic metabolites, were isolated from the

bryozoans *Bugula neritina*[32], blocking the protein kinase C, (**12**) or the synthesis of macromolecules. Bryostatins also isolated from *Amathia convulata*[33], were used as a partial antagonist of protein kinase C (PKC), so that bryostatin 1 (**12**) is currently in phase II clinical trials.



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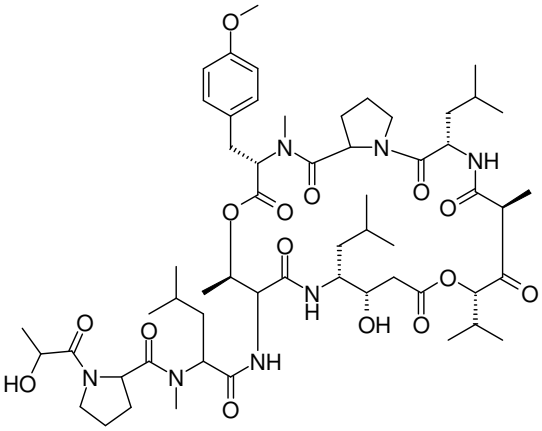
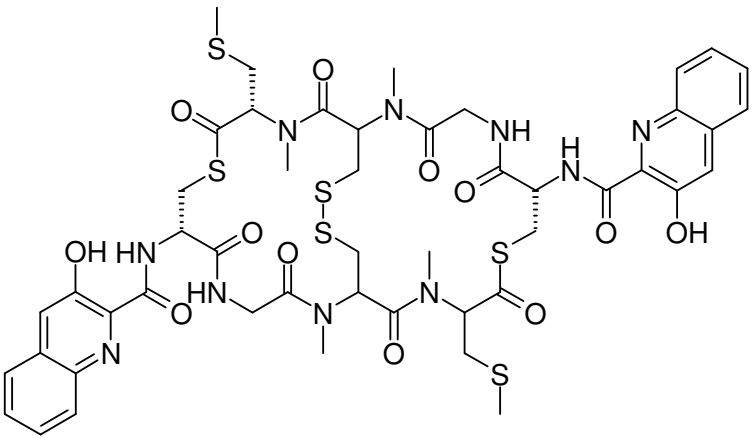


13

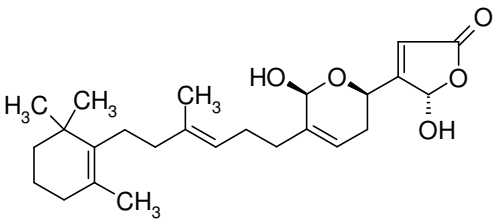
Didemnin B (**13**), a depsipeptide, was isolated from the Caribbean tunicate *Tridemnum solidum*[34], inhibiting the synthesis of RNA, DNA and proteins in various cancer cell lines. It shows anti-viral and immunosuppressive activities as well as being an effective agent in treatment of leukaemia and melanoma. However, due to its toxicity, it was withdrawn from phase II clinical trials[35,36].

Thiocoraline (**14**), a novel depsipeptide was isolated (1997) from the mycelial extract of the bacterium *Micromonospora marina,* associated with a marine soft coral in the Indian Ocean. Thiocoraline (**14**) showed potent cytotoxic activity at a nanomolar concentration against several tumor cell lines. It inhibits DNA polymerase[37, 38]. It is currently in preclinical phase by PharmaMar.



14

Manoalide (**15**), a sesterpenoid, was isolated from the sponge *Luffariella variabilis*[39], inhibiting irreversibly the release of arachidonic acid from membrane phospholipids. Subsequently, it inhibits the inflammatory reactions[40]. The work on this compound was however, discontinued in phase II clinical trial due to formulation problems[38].



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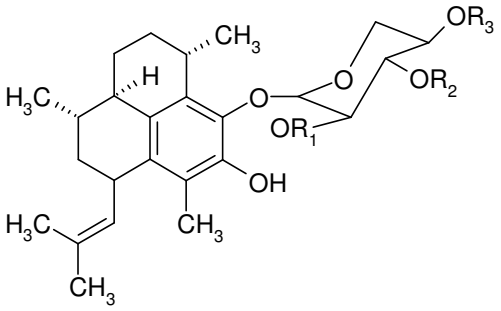
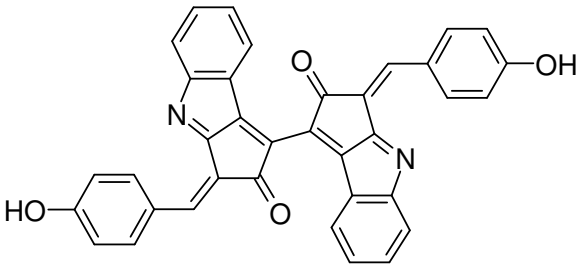


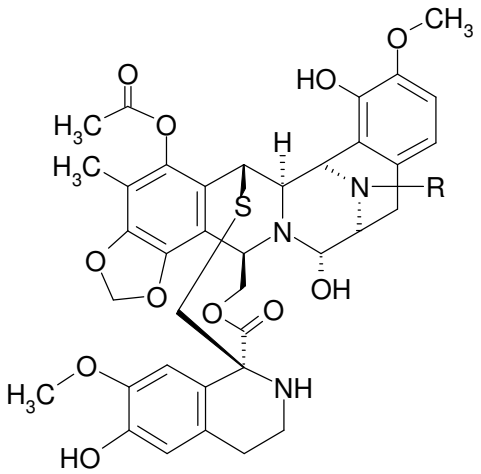
16: $R_1 = R_2 = R_3 = H$
17: $R_1 = COCH_3, R_2 = R_3 = H$
18: $R_1 = R_3 = H, R_2 = COCH_3$
19: $R_1 = R_2 = H, R_3 = COCH_3$

Pseudopterosins A-D (**16-19**), tricyclic diterpene glycosides, were isolated from the dinoflagellate symbiont *Sympoidinium* sp. localized within the tissues of the Caribbean Sea whip *Pseudopterogorgia elisabethae*. They possess *in vivo* anti-inflammatory and analgesic activities[42] and were used as additive to prevent irritation caused by exposure to sun or chemicals (i.e. cosmetic) with name Resiliene®. Finally, scytonemin (**20**) was isolated from the sheath of many cyanobacteria[43], and was recently patented as anti-inflammatory agent.

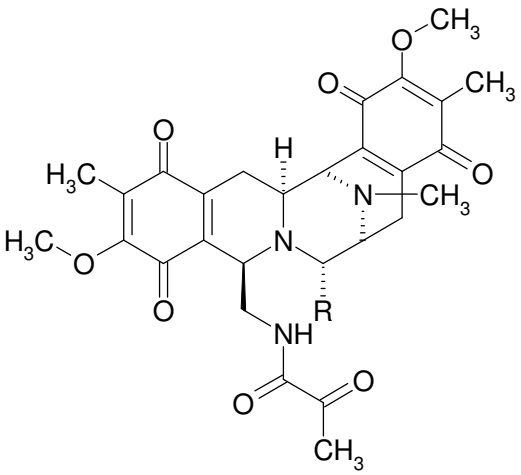


20

The low amounts produced from the above mentioned compounds as well as the striking structural similarities between some pharmaceutically active agents and known microbial metabolites addressed the question about their biosynthetic origin. Inspection of structural features of ecteinascidin-743 (ET-743; **21**) and ecteinascidin-729 (ET-729; **22**) from tunicate reveals similarities to saframycins A (**23**) and B (**24**) isolated from *Streptomyces lavendula*[44] and safracin isolated from *Pseudomonas fluorescens*[45]. Such observation represents one of several clues on the microbial origin of these chemicals[47]. Ecteinascidin-743 (**21**) and saframycin B (**24**) are examples on the structural similarities between invertebrate metabolites and microbial compounds.



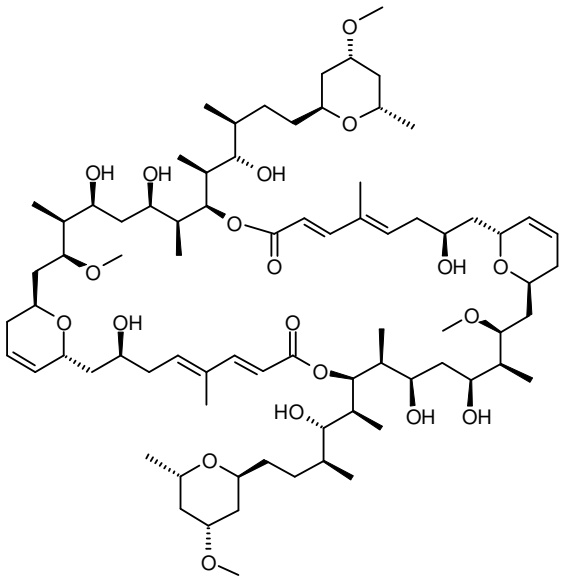
21: R = CH_3, **22:** R = H



23: R = CN, **24:** R = H

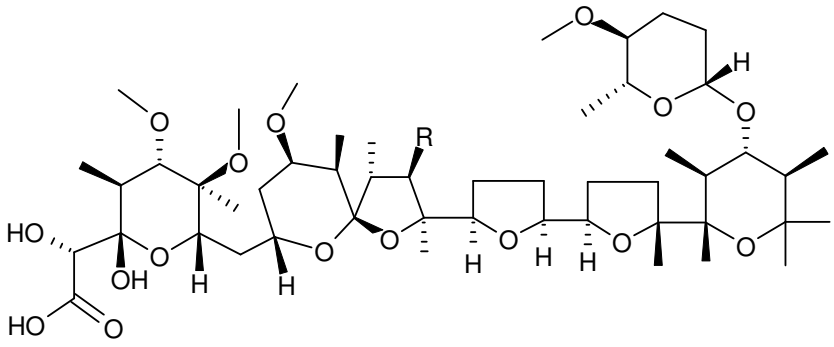
Bacteria are regularly observed in unique microhabitats on surfaces and internal spaces of marine invertebrates. The cytotoxic macrolide swinholide A (**25**), isolated from the sponge *Theonella swinhoei*, was found to be produced by the symbiotic unicellular bacteria inhabiting the endosome of this sponge[48]. Sponge bacterial associations are probably the most thoroughly described. Several studies showed that

the associated bacteria could be distinct from those in the surrounding sea water (specific association)[49].



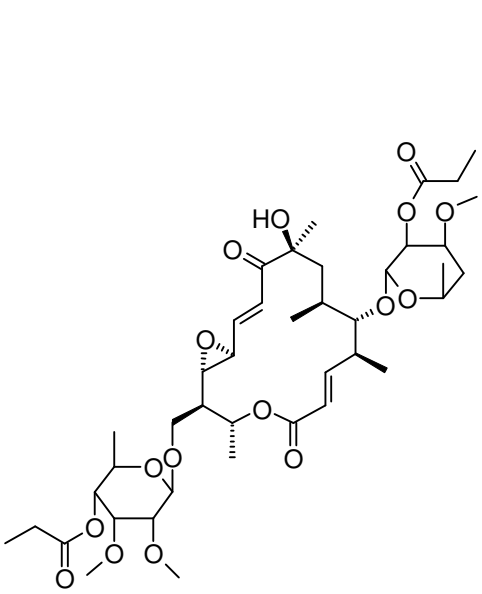
25

CP-96,797 (**26**)[50] a polyether structural analogue of K-41A (**27**)[51], was produced by the *Streptomyces* sp. ATCC 55028, exhibiting an activity against Gram-positive bacteria. The absolute stereochemistry of **26** was determined by X-ray crystallography.



26: R = H, **27:** R = OCH_3

The culture broth of the marine isolate *Streptomyces* sp. B7064 was a source for the new macrolide, chalcomycin B (**28**) in our research group. Compound **28** exhibited strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* with MIC values of 0.39 μg/ml, >50 μg/ml, and 6.25 μg/ml, respectively[52].



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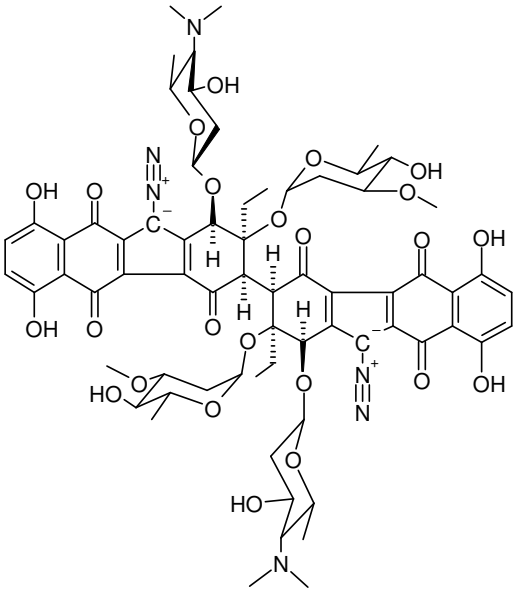
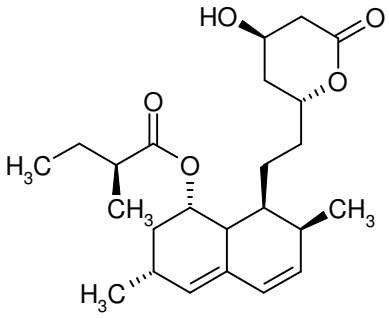


29

Lovastatin (**30**), an interesting polyketide, was delivered from *Aspergillus terreus*[53]. Lovastatin (**30**) experienced a wide use as a cholesterol lowering agent (Mevacor®). The brevetoxins (e.g. brevetoxin A, **31**)[54] were produced by *Gymnodinium breve* (or *Ptychodiscus brevis*, red tide organisms). While they are potent ichthyotoxins against fish, brevetoxins do not present any significant human toxicity. These compounds bind to the sodium channel and cause persistent activation, increasing sodium ion flux, and depolarisation of excitable cells.



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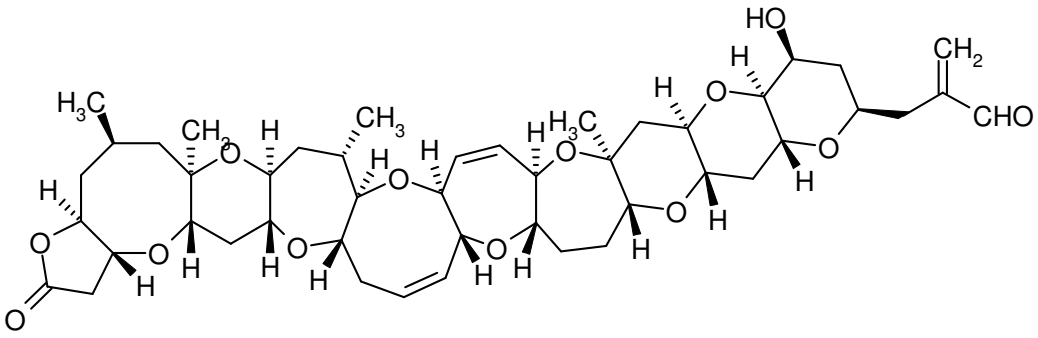


31

A rare actinomycete was isolated from sea squirts, *Micromonospora lomaivitiensis* producing lomaiviticin A (**29**) and B. They are antibiotics, exhibiting high activities against *Staphylococcus aureus* at 10 pg/mL[55] besides their high antitumor activities against a number of cell lines.

It was remarked that symbiotic and endophytic microbes, associated with terrestrial and marine macroorganisms, were mostly responsible for metabolites in their hosts. Accordingly, it is suggested that many natural marine chemicals are not produced by the source invertebrate, but rather attributed to symbiotic microorganisms living within the tissues of the invertebrate. The studies on these associated bacteria have a considerable meaning, because it is easier to get mass production from cultures of microorganisms than those from macroorganisms.

1.4.2 Marine Bacteria as Prolific Source of Natural Products

The oceans are massively complex and consist of diverse assemblages of life forms. The water column of the oceans contains approximately 10^6 bacterial cells per ml[56]. Marine bacteria and other marine microorganisms develop unique metabolic and physiological capabilities. These capabilities enable them to survive in extreme habitats and to produce compounds that might not be produced by their terrestrial counterparts. Since 1990, the number of bioactive metabolites from marine bacteria has exponentially increased (Figure 3)[10,28, 30,57-59].

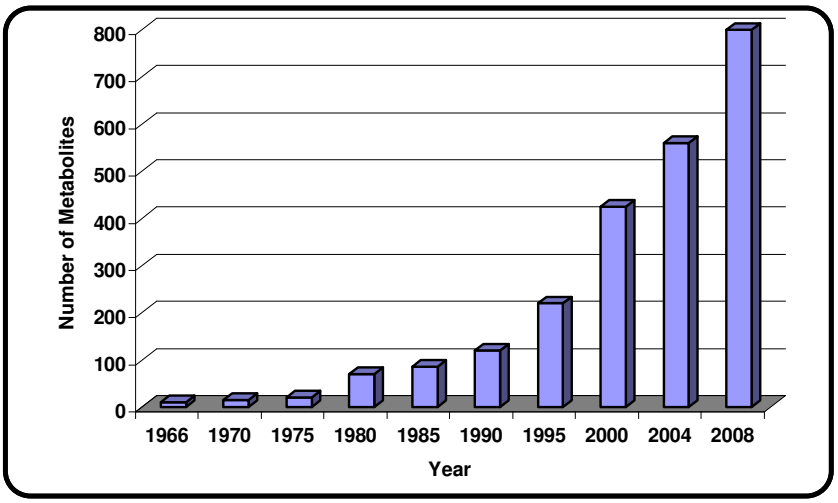


Figure 3: Annual increase in the number of marine bacterial metabolites, according to Laatsch[60] and AntiBase[61].

Biological activity, a second point of comparison, is classified into eight areas. They are anticancer, antibiotic (including antibacterial, antifungal and antimalarial), anti-inflammatory, antiviral, immunomodulatory, agricultural, methodology and other. Figure 4 shows the types of testings carried out up to 2005, suggestive that the "catchall" category of methodology was increasing. Testing for anticancer activity remains one of the least used assays. Increasingly results are appearing in the literature for antimalarial, antitubercular and antiinfective assays against drug-resistant microorganisms. There can be no doubt that there is an urgent need for new therapeutics in those areas, but also in agricultural areas where resistance to the standard anthelmintics is becoming a serious problem[59].

The search for new bioactive chemicals from marine organisms resulted in the isolation of about 10000 metabolites [62], many of which are potential biomedicals. These agents show a broad spectrum of biological activities. Up to now, bioactive agents were isolated extensively from *Streptomyces*, *Alteromonas/Pseudoalteromonas, Bacillus, Vibrio, Pseudomonas,* and *Cytophaga* (Figure 5). These microorganisms were isolated from seawater, sediments, algae and marine invertebrates. They are able to produce quinones, polyenes, macrolides, alkaloids, peptides and to a lesser extent terpenoids.

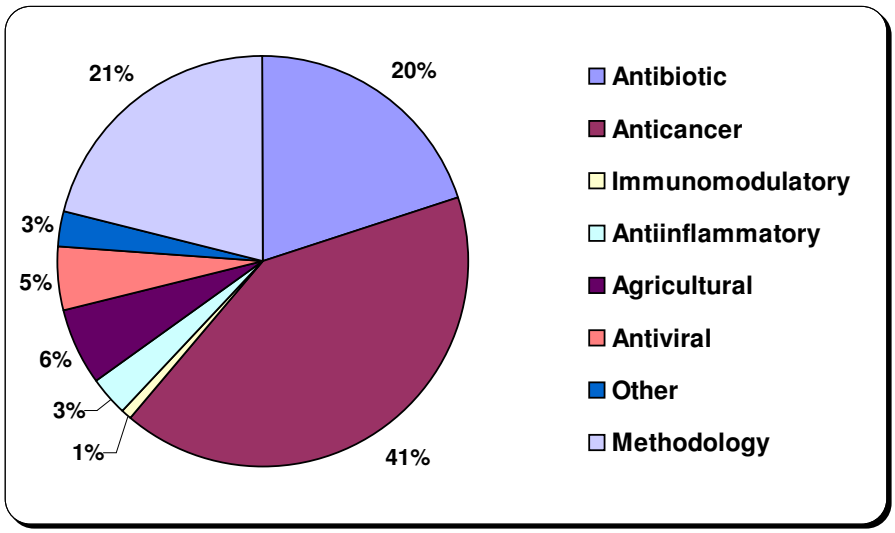


Figure 4: Reported distribution of biological testing carried out on marine natural product extracts and isolated compounds to 2004.

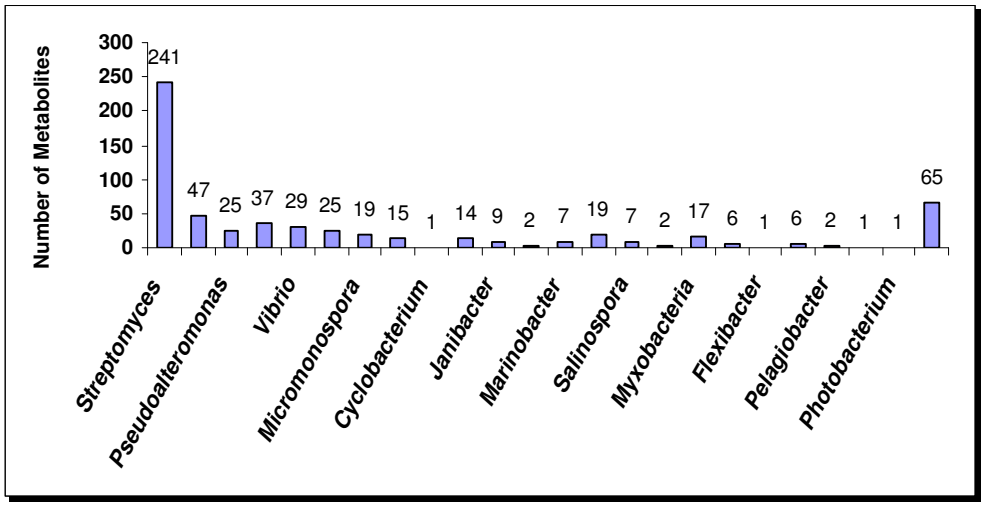
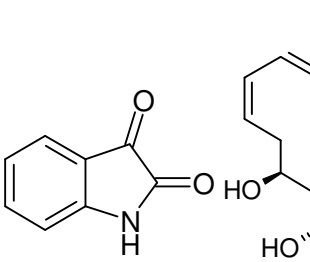


Figure 5: Number of secondary metabolites isolated from some marine bacteria according to their taxonomic origin since 1966 till 2004[60].

The pioneering work of Okami and co-workers represents the first building unit in the knowledge of the chemistry of marine derived bacteria, in particular of Actinomycetes. They have reported the isolation of a benzanthraquinone antibiotics SS-228Y (**34**) and SS-228R (**35**) from the actinomycete *Chainia purpurogena*[63]. On othe ther hand, they isolated istamycins A (**36**) and B (**37**) antibiotics from *Streptomyces tenjimariensis.* Isatin; 3,2-indolinedione (**32**), as one of the early marine metabolites, was produced by a bacterium colonizing the surface of the embryos of the

shrimp *Palaemon macrodactylus.* It is responsible for the protection of eggs against the pathogen fungus *Lagenidium callinectes*[64]. Macrolactin A (**33**), a new macrolide, having antibacterial, antiviral and cytotoxic activities, was isolated from an unidentified unicellular deep sea bacterium[65].



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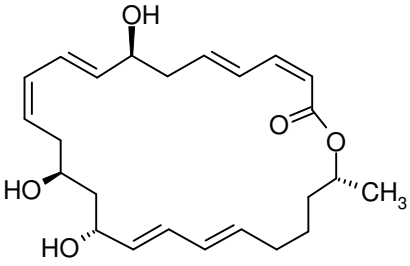

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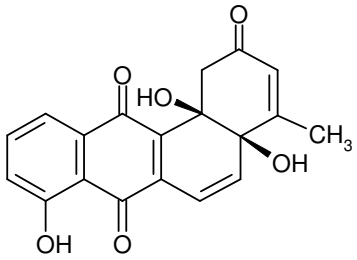

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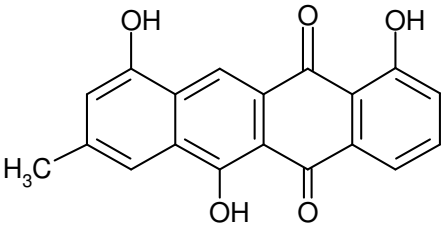

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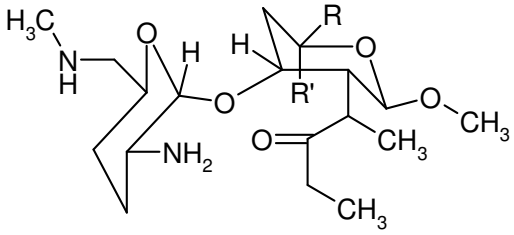
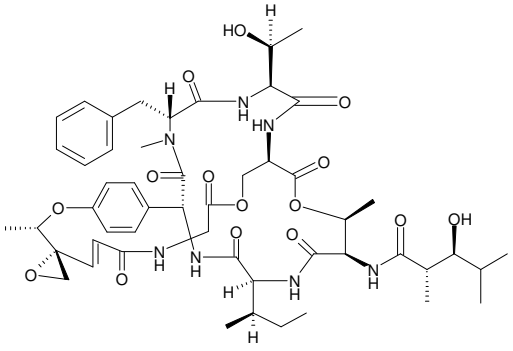


36: R = H, R' = NH_2; **37:** R = NH_2, R' = H



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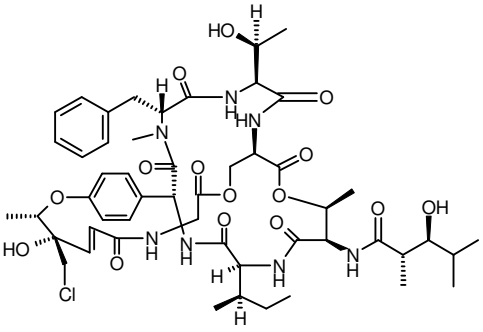
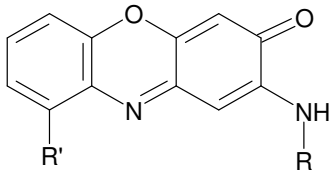


39

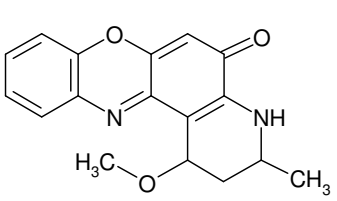
Two bicyclic depsipeptides, salinamide A (**38**) and B (**39**), were produced by a *Streptomyces* sp., isolated from the surface of the jellyfish *Cassiopeia xamachana.* They are potent topical anti-inflammatory in chemically induced mouse ear oedema assays[66,67].

1.4.2.1 Newly Described Metabolites from Marine Bacteria

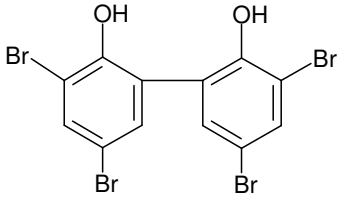
Since the beginning of this century nearly 250-300 marine bacterial compounds have been described. Interestingly, within the same period the number of described metabolites produced by terrestrial bacteria did not exceed 150 compounds (Laatsch, pers. Comm.). Nearly 100 marine compounds from bacterial origin were isolated within the year 2004, most of them are belonging to actinomycetes. For example, chandrananimycins A-C (**40a-c**), novel anticancer and antibacterial agents, were isolated in our research group from *Actinomadura* sp.[68].



40a: R = $COCH_3$, R' = OH;
40b: R = $COCH_2OH$, R' = H



40c



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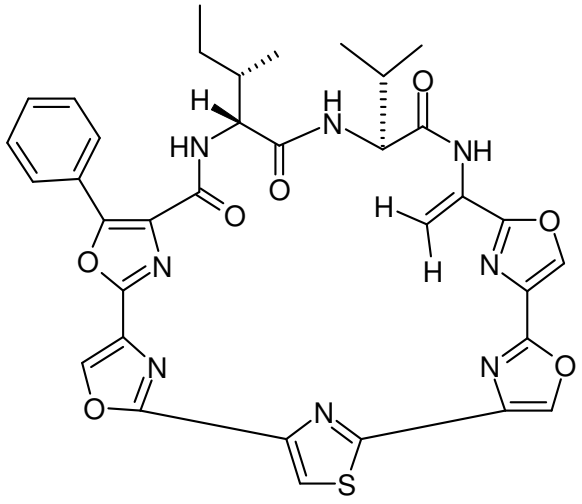
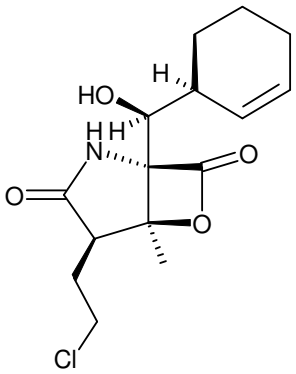


42

MC21-A (**41**), a brominated anti-MRSA, was isolated from the new species *Pseudoalteromonas phenolica*. It rapidly permeabilizes the cell membranes of MRSA, while it has no lytic activity against bacterial cells or human erythrocytes[69]. Mechercharmycin A (**42**), a cyclic-peptide, was recently isolated from the bacterium

Thermoactinomyces sp. and shows a cytotoxic activity against human lung carcinoma and human leukaemia[70].

Salinosporamide A (**43**), a highly cytotoxic proteasome inhibitor, was isolated from *Salinospora* sp. representing one of the remarkable studies in this century. This new genus belongs to a group of rare obligate marine actinomycetes isolated from the ocean sediments[71]. The cytotoxic activity of 48 against human colon carcinoma, was attributed to the inhibition of the 20S proteasome[72]. Moreover, sporolides A-B (**44**, **45**), halogenated macrolides, were isolated from *Salinospora tropica*[73].



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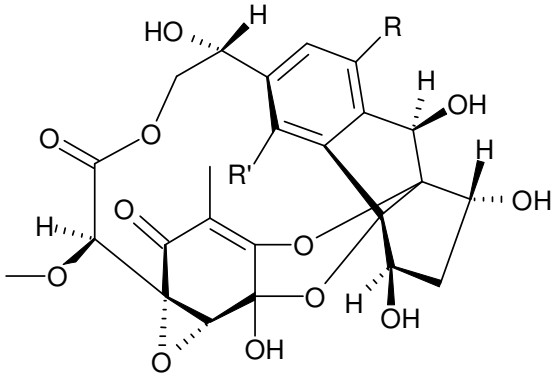
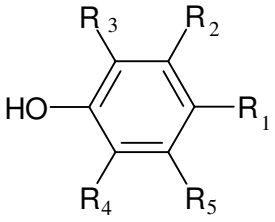
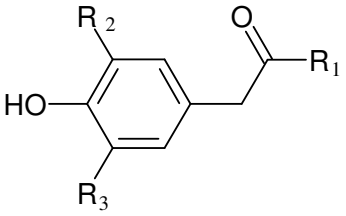


44: R = H, R' = Cl; **45:** R = Cl, R' = H

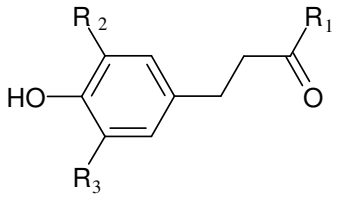
The marine *Salegentibacter* sp. T436 from the Eastern Weddell Sea delivered 20 nitro-derivatives, classified into mono/dinitro-4-hydroxy-phenyl and/ or mono/dinitro-genistein derivatives (**46a~53**)[74-77]. Most of the 20 compounds exhibited an activity against Gram-positive bacteria.

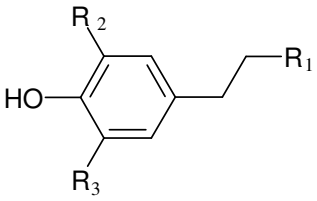


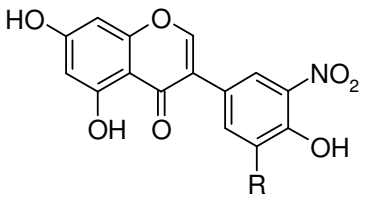


46a: $R_1 = NO_2$, $R_2 = R_3 = R_4 = R_5 = H$
46b: $R_1 = COOH$, $R_2 = R_3 = R_5 = H$, $R_4 = NO_2$
46c: $R_1 = NO_2$, $R_2 = R_5 = H$, $R_3 = NO_2$, $R_4 = OCH_3$
46d: $R_1 = R_2 = NO_2$, $R_3 = R_4 = H$, $R_5 = OCH_3$

47a: $R_1 = OCH_3$, $R_2 = NO_2$, $R_3 = H$
47b: $R_1 = OCH_3$, $R_2 = R_3 = NO_2$
47c: $R_1 = OH$, $R_2 = NO_2$, $R_3 = H$
47d: $R_1 = OH$, $R_2 = R_3 = NO_2$



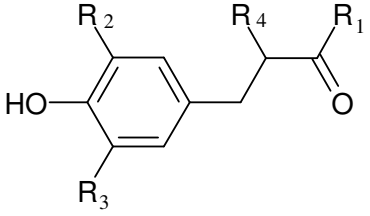


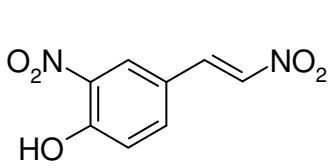


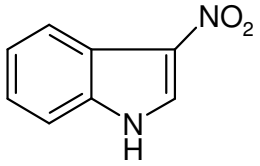
48a: $R_1 = OCH_3$, $R_2 = R_3 = NO_2$
48b: $R_1 = OH$, $R_2 = NO_2$, $R_3 = H$
48c: $R_1 = OH$, $R_2 = R_3 = NO_2$

49 a: $R_1 = OH$, $R_2 = NO_2$, $R_3 = H$
49b: $R_1 = Cl$, $R_2 = R_3 = NO_2$
49c: $R_1 = OH$, $R_2 = NO_2$, $R_3 = H$

50a: $R = NO_2$
50b: R = H







51a: $R_1 = OCH_3$, $R_2 = R_3 = NO_2$, $R_1 = Cl$
51b: $R_1 = OCH_3$, $R_2 = NO_2$, $R_3 = H$, $R_4 = OH$

52

53

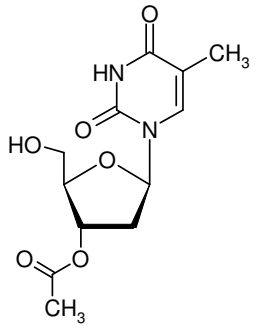
Despite of the high interesting metabolites delivered from marine derived bacteria, several restrictions are present: a) less than 5% of marine bacteria isolated from marine samples are amenable to be cultured on the bases of normal microbiological techniques[78], b) taxonomy of the marine bacteria is very poorly defined, c) The afforded yields of bacterial extracts are very low, and in some cases, they are below 1 mg/litre. To overcome such complications, microbiologists developed PCR-based screening assays. This technique might be able to increase the screening efficiency for bioactive compounds. Furthermore, the knowledge of genes involved in the biosynthesis of secondary metabolites has been enlarged. Consequently, understanding of different biosynthetic systems (e.g. polyketide synthetases (PKS), nonribosomal polypeptide synthetases (NRPSs), halogenases) allows new approaches, such as combinatorial biosynthesis. Such insight led to the discovery of the bacterial origin of bryostatins[79]. Additionally, novel antibiotics have been explored as the discovery of recent numerous techniques are able to elucidate the metabolites structures much easier[80,81].

1.4.2.2 Marine Metabolites from North Sea Bacteria

The German North Sea is a special ecological area due to the dynamic tidal water. Therefore, its microbiological and chemical features might be differ from those of other marine environments[82]. Screening of numerous crude extracts of North Sea

bacteria on the bases of agar diffusion and toxicity tests against brine shrimps as well as human cell lines showed significant activities. Structures of most of the isolated metabolites were, however, not complex and seem to be derived from the amino acid pathways.

The isolation of a new nucleoside, 3´-acetoxy-2´deoxythimidine (**54**), from strain Bio134, and of the plant metabolite isoxanthohumol (**55**) from the bacterium Pic009 were reported in our research group[83]. The latter (**55**) is known for its anti-carcinogenic and antifungal properties. Moreover, two new antibacterial agents, quinoline-2-one-4-carboxylic acid methyl ester (**56**) and 3-pyridinecarboxamide (**58**) were obtained from the strain Hel59b[83]. Two new indole alkaloids, 3,3-bis-(3-indolyl)-butan-2-one (**59**) and 3,3',3"-trisindolyl-methane (**60**) were also reported from *Vibrio parahaemolyticus* Bio249 by our group[84].



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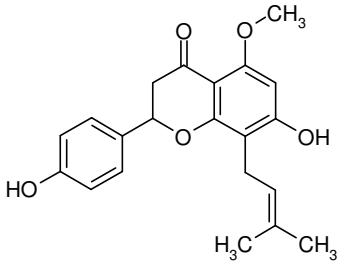

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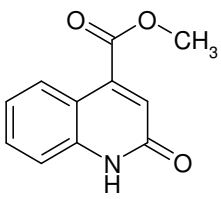

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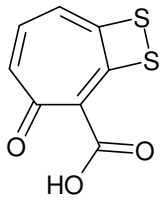

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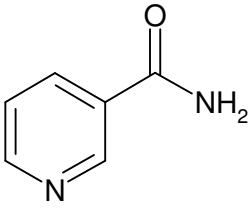

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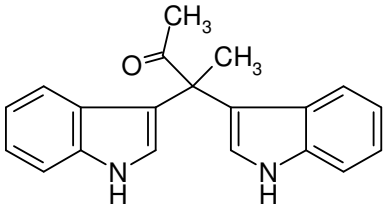

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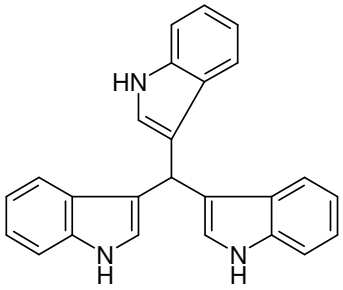
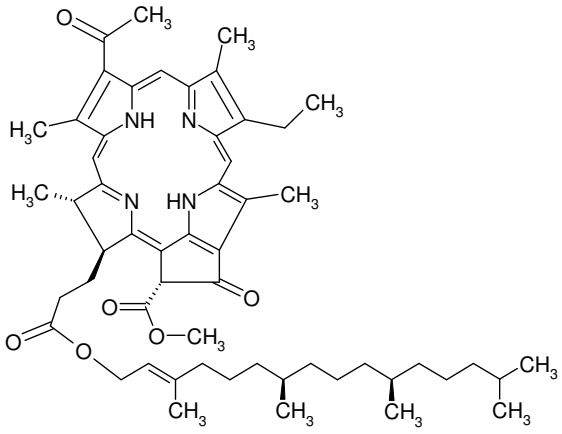


60

Zeeck and his co-workers[82] reported the isolation of tropodithietic acid (**57**), a sulphur containing compound. This was together with 3-(4´-hydroxyphenyl)-4-phenylpyrol-2,5-dicarcoxylic acid (**62**) and 3,4-di(4´-hydroxyphenyl) pyrrole-2,5-

dicarcoxylic (**63**) from the strain RK377. Bacteriopheophytin a_L (**61**) was isolated by the same research group for the first time from a marine bacterial strain.



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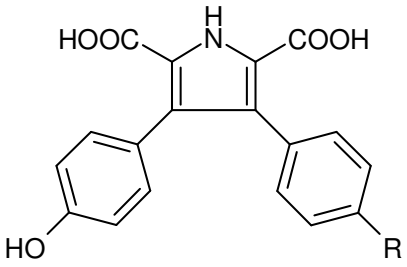
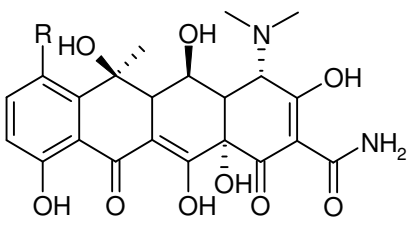


62: R = H, 63: R = OH

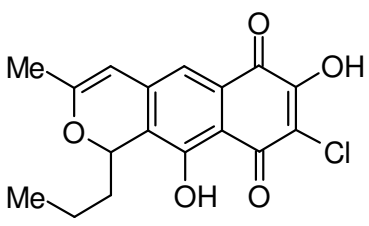
1.5 Importance of Bacteria for the Development of Drug Research

Several classes of newly developed drugs currently on the market, came originally from bacteria. Erythromycin A (**4**)[85] e.g. is the main component of the erythromycin group of macrolide antibiotic produced by *Streptomyces erythreus*. Erythromycin A (**4**) is used mainly against Gram-positive bacterial infections, particularly caused by penicillin-resistant bacteria.

The tetracyclines **64**, **65**[86] are broad-spectrum antibiotics and were produced by different *Streptomyces* species. They have activity against both Gram-positive and Gram-negative bacteria. A member of the related anthracycline group is doxorubicin (**67**)[87], a widely used antitumor agent, which is active particularly against solid tumors. Amphotericin B (**6**)[88], a polyene antibiotic, is produced by *Streptomyces nodosus*, and is used primarily as an antifungal agent. Chloroquinocin (**66**) is a novel chlorinated naphthoquinone antibiotic isolated from *Streptomyces* sp. LL-A9227. It exhibits a moderate *in vitro* activity against Gram-positive bacteria, including methicillin-ressistant *Staphylococcus aureus* [89].



64: R = H; **65:** R = Cl



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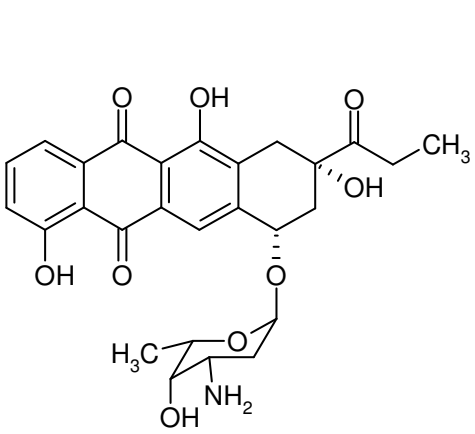

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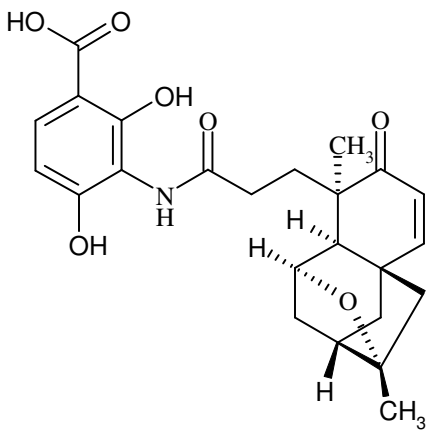
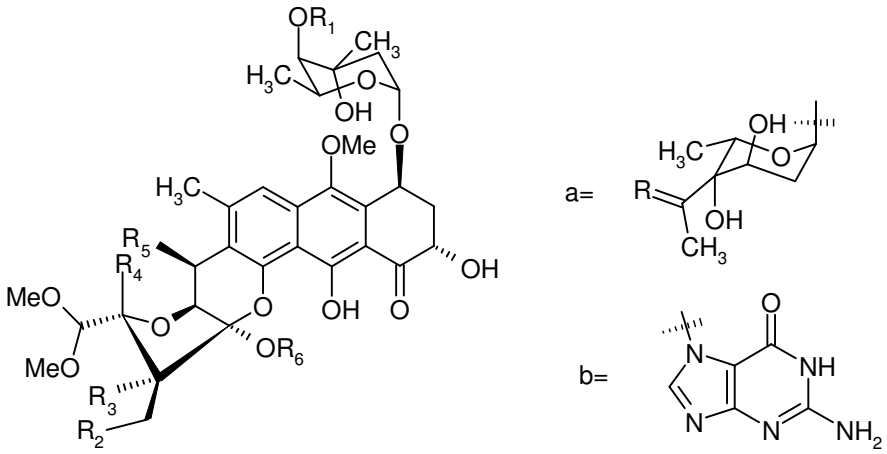


68

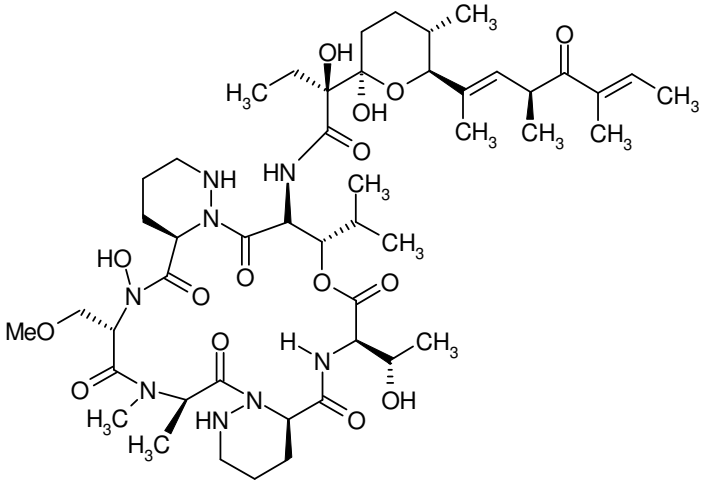
BS-46 (**68**) having a novel carbon skeleton was isolated from a terrestrial *Streptomyces* sp. in Spain, and elucidated in our group (2004) using conventional spectroscopic and synthetic methods. BS-46 (**68**) displayed potent antibacterial activities against *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57) at 10 µg/disc [90]. BS-46 was recently patented by Wang *et al*. (2006) as cin[91,92]. Due to its highly interesting structure and activity as an inhibitor of FabF/B condensing enzymes, **68** was finally totally synthesized as racemic mixture by Nicolaou *et al*.[93].

Gutingimycin (**69g**)[94] and the trioxacarcines A-F (**69a-f**), highly oxygenated antibiotics with complex structures, were isolated in our group from the marine *Streptomyces* B8652. Trioxacarcins exhibited anti-tumor, antibacterial and high antimalaria activity[95,96], while trioxacarcine D (**69d**) possesses extremely high antiplasmodial activity.



69a :	R_1= $COCH_3$	R_2-R_3 = O	R_4-R_5 = O	R_6 = a	R = O
69b:	R_1= $COCH_3$	R_2 = R_3 = OH	R_4-R_5= O	R_6 = a	R = O
69c:	R_1= $COCH_3$	R_2-R_3= O	R_4-R_5= O	R_6 = a	R = OH, H
69d:	R_1= H	R_2-R_3= O	R_4-R_5= O	R_6 = a	R = O
69e:	R_1= $COCH_3$	R_2 =R_3= OH	R_4 = R_5= OH	R_6 = H	-
69f:	R_1= $COCH_3$	R_2 =R_3= OH	R_4 = R_5= OH	R_6 = a	R = O
69g:	R_1= $COCH_3$	R_2 = b, R_3= OH	R_4 - R_5= O	R_6 = a	R = O

Kettapeptin (**70**) belongs to the group of cyclic hexadepsipeptides, which was isolated from the terrestrial *Streptomyces* sp. GW 99/1572 by Maskey[97, 98]. It is a potent antibacterial agent against Gram positive and negative bacteria, more active than bacitracin A. It is additionally highly active against human cancer cell lines LXFA 629L and LXFL 529L (lung cancer), MAXF 401NL (breast tumor), MEXF 462NL (melanoma), RXF 944L (kidney tumor) and UXF 1138L (uterus tumor) with IC_{70} value of <0.6 μg/ml.



70

1.6 Conclusion

The early development of natural product chemistry was largely restricted to terrestrial plants. But since most (easily accessible) plants worldwide have been studied, it is necessary to search for other sources of medicinally useful compounds. For sponges, tunicates, soft corals etc., disturbance of the ecological balance and difficult access are still representing a challenge to the chemists. Consequently, the search focus has been shifted towards bacteria as a new source of natural products. They were and are still a creative sources for the production of bioactive metabolites indeed. Moreover, they are favoured over plants and animals due to the possibility of cultivation in any quantity and at any time.

Bacteria are symbiotically important for animals and plants, and it can be expected that this may induce interesting metabolic pathways. Moreover, bacteria are also being found in surprisingly extreme environments: polar ice, geothermal vents, and deep-sea sites, where the habitat may induce unusual structures. It should be kept in mind that most natural antibiotics came from bacteria and fungi, so that the chances for bioactive drugs from these sources are high.

2 Aim of the Investigation

In the beginning of the era of antibiotics, it seemed that all diseases could be cured soon, however, extended use of antibiotics made them insensitive, due to drug resistance phenomena. For this reason, there is a worldwide urgent need now of new medicinal sources.

Despite of the short period of research on marine natural products for only ~40 years, more than 7000 compounds have been published[99,61]. Many of them possess unique functional groups or skeletons and also potent biological activities. It is noteworthy that presently published new compounds with novel or rare skeletons are almost exclusively found in marine organisms. Some of them are already in the preclinical phase and may lead to pharmaceutical products[100]. So, exploring marine organisms, especially microorganisms, will be one of the essential focuses in the next years and should be more and more successful.

Accordingly, the main objective of the present investigation is concerned with the isolation and structure elucidation of new and preferably biologically active secondary metabolites from bacteria. This study is focussed mainly on the genus *Streptomyces* collected from terrestrial and marine sources, including the North Sea.

To achieve this aim, chemical and biological screenings should be applied in such a way, that in a minimum of time a maximum of results is obtained. For this reason, a 'horizontal screening' should be applied: With a few biological tests using Gram-positive and Gram-negative bacteria, fungi and yeasts, microalgae and brine shrimps, the antibacterial, antifungal, phytotoxic and cytotoxic activities are covered, and results can be used as a lead for further detailed investigations. The established chemical screening should be applied additionally, extended by using an HPLC/MS screening (Figure 6).

The individual steps can be summarized as follows:

- *Microbiological part*: Collection of bacterial cultures from carefully selected habitats, preferably in cooperation with microbiologists. Optimisation of fermentation conditions and small scale cultivations.
- *Pre-screening*: Fermentation in a 1 L-scale and horizontal screening. Selection of suitable strains for in-depth explorations.

- *Up-scaling*: A large-scale fermentation of selected strains is usually required to obtain sufficient amounts of metabolites; for strains with low productivity, an optimisation (pH, fermentation time, medium type) may be needed.
- *Isolation*: The fermentation broths are subjected to exhaustive extraction using either liquid-liquid (solvents) or liquid-solid (Amberlite-XAD) extraction techniques. Chromatographic methods are used to deliver the pure constituents.
- *Dereplication*: Chemical tests (colour reactions), ^{1}H NMR and mass spectra are used to gain preliminary structural information for a dereplication of known compounds with data bases.
- *Structure Elucidation*: Novel compounds will be elucidated preferably by using spectroscopic techniques and – if possible – crystal structure analysis.
- *Biological Activity*: Depending on the results of our horizontal screening, new and promising known compounds will be investigated in cooperation with industrial partners for a potential use in medicine or agriculture.

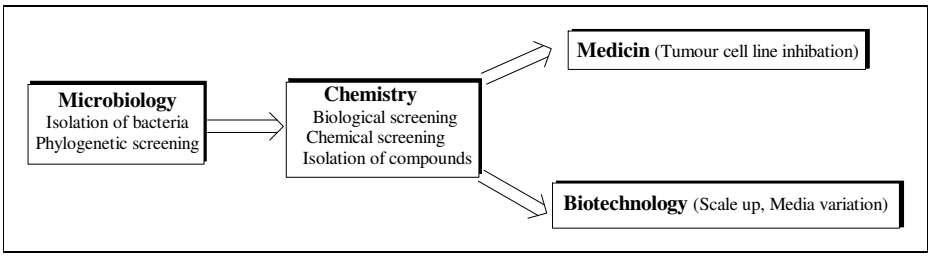


Figure 6: Integrated approach to explore the metabolic capabilities of bacteria for the production of bioactive compounds.

3 General Techniques

3.1 Collection of Strains

In this study, the search for bioactive constituents from streptomycetes was continued, as the latter represents a source of about ~75% of the current antibiotics. For this purpose, the bacterial strains were obtained in cooperation with the following microbiological institutions:

- The terrestrial *Streptomyces* spp. (code beginning with GW- and Bl-) were obtained from the strain collection of bioLeads in Heidelberg.
- The marine *Streptomyces* spp. (B series) and *Actinomyces* spp. (Act series) were obtained from the collection of E. Helmke, Alfred-Wegener Institute for Polar and Marine Research, Bremerhaven.
- North Sea bacteria (Mei strains) were collected in the group of Prof. Meiners, Fachhochschule Emden.
- Some further cultures were obtained from our partners in Tunisia, Pakistan and Egypt and will be discussed later.

The bacteria will be described at the beginning of each chapter on the basis of colour, morphological characteristics, mucus, etc. In some cases, the taxonomy was fully determined on the RNA level.

3.2 Pre-screening

Around 30% among the received strains are usually able to produce bioactive metabolites or further interesting properties. To select these strains, a so-called pre-screening is performed. In this method, strains are selected according to a number of suitable qualitative or quantitative criteria, e.g. biological, chemical or physical interactions of metabolites with test systems.

Initially, the strains are sub-cultured on agar plates for 3-7 days and microscopically examined for contaminations. Small pieces of the agar culture are then used to inoculate 1 L Erlenmeyer flasks, each containing ~250 ml of medium, for a small scale cultivation, followed by incubation on a rotary shaker at 28 °C. The culture broth is then lyophilised, and the dried residue is extracted with ethyl acetate. The obtained crude extract is used for biological, chemical (HPLC-MS and TLC scanner)

and pharmacological screenings (Figure 7). Promising strains are selected for chemical in-depth investigation.

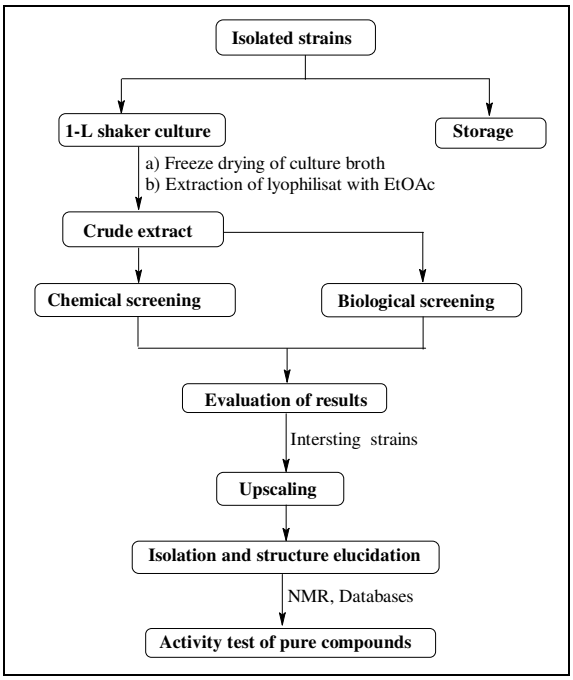


Figure 7: General screening and working up of the selected bacterial strains

3.2.1 Chemical Screening

The isolation of pure bioactive compounds from bacterial strains is a multiple stage procedure and an expensive task. For this reason, it is important to avoid the re-isolation of known metabolites from the crude extract or from a partially purified fraction. Chemical screening is one of economical methods, which allows reaching this aim at an early stage of separation.

The thin layer chromatography (TLC) is one of the simplest and cheapest methods for such a screening. In comparison with other methods (like HPLC-MS), TLC is easier, quicker, and simpler to perform, and is sufficiently reproducible. A spot of the crude extract is developed on a TLC with a suitable solvent system. The developed TLC plate is visualized under UV light, and interesting zones are further localized by exposure to spray reagents. Several spraying reagents are available for the detection, some specific, other universal[101]. In our research group, the following reagents are used intermittently:

- Anisaldehyde/sulphuric acid spray reagent gives different colour reactions with many structural elements and is a universal reagent. Modified colours can be obtained with orcin, vanillin, or *p*-methoxycinnamaldehyde instead of anisaldehyde.
- Ehrlich's reagent is a specific reagent for indoles, which turn pink, blue or violet. Some other groups of compounds are giving less specific colour reactions: pyrroles become brown, anthranilic acid derivatives change to yellow.
- Concentrated sulphuric acid is useful for polyenes: short conjugated chains are turning to brown or black, while carotenoids develop a blue or green colour.
- Palladium(II)-chloride is used as specific reagent for sulphur compounds.
- Ninhydrin is used to detect terminal amino groups of amino acids and peptides.
- Sodium or potassium hydroxide solutions serve to identify *peri*-hydroxyquinones, which turn to red, blue or violet, while the intensively red prodigiosin salts are forming the yellow free base.
- Chlorine/*o*-dianisidin is a universal reagent used for the detection of peptides.

3.2.2 Pharmacological and Biological Assays

As there is no general way to derive the biological activity of metabolites solely from their structures, a multitude of pharmacological test systems has been developed. They can be subdivided into defined and complex systems: The defined systems are often using highly sophisticated receptor tests; they are often expensive, sometimes highly specific and therefore are giving low response rates, sometimes as low as 1:20.000 and even 1:80.000 (as in the case of platensimycin). Receptor tests are therefore less suitable for use in universities, where a quick, sensitive affordable test is required. The selectivity should be low, so that broad ranges of activities can be covered; a response rate of 10-20 % is suitable. The resulting 'horizontal screening' can be realized best with (micro)organisms, i.e. by use of complex systems.

Based on agar diffusion method, the crude extracts are tested in our group against Gram-positive (*Bacillus subtilis, Staphylococcus aureus, Streptomyces viridochromogenes* (Tü 57), Gram-negative (*Escherichia coli*), fungi (*Mucor miehei*),

yeast (*Candida albicans*), as well as microalgae (*Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus subspicatus*).

The brine shrimp toxicity has a strong correlation with cellular cytotoxicity and is therefore a good indicator for potential anticancer activity. Positive extracts can then be tested against human cancer cell lines, as we do it in cooperation with other research groups. The bio-autography by TLC is also a very convenient and simple way for testing the bacterial extracts and pure substances against microorganisms. The latter technique gives simultaneously guiding information about the number and polarity of unknown bioactive components present in the crude extract.

3.3 Scale-up, Cultivation and Extraction

Generally, there is a minimum amount needed for structure elucidation of a new compound, which is with our equipment near 1-5 mg, depending on the complexity of the structure. As additional quantities are needed for biological tests, 5-10 mg are desirable. As most strains are producing only metabolite concentrations of 0.1 – 1 mg/L, fermentation in a 20~50 L scale is necessary. A systematic optimisation of the fermentation conditions and nutrient composition is usually not done for routine experiments. It becomes essential, however, if large amounts are needed or incorporation experiments should be performed.

Two cultivating techniques were used in this investigation: a) shaker fermentations in Erlenmeyer flasks (sufficient for up to 25 L), and b) jar fermentor (20 L, 25 L and 50 L). In case of the latter, a pre-culture of 2~5 L is used for the inoculation. After harvesting, the culture broth is mixed with Celite, followed by filtration using a filter press. The water phase can be extracted with ethyl acetate, but it is highly recommend to use a solid phase extraction (Amberlite XAD-2 resin). Extraction with XAD has several advantages in comparison with the commonly used ethyl acetate extraction: the costs are considerably less than for ethyl acetate, also rather polar water-soluble compounds can be obtained, good recovery rates are obtained, the resin is easily recovered and purified, and not harmful. The mycelium is extracted with ethyl acetate and acetone. The organic phases are evaporated to dryness, giving the desired residue for further separations. Storing extracts in the moist ethyl acetate at room temperature could result in decomposition products due to the formation of

acetic acid. So, solutions should be evaporated as soon as possible, and it is strongly recommended to store the residues at low temperature to minimize degradation reactions.

3.4 Isolation Methods

Methods of separation depend largely on the polarity of the entire metabolic constituents and their amounts in the crude extract. The latter is examined mainly by TLC monitoring (using eluents of variable polarities). Generally, the crude extract is firstly defatted by extracting the methanol solution with cyclohexane, and then two preliminary separation systems are commonly used:

- Flash silica gel chromatography using a gradient of increasing polarity of various solvent systems. However, the contact with silica gel may rearrange, oxidise, cleave or even destroy the adsorbed metabolites.
- Size-exclusion chromatography (Sephadex LH-20), where the compounds are separated depending on molecular size differentiation. This technique offers the advantage of a higher recovery rate than silica gel and minimizes the destruction of compounds. It is used preferentially when the amount of the crude component is less than 3 mg.

Fractions obtained from flash silica gel chromatography or Sephadex LH-20 are monitored by TLC to decide the next isolation steps. The next stages of isolation could be achieved using PTLC, silica gel column chromatography, Sephadex LH-20 and HPLC. Further purification can be done with the aid of HPLC and RP-18 columns.

3.5 Dereplication Concept and Partial Identification

It is noticeable that despite the existence of modern methods of identification, still the isolation and structural elucidation of natural compounds is time-consuming and expensive. Natural product chemists have to face the steadily increasing problem of how to optimise the discovery of new compounds and to minimize the re-isolation of known metabolites. It is important to distinguish between known compounds and unknowns from a partially purified mixture, and subsequently exclude known compounds at an earlier stage.

The complementary processes of rapid identification of known compounds or the partial elucidation of unknown compounds have come to be termed "dereplication"[102]. Dereplication became much more reliable after the combination of databases with the UV-multi-wavelength TLC-scanner[103] (Figure 8). The last property is useful in identifying the principle chromophors located in the metabolic constituents of the desired crude extracts. Databases were, recently, combined with MS and HPLC and appropriate reference data collections. At the present time, some databases are supported with various spectroscopic data (NMR, MS,…etc), where derived sub-structures or physico-chemical properties can be rapidly found out using computers[104]. The most useful and comprehensive tools for our purpose are the data collection AntiBase[61] and the Dictionary of Natural Products (DNP)[105]. The DNP allows the dereplication of all naturally occurring compounds, while AntiBase is specific for microbial products, and is much easier to use. These databases are not only helpful for to find out the known compound but also important for the identification of new metabolites with respect to compound classes and chromophors.

Figure 8: Multi-wavelength TLC-scanner which is a developed UV-Spectrometer with X-Y scanning ability for TLC plates

Dereplication can be also achieved using the combination of liquid chromatography/NMR spectroscopy (HPLC-NMR) and/or the tandem mass spectrometry (HPLC-MS/MS). In such a case, the biological matrices (extracts from marine microorganisms[106] or plants[107]) are screened to find out the most plausible structural data for the known constituents at lowest material amount. Currently, ESI MS/MS spectra of more than 500 frequently isolated substances are included in AntiBase. HPLC-ESI-MS/MS is the method of choice to identify known molecules from multicomponent mixtures with high selectivity and sensitivity [108]. This method needs

only negligible sample amounts and affords reliable results, if authentic samples are available to measure the reference data. It has the disadvantage, however, that reference spectra of authentic samples must be measured under identical conditions.

UV data and MS fragmentation patterns are also useful to identify unknown metabolites, if these show similar chromophors or fragmentation patterns as known analogues.

The NMR spectroscopy and mass techniques don't allow a chiral differentiation of the metabolites' configuration. However, this information is available, if the circular dichroism (CD) spectroscopy is combined with HPLC separations[109].

4 Terrestrial *Streptomyces* sp.

4.1 Terrestrial *Streptomyces* sp. GW58/450

In the primary screening, the ethyl acetate extract of the terrestrial *Streptomyces* isolate GW58/450 showed interesting characteristics during TLC: It showed five middle polar UV fluorescent bands, three among them exhibited a green fluorescence, while the other two showed blue fluorescence. These bands displayed no colour with anisaldehyde/sulphuric acid or Ehrlich's reagent. Moreover, the extract showed a moderate activity against *Bacillus subtilis* and *Escherichia coli*, and strong activity against *Streptomyces viridochromogenes* (Tü 57), however, no activity against fungi, yeasts or microalgae (Table 47).

For further investigation, the fermentation of this strain was upscaled using a 25 L jar fermentor. After usual working-up, based on TLC, three fractions were obtained. Further separation of the less polar fraction I using PTLC and Sephadex LH-20 afforded the karamomycins A~C (**73**, **75**, **78**), 1-acetyl-β-carboline (**228**)[152] and actinomycin C_2. Purification of the middle polar fraction II by Sephadex LH-20 yielded 1-hydroxy-4-methoxy-2-naphthoic acid (**71**). From fraction III, only uracil was obtained (Figure 9).

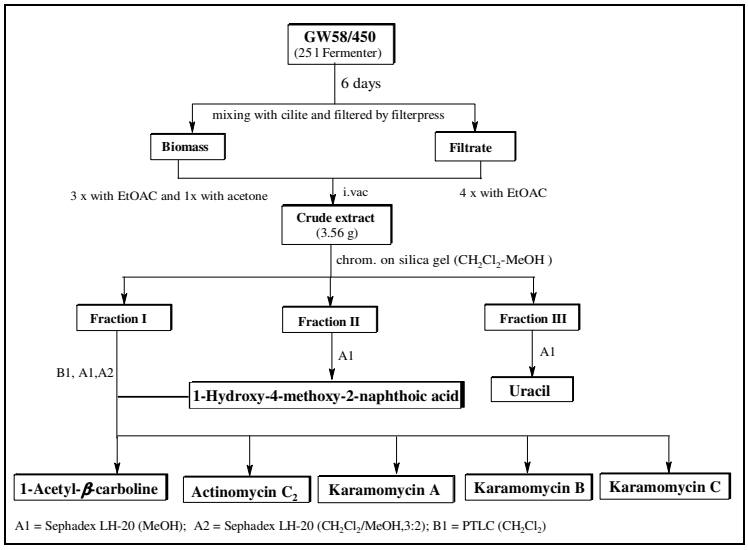


Figure 9: Working up scheme of the terrestrial *Streptomyces* sp. isolate GW58/450

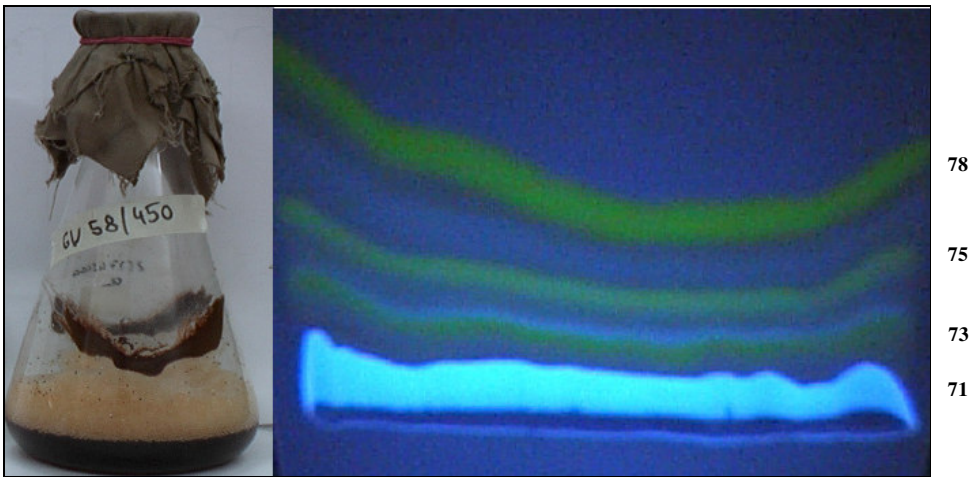


Figure 10: Karamomycins A-C (**73**, **75**, **78**) and 1-Hydroxy-4-methoxy-2-naphthoic acid (**71**) on PTLC (CH_2Cl_2) under UV light (366 nm).

4.1.1 1-Hydroxy-4-methoxy-2-naphthoic acid

Compound **71** was isolated from fraction II as colourless solid. On TLC, it gave an intense blue UV fluorescence; the zone turned light brown with anisaldehyde/sulphuric acid. The molecular weight of compound **71** was determined as 218.0 Dalton by EI MS. HREI-MS deduced the molecular formula of **71** as $C_{12}H_{10}O_4$.

The proton NMR spectrum of compound **71** showed a downfield 1H singlet of a chelated hydroxy group at δ 16.40. The typical pattern of four *ortho*-coupled protons (two doublets and two triplets) indicated a 1,2-disubstitued aromatic system. A 1H singlet (7.25) indicated a *penta*-substituted aromatic ring. Finally, the singlet of an sp^2-bound methoxy group was located at δ 3.91.

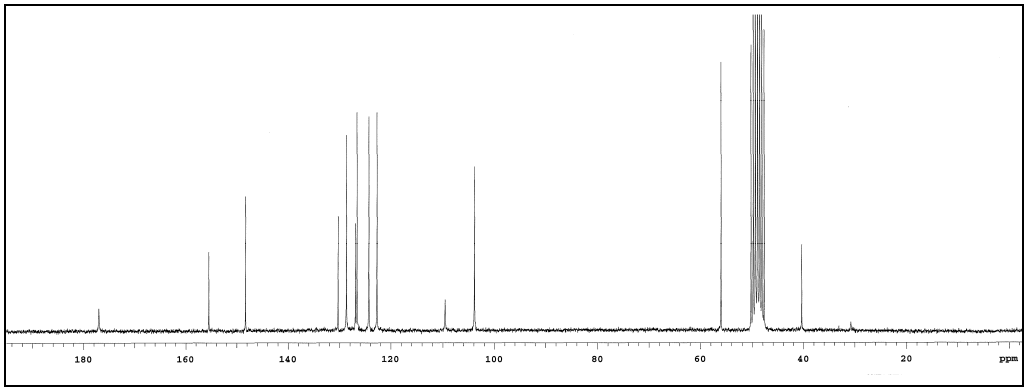
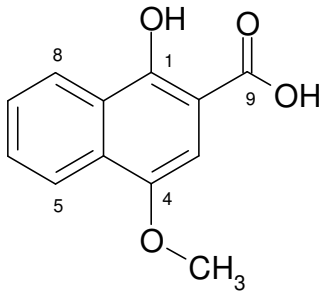


Figure 11: ^{13}C NMR spectrum (CD_3OD, 50 MHz) of 1-Hydroxy-4-methoxy-2-naphthoic acid (**71**).



71

Based on the spectroscopic data and a search in AntiBase[61], this compound was determined as 1-hydroxy-4-methoxy-2-naphthoic acid (**71**) and confirmed by comparison with a previously prepared sample[110].

Compound **71** was further synthesized by treatment of 1-hydroxy-4-methoxy-naphthaline (**71**) with carbon dioxide/anhydrous potassium carbonate[110,111]. Based on NMR spectroscopy, both the synthetic and the natural acid differed in their ^1H and ^{13}C NMR shifts (Table 2). A 1:1 mixture of both components afforded, however, NMR signals located in between of those of the individual spectra (Figure 12).

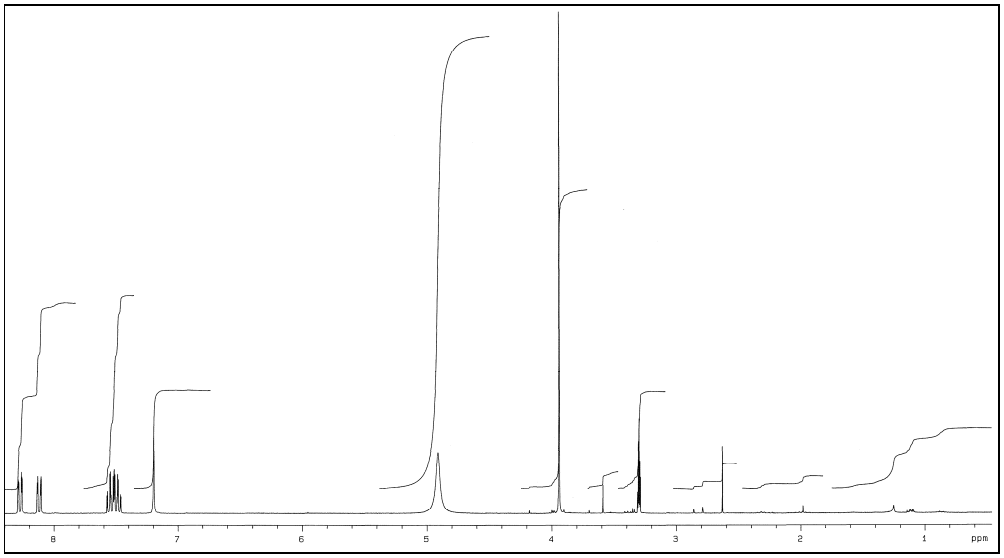


Figure 12: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of a 1:1 mixture of natural and synthetic 1-Hydroxy-4-methoxy-2-naphthoic acid (**71**).

Table 1: ^{13}C and ^{1}H NMR spectroscopic data of 1-Hydroxy-4-methoxy-2-naphthoic acid (**71**) in comparison with the reported data[112] in CD_3OD.

Position	1-Hydroxy-4-methoxy-2-naphthoic acid (**71**), (*J* in [Hz]).			
	δ_H (300 MHz) Exp.	δ_H (300 MHz) Lit.	δ_C (75 MHz) Exp.	δ_C (lit.)
1	16.40 (s) ([D_6]DMSO)	-	155.5	155.3
2	-	-	109.6	125.8*
3	7.25 (s)	7.19 (s)	103.9	101.8
4	-	-	148.3	147.6
4a	-	-	130.3	129.8
5	8.09 (dd, 8.9, 1.5)	8.18 (d, 8.1)	122.7	121.8
6	7.52 (td, 7.9, 6.9, 1.4)	7.62 (dd, 6.9, 1.2)	128.7	128.4
7	7.46 (td, 7.9, 7.0, 1.2)	7.56 (dd, 8.0, 7.1)	126.6	126.1
8	8.25 (dd, 7.5, 1.5)	8.33 (d, 8.0)	124.3	123.4
8a	-	-	126.9	125.8
9	-	-	177.0	173.9
4-OCH_3	3.91 (s)	3.96 (s)	56.0	55.1

*Wrong value reported in literature

Table 2: Comparison of the ^{1}H & $^{13}CNMR$ ([D_6]DMSO) of both natural and synthetic 1-Hydroxy-4-methoxy-2-naphthoic acid (**71**), (*J* in [Hz]).

Position	1-Hydroxy-4-methoxy-2-naphthoic acid (**71**)				
	δ_H (Nat.)[a]	δ_H (Synth.)[a]	δ_H (Nat. + Synth.)[b]	δ_C (Natural)[c]	δ_C (Synthetic)[c]
1	16.40 (s)	12.6 (brs)	-	155.1	154.6
2	-	-	-	110.7	104.8
3	7.33 (s)	7.07 (s)	7.20 (s)	104.5	101.2
4	-	-	-	144.7	146.8
4a	-	-	-	127.7	128.8
5	8.05 (dd, 8.9, 1.5)	8.15 (dd, 8.1, 1.5)	8.12 (dd, 8.9, 1.7)	122.7	121.5
6	7.45 (td, 7.9, 6.9, 1.4)	7.55 (m)	7.55 (m)	126.6	129.0
7	7.40 (td, 7.9, 7.0, 1.2)	7.55 (m)	7.49 (m)	123.1	126.5
8	8.22 (dd, 7.5, 1.5)	8.30 (d, 8.1)	8.28 (dd, 8.5, 1.5)	121.0	123.1
8a	-	-	-	124.7	124.7
9	-	-	-	173.0	172.7
4-OCH_3	3.85 (s)	3.92 (s)	3.94 (s)	55.4	55.5

[a](300 MHz); [b](MeOH, 300 MHz); [c](75 MHz)

4.1.2 Karamomycin A

Compound **73** was isolated as pale yellow solid from the less polar fraction I. During TLC, compound **73** appeared as green fluorescence band under UV. On spraying with $PdCl_2$, it was stained brown, as indication for a sulphur containing compound. Its molecular weight was established by ESI and EI spectra as 289 Dalton. ESI and EI-HRS mass spectrometry determined the molecular formula as $C_{15}H_{15}NO_3S$, bearing 9 double bond equivalents.

The proton NMR spectrum of compound **73** established the presence of the naphthalene moiety containing a 1,2-disubstituted aromatic ring (δ8.38~7.55), a chelated 1-hydroxy group (δ13.44), the signal of 3-H (δ6.65, s), as well as an aromatic methyl ether group (δ3.98) and the AA',BB' system. In the aliphatic region, two sets of downfield shifted methylene protons were visible at δ4.03 (dd) and 3.87 (dd), while the other one appeared at δ3.48 (dd) and 3.44 (dd). The high coupling constant (~11 Hz) of both explained their location in a ring system. Moreover, a multiplet signal of a hetero-bound methine proton was visible at δ4.93.

The ^{13}C NMR/APT spectra indicated all 15 carbon signals, among them a carbonyl of an ester, amid or thioimine (174.2), two oxy-sp^2 carbons (152.5 and 147.7) and five sp^2 methines. In the aliphatic region, the methoxy signal appeared at δ55.8, while the two methylene carbons were visible at δ64.2 and 33.0, along with a signal

at δ 77.2 for a hetero bound methine. The carbon of the first methylene (64.2) was linked to oxygen or of an imine-system[113, 114], while the other one (δ 33.0) could be connected to sulphur. Based on the above spectroscopic data, the molecular formula and spectra simulations, two structures **72** and **73** were proposed.

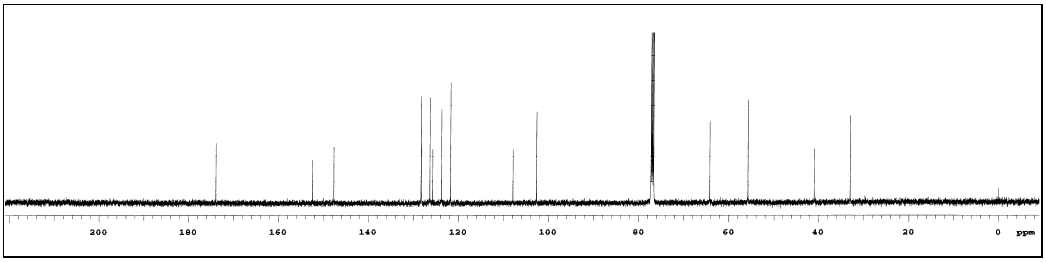
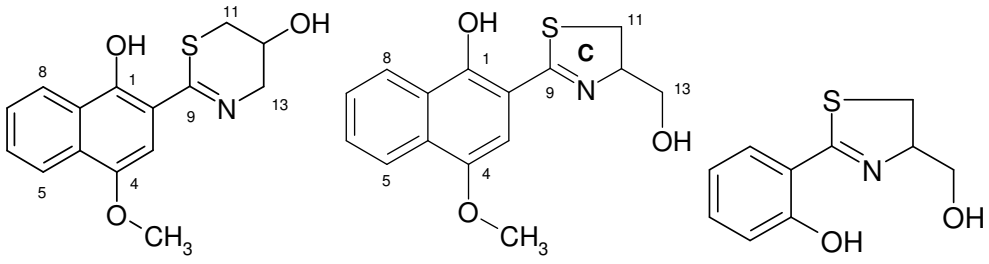


Figure 13: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of Karamomycin A (**73**).

Based on HMBC and H,H COSY correlations (Figure 14), the 2-substituted 4-methoxy-α-naphthol skeleton was confirmed. The S-methylene protons (δ 3.48 and 3.44) exhibited a HMBC ^3J coupling with the carbon C-9 (δ 174.2). Additionally, the aromatic singlet of H-3 showed two strong correlations (^3J) to C-9 (δ 174.2) and the oxy-carbon C-1 (δ 152.5), confirming the existence of a thio-imino carbon at position 2 of the naphthalene moiety, and establishing the free hydroxy group in position-1, as in 1-hydroxy-4-methoxy-2-naphthoic acid (**71**). The isopropanol partial structure -CH_2-CH(N/O)-CH_2-(N/O) was further established by the H,H COSY data, in addition to ^3J cross signals between both methylene groups in the HMBC spectrum. On the other hand, there was no coupling between the methylene protons of 13-H_2 (δ 4.03 & 3.87) and C-9, excluding structure **72**, and further confirming structure **73**. The ring C was further confirmed by comparison with an authentic sample of aerugine (**74**) from our collection. The resulting 2-(4-hydroxymethyl-4,5-dihydro-thiazol-2-yl)-4-methoxy-naphthalen-1-ol (**73**) is isolated here as a novel natural product, and was named as karamomycin A (Figure 14).



72 **73** **74**

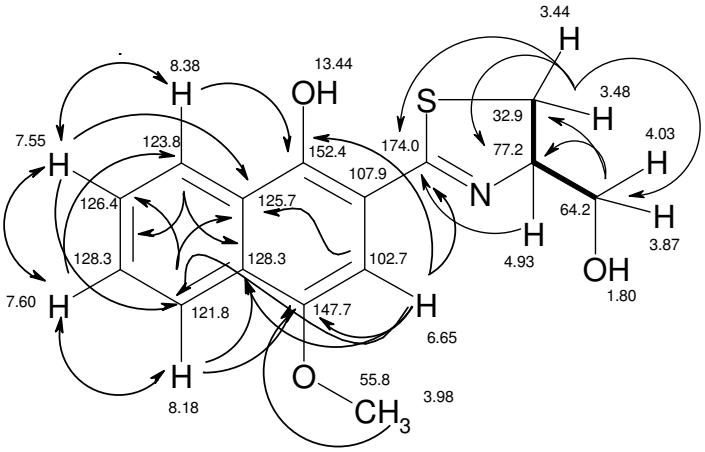


Figure 14: H,H COSY (▬, ↔) and HMBC(→) connectivities of Karamomycin A (**73**).

The absolute configuration at C-12 in **73** was deduced using the optical rotation, CD and NOESY experiments. Based on NOESY experiments, the amino-methine proton (H-12) exhibited a coupling with one of the methylene protons δ3.48 ($\delta_{C\text{-}11}$ 33.0), Figure 15. Furthermore, comparison of the obtained negative specific optical rotation $[\alpha]_D$ (-100°) of karamomycin A (**73**) with reported data of (S)-dihydroaeruginoic acid (**76**) ($[\alpha]_D$ +47)[115,116] and authentic CD spectrum of aerugine (**73**), confirmed the (-)-*R*- stereochemistry at C-12.

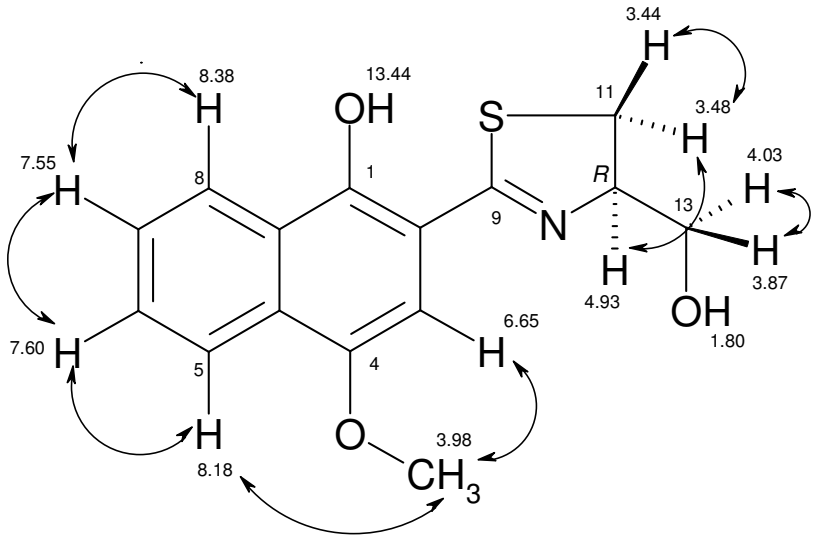


Figure 15: NOESY (↔) correlations of Karamomycin A (**73**).

Table 3: ^{13}C and ^{1}H NMR spectroscopic data of Karamomycins A (73) and B (75) in $CDCl_3$.

Position	Karamomycin A (**73**)		Karamomycin B (**78**)	
	δ_H (J in [Hz])[a]	δ_C[b]	δ_H[a]	δ_C[c]
1	-	152.4	-	148.5
1-OH	13.44 (s)	-	12.71 (s)	-
2	-	107.9	-	108.6
3	6.65 (s)	102.7	6.79 (s)	100.5
4	-	147.7	-	148.5
4-OCH_3	3.98 (s)	55.8	4.00 (s)	55.8
4a	-	128.3	-	127.7
5	8.18 (dd, 7.2, 1.6)	121.8	8.17 (m)	121.9
6	7.60 (td, 6.9, 1.5)	128.3	7.55 (m)	127.6
7	7.55 (td, 7.5, 1.6)	126.4	7.55 (m)	126.6
8	8.38 (dd, 7.6, 2.2)	123.8	8.38 (m)	123.5
8a	-	125.7	-	126.2
9	-	174.0	-	170.3
11	3.48 (dd, 10.7, 9.0), 3.44 (dd, 10.6, 8.2)	32.9	7.17 (s)	102.0
12	4.93 (m)	77.2	-	155.2
13	4.03 (dd, 11.3, 4.9), 3.87 (dd, 11.7, 4.3)	64.2	4.86 (s)	61.0
13-OH	1.80 (s br)	-	2.03 (s br)	-

[a](600 MHz); [b](125 MHz); [c](150 MHz)

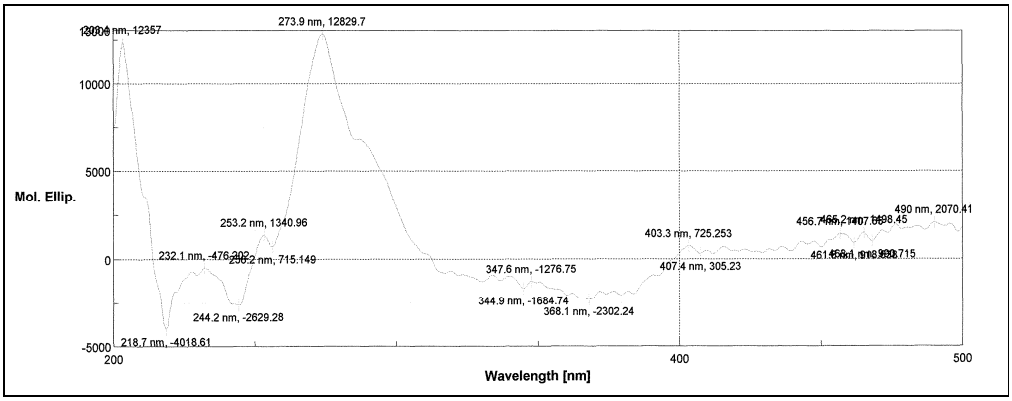


Figure 16: CD spectrum of Karamomycin A (**73**).

4.1.3 Karamomycin B

Compound **75** was obtained as yellow solid from the same fraction I as compound **73**, and also exhibited a similar fluorescence. The molecular weight was established by EI MS spectra as 287 Dalton; HRESI MS delivered the molecular formula $C_{15}H_{13}NO_3S$, containing 10 double bond equivalents.

The ^{1}H NMR spectrum established the carbon skeleton of karamomycin A (**73**) also in **75**: The chelated OH group appeared at δ12.71, along with the four signals of 1,2-disubstituted aromatic system (δ8.38~7.55), a singlet of H-3 (δ6.79) and of 4-OCH_3 (δ4.00). In the aliphatic region, two singlets for an aromatic/olefinic methine (δ7.17) and oxymethylene (δ4.86) were observed. The difference between the molecular weights of **73** and **75** (Δm = 2 amu) can be interpreted as dehydrogenation between the methine CH-12 and one of the surrounding methylenes (S-CH_2-CH(N)-CH_2-OH) in compound **73**.

The ^{13}C/HMQC spectra indicated the presence of 15 signals as in **73**. Two aromatic signals for C-11 (or 13) and C-12 appeared at δ102.0 and δ155.2, while those of C-11 (δ32.9) and C-12 (δ77.2) in karamomycin A (**73**) had disappeared.

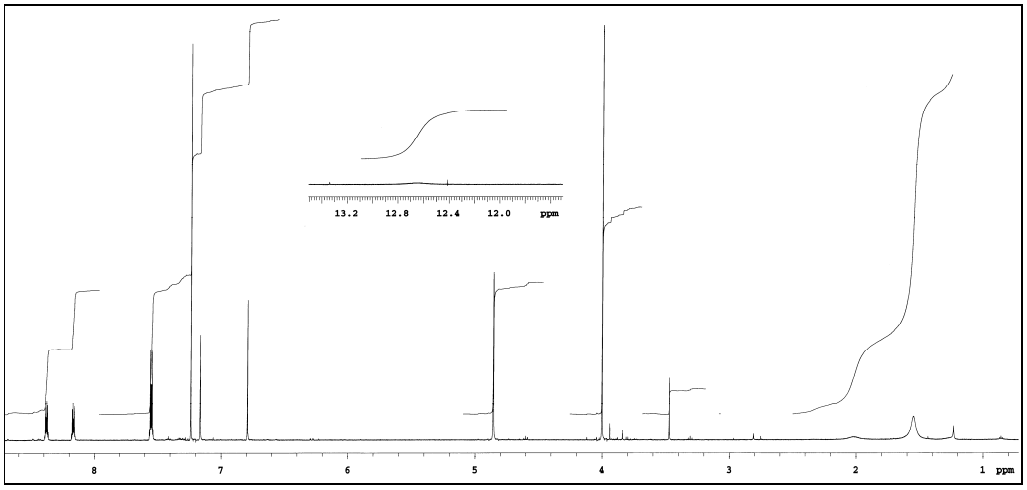


Figure 17: ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of Karamomycin B (**75**).

Based on 2D (H-H, C-H) correlations (Figure 18), the 2-substituted-4-methoxy-α-naphthol skeleton was further fixed. The methine proton of H-10 (δ7.17) exhibited ^{3}J couplings with the thio-imino carbon C-9 (δ170.3) and the hydroxymethyl of C-13 (δ61.0) along with a ^{2}J coupling with the quaternary C-12 (δ155.2). As in compound **73**, the singlet of the aromatic proton H-3 showed two strong correlations (^{3}J) with C-9 (δ 170.3) and C-1 (δ 148.5), identifying the connection of C-9 (thio-imino carbon) with position 2 of the naphthalene moiety.

Finally, the structure was assigned as 2-(4-hydroxymethyl-thiazol-2-yl)-4-methoxy-naphthalen-1-ol (**75**), where the thiazole ring (C) was further confirmed by comparison with aerugine (**74**)[116-118], dihydroaeruginoic acid (**76**)[117,118] and aeruginoic acid (**77**)[119]. The new compound **75** was named as karamomycin B (Figure 14).

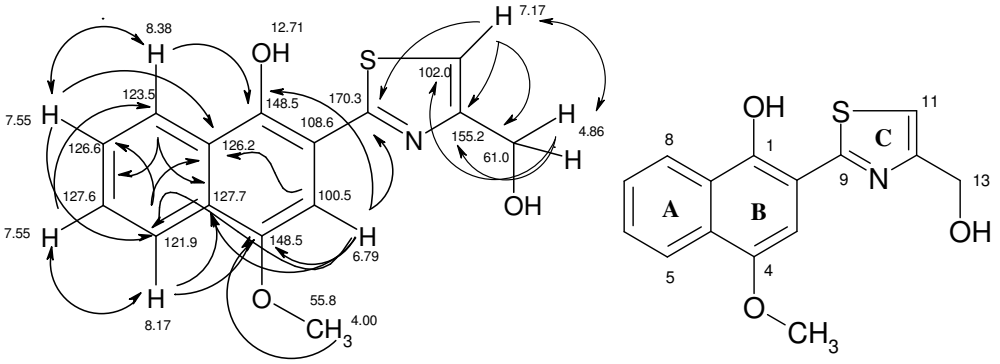
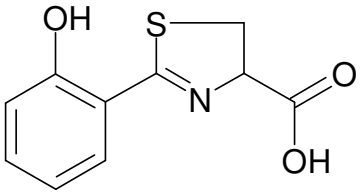


Figure 18: H,H COSY (↔) and selected HMBC(→) coupling in Karamomycin B (**75**).



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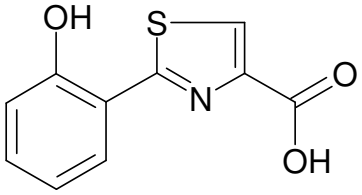


77

4.1.4 Karamomycin C

Along with karamomycins A~B (**73**, **75)**, a third yellow solid was obtained from fraction I, showing the same green fluorescence as **73** and **75**. The molecular weight of compound **78** was established as 487 Dalton by ESI and EI mass spectra. HRESI-MS delivered the molecular formula $C_{23}H_{25}N_3O_3S_3$, containing 13 double bond equivalents.

The proton NMR spectrum of compound **78** indicated again the same 1-hydroxy-2-substituted-4-methoxynaphthalene sub-unit present in karamomycins A and B (**73**, **75**). Accordingly, signals of a 1,2-disubstituted aromatic system, of 1-OH (13.55), 2-H (δ 6.61) and 4-OCH_3 (δ 3.95) were observed. In the aliphatic region, singlets of two methyls were found at δ 2.27 and 1.55, one of them (δ 2.27) was probably located at nitrogen or sulphur. Signals of a methylene group in a ring (dd, δ 3.51 and 3.47, *J*~ 11, 6~8 Hz), formed an ABX system, while a second methylene gave AB doublets at δ 3.33 and 2.88. A third methylene group gave a multiplet at δ 2.98, while three signals of oxygenated methines were appearing at δ 5.80 (d) 4.92 (m) and 4.54 (s).

The ^{13}C NMR/APT spectra of compound **78** exhibited 23 carbon signals, among them two carbonyls (δ 174.2 and 169.8) of ester, amide and/or formimidic carbons (-S-C=N-/-O-C=N-). In the aromatic region, two oxy-carbons (δ 152.9 and 147.5), five methines, three quaternary carbons and the 4-methoxy carbon (δ 55.8) were observed, representing the 2-substited naphthalene moiety, as in karamomycins A and B (**73**, **75**). In the aliphatic region, three methine carbons (δ 80.0, 74.0 and 61.5) and one quaternary (δ 74.1) were shown, where most of them were oxygenated. Finally, three methylenes (δ 41.0, 34.6, 33.9) and two methyls (δ 37.9 and 18.0) were visible.

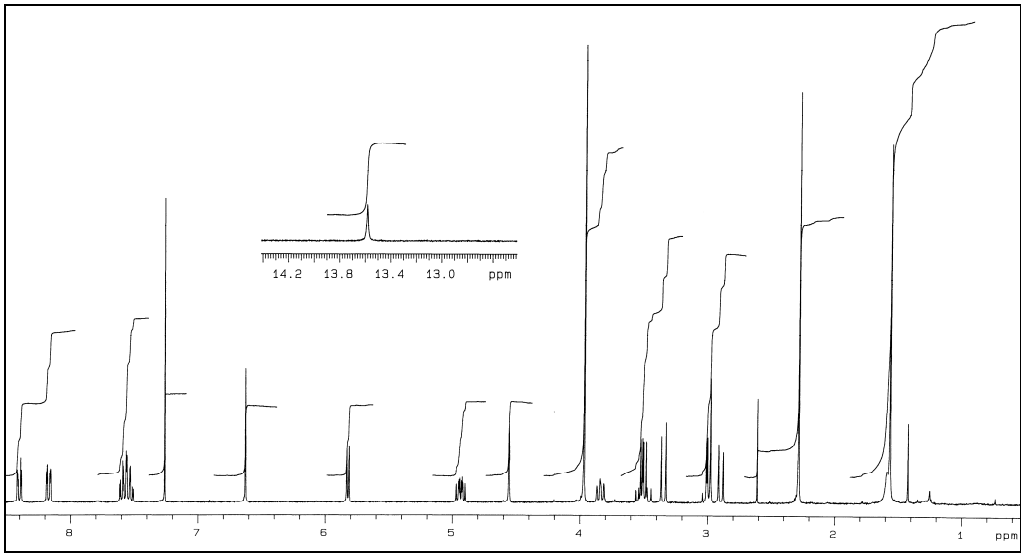


Figure 19: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Karamomycin C (**78**).

Based on 2D H,H correlations, protons of the methylene group H_2-11 (δ_H 3.51/3.47) were correlated via ^{3}J with the methine multiplet H-12 (δ_H 4.92) confirming their direct linkage. Also, the methine H-12 was adjacent to the methine proton H-13 (δ_H 5.80). Based on HMBC connectivities, protons of H_2-11 and the nitrogen-bound methine H-12 exhibited ^{3}J cross-signals with the carbon C-9 (δ 174.2). The oxygenated methine proton H-13 exhibited no cross-section at C-9, which was the same as for karamomycin A (**73**). So, the partial structure **A** (karamomycin A, **73**) substituted at the oxy-methine carbon C-13 (δ 61.5) (Figure 20) was established.

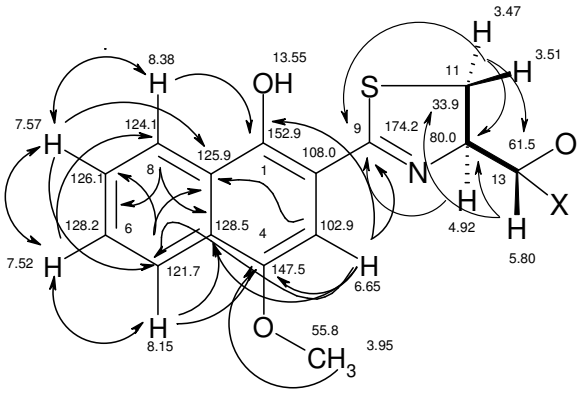


Figure 20: H,H COSY (↔, ▬) and HMBC(→) correlations of fragment A in Karamomycin C (**78**).

In the remaining fragment, the methylene protons H_2-22 (δ_H 2.98) and the two methines H-17 (δ_H 3.82) and H-21 (δ_H 4.54) were confirmed to be vicinal by the H,H

COSY coupling, representing a -CH-CH_2-CH- fragment. This sequence was reconfirmed by HMBC correlations, in which the methine proton H-17 (3.82) displayed two cross signals with C-22 (δ 34.6) and C-21 (δ 74.0), respectively. Moreover, the methine proton H-21 (δ 4.54) displayed three relevant ^3J correlations towards the methyl singlet 20-NCH_3 (δ 37.9), the quaternary carbon C-19 (δ 74.1) and the methylene carbon C-24 (δ 41.0). The methyl 19-CH_3 (δ 18.0, s) was attached to C-19 (δ 74.1) as shown by the ^2J coupling. The latter methyl (19-CH_3) exhibited ^3J correlations to the carbon signals at δ 169.8 (C-15) and C-24 (δ 41.0). So, two bicyclic partial structures were purposed: a 1,9-dimethyl-7-thia-3,9-diaza-bicyclo[4.2.1]non-ene-2-thiol (**B**) and 1,7-dimethyl-9-thia-3,7-diaza-bicyclo[4.2.1]non-ene-2-thiol (**C**) (Figure 21).

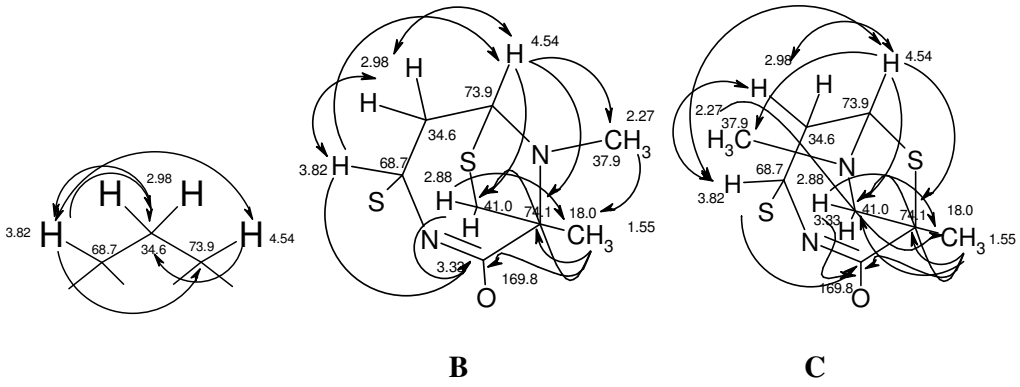
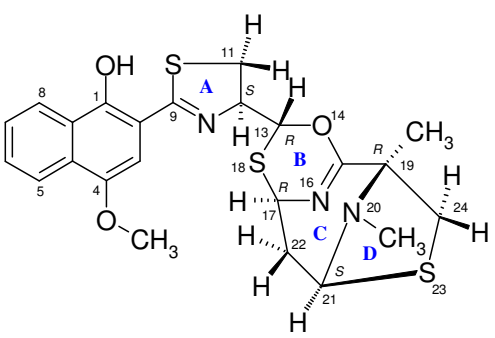


Figure 21: Proposed heterocyclic rings fragments B and C of Karamomycin C (**78**).

In the HMBC spectra, the methine H-13 (δ 5.80) displayed three significant cross signals towards C-17 (δ 68.7, ^3J), the formimidic carbon C-15 (δ 169.8, ^3J), and the methylene C-22 (δ 34.6, ^4J). This established the direct attachment of the partial structures **B** or **C** with the partial structure **A**. This ring closure was carried out *via* an –O/S- bonds of **B** or **C** and the oxy-carbon (C-13) of the partial structure **A**. In accordance, four possible structures were suggested (**78~81**).



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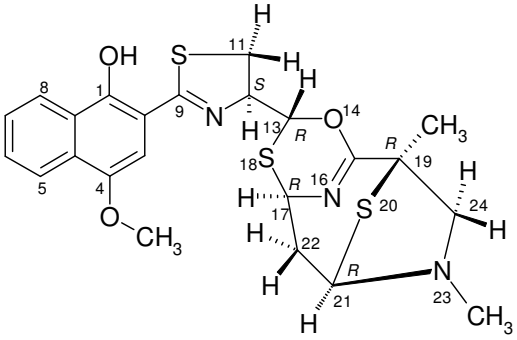

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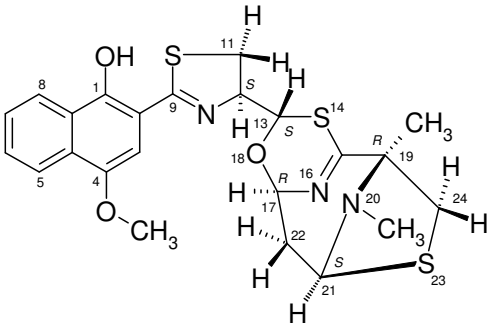

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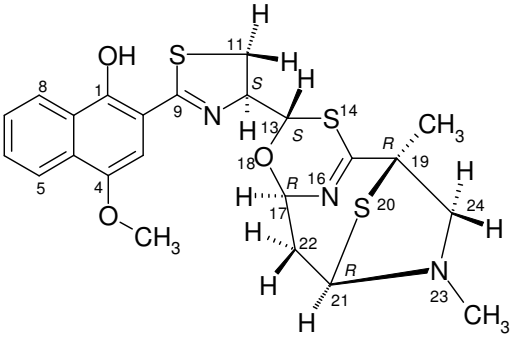


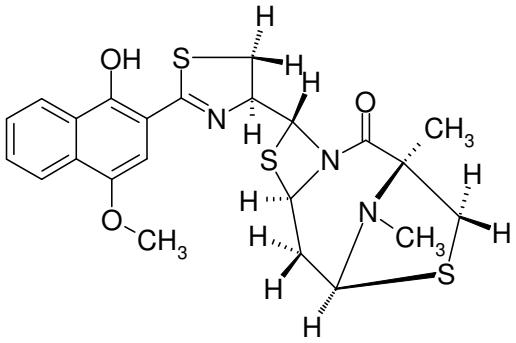
81

Based on the chemical shifts, the partial structure **B** in compounds **78** and **80** were more reasonable, as the N-methyl showed NOE as well as a ^{4}J couplings at the methyl singlet (19-CH_3, δ18.0), while in case of **C** (**79**, **81**), it is unusual ^{5}J coupling. Besides, the formimidic carbon (δ169.8) was expected to be at δ164 for **B** and at δ 176 for **C**. Further more, The N-methylene carbon in **C** was downfield shifted at δ 60, while the S-methylene in **B** was mainly between 36~42[120], and much more satisfactory.

So, the hypothetical structures **79** and **81** were eliminated. The possibility of **80** was also excluded as the estimated chemical shift of CH-17 for O-CH-N= (δ 89.0) was much higher than those of S-CH-N= (δ 60.7). Hence, the compound was recognized as **78**.

Based on calculations with COCON[121], 16000 possible structures were found in accordance with the observed couplings, after exclude the (-S-S-) containing structures, 18 alternative structure containing the dimethyl-thiazolidine ring (D) were ob-

tained. A calculated for karamomycin C (**78**), only five of them could be included (**78**, **82~86**) and none are known up till now. The compound's structure was applied to a search in AntiBase, DNP and chemical abstract, confirming its novelty. So, we have assigned it as karamomycin C (**78**), a hexacyclic structure, containing 5 chiral centres (at C-12, 13, 17, 19 and 21).



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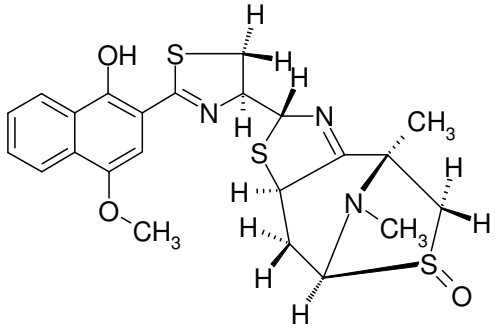

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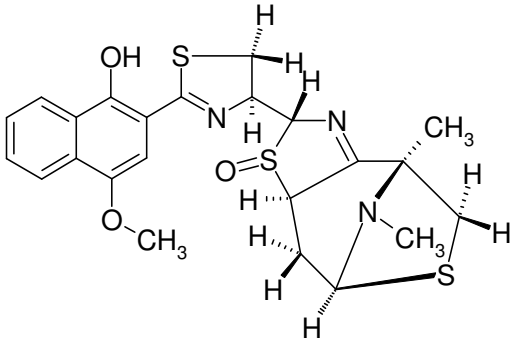

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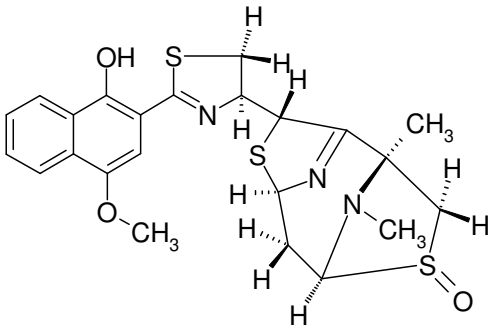

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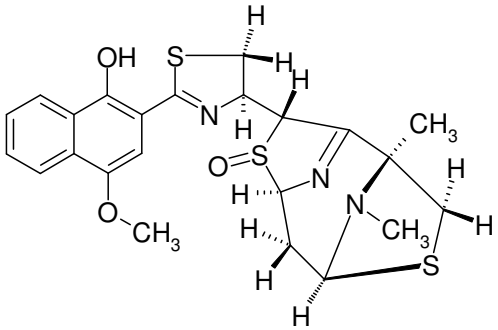

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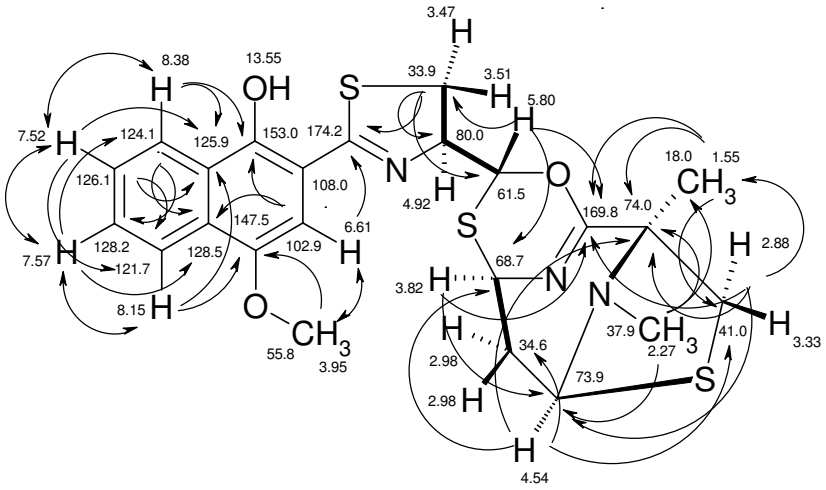


Figure 22: H,H COSY (↔,▬) and selected HMBC (→) connectivities of Karamomycin C (**78**).

Table 4: ^{13}C and ^{1}H NMR spectroscopic data of Karamomycin C (**78**) in $CDCl_3$.

Karamomycin C (**78**)					
Position	δ_H (*J* in [Hz])[a]	δ_C[b]	Position	δ_H (*J* in [Hz])[a]	δ_C[b]
1	-	153.0	9	-	174.2
1-OH	13.55 (s)	-	11	3.51 (ABX, *J* = 11.1, 6.6), 3.47 (ABX, *J* = 11.0, 8.7)	33.9
2	-	108.0	12	4.92 (m)	80.0
3	6.61 (s)	102.9	13	5.80 (d, *J* = 5.4)	61.5
4	-	147.5	15	-	169.8
4-OCH_3	3.95 (s)	55.8	17	3.82 (dd, *J* = 9.7, 6.2)	68.7
4a	-	128.5	19	-	74.0
5	8.15 (dd, *J* = 7.7, 0.5)	121.7	19-CH_3	1.55 (s)	18.0
6	7.57 (td, *J* = 8.2, 1.3)	128.2	20-N-CH_3	2.27 (s)	37.9
7	7.52 (td, *J* = 8.2, 1.3)	126.1	21	4.54 (sbr)	73.9
8	8.38 (dd, *J* = 8.2, 0.6)	124.1	22	2.98 (m)	34.6
8a	-	125.9	24	3.33 (d, *J* = 10.8), 2.88 (d, *J* = 10.8)	41.0

[a](600 MHz); [b](150 MHz)

The relative stereochemistry of karamomycin C (**78**) was confirmed on the bases of the CD spectra and NOESY experiments. The CD spectra as well as specific rotation $[\alpha]_D$ -336 pointed to the compound is mostly of negative rotation, at which, the CD spectra exhibited three bands in the negative region (λ_{max} 240, 326 and 223 nm), while only one band was in the positive region at λ_{max} 210 nm. On the bases of NOESY experiments (Figure 24), H-12 (δ 4.92) showed coupling with both protons of H_2-11 (δ 3.51, 3.47), and both methines H-13 (δ 5.80) and H-17 (δ 3.82). However,

the last two methines (H-17 & H-13) displayed weak NOESY to each other, pointing to their existence in anti-sides. The methine proton H-17 (δ 3.82) exhibited a further coupling with the methine proton H-21 (δ 4.54) along with the protons of H_2-22 (δ 2.98). The last methine (H-21) showed in turn a coupling with the N-methyl protons (20-CH_3, δ 2.27). Accordingly, the protons H-12, 17, 21 and 19-CH_3 have the same direction. So, the positions of C-12 and C-21 were of S-configuration, while C-13, C-17 and C-19 were of *R*-configuration. From the NOESY spectrum, compound **78** was predominantly of *R**-configuration (i.e. three (*R**)-stereo-centres and two *S**-stereo centres) (Figure 23). Few (*ca.* ~10) naturally occurring 1-hydroxy-4-methoxy-naphthalene derivatives were reported[61,105], some of them are characterised by their cytotoxicity[122] and herbicidal activity[112].

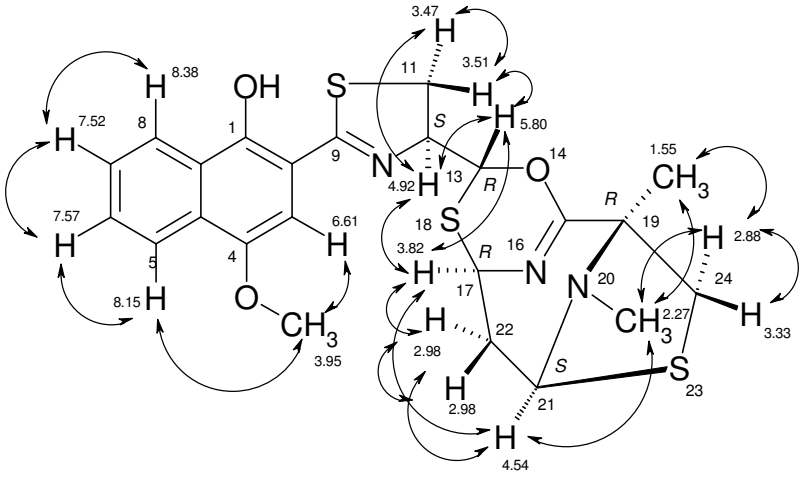


Figure 23: Selected NOESY coupling in Karamomycin C (**78**).

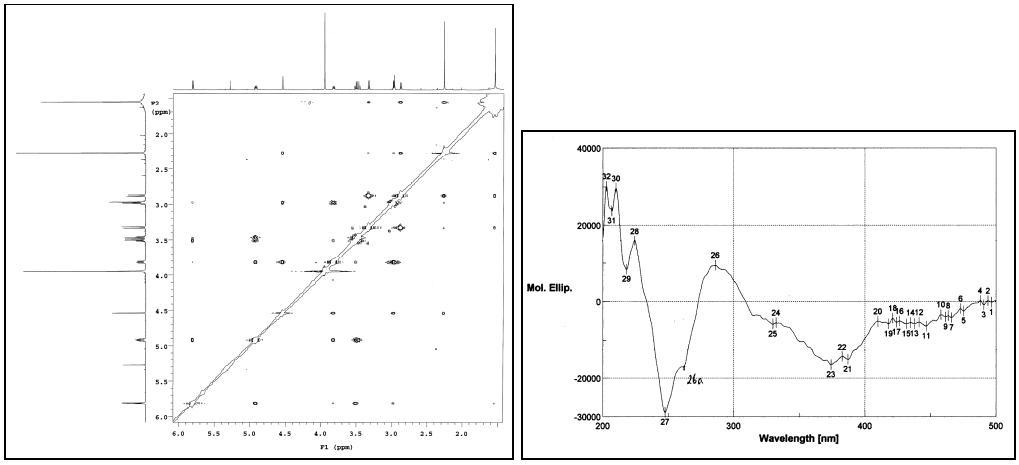


Figure 24: NOESY ($CDCl_3$, 600 MHz) and CD spectra of Karamomycin C (78).

4.1.5 Hypothetic Biosynthetic Pathway of Karamomycins

Feeding experiments to elucidate the biosynthesis of the karamomycins have not been performed so far, so that the following considerations are highly speculative. In a first step, the naphthalene carboxylic acid **71** could be combined with cysteine (**86**) followed by cyclization and reduction to give karamomycin A (**73**). On dehydrogenation, the latter would yield karamomycin B (**75**). Additionally, **87** was combined with the β-amino acid (**88**) followed by cyclization to deliver the analogue **89**. The latter was further combined with dimethyl-cystein (**90**) followed by cyclization to give karamomycin C (**78**) (Figure 25). Dimethylcystein (**90**) was reported in many natural compounds including thiazostatins A-B (**91**-**92**)[123] and watasemycins A-B (**93**-**94**)[124], which could be also present in karamomycin C (**78**). While, the amino acid **87** was not reported so far as natural product, which may be exchanged with an cystein analogue.

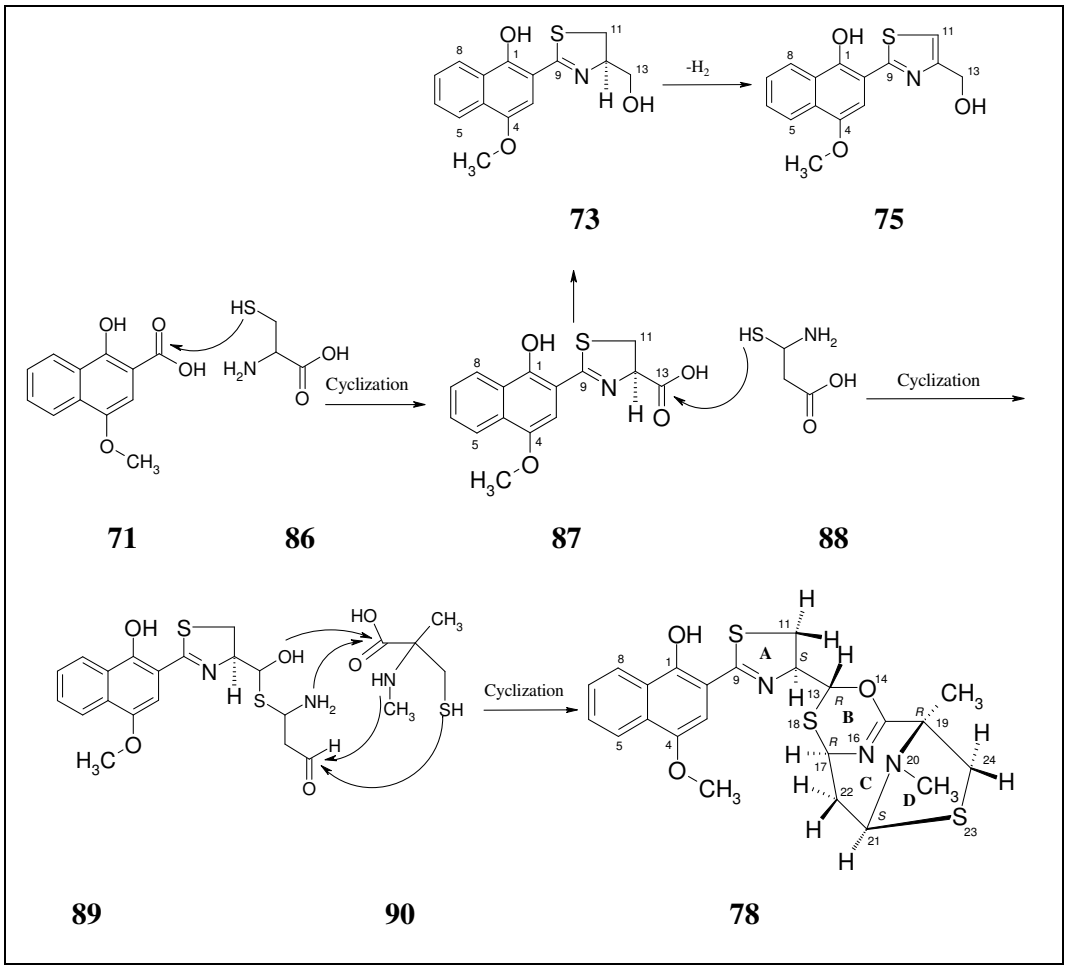
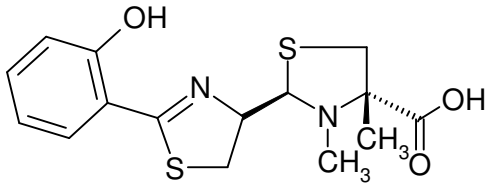
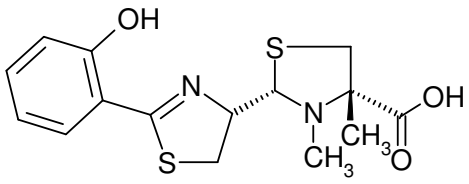


Figure 25: Proposed biosynthesis of Karamomycins A (**73**), B (**75**) and C (**78**).



91



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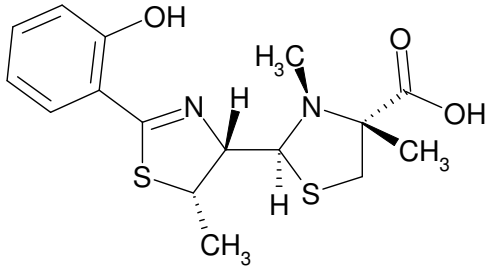

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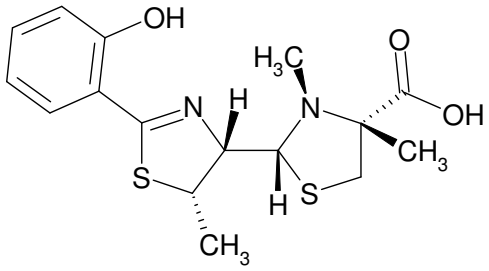


94

4.1.6 Biological Properties

Using the agar diffusion disk method (40 μg/disk), the karamomycins A~C (**73**, **75** and **78**) were inactive against selected bacteria, fungi and micro algae. Karamomycin A (**73**) displayed, however, an unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 6.78 μM (mean IC_{70} = 11.20 μM) (Table 48). The antitumor activity of karamomycins B (**75**) and C (**78**) are still under investigation.

4.2 Terrestrial *Streptomyces* sp. TN58

The terrestrial *Streptomyces* sp. TN58 was isolated from a Tunisian soil sample and taxonomically identified by L. Mellouli[125] as a new aerobic Gram-positive bacterium. A white aerial mycelium was formed on incubation on M_2^+ medium for 4 days at 28 °C, as it is characteristic for streptomycetes. This strain was selected, because a potent antibacterial activity against a set of Gram-positive and Gram-negative bacteria was observed. Moreover, the strain was used for genetic transformations[126]. As we have already published most of the chemical results[127], they will be discussed only briefly in the following text.

In the chemical screening using TLC, the extract showed numerous UV absorbing and blue florescence bands. On spraying with anisaldehyde/ sulphuric acid and heating for staining three major bands appeared, two of them as dark-green, while the other remained as dark red.

Therefore, the strain was applied to fermentation on large-scale cultivation (25 l shaker) using M_2^+ medium for isolation of its bioactive constituents. A brown crude extract (1.30 g) was obtained after a series of working up. The extract was analysed by TLC, showing a similarity with those described in primary chemical screening. An application of the extract to silica gel column chromatography monitored by TLC, followed by purification on Sephadex LH-20 column chromatography delivered six compounds. They were brevianamide F [*cyclo*(tryptophanyl-prolyl, **95**][128,129], N^{β}-acetyltryptamine (**96**)[130,131], thiazolidomycin (acidomycin, **97**), 1-O-(2-aminobenzoyl)-α-L-rhamnopyranoside (**99**), 4-hydroxybenzoyl-α-L-rhamnopyranoside (**100**) and uracil (Figure 26). Dereplication of trivial compounds was done with AntiBase and is not discussed here.

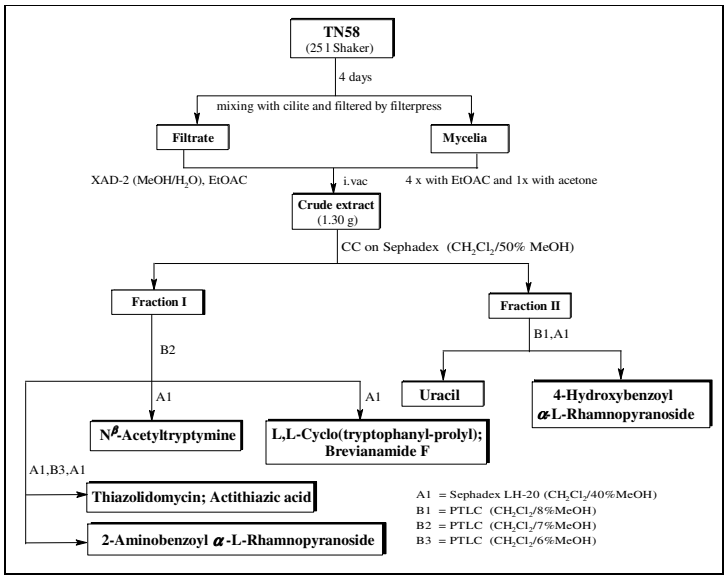
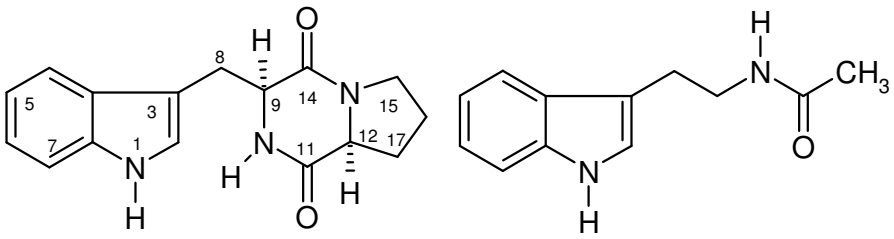


Figure 26: Work-up procedure of the terrestrial *Streptomyces* sp. TN58.



95 **96**

4.2.1 Thiazolidomycin; Acidomycin

Beside **95** and **96**, sub-fraction I delivered compound **97** as white crystals, using PTLC followed by Sephadex LH-20. It was a non UV-absorbing substance, which turned dark reddish brown by anisaldehyde/sulphuric acid reagent. The molecular weight of compound **97** was determined by EI and ESI mass spectra as *m/z* 217, and the corresponding molecular formula $C_9H_{15}NO_3S$ was established by HRESI MS, containing 3 double bond equivalents. This pointed to **97** as compound with no aromatic properties.

The ^{1}H NMR spectra showed a 1H multiplet (δ 4.75) of a methine proton linked to a hetero atom (oxygen and/or nitrogen). Additionally, two signals each of two methylene protons appeared at δ 3.51 (s) and 2.27 (t); their high shift value indicated again their connection with hetero atoms and/or carbonyl groups. Four additional methylene groups were recognized in the region of δ 1.30-190.

The ^{13}C/APT NMR spectrum revealed 9 carbon signals corresponding to the molecular formula. They were classified into two carbonyls of carboxylic acids or amides (δ 178.2 and 176.9), one sp^3 methine (δ 59.1), and six methylene signals (δ 39.6- 25.9).

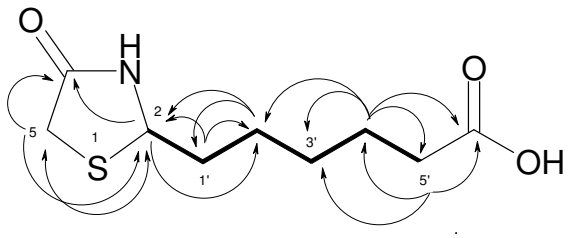
Based on the spectral data and the molecular weight, a search in AntiBase delivered the structure of thiazolidomycin (**97**). As there were no spectroscopic data reported for **97**, we assigned the structure here by detailed 2D NMR experiments (H,H COSY, HSQC and HMBC) (Figure 27).

Table 5: ^{13}C and ^{1}H NMR data of Thiazolidomycin (**97**) in CD_3OD.

Position	Thiazolidomycin (**97**)	
	δ_C[a]	δ_H (J in [Hz])[b]
2	59.1	4.75 (m)
4	176.9	-
5	32.7	3.51 (s)
1'	39.6	1.89 (m, H_a), 1.73-1.56 (m, H_b)
2'	25.9	1.50-1.34 (m)
3'	29.8	1.50-1.34 (m)
4'	26.0	1.73-1.56 (m)
5'	35.2	2.27 (t, 7.3)
6'	178.2	-

[a] (75.4 MHz); [b](300 MHz);

Compound **97** belongs to the actithiazic acid group, isolated from numerous microorganisms, e.g. *Streptomyces cinnamonensis*, *S. acidomyceticus*, *S. lydicus*, *S.virginiae*, *S. lavendulae* and *S. roseochromogenes*[132]. The compound was reported as antibacterial, anti-yeast and antitumor agent[133].



97

Figure 27: H,H COSY (▬, ↔) and HMBC (→) correlations of Thiazolidomycin (**97**).

4.2.2 1-O-(2-Aminobenzoyl)-α-L-rhamnoside

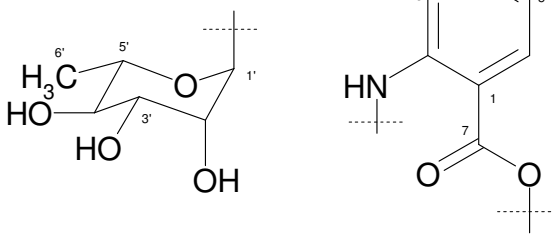
Using same methods as outlined above, compound **99** was isolated from subfraction I as white solid. It showed a bright blue fluorescence under UV light, and turned dark green after spraying with anisaldehyde/sulphuric acid. The molecular weight of **99** was established by EI mass spectra as *m/z* 283.

The ^{1}H NMR spectra showed four aromatic protons (δ 7.77~ 6.57), which were characteristic for a 1,2-disubstituted benzene system. In the aliphatic and sugar region, five oxygenated methines were displayed at δ 6.15 (1H, d) and 3.93-3.49 (4H,

m), revealing the presence of a sugar moiety, in addition to a methyl doublet at δ 1.28.

Based on the ^{13}C/APT NMR spectrum, 13 carbon signals were deduced, and grouped into the following categories: one carbonyl signal appeared at δ 167.3, which could be assigned to a carboxylic acid, ester or amide, two quaternary carbons (δ 153.5 and 110.0), four methine carbon signals (δ 135.7, 131.8, 117.9 and 116.5), constructing a 1,2-disubstituted aromatic system. The remaining six sp^3 carbons belonged to a sugar moiety; five of them were of oxygenated methines (δ 94.9, 73.5, 72.4, 72.3 and 71.4), and the remaining one was of a methyl signal (18.1). This confirmed the sugar system as rhamnose (**A**) or a stereoisomer.

The molecular weight of compound **99** was confirmed by EI mass spectra as *m/z* 283. The odd mass revealed the presence of an odd number of nitrogen atoms in the molecule. On EI MS, a fragment peak at *m/z* 137 corresponded to an anthranilic acid unit (**B**), which was attributed to expulsion of rhamnose from the whole molecule ([M– rhamnose]). Hence compound **99** contained the partial structures of rhamnose (**A**) and anthranilic acid (**B**), which were further established by H,H COSY correlations. With respect to the connection between the sugar moiety and the acid, two structures, **98** and **99** were plausible.

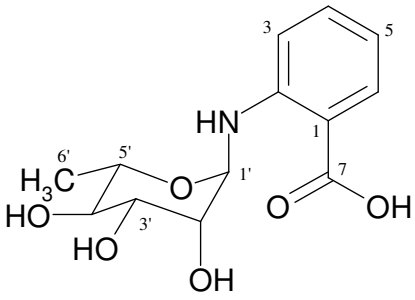


A **B**

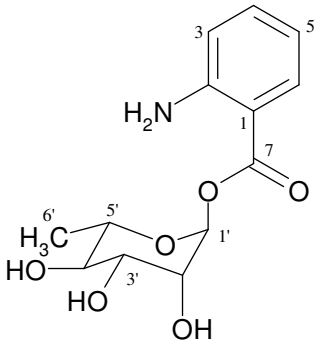
Based on the ^3J coupling between the anomeric proton at δ 6.15 and the acid carbonyl (δ 167.3) in an HMBC experiment, the recognized as 1-O-(2-aminobenzoyl)-α-L-rhamnoside (**99**).

1-O-(2-Aminobenzoyl)-α-L-rhamnoside (**99**) had already been obtained after feeding a streptomycete culture Tü 3634 with anthranilic acid[134].The structure of **99**including the configuration of the sugar was further confirmed by comparison with

authentic spectra from Grond *et al.*[135]. As no further spectroscopic data were reported for **99**, a complete structure assignment is done here for the first time using 2D NMR spectroscopy (Table 6 and Figure 28).



98



99

Table 6: ^{13}C and ^{1}H NMR (CD_3OD, *J* in [Hz]) data of 1-O-(2-Aminobenzoyl)-α-L-rhamnoside (**99**).

Position	1-O-(2-Aminobenzoyl)-α-L-rhamnoside (**99**)[a]	
	δ_C (150 MHz)	δ_H (300 MHz)[c]
1	110.0	-
2	153.5	-
3	117.9	6.75 (dd, 8.4, 0.6)
4	135.7	7.26 (td, 7.0, 1.6)
5	116.5	6.57 (td, 7.1, 1.1)
6	131.8	7.77 (dd, 8.1, 1.6)
7	167.3	-
1’	94.9	6.15 (d, 1.8)
2’	71.4	3.93 (dd, 3.4, 1.9)
3’	72.3	3.83 (dd, 9.5, 3.4)
4’	73.5	3.49 (t, 9.5)
5’	72.4	3.73 (m)
6’	18.1	1.28 (d, 6.2)

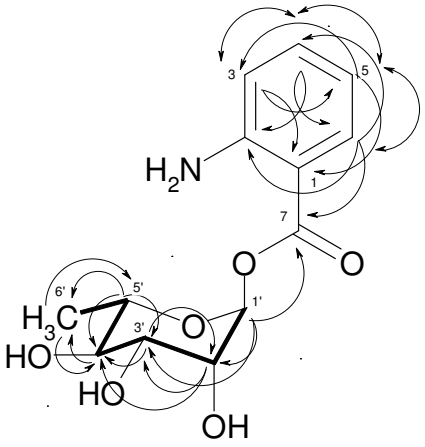


Figure 28: H,H COSY (▬, ↔) and HMBC (→) correlations of 1-O-(2-aminobenzoyl)-α-L-rhamnoside (**99**).

4.2.3 4-Hydroxybenzoyl α-L-rhamnopyranoside

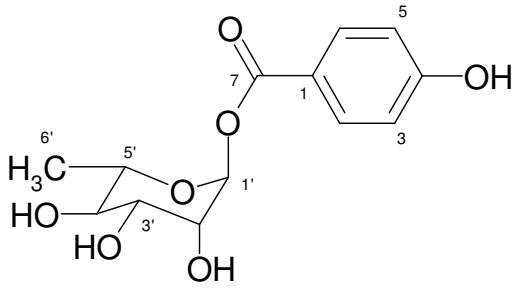
An additional white solid was isolated from fraction II using PTLC followed by Sephadex LH-20. It showed similar chromatographic properties as **99**, but had a higher polarity.

The ^{1}H NMR spectra displayed two 2H doublets at δ7.06 and 6.68 (^{3}J = 8.3 Hz) corresponding to a 1,4-disubstituted benzene ring. In the aliphatic region, 5 oxygenated methine protons and one methyl doublet (δ1.28) were visible, characteristic for the rhamnose moiety as in **99**.

(-)-ESI MS exhibited three *quasi*-molecular ion peaks at *m/z* 589 ([2M-2H+Na]$^{-}$), 567 ([2M-H]$^{-}$) and 283 ([M-H]$^{-}$), establishing the molecular weight as 284 Dalton, 1 amu higher than that of **99**. The even number of the molecular weight proved the replacement of the amino group in **99** by a hydroxyl group in **100**. This is coincident with 4-hydroxybenzoyl-α-L-rhamnopyranoside (**100**). A search in AntiBase led to **100** as sole consistent structure, which was further confirmed by comparison with the literature[134, 136]. Compound **100** is known as an inhibitor of 3α-hydroxysteroid-dehydrogenase (3α-HSD)[136].

The compound was first isolated from *Streptomyces* sp. GT 61150 by Hu *et al.* in 2000. Biosynthetically, it was recently investigated by Grond *et al.* by feeding of 4-hydroxybenzoic acid[134~137].

According to the literature, rhamnopyranoside derivatives possess inhibitor activity against the enzyme 3α-hydroxysteroid-dehydrogenase (3α-HSD) but not antimicrobial activities. According to our biological tests, we have noted a weak antibacterial activity of the two rhamnopyranosides against *S. aureus*; this one of compound **99** was with 30 μg/disc slightly higher[127].



100

4.3 Terrestrial *Streptomyces* sp. GW19/5671

The terrestrial *Streptomyces* sp. GW19/5671 was selected because of its behaviour in the pre-screening: The extract showed no coloured bands on TLC at daylight,

however, spraying with anisaldehyde/ sulphuric acid led to a middle polar violet coloured band, which was no observed in other strains and in another media. The produced secondary metabolites showed a high activity against *Staphylococcus aureus,* moderate activity against *Bacillus subtilis* and *E. coli*, and weak activity against *Candida albicans* (Table 49).

Consequently, the strain was applied to fermentation as 20 l shaker culture using casein medium for 10 days affording yellowish-brown crude extract. After a series of chromatographic methods, the new isrocin (**101**) along with uracil were isolated (Figure 29).

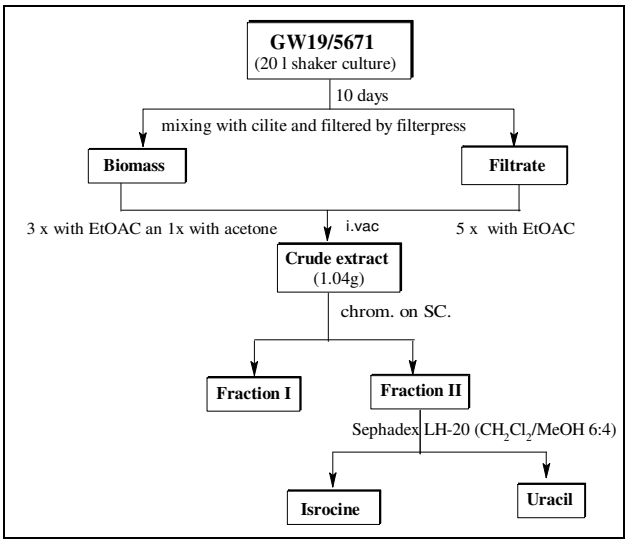


Figure 29: Work-up procedure of the terrestrial *Streptomyces* sp. GW19/5671

4.3.1 Isrocine; N-[3-Hydroxy-1-(4-hydroxy-phenyl)-propyl]-acetamide

Fraction II showed a faintly UV absorbing zone at 254 nm, which turned violet after spraying with anisaldehyde/sulphuric acid. The component was extracted and further purified on Sephadex LH-20 and by PTLC to afford an oily colourless substance.

The UV spectrum (MeOH) revealed a high peak at λ_{max} 276 nm characteristic for an aromatic compound without further conjugation. The IR spectrum (KBr) showed a band at ν 3279 cm^{-1} characteristic for a hydroxyl or secondary amino group. The spectrum displayed additionally a sharp band at ν 1666 cm^{-1} characteristic for an amide carbonyl group. It showed also several signals between 1650-1516

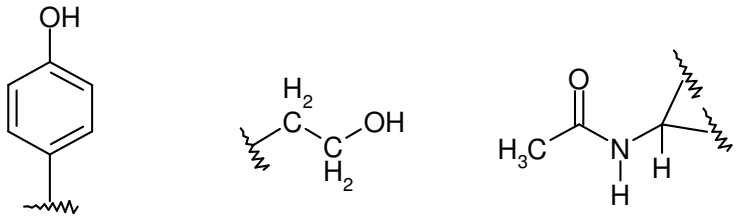
cm^{-1}, as well as a band at 1456 characteristic for methylene bond vibration. It showed additionally bands at ν 1263 and 1061 due to C-N or C-O bonds as well as a band at ν 834 due to a *p*-substituted aromatic system.

The ^{1}H NMR spectra showed an exchangeable broad singlet with an intensity of 1 H at δ 9.15, which could be due to a phenolic hydroxyl group. It revealed additionally another exchangeable 1 H doublet at δ 8.1 (^{3}J = 7.2 Hz). A 1,4-disubstituted aromatic ring was displayed in the spectrum by the presence of two 2H doublets at δ 7.06 and 6.68 (^{3}J = 8.3 Hz). In the aliphatic region, it showed four signals, of which a 1H quartet at δ 4.78 (^{3}J = 7.2 Hz) changed to a triplet after H/D exchange. A broad exchangeable signal at δ 4.42 suggested a further OH group, a multiplet with the intensity of 2 H at δ 3.33 indicated an oxygenated aliphatic methylene group, and the singlet at δ 1.78 indicated a methyl group attached to an sp^2 carbon atom either of an olefin or a carbonyl group. Another 2 H multiplet at δ 1.76 was due to a methylene group.

The ^{13}C/APT NMR spectrum showed two different types of carbon signals. The first group was displayed in the aromatic or olefinic region including two methine carbon signals at δ 127.5 and 114.9 and two quaternary carbon atoms at δ 155.5 due to a phenol and at δ 134.0 due to the attachment of a second substituent, forming a 1,4-disubstituted aromatic system. The spectrum showed a carbonyl signal at δ 168.3 which could be assigned to a carboxylic acid, an ester or amide. The second group showed four sp^3 carbon signals in the aliphatic region, one of which (δ 48.9) was due to a methine carbon connected to a hetero atom, namely nitrogen. A carbon signal at δ 57.9 represented a methylene group attached to oxygen, while a second methylene signal was at δ 39.8. The fourth signal at δ 22.7 was that of a methyl group attached to a sp^2 carbon atom.

The (+)-ESI mass spectrum showed two *quasi*-molecular peaks at *m/z* 441 ($[2M+Na]^+$) and 232 ($[M+Na]^+$) indicating a molecular weight of 209 Dalton. This was further confirmed by the EI mass spectrum which showed a molecular ion at *m/z* 209 followed by the loss of CH_2CH_2OH to give a peak at *m/z* 164 and followed by a further fragmentation to give a base peak at *m/z* 122 that is, loss of an acetyl group. High resolution EI MS afforded the molecular formula $C_{11}H_{15}NO_3$.

According to the revealed spectroscopic data, the compound consisted of an 1,4-disubstituted aromatic ring **A**, an ethanol group (**B**), and an acetamide fragment attached to a methine carbon (**C**).



A **B** **C**

The fragments above were established by H,H COSY correlations, which also confirmed the direct attachment of the ethanol fragment to the amide unit.

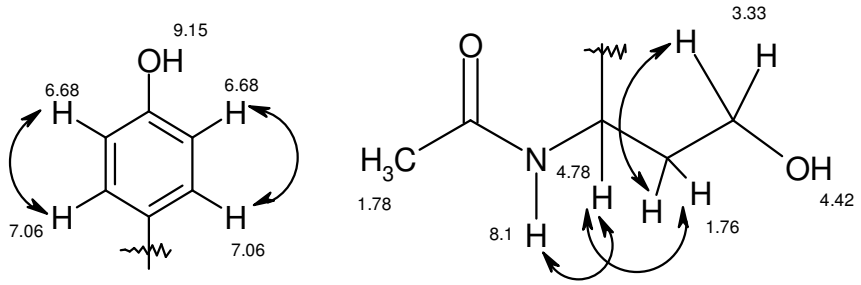
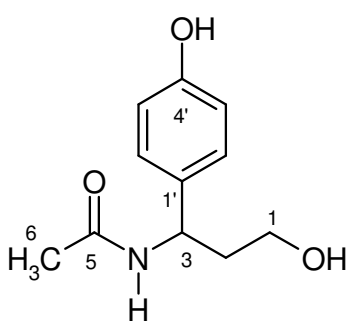


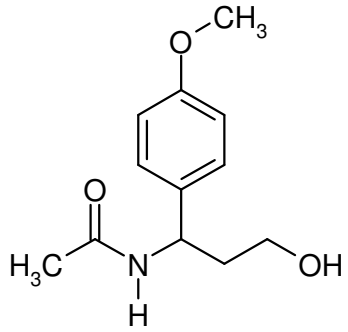
Figure 30: H,H COSY (↔) correlations of structural constituents of Isrocine (**101**).

Additionally, ^{3}J and ^{2}J couplings directing from the methine carbon in the aliphatic fragment to the aromatic carbons at positions 1 and 2/6 of the *para*-disubstituted phenolic moiety confirmed its direct attachment at position 1 of the aromatic ring. This was further confirmed by other 2D correlations, resulting in the new structure of N-[3-hydroxy-1-(4-hydroxy-phenyl)-propyl]-acetamide (**101**), which was named isrocine. A search for structurally related derivatives resulted in its methyl ether[138] **102**, which showed a close spectroscopic similarity with **101** and further confirmed the structure of isrocine.

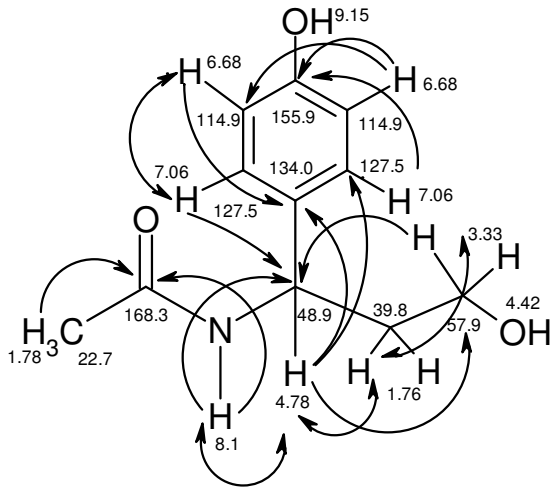
The methoxy derivative **102** was isolated from the cultures of *Streptomyces michiganensis* isolate Tü1074 by Corbaz *et al*. The strain was isolated from a soil sample of EL Djem (Tunesia); compound **102** was antibiotically inactive[138].



101



102



101

Figure 31: H,H COSY (↔) and HMBC (→) correlations of Isrocine (**101**).

4.3.2 Cultivation on M_2 Medium

The production of isrocine (**101**) on casein-medium and the antimicrobial activity encouraged us to cultivate the strain under other nutrient conditions to search for further active metabolites. The extract obtained from a 20-liter shaker culture on M_2 medium showed on TLC two slightly nonpolar yellow/green bands at day light as well as at 254 nm, in addition to middle UV absorbing zone at 254 nm.

The antimicrobial activity of the extract was higher against *Staphylococcus aureus* than that from the casein medium, moderately active against *Bacillus subtilis*, and *E. coli*, and again weakly active against *Candida albicans* (Table 49). Column chromatography on silica gel afford three compounds. Two of them were yellow solids and the third one was colourless (see Figure 32).

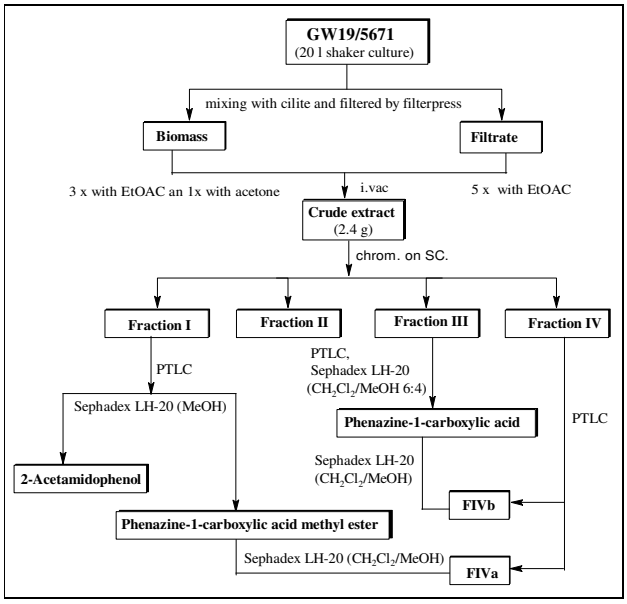
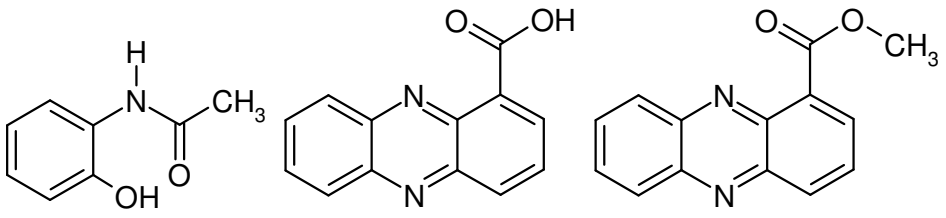


Figure 32: Work-up procedure of the terrestrial *Streptomyces* sp. GW19/5671 grown on M_2 medium.

4.3.2.1 *o*-Hydroxy-acetanilide (2-Acetamidophenol)

Preparative thin layer chromatography (PTLC) of fraction I followed by purification using Sephadex LH-20 (MeOH) lead to a colourless solid, whose molecular weight was established as 151 Dalton by ESI MS, which delivered the molecular formula $C_8H_9NO_2$ by HRMS.

The ^{1}H NMR spectrum revealed two broad singlets (δ9.40, 9.28), four aromatic protons (7.39~6.78) corresponding to a 1,2-disubstitiuted benzene ring, and an sp^2 bound methyl singlet (δ 2.22). Dereplication and comparison with authentic spectra confirmed this compound as 2-acetamidophenol (**103**)[139].



103 **104** **105**

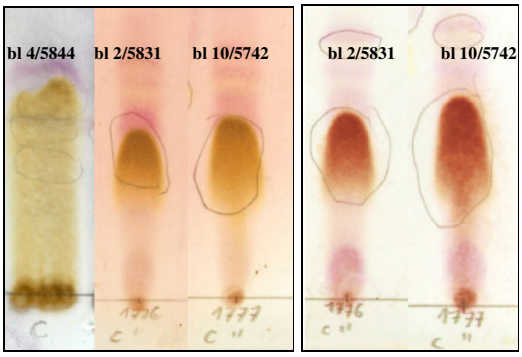
4.3.2.2 Phenazine-1-carboxylic acid and the Methyl ester

Compounds **104** and **105** were isolated by PTLC followed by Sephadex LH-20 as two yellowish-green solids with molecular weights of 224 and 238 Dalton, respectively. The ^{1}H NMR spectra confirmed the existence of five aromatic protons with high chemical shifts (δ8.98~8.26) as indicative for mono-substituted phenazine moieties. For **105**, an additional methyl ether signal at δ4.15 was displayed. Accordingly, a search in AntiBase and comparison with authentic spectra, the two compounds were identified as phenazine-1-carboxylic acid (**104**)[140] and methyl 1-phenazinecarboxylate (**105**®), respectively[141].

Phenazine-1-carboxylic acid (**104**) was isolated previously from several microorganisms, e.g. *Pseudomonas* sp., *Streptomyces cinnamonensis*, *Streptomyces misakiensis* and *Actinomadura dassonvillei*: It exhibits weak activity against Gram-positive bacteria, while it exhibits a moderate activity against both *Mycobacterium tuberculosis* BCG and *Mycobacterium tuberculosis* H_{37}Rv (streptomycin resistant)[140]. Methyl 1-phenazinecarboxylate (**105**) was also isolated from *Streptomyces luteoreticuli* as well as *Streptomyces misakiensis* and exhibited similarly activity against *Mycobacteria*[141].

4.4 Terrestrial *Streptomyces* spp. bl 2/5831, bl 4/5844 and bl 10/5742

On TLC, the terrestrial *Streptomyces* spp. isolates bl 2/583; bl 4/5844 and bl 10/5742 delivered a single major UV absorbing band (254 nm), which turned to brown and reddish-brown by spraying with anisaldehyde/sulphuric acid and Ehrlich's reagents, respectively (Figure 33). All three extracts exhibited a similar moderate antibacterial activity against Gram-positive bacteria (*B. subtilis, S. aureus*) in addition to a high anti-algal activity (*Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*) (Table 50).



a **b**

Figure 33: TLC of the crude extract from bl 2/5831, bl 4/5844 and 10/5742 after spraying with anisaldehyde/sulphuric acid (**a**) and Ehrlich's reagent (**b**).

To isolate this component, strains bl 2/5831 and bl 4/5844 were cultivated on C-medium as 2 L and 4 L cultures, respectively, using a rotary shaker for 6 days (28 °C). After working up, both strains afforded yellowish-brown extracts (0.40 g and 0.85 g). The two extracts were individually subjected to silica gel followed by Sephadex LH-20. Consequently, an identical colourless solid from both extracts was obtained, and was identified as pyrrol-2-carboxylic acid. Pyrrol-2-carboxylic acid is commonly isolated from *Streptomyces* and was isolated to first time from *Streptomycetes griseoflavus* in 1983[142]; it was responsible for the brown spot on TLC.

4.4.1 Optimization of the Terrestrial *Streptomyces* sp. bl 2/5831

The prolific productivity of pyrrol-2-carboxylic acid from these three terrestrial *Streptomyces* spp. isolates bl 2/5831, b1 4/5844 and bl 10/5742, prompted us to select the isolate bl 2/5831 for optimization, in an effort to find further interesting bioactive metabolites. Therefore, the strain bl 2/5831 was cultivated (6 days) using five culture media: Meat extract (C-medium) containing 50% sea water, C-medium with tap water, soja-mannite (SM-medium), fish powder (F-medium) and M_2 (A-Medium), at different pH (7.8 and 6.5), temperature (28 and 35 °C), and shaking rate (95, 110 rpm); (Table 51). TLC screening of the extracts obtained (Figure 34) indicated that the C-medium with 50% sea water (pH 7.8, 28 °C and 95 rpm) afforded a different main component in a yield of 130 mg/l, while the previously reported pyrrol-2-carboxylic acid was not present.

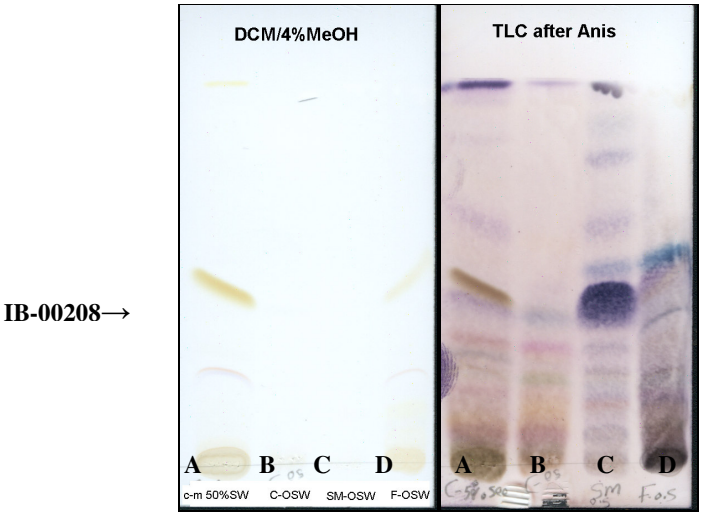


Figure 34: TLC of optimization after 10 days in 4 medium: A = C-medium with 50% sea water; B = C-medium; C = SM-medium; D = F-medium

A 4 L culture was harvested after 6 days, the culture broth was extracted with ethyl acetate and the crude extract (4.20 g) was purified by silica gel column chromatography to afford **106** as orange solid (520 mg, i.e. 130 mg/L) (Figure 35).

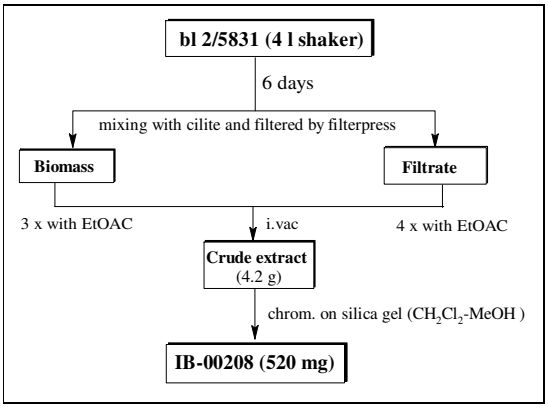


Figure 35: Work-up procedure of the terrestrial *Streptomyces* sp. bl 2/5831 using meat extract medium with 50% sea water

4.4.2 IB-00208

Compound **106** was obtained as an UV absorbing orange powder, which showed no colour change after treatment with sulphuric acid or spraying with anisaldehyde/sulphuric acid reagent. The colour was changed, however, to light red by treatment with dil. sodium hydroxide, which is indicative of *peri*-hydroxyquinones. The

molecular weight was deduced by (+)-ESI MS as 690 Dalton, and the corresponding molecular formula was $C_{36}H_{34}O_{14}$, containing ten double bond equivalents.

The IR spectrum of **106** indicated the presence of hydroxyl (3444 cm^{-1}) and conjugated carbonyl (1697, 1650 cm^{-1}) functionalities. The UV spectrum exhibited signals (λ_{max} 390, 321, 251 and 225 nm), characteristic for a polycyclic xanthone moiety[143].

The ^1H NMR spectrum displayed four 1H signals in the sp^2 region, representing two *o*-coupled benzenoide AB systems. The first set was visible at δ8.58 and 8.28 (J = 8.8 Hz), while the second one was found at δ7.46 and δ7.38 (d, J = 9.2 Hz), in addition to a singlet of a chelated hydroxyl group at δ12.71. In the aliphatic region, six oxy-methines, one methylene and five singlet oxy-methyl signals were detected. Additionally, two methyl doublets were observed at δ1.56 and 1.15; (Table 7).

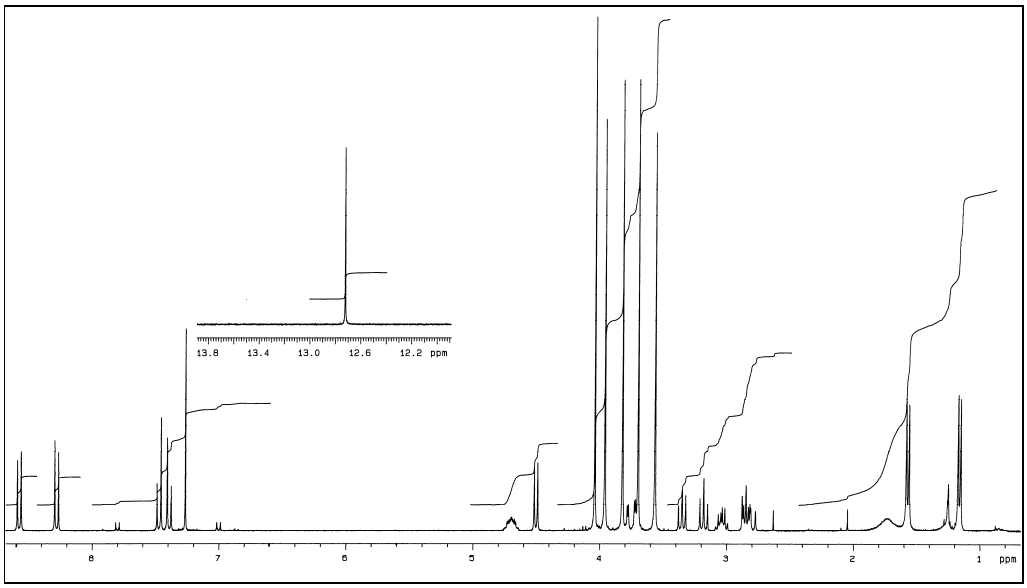


Figure 36: ^1H NMR spectrum ($CDCl_3$, 300 MHz) of IB-00208 (**106**).

The ^{13}C NMR/ APT spectra and HMQC experiments of compound **106** revealed 36 carbon signals (Table 7), i.e. 18 quaternary sp^2 carbon atoms (δ178~104) including four carbonyl signals (δ181.5~170.1), and four sp^2 methines (δ127~114.4). Additionally, six sp^3 oxy-methines (δ105~71) were displayed, among which one signal (δ 105.0) was assigned as an acetal H. Moreover, five methyl ether signals (δ

62.0~56.8), one methylene (δ30.2) together with two methyl signals (δ20.9 and 17.5) were visible (Table 7).

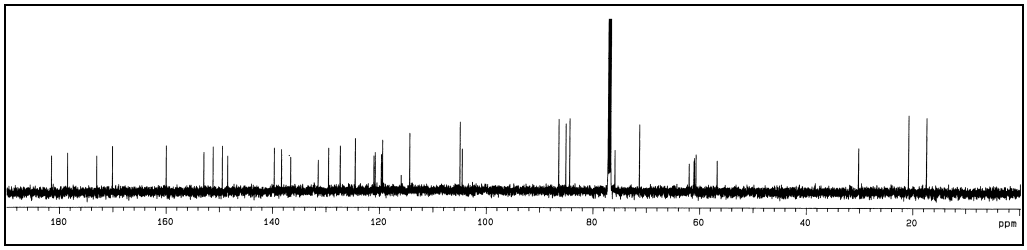
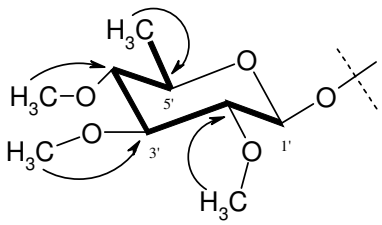
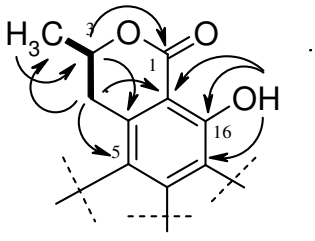


Figure 37: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of IB-00208 (**106**).

Compound **106** was established to bear a rhamno-pyranosyl moiety based on the H,H COSY and the coupling constants of the attached protons (J= 8~9 Hz). Accordingly, the anomeric methine C-1' (δ_H 4.51; δ_C 105.0) was of β–configuration (J~8 Hz). The three hydroxy groups of the sugar at C-2', C-3' and C-4' were replaced by three methoxy groups, at where protons of the three methoxy groups (δ 3.81, 3.69 and 3.56) showed a ^3J correlation in HMBC at the corresponding carbons (C-2': 61.4, C-3': 61.3, C-4': 61.0), respectively. Subsequently, the double methyl 5'-CH_3 (1.15) of trimethoxy-rhamnose was recognized as its directed ^2J correlation towards C-5' (71.7). The whole structure of sugar system was further deduced by H, H COSY giving the partial structure of A. As rather confirmation, the ESI MS^2 exhibited a peak fragment at *m/z* 525.2 due to the splitting of the glycoside moiety (A) from the whole molecule (*m/z* 713.2, $[M+Na]^+$) (Figure 40). The long-range coupling from H-1' to C-5 (δ139.7) shown by HMBC confirmed the attachment of the sugar at C-5 of ring B.



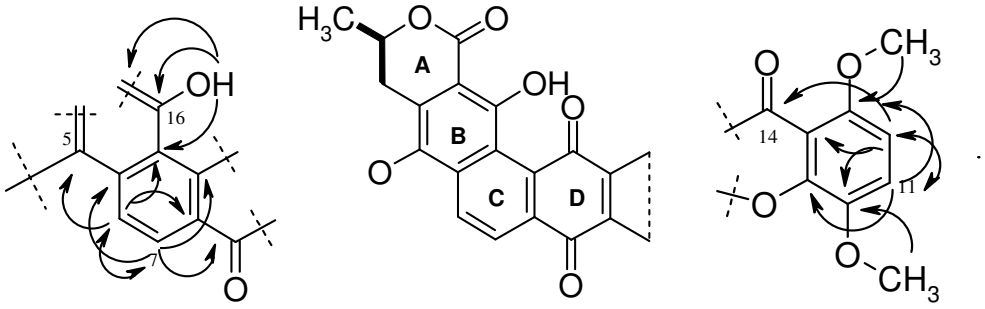
A



B

Additionally, the methylene protons of H_2-4 (δ 3.74, 2.80) exhibited five cross-sections in HMBC towards C-3 (δ 75.9, ^2J), 3-CH_3 (δ20.9, ^3J), C-4a (δ131.5, ^2J), C-5 (δ139.7, ^3J) and C-16a (δ104.5, ^3J). On other hand, the 3-CH_3 (δ1.57) directed

two important correlations at C-3 (^2J), and the lactonic carbonyl C-1 (δ170.1, ^4J) along with those at C-4 (^3J). This lead to construction of a lactone moiety fused with an aromatic residue (fragment B).

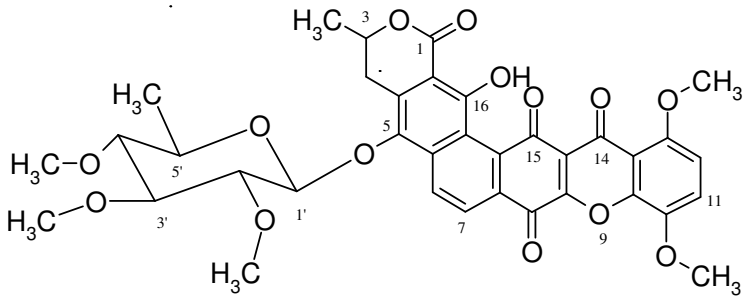


C **D**

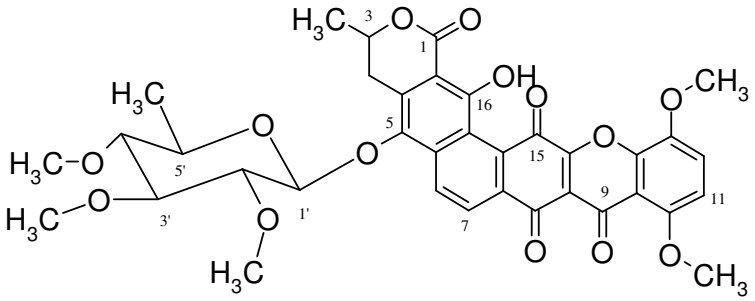
The singlet chelated OH signal (δ12.71, 16-OH) displayed three significant connectivities versus C-15b (119.9, ^3J), C-16 (160.3, ^2J), and C-16a (104.8, ^3J) as indicative of its joining at C-16 (ring **B**) in *peri*-position to the lactonic carbonyl (C-1, ring **A**). Alternatively, the two *ortho*-coupled protons (J~8.8 Hz) of H-6 (δ8.57) and H-7 (8.26) showed relevant cross-sections against C-5 (139.9), C-5a (138.6), C-7a (129.8), C-15a (136.9), C-15b (119.9) as well as to those of the quinone carbonyls, C-8 (178.8) and C-15 (181.8). Hence, the three rings (**B**, **C** and **D**) allowed the construction of the phenanthrone moiety fused with the lactone ring of **A**.

The remaining molecular formula ($C_9H_8O_4$) was constructed by HMBC correlations as follows: the residual ortho-coupled protons, H-11 (δ7.40) and H-12 (δ7.47) showed interchangeable correlations at C-9a (δ149.5), C-10 (δ148.5), C-11 (δ119.5), C-12 (δ114.4), and C-13 (δ151.2), C-13a beside to a γ-lactonic carbonyl (C-14, δ173.1). In addition, the residual methyl-ether groups at δ 4.04 and 3.96 displayed connectivities at C-10 and C-13, respectively, confirming their location at the assigned carbons. This supported the assignment of F ring (fragment D). The ^4J coupling from H-12 at C-14 gave an evidence for the location of the carbonyl of γ-pyrone system. Alternatively, this F fragment was connected to the ring C of phenanthrone moiety *via* the pyrone ring E. Hence two alternatives were suggested: 16-hydroxy-10,13-dimethoxy-3-methyl-5-(3,4,5-trimethoxy-6-methyl-tetrahydro-pyran-2-yloxy)-3,4-dihydro-2,9-dioxa-hexaphene-1,8,14,15-tetraone (**106**) and 16-hydroxy-

10,13-dimethoxy-3-methyl-5-(3,4,5-trimethoxy-6-methyl-tetrahydro-pyran-2-yloxy)-3,4-dihydro-2,14-dioxa-hexaphene-1,8,9,15-tetraone (**107**).



106



107

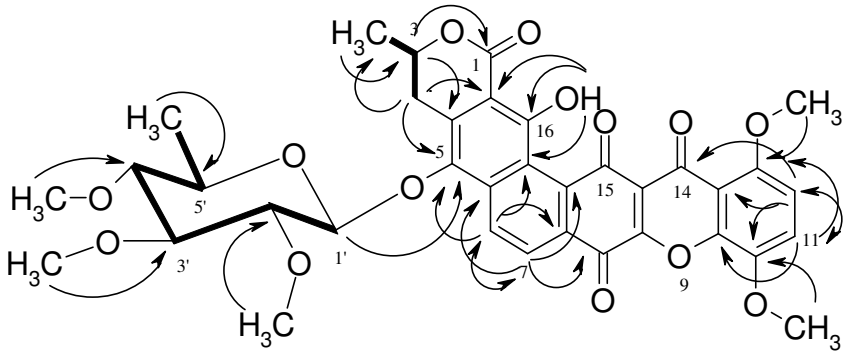


Figure 38: H,H COSY (↔, ▬) and selected HMBC (→) connectivities of IB-00208 (**106**).

Based on the similarity of NMR data of the two isomers, the final structure could not be determined. Also, additional spectroscopic techniques (e.g. NOE, NOESY and/or CD experiments), could not give a final confirmation of the structure. Therefore, the compound could be subjected to some chemical reactions in order to determine the correct isomer. Since this compound isolated, searching in all data-

bases resulted no hits, indicating the novelty of **106**. However one month later, searching in AntiBase using the deduced substructures (Figure 39) identified the compound **106** as IB-00208[144,145], which is coincided with the reported data (Table 7) but still may be not the correct structure.

Table 7: ^{1}H and ^{13}C NMR assignments for IB-00208 (106) in comparison with literature data in $CDCl_3$.

Position	Litrature[144,145]		IB-00208 (**106**)	
	δ_C [a]	δ_H (J in[Hz]) [b]	δ_C [c]	δ_H (J in [Hz]) [d]
1	170.4	-	170.1	-
3	76.2	4.68 (m)	75.9	4.70 (m)
3-CH_3	21.1	1.56 (d, 6.4)	20.9	1.57 (d, 6.3)
4	30.5	2.80 (dd, 16.2, 11.2) 3.74 (dd, 16.4, 3.0)	30.2	2.82 (dd, 16.6, 11.3) 3.75 (dd, 16.7, 2.9)
4a	131.8	-	131.5	-
5	139.9	-	139.7	-
5a	138.6	-	138.4	-
6	127.6	8.56 (d, 8.8)	127.4	8.57 (d, 8.8)
7	124.8	8.26 (d, 8.8)	124.6	8.26 (d, 8.8)
7a	129.8	-	129.6	-
8	178.8	-	178.6	-
8a	153.2	-	153.0	-
9a	149.8	-	149.5	-
10	148.7	-	148.5	-
10-OCH_3	62.3	4.03 (s)	62.0	4.04 (s)
11	119.7	7.38 (d, 9.2)	119.5	7.40 (d, 9.2)
12	114.6	7.46 (d, 9.2)	114.4	7.47 (d, 9.2)
13	151.5	-	151.2	-
13-OCH_3	57.0	3.95 (s)	56.8	3.96 (s)
13a	121.1	-	120.8	-
14	173.3	-	173.1	-
14a	121.3	-	121.1	-
15	181.8	-	181.5	-
15a	136.9	-	136.7	-
15b	119.9	-	119.7	-
16	160.3	-	160.0	-
16-OH	-	12.71 (s)	-	12.71 (s)
16a	104.8	-	104.5	-
1'	105.2	4.49 (d, 8.0)	105.0	4.51 (d, 7.9)
2'	84.7	3.34 (dd, 9.1, 8.0)	84.4	3.36 (dd, 9.2, 7.9)
2'-OCH_3	61.4	3.81 (s)	61.1	3.82 (s)
3'	86.7	3.17 (t, 9.0)	86.5	3.19 (t, 8.9)
3'-OCH_3	61.3	3.69 (s)	61.0	3.70 (s)
4'	85.4	2.84 (t, 9.0)	85.1	2.85 (t, 9.0)
4'-OCH_3	61.0	3.56 (s)	60.7	3.57 (s)
5'	71.7	3.02 (dd, 9.2, 6.0)	71.4	3.05 (m)
5'-CH_3	17.7	1.15 (d, 6.0)	17.5	1.17 (d, 6.0)

[a] (100 MHz); [b](400 MHz); [c](150 MHz); [b](300 MHz)

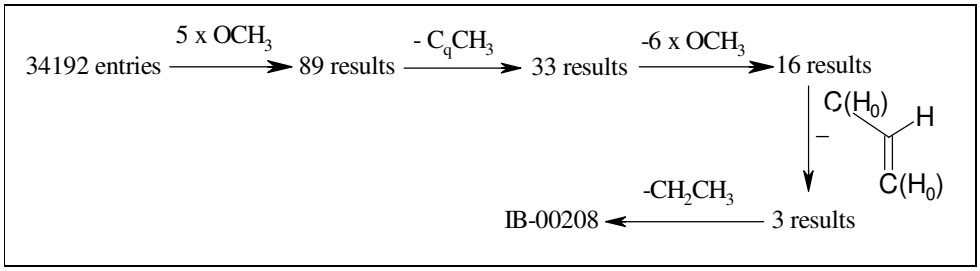


Figure 39: AntiBase search of IB-00208 (**106**).

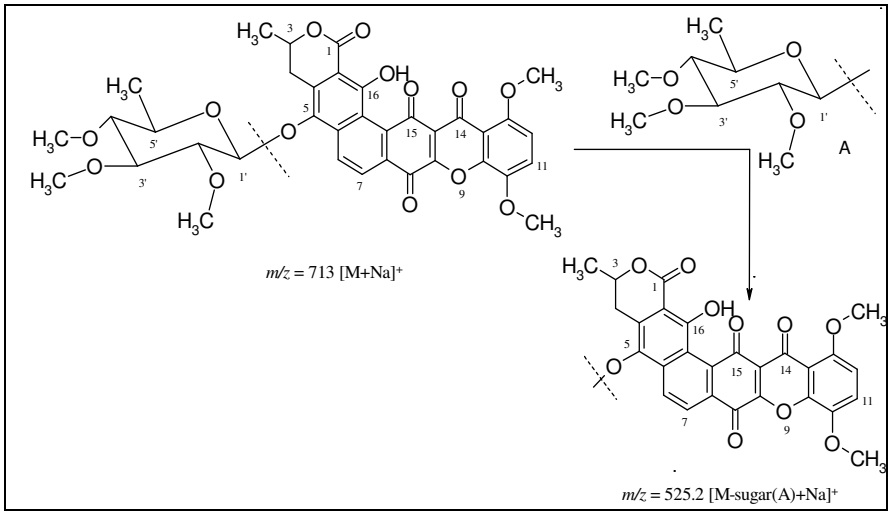


Figure 40: (+)-ESI-MS/MS of IB-00208 (**106**).

IB-00208 (**106**), a polycyclic compound possessing a xanthone unit, exhibit potent cytotoxic and antibiotic activity. This agrees well with our own results, determined in a monolayer cell proliferation assay using a panel of 36 human tumor cell lines, with a mean IC_{50} of 0.002 μM (mean IC_{70} = 0.007 μM), Table 53. Compound **106** is structurally related to a small family of polycyclic antibiotics including cervinomycins[146], citreamicins[148], simaomycins[149] and the highly cytotoxic polycyclic xanthones actinoplanones A~G; which have a lactam with partial reduction at ring F [150]. They were isolated from terrestrial *Micromonospora*, *Streptomyces*, *Actinomadura* and *Actinoplanes* strains, respectively.

4.5 Terrestrial *Streptomyces* sp. bl 47/4455

The terrestrial *Streptomyces* sp. bl 47/4455 drew attention due to its high antimicrobial activity the against Gram-positive bacteria *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57), as well as the fungus *Mucor miehei* (Table 54). On TLC screening, the extract exhibited various UV absorbing bands, some of which

were stained to reddish-brown, another one gave an intense yellow spot with anisaldehyde/sulphuric acid. Accordingly, the extract showed several components on HPLC/MS analysis with molecular weights in the region of *m/z* 604~915.

The TLC profiles of the extracts from strain bl 47/4455 grown on M_2 and C-media were similar, however, the productivity in C-medium was higher than in M_2 medium. Therefore, meat extract medium was used for a 20 L culture at standard conditions[151]. After 10 days, the broth was extracted using XAD-16 for the supernatant, and acetone for the mycelial cake. Extracts of both phases were evaporated *in vacuo* and the resulting water residue was re-extracted by ethyl acetate. The organic extract was concentrated and chromatographically separated. Eight bafilomycin derivatives **108~115** were isolated, among them the new bafilomycin K (**115**) (Figure 41). Structures of the isolated new bafilomycin K (**115**) and the known bafilomycin analogues; A_1 (**108**), A_2 (**109**), B_1 (**110**), B_2 (**111**), C_1 (**112**), TS155 (**113**) and D (**114**) were deduced by intensive studies of their NMR (1D&2D) and MS spectroscopic data. Bafilomycins A_2 (**109**), B_1 (**110**) and B_2 (**111**) were recently described by us in detail[152].

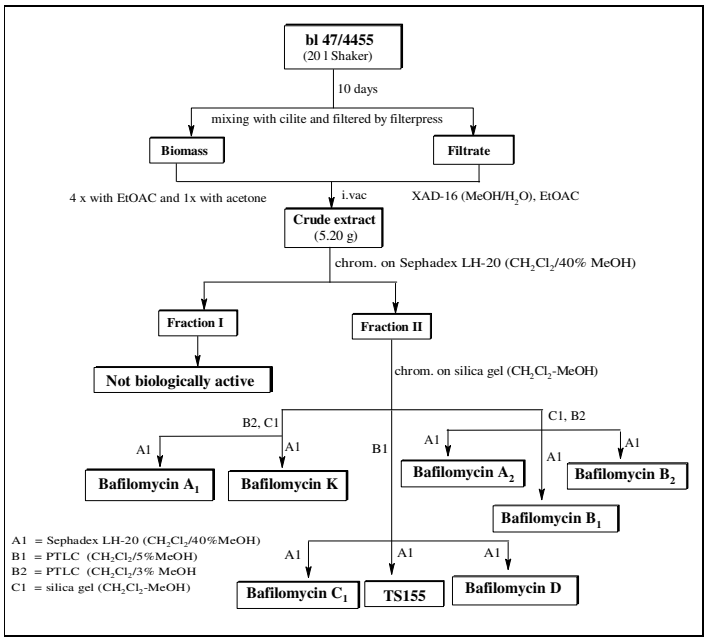
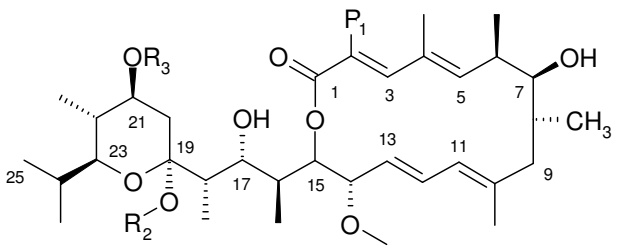
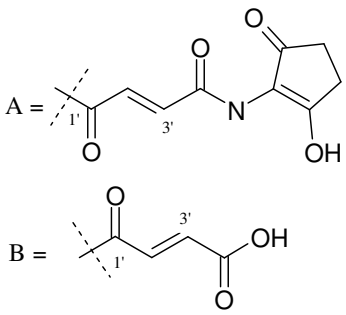


Figure 41: Work-up scheme of the terrestrial *Streptomyces* sp. bl 47/4455





108: $R_1 = OCH_3$ $R_2 = R_3 = H$
109: $R_1 = OCH_3$ $R_2 = CH_3$ $R_3 = H$
110: $R_1 = OCH_3$ $R_2 = H$ $R_3 = A$
111: $R_1 = OCH_3$ $R_2 = CH_3$ $R_3 = A$
112: $R_1 = OCH_3$ $R_2 = H$ $R_3 = B$
113: $R_1 = CH_3$ $R_2 = H$ $R_3 = B$
115: $R_1 = CH_3$ $R_2 = R_3 = H$

4.5.1 Bafilomycin A_1

Compound **108** was obtained as a colourless powder, which stained reddish-brown on TLC by spraying with anisaldehyde/sulphuric acid and heating. Based on positive and negative mode ESI spectra, the molecular weight of **108** was 622 Dalton.

The proton NMR spectrum displayed five olefinic protons located at δ 6.10 (s), 6.55 (dd), 5.80 (td, 2H), 5.15 (dd) along with two exchangeable broad singlets (δ 5.59 and 4.68). Five oxymethine protons appeared between δ 4.96~3.30; one of them gave a dd (4.96), while the others were multiplets. Four methyls singlet were visible, two them were of methoxy groups (δ 3.63 and 3.22), while the other two (δ 1.98 and 1.93) were bound to olefinic systems. Additionally, seven methyl doublets were located in the range of δ 1.08-0.70.

The ^{13}C/APT NMR spectra of **108** displayed 35 carbons signals, among them one carbonyl (δ 167.3) of acid, ester or amide, eight sp^2 carbons (δ 143.1~125.0), a hemiketal carbon (δ 98.9) and five oxygenated methines (82.2~70.6). Nine of the remaining carbons belonged to methyls (δ 59.9~7.0), of which two were methoxyls (δ 59.9 and 55.5).

A search with the discussed spectroscopic data and the molecular weight of **108** in AntiBase[61] pointed to bafilomycin A_1. The structure was further confirmed by comparison with authentic spectroscopic data from the literature[153]. Bafilomycin A_1 (**108**) is reported many times with wrong carbon assignments; its carbon assignments

were corrected here by comparison with the recent literature as well as in comparison with the new isolated bafilomycin analogue, bafilomycin K (**115**); Table 9.

Bafilomycin A_1 (**108**) is a 16-membered lactone, which was previously reported from *Streptomyces griseus* sp. s*ulphurus*[153-155]. Compound **108** and its analogues are strongly active against Gram-positive bacteria and fungi. Bafilomycin A_1 (**108**) was also reported as potent vacuolar Na^+, K^+, H^+-ATPase inhibitor[156], and inhibits cell growth by apoptosis[157]. Therefore, the compound was also of interest for synthetic chemistry, and it was totally synthesized by Toshima *et al.*[158] and Roush *et al.*[156].

4.5.2 Bafilomycin C_1

Compound **112**, was isolated as white solid from sub-fraction II using PTLC followed by Sephadex LH-20. The compound showed similar chromatographic properties as **108**. The ESI mass spectra delivered a molecular weight of 720 Dalton for **112**.

The ^{1}H NMR spectrum of **112** showed a similar pattern as bafilomycin A_1 (**108**), except that two 1H doublets (J = 15.0 Hz) of *trans*-protons at δ 6.78 and 6.40 were present in **112** but not in **108**. In accordance, a search in AntiBase referred to bafilomycin C_1 (**112**) as sole possible structure, which was further confirmed by a comparison with the literature[153,159]. Bafilomycin C_1 (**112**) was reported to exhibit higher[153] antibacterial activity than bafilomycins A_1 (**108**), A_2 (**109**), B_1 (**110**), and B_2 (**111**). Along with its insecticidal and immunosuppressive activity, bafilomycin C_1 (**112**) was reported as ATPase inhibitor. Particularly, compound **112** exhibited higher activity against fungi using the agar diffusion assay than antibacterial activity (Table 55).

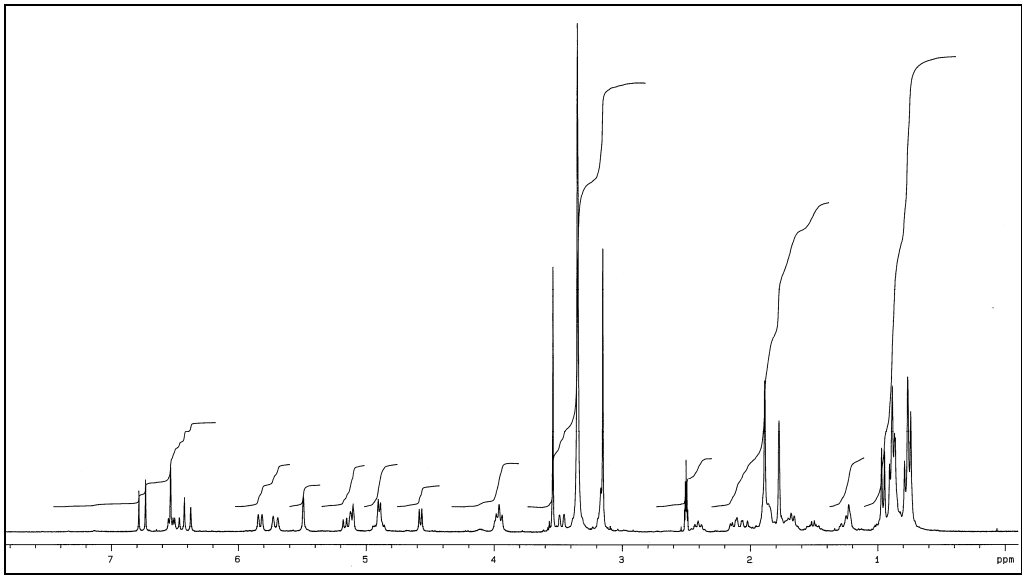


Figure 42: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of Bafilomycin C_1 (**112**).

4.5.3 TS155

The compound **113** showed the same physical and chemical properties as **112**, and also the ^{1}H NMR spectrum was very similar to that **112**. Whereas, both **108** and **112** had two methoxy groups, the ^{1}H NMR spectrum of **113** displayed only one methoxy signal (δ 3.08, 14-OCH_3). The missing methoxy at C-2 position (normally present at δ ~3.60) was replaced by a methyl signal located at δ 1.95 in **113** (Table 8). This assignment was supported by ESI MS, where the molecular weight of **113** was at *m/z* 704 with a corresponding molecular formula of $C_{39}H_{60}O_{11}$ with one oxygen less than in **112**. Correspondingly, the missing 2-methoxy carbon signal was replaced by a methyl signal at δ 13.3 in the ^{13}C NMR spectrum, while the sole methoxy carbon remained at the position of compounds **108** and **112** (δ 59.9).

Based on these data, compound **113** was identified as TS155[160], which had been reported in a patent. No spectroscopic data were reported for comparison; therefore, structure of the compound was here fully assigned using 2D experiments (H,HCOSY, HMQC and HMBC;) (Table 8). TS155 (**113**) inhibits the thrombin-induced calcium influx, which is a useful feature for manufacturing pharmaceuticals[160].

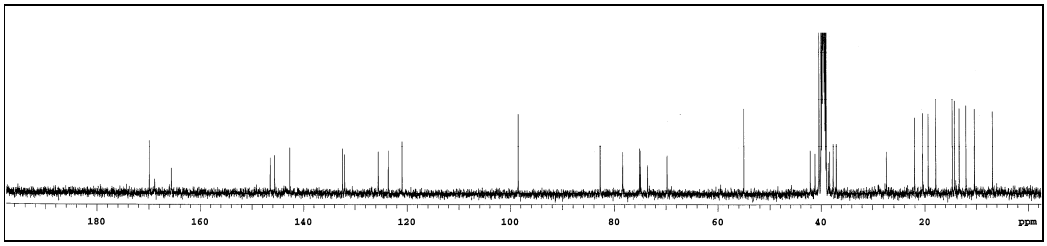


Figure 43: ^{13}C NMR spectrum (DMSO-d_6, 125.7 MHz) of TS155 (**113**).

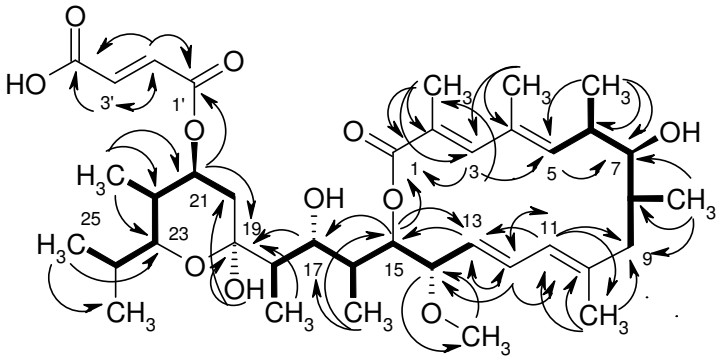
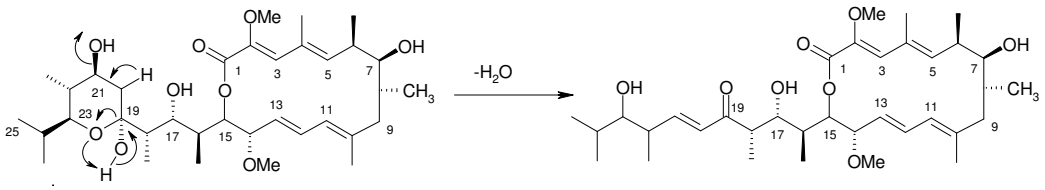


Figure 44: H,H COSY (▬, ↔) and selected HMBC (→) correlations of TS155 (**113**).

4.5.4 Bafilomycin D

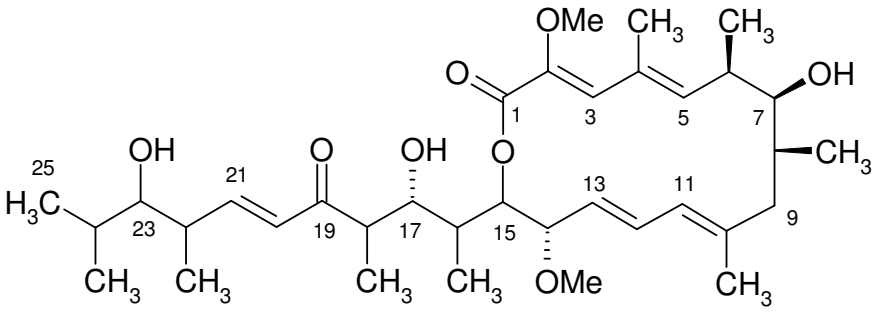
Compound **114** was isolated as yellow amorphous solid from sub-fraction II. It was a UV absorbing (254 nm) substance, which turned intensive yellow after spraying with anisaldehyde/sulphuric acid and heating. The molar mass of compound **114** was derived as 604 Dalton by (+)-ESI MS from the *quasi*molecular ions at *m/z* 1231 and 627 corresponding to $[2M+Na]^+$ and $[M+Na]^+$. Based on the ^{1}H NMR spectrum, structure of compound **114** was closely related to bafilomycin A_1 (**108**). The spectrum showed two doublets (J = 15.0 Hz) of *trans*-protons at δ 6.95 and 6.30. Furthermore, a ring opening of the tetrahydropyran moiety followed by dehydration at positions 20 and 21 in compound **108** lead to **114** (Figure 45).



108 **114**

Figure 45: Structural correlation between Bafilomycin A_1 (**108**) and Bafilomycin D (**114**).

The lower molar mass of **114** by 18 amu than **108** was attributed to the loss of a water molecule. This resulted in an α,β-unsaturated ketone, while the methylene (CH_2-20, δ 2.31-1.16) and its vicinal oxy-methine (δ3.69 (m) in **108** were replaced by a *trans*-olefinic bond. The acetonide carbonyl (C-19) and its conjugated α,β-en carbons (C_{20}-C_{21}) were assigned by APT/^{13}C NMR at δ203.2, 129.2 and 148.7, respectively (Table 8). The hemi-ketal carbon (δ98.9), and both the oxy-methine (C-21, δ70.8) and methylene (C-20, 43.4) signals seen in **108** were absent. According with the literature, the structure was established as bafilomycin D (**114**)[154,161]. Bafilomycin D (**114**) was previously obtained from *Streptomyces* sp.[161]. It has insecticidal and herbicidal activities, and is a K-dependent ATPase inhibitor[161].



114

Table 8: ^{1}H and ^{13}C NMR assignments of Bafilomycins C_1 (**112**), D (**114**), and TS155 (**113**).

	Bafilomycin C_1 (**112**)[a]	TS155 (**113**)[a]		Bafilomycin D (**114**)[b]		
Position	δ_H (J in [Hz])[c]	δ_C[d]	δ_H (J in [Hz])[e]	δ_C Lit[161]	δ_C[f]	δ_H (J in [Hz])[c]
1	-	170.0	-	166.5	166.4	-
2	-	121.0	-	141.6	141.3	-
2-CH_3	-	13.3	1.95 (s)	-	-	-
2-OCH_3	3.58 (s)	-	-	60.3	60.1	3.68 (s)
3	6.60 (s)	145.7	7.16 (s)	133.0	133.1	6.65 (s)
4	-	132.4	-	132.9	132.8	-
4-CH_3	1.90 (s)	14.6	1.90 (s)	13.9	13.9	1.98 (s)
5	5.85 (d, 10.0)	146.5	5.90 (d, 10.0)	142.3	142.5	5.78 (td, 9.2)
6	2.40 (m)	37.0	2.43 (m)	37.0	36.7	2.57 (m)
6-CH_3	1.00 (d, 7.0)	17.9	1.00 (d, 7.0)	17.5	17.3	1.07 (d, 7.0)
7	3.29 (m)	78.4	3.18 (m)	81.4	81.1	3.31 (dd, 6.5, 2.0)
8	1.97 (m)	39.9	1.91 (m)	39.9	39.8	1.91 (m)
8-CH_3	0.93 (d, 6.5)	22.0	0.93 (d, 6.5)	22.0	21.8	0.94 (d, 6.5)
9	2.17 (dm, 14.0); 1.94 (dd, 14.0, 11.5)	41.2	2.00 (dm, 14.0); 1.94 (dd, 14.0, 11.5)	41.4	41.2	2.13 (m)
10	-	142.7	-	142.9	142.9	-
10- CH_3	1.80 (s)	20.5	1.80 (s)	20.0	19.9	1.91 (s)
11	5.78 (d, 10.0)	123.6	5.70 (d, 10.0)	125.4	125.1	5.82 (d, 10.5)
12	6.55 (dd, 15.0, 10.5)	132.0	6.55 (dd, 15.0, 10.5)	132.8	132.8	6.48 (dd, 15.0, 10.5)
13	5.15 (dd, 15.0, 10.5)	125.5	5.10 (dd, 15.0, 10.5)	127.2	126.9	5.18 (dd, 15.0, 9.5)
14	3.98 (dd, 9.5, 8.7)	82.8	4.00 (dd, 9.5, 8.7)	83.5	83.3	3.81 (dd, 9.5, 8.5)
14-OCH_3	3.18 (s)	55.0	3.18 (s)	55.7	55.6	3.22 (s)
15	4.96 (dd, 8.7, 1.2)	74.9	5.03 (dd, 8.7, 1.2)	76.5	76.2	5.06 (dd, 8.7, 1.2)
16	2.13 (m)	37.6	2.13 (m)	38.7	38.4	2.06 (m)
16- CH_3	0.84 (d, 6.7)	10.4	0.80 (d, 6.7)	10.8	10.6	0.94 (d, 6.7)
17	4.13 (m)	69.8	3.90 (m)	72.8	72.5	3.76 (m)
17-OH	4.60 (sbr)	-	4.68 (d, 4.2)	-	-	3.63 (s br)
18	1.78 (m)	42.1	1.78 (m)	46.5	46.2	2.98 (m)
18-CH_3	0.95 (d, 7.2)	6.8	0.88 (d, 7.2)	10.4	10.1	1.21 (d, 7.2)
19	-	98.5	-	203.2	203.2	-
19-OH	5.59 (s br)	-	5.59 (s br)	-	-	-
20	1.88 (m); 1.16 (m)	38.3	2.10 (dd, 11.9, 4.7); 1.70 (dd, 11.9, 11.0)	129.4	129.2	6.29 (d, 16.0)
21	3.59 (m)	73.5	4.90 (m br)	148.6	148.7	6.92 (dd, 16.0, 14.2)
22	1.43 (m)	39.8	1.53 (m)	40.1	39.9	2.57 (m)
22-CH_3	0.94 (d, 6.5)	12.0	0.90 (d, 6.5)	16.7	16.8	1.07 (d, 6.5)
23	3.48 (dd, 10.2, 2.2)	75.1	3.48 (dd, 10.2, 2.2)	79.9	79.7	3.18 (dd, 10.2, 2.2)
24	1.89 (m)	27.4	1.79 (m)	31.1	30.9	1.72 (dd, 12.0, 8.0)
24-CH_3	0.90 (d, 6.7)	19.4	0.90 (d, 6.7)	19.8	19.6	0.90 (d, 6.7)
25	0.76 (d, 6.7)	14.2	0.76 (d, 6.7)	16.9	16.6	0.94 (d, 6.7)
1'	-	165.7	-	-	-	-
2'	6.78 (d, 15.1)	nd	6.78 (d, 15.1)	-	-	-
3'	6.40 (d, 15.0)	nd	6.40 (d, 15.0)	-	-	-
4'	-	168.9	-	-	-	-

[a]DMSO-d_6; [b] $CDCl_3$; [c] 300 MHz; [d] 125.7 MHz; [e] 600 MHz; [f] 75.5 MHz; nd = not detected

4.5.5 Bafilomycin K

Compound **115** was obtained as a colourless solid as well. It showed the same reddish-brown colour reaction with anisaldehyde characteristic for bafilomycins on

TLC. The molecular weight **115** was determined as 606 Dalton by ESI MS, and (+)-HRESI MS (629.402290, $[M+Na]^+$) confirmed the molecular formula as $C_{35}H_{58}O_8$.

The ^1H NMR spectrum established again a structure closely related to bafilomycin A_1 (**108**). A major difference between both structures was that one of the two methoxy signals in **108** was lost in **115**. Additionally, one olefinic bound methyl signal was observed in **115** at δ2.03, beside to one methyl ether signal at δ3.25. As the 2-OMe signal at δ3.63 was absent in **115**, it might be replaced by the additional olefinic methyl singlet (δ2.03). This conclusion was further supported by ^{13}C and APT NMR spectra in comparison with compound **108** (Table 9). Accordingly, 35 carbons signals were observed, among them only one methoxy signal (δ55.5) and the extra olefinic methyl carbon (δ15.2).

In the HMBC experiment, a relevant correlation between 3-H (δ7.22) and 2-CH_3 (δ15.2) was observed. Moreover, protons of this methyl group exhibited three important correlations, to the lactone carbonyl C-1 (172.0, ^3J), the directly attached C-2 (127.4, ^2J) and its olefinic partner C-3 (144.7, ^3J) (Figure 46). Based on these features, the methyl was confirmed at C-2. This replacement had an effect on the chemical shifts of directly attached and the neighbouring olefinic partner, where C-2 was shifted upfield to δ127.4 (δ141.3 in **108**), while H-3 was downfield shifted at δ 7.22 (δ6.71 in **108**). No corresponding structures were detected in databases, pointing to **115** as a novel bafilomycin analogue, which was named as bafilomycin K.

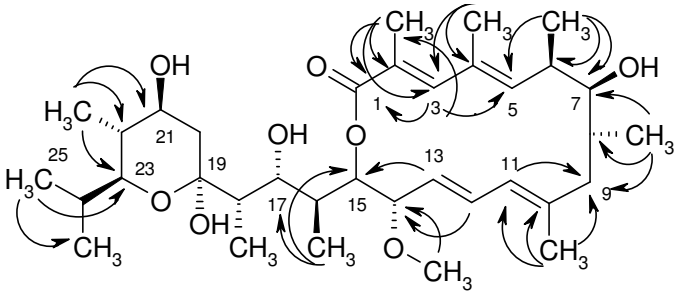


Figure 46: Selected HMBC (→) correlations of Bafilomycin K (**115**).

Bafilomycins are 16-membered macrolides, to which various side chains are attached (at the lactone terminus C15), leading to the generic term bafiloid [162]. To this class, numerous structural groups are belonging: the concanamycins[163], forma-

micins[164], hydrolidins[165], leucanicidins[166], PD-118576[167] and micromonospolides[168].

These metabolites were exclusively found so far in *Streptomyces* sp. and are characterized by their broad antibacterial, antifungal and antitumor activities[169]. Additionally, they are reported as Na^+, K^+ dependant ATPase inhibitors[153,159,]. Recently, these structural analogues were discovered as potent nematocidal agents [171]. Bafilomycins have been suggested as possible therapeutic agents in the treatment of peptic ulcera due to their virtue of inhibiting cell vacuolization induced by the bacterium *Helicobacter pylori*[172].

Table 9: The ^{13}C & ^{1}H NMR ($CDCl_3$) of Bafilomycins A_1 (**108**) and K (**115**).

Position	Bafilomycin A_1 (**108**)			Bafilomycin K (**115**)	
	δ_C Lit.[155]	δ_C[a]	δ_H (*J* in [Hz])[b]	δ_C[a]	δ_H (*J* in [Hz])[b]
1	167.3	167.3	-	172.0	-
2	141.3	141.3	-	127.4	-
2-CH_3	-	-	-	15.2	2.03 (s)
2-OCH_3	59.9	59.9	3.63 (s)	-	-
3	141.3	143.0	6.71 (s)	144.7	7.22 (s)
4	143.0	133.6	-	134.4	-
4- CH_3	14.0	13.9	1.98 (s)	13.7	1.99 (s)
5	125.1	143.0	5.80 (td, 10.0 1.2)	142.7	5.84 (br d, 8.9)
6	36.8	36.6	2.56 (m)	36.7	2.55 (m)
6-CH_3	17.2	16.0	1.06 (d, 7.0)	17.6	1.08 (d, 7.0)
7	81.0	81.0	3.29 (m)	81.2	3.29 (br d, 5.3)
7-OH	-	-	5.59 (s br)	-	5.64 (d, 2.0)
8	40.1	40.0	1.87 (m)	41.0	1.89 (m)
8-CH_3	14.5	14.3	0.93 (d, 6.5)	14.3	1.09 (d, 7,5)
9	41.3	41.2	2.17 (dm, 14.0); 1.94 (dd, 14.0, 11.5)	41.2	2.18 (m) 1.90 (m)
10	132.8	143.1	-	146.5	-
10- CH_3	20.1	20.1	1.93 (s)	20.1	1.94 (s)
11	142.7	125.0	5.80 (td, 10.0 1.2)	125.2	5.77 (br d 9.8)
12	133.0	133.0	6.55 (dd, 15.0, 10.5)	132.5	6.50 (dd, 14.9, 10,6)
13	127.0	126.9	5.15 (dd, 15.0, 10.5)	125.2	5.19 (dd, 15.0, 9.2)
14	82.3	82.2	3.89 (dd, 9.5, 8.7)	82.6	3.89 (q, 8.3)
14-OCH_3	55.5	55.5	3.24 (s)	55.5	3.25 (s)
15	76.8	76.7	4.96 (dd, 8.7, 1.2)	76.0	4.96 (dd, 8.2, 1.3)
16	37.2	37.1	2.13 (m)	37.6	2.11 (m)
16- CH_3	9.8	9.7	0.84 (d, 6.7)	9.8	0.81 (d, 6.8)
17	70.6	70.6	4.13 (ddd, 11.0, 4.1, 2.0)	70.4	4.11(m)
17-OH	-	-	4.68 (s br)	-	4.85 (d, 4.2)
18	42.1	42.0	1.78 (qm, 7.2, 0.9)	41.8	1.74 (m)
18-CH_3	7.0	7.0	1.04 (d, 7.2)	6.9	1.03 (d, 7.2)
19	98.9	98.9	-	98.8	-
20	43.5	43.4	2.31 (dd, 11.9, 4.7); 1.16 (ddd, 11.9, 11.0, 2.2)	43.4	2.29(dd, 11.9, 4.5) 1.17 (m)
21	70.8	70.8	3.69 (ddd, 11.0, 9.9, 4.7)	71.0	3.69 (m)
22	41.0	41.0	1.33 (m)	41.2	1.30 (m)
22-CH_3	21.7	21.6	0.94 (d, 6.5)	21.5	0.94 (d, 6.4)
23	75.9	75.8	3.48 (dd, 10.2, 2.2)	75.7	3.48 (dd, 10.2, 2.0)
24	27.9	27.8	1.89 (sept. d, 6.7, 2.2)	27.9	1.90 (m)
24-CH_3	12.1	12.1	0.90 (d, 6.7)	12.1	0.90 (d, 6.8)
25	21.2	21.1	0.76 (d, 6.7)	20.7	0.76 (d, 6.8)

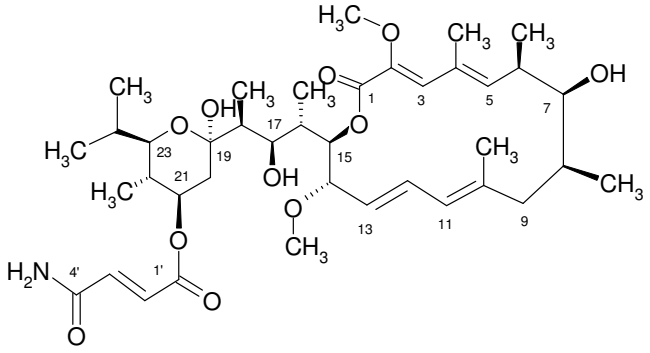
[a](150.82 MHz); [b](300 MHz)

4.5.6 Bafilomycin C_1-amide

Compound **116**, was isolated from the marine *Streptomyces* sp. Mei6-1,2 as a colourless amorphous solid (see p. 237). It was obtained from fraction IV and showed an UV absorbing band, which stained brown by spraying with anisaldehyde/sulphuric acid. The molecular weight was determined by (+)-ESI and (-)-ESI mass spectra as 719 Dalton. In the ^{1}H NMR spectrum, eight olefinic methines with an intensity of 7H were observed in the region of δ 7.00~ 5.18, two among them (δ7.00, 6.82) were belonging to an olefinic *trans* double bond (J ~ 15 Hz). In the aliphatic region, the pattern was very complex: a dd signal of an oxymethine appeared at δ4.96. In addition, five multiplets in the region of δ4.10-3.30 were attributed to five oxymethines. Two methyl ether signals were observed at δ 3.63 and 3.22, along with two further sp^2 bound methyl singlets (δ 1.98 and 1.93) as well as seven methyl doublets between δ1.08-0.70.

The ^{13}C/APT NMR spectra of component **116** displayed 39 carbon signals, including three carbonyl signals (δ 167.3, 165.7 and 164.9) of carboxylic acid derivatives. A further three sp^2 quaternary and seven methine carbons were observed in the region of δ 143.1-125.2, attributing most likely to five olefinic double bonds. Additionally, one quaternary carbon at δ98.9 was of an oxygenated sp^3 carbon atom. Further, five oxymethines (δ82.2-70.6) and two methoxy signals (δ 55.5, 50.0) were observed together with two methylenes (δ42.9, 40.0), five non oxygenated methines (δ42.0-27.8) and nine methyl carbons (δ21~7).

These data were in full accordance with bafilomycin C_1-amide (**116**), which was further confirmed by direct comparison with the literature[173].



116

Table 10: ^{13}C NMR experimental spectroscopic data for bafilomycin C_1-amide (**116**), in comparison with literature[173].

Position	Exp.[a]	Lit.[b]		Exp.[a]	Lit.[b]
	δ_C (150 MHz)	δ_C (125 MHz)	Position	δ_C (150 MHz)	δ_C (125 MHz)
1	167.3	167.7	15	76.6	77.3
2	141.2	141.8	16	37.1	38.2
2-OCH_3	60.0	60.1	16-CH_3	9.8	10.3
3	133.6	134.1	17	70.6	71.5
4	133.0	132.7	18	41.9	42.9
4-CH_3	14.0	14.1	18-CH_3	7.0	7.4
5	143.8	145.6	19	98.8	99.7
6	38.1	38.0	20	40.0	40.6
6-CH_3	17.3	17.7	21	75.6	75.7
7	81.1	80.3	22	38.7	39.0
8	41.1	41.7	22-CH_3	12.2	12.5
8-CH_3	21.6	22.2	23	76.6	76.4
9	42.9	42.2	24	27.9	28.7
10	143.1	144.7	24-CH_3	21.0	21.6
10-CH_3	20.1	20.3	25	14.2	14.6
11	125.2	125.1	1’	164.9[C]	165.3[C]
12	135.3	134.3	2’	131.9	130.6
13	127.1	126.9	3’	135.4	137.8
14	82.2	83.3	4’	165.7[C]	165.5[C]
14-OCH_3	55.5	55.6	-	-	-

[a] $CDCl_3$; [b] acetone-d_6; [C] Interchangeable assignments,

4.5.7 Antimicrobial activities

The isolated bafilomycins A_1 (**108**), K (**115**), C1 (**112**) and TS155 (**113**) were applied to antibacterial, antifungal and anti-micro algal assays, using the agar diffusion method, at concentrations of 40 μg/9 mm paper disc. All compounds were found to exhibit activity against *Mucor miehei*, *Staphylococcus aureus, Chlorella sorokiniana,* and *Scenedesmus subspicatus* (Table 55).

Bafilomycin C_1-amide is known for its cytotoxic activity against a number of human tumor cellular models using SRB method. It is extremely cytotoxic against non-small cell lung cancer, ovarian cancer, melanoma, central nervous system cancer, and colon cancer cell lines at concentrations of 0.20 - 1.08 ng/ml. It is over 100-fold more potent than adriamycin. The presence of the fumarate moiety at C-21 seems to responsible for this high cytotoxicity[173].

4.6 Terrestrial *Streptomyces* sp. GW6225

The terrestrial *Streptomyces* sp. GW6225 was intensively studied as its high of interest on the bases of chemical, HPLC-MS and biological screenings: Chemical screening during TLC of the terrestrial *Streptomyces* sp. GW6225 extract exhibited several middle polar UV-absorbing bands (254 nm). These bands turned reddish-brown by spraying with anisaldehyde/sulphuric. Additionally, one non UV-absorbing was detected as violet by the previous reagent, together with a highly polar band which showed intensive yellowish-green fluorescence (366 nm). Finally, three yellow bands were observed, which on treatment with dilute sodium hydroxide, changed to red, pointing to *peri*-hydroxy quinones. The HPLC-MS analysis of the extract showed three ion-peak signals corresponding to 750, 764 and 770 Daltons.

In spite of the highly interesting TLC screening of the extract delivered from the terrestrial *Streptomyces* sp. GW6225, it was weakly to moderately active as antibacterial on the basis of agar diffusion method. The strain showed activity against Gram-positive (*Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57) and Gram-negative bacteria (*Escherichia coli, Bacillus subtilis*). The extract showed no activity against fungi, yeast or microalgae.

So, it was applied to a large-scale fermentation, followed by working up and isolation using a series of chromatographic techniques. The following unusual compounds were isolated: N-Phenyl-β-naphthylamine (**129**), 4-acetylchrysophanol (**119a**), julichromes $Q_{1\cdot2}$ (**117b**), $Q_{1\cdot5}$ (**117a**), $Q_{3\cdot5}$ (**118**), julichrome Q_6 glucuronide (**120**)[174], and nosiheptide (**127**) (Figure 47). All compounds were assigned on the bases of elevated spectrocscopic techniques and discussed in detail in our recently published article[174].

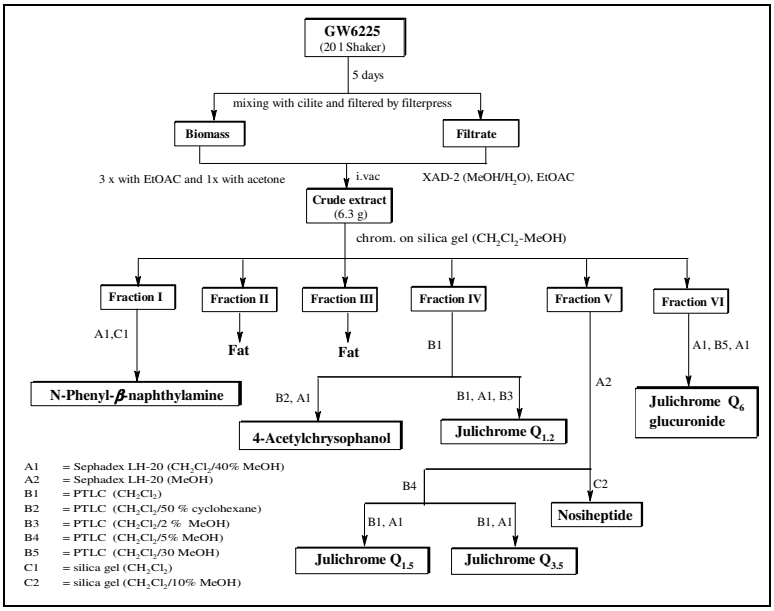


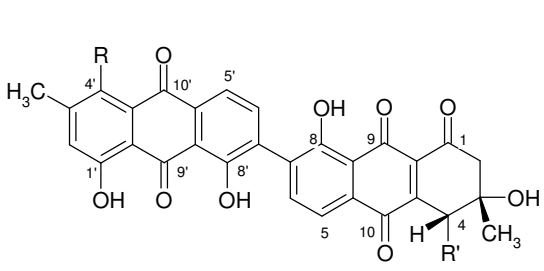
Figure 47: Work-up procedure of the terrestrial *Streptomyces* sp. GW6225

4.6.1 Julichrome $Q_{1\cdot5}$ and Julichrome $Q_{3\cdot5}$

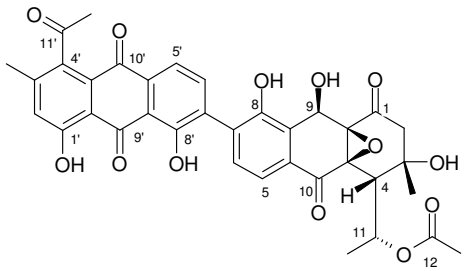
Compounds **117a** and **118** were obtained as UV-absorbing polar red solids, which turned blue-violet with diluted sodium hydroxide (indicative for *peri*-hydroxyquinones) and brown with conc. sulphuric acid. According to ESI and HRESI MS, the molecular weights of **117a** and **118** were established, respectively as 652 and 670 Daltons with the corresponding molecular formulas $C_{36}H_{28}O_{12}$ and $C_{36}H_{30}O_{13}$. The NMR spectra of both compounds confirmed them to have three sp^2-bound chelated hydroxy groups, three sets of aromatic systems (two of them are belonging to 1,2,3,4-tetrasubstituted systems, and the third was pentasubstituted). Both compounds exhibited two sp^3 methines, a singlet methylene and two methyls. Compound **118** showed the same pattern as **117a**, except that one of the quinone carbonyls in **117a** was replaced by an oxy-methine, and two of the quaternary bridgehead sp^2-atoms were replaced by two quaternary oxygen-connected sp^3 carbons constructing an epoxide ring. According to the revealed spectroscopic data and a search in AntiBase, julichromes $Q_{1\cdot5}$ and $Q_{3\cdot5}$ **118** were fixed as the sole coincident structures[175,176].

Both julichromes $Q_{1\cdot5}$ and $Q_{3\cdot5}$ (**117a** and **118**) have been described previously[175,176], however, with no spectroscopic data were reported. So, the two com-

pounds were unambiguously assigned here for the first time using 2D (H,H COSY, HMQC and HMBC) NMR experiments (Figure 48, Figure 49 and Table 11).



117a: R = $COCH_3$, R' = $CH(CH_3)OCOCH_3$
117b: R =R' = $CH(CH_3)OCOCH_3$



118

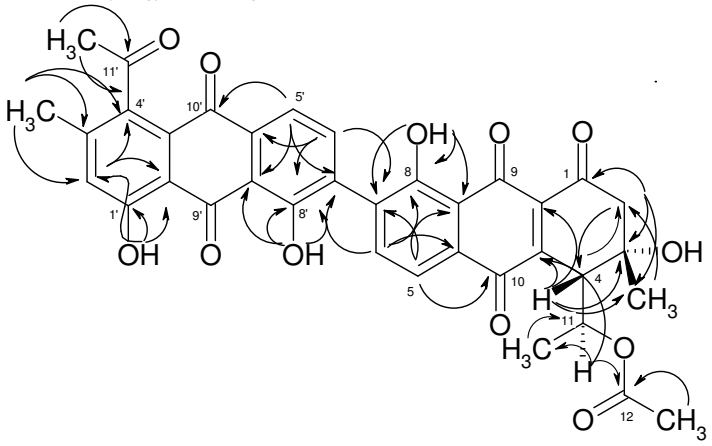


Figure 48: Selected HMBC (→) couplings of Julichrome $Q_{1\text{-}5}$ (**117a**).

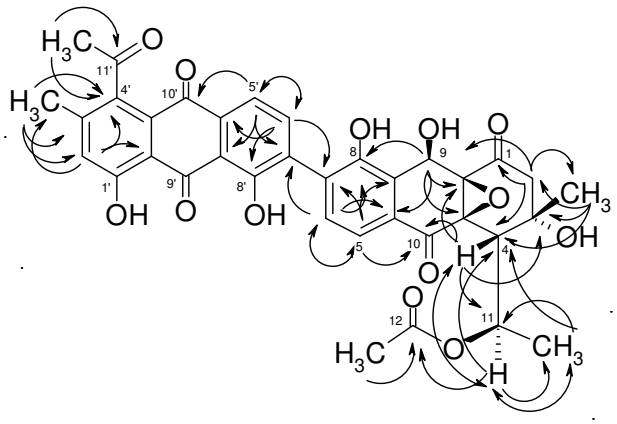


Figure 49: $^{1}H,^{1}H$ COSY (↔) and selected HMBC (→) couplings of Julichrome $Q_{3\text{-}5}$ (**118**).

Table 11: ^{13}C and ^{1}H NMR spectroscopic data for Julichromes $Q_{1\cdot5}$ (**117a**), $Q_{3\cdot5}$ (**118**) and 4-Acetylchrysophanol (**119a**) in $CDCl_3$, (*J* in [Hz]).

Position	Julichrome $Q_{1\cdot5}$ (**117a**)		Julichrome $Q_{3\cdot5}$ (**118**)		4-Acetylchrysophanol (**119a**)	
	δ_C [a)]	δ_H [b)]	δ_C [a)]	δ_H [c)]	δ_C [a, d)]	δ_H [c)]
1	194.7	-	204.1	-		
2	50.7	2.78 (s)	50.4	2.78 (d, 18.5, Ha), 2.74 (d, 18.9, Hb)		
3	70.4	-	69.5	-		
3-CH_3	31.4	1.44 (s)	33.8	1.65 (s)		
4	48.1	3.58 (d, 1.5)	45.2	3.30 (d, 3.8)		
4a	153.1	-	62.7	-		
5	119.0	7.81 (brs)	120.5	7.83 (d, 8.1)		
6	138.1	7.81 (brs)	132.0	7.46 (d, 8.1)		
7	131.3	-	130.7	-		
8	159.6	-	153.0	-		
8a	114.9	-	126.0	-		
9	186.8	-	60.3	6.01 (s)		
9a	133.5	-	63.7	-		
10	184.4	-	187.4	-		
10a	132.8	-	128.5	-		
11	68.1	5.82 (m)	68.3	5.60 (m)		
11-CH_3	20.6	1.22 (d, 6.6)	21.9	1.38 (d, 6.5)		
12	170.5	-	170.6	-		
13	21.3	1.91 (s)	21.4	1.80 (s)		
1'	162.5	-	162.6	-	162.4	-
2'	126.2	7.20 (s)	126.3	7.17 (s)	126.0	7.15 (d, 0.7)
3'	144.9	-	145.2	-	144.6	-
3'-CH_3	19.6	2.36 (s)	19.6	2.33 (s)	19.6	2.32 (d, 0.7)
4'	137.3	-	137.4	-	137.2	-
4a'	129.6	-	129.5	-	129.7	-
5'	119.8	7.88 (d, 7.8)	120.5	7.86 (d, 7.8)	120.4	7.75 (dd, 7.5, 1.1)
6'	138.7	7.78 (d, 7.8)	139.3	7.75 (d, 7.8)	137.4	7.59 (dd, 8.5, 7.5)
7'	131.6	-	133.3	-	125.0	7.30 (dd, 8.4, 1.1)
8'	160.0	-	158.7	-	162.4	-
8a'	115.7	-	115.7	-	115.4	-
9'	192.5	-	192.7	-	192.5	-
9a'	113.5	-	113.3	-	113.5	-
10'	181.9	-	181.7	-	182.3	-
10a'	133.0		132.7	-	132.9	-
11'	204.7	-	204.8	-	204.8	-
11'-CH_3	31.1	2.55 (s)	31.1	2.52 (s)	31.1	2.51 (s)
8-OH	-	12.54	-	-	-	-
1'-OH	-	12.14	-	12.08 (s)	-	12.19 (s)
8'-OH	-	12.53	-	12.88 (brs) 4.15 (brs, OH)	-	11.98 (s)

a) 125 MHz; b) 300 MHz; c) 600 MHz
d) The atom numbers are corresponding to the dashed atom numbers in **117a** and **118**.

4.6.2 Julichrome $Q_{1\cdot2}$

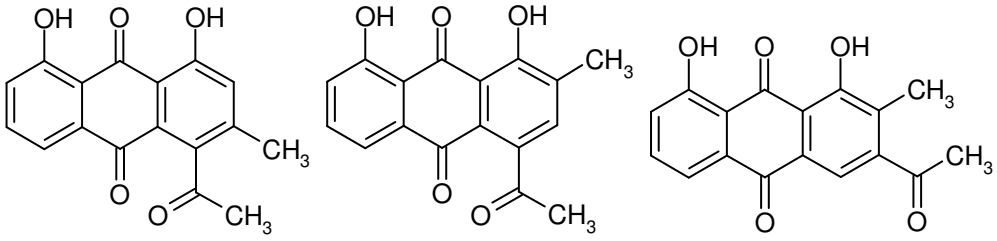
Compound **117b** was obtained as a red solid from sub-fraction IV. The molecular weight (696 Dalton) was determined by ESI mass spectra. High resolution ESI

MS of the molecular ion (697.1916680 $[M+H]^+$) afforded the molecular formula $C_{38}H_{33}O_{13}$, which differed by C_2H_4O from that of **117a**.

The ^1H NMR spectrum of **117b** showed the same signal pattern of **117a** except for the presence of an additional methyl doublet (δ1.22) together with an oxymethine (δ5.82, m), affording a CH_3-CH-O group. A search in AntiBase[61] using the above data, resulted in julichrome $Q_{1\cdot2}$ (julimycin-B-I, **117b**), which was further confirmed by comparison of the spectroscopic data with the literature[176].

4.6.3 4-Acetylchrysophanol

Compound **119a** was obtained as a yellow solid, exhibiting UV-absorbance and a yellow fluorescence. It turned red on treatment with sodium hydroxide pointing to a *peri*-hydroxyquinone. The molecular weight of **119a** was established as 296 Dalton, with a corresponding molecular formula of $C_{17}H_{12}O_5$. Based on the NMR data, **119a** is constituted of two aromatic rings, namely a 1,2,3-trisubstituted and a pentasubstituted benzene ring, connected by carbonyl groups forming an anthraquinone. In addition, two sp^2 attached methyls were recognized, one of which blonging to an acetyl group. Accordingly, three acetylchrysophanol isomers were suggested, **119a**, **119b** and **119c**.



119a **119b** **119c**

Based on 2D couplings (HMBC, Figure 50), the structure was identified as 3-acetyl-chrysophanol (**119a**). 4-Acetylchrysophanol (**119a**) is a logical precursor in the julichrome biosynthesis.

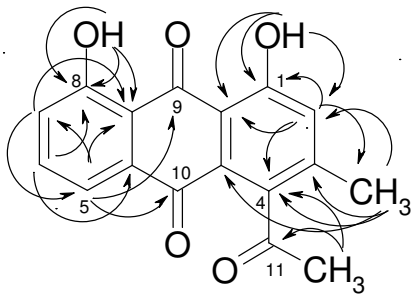


Figure 50: HMBC (→) correlations of 4-Acetylchrysophanol (**119a**)

4.6.4 Julichrome Q_6 glucuronide

Compound **120** was a polar yellowish-green solid, showing a green UV fluorescence (Figure 54). There was no colour changing with conc. sulphuric acid or sodium hydroxide solution, i.e. a *peri*-hydroxyquinone or benzopyrone were excluded. The molecular weight was established as 520 Dalton and a molecular formula $C_{25}H_{28}O_{12}$ resulted.

The $^{1}H/^{13}C$ NMR spectra and HMQC experiments of **120** confirmed the existence of an acidic proton (δ 14.79), a 1,2,3-trisubstituted and a pentasubstited aromatic residue. In the aliphatic region, six oxygenated sp^3 methines, a methylene group (δ 2.85, 2.60) involved in a ring system, and four methyl signals were confirmed. Among the revealed carbons, a ketone (δ 204.0) and two ester/acid carbonyls (δ 170.2 and 169.1) beside two signals of oxygenated sp^2 quaternary carbons and an anomeric oxy-methine (δ 100.5) were deduced.

The compound was intensively studied by 2D experiments, including NOESY (Figure 52) as well as MS-MS fragmentation (Figure 51), as no related structures were found in databases.

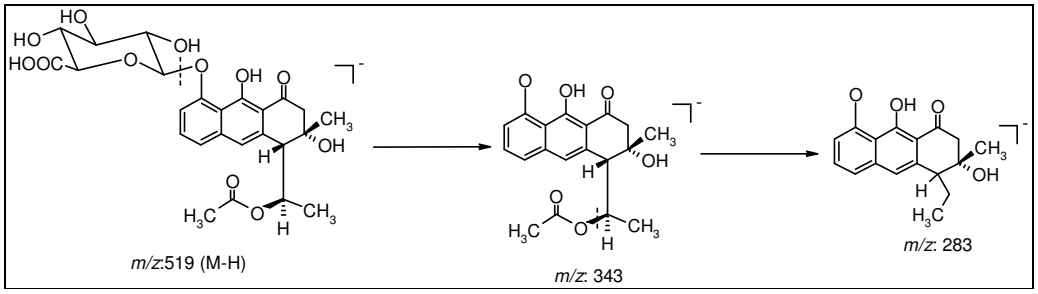


Figure 51: (-)-ESI MS/MS of Julichrome Q_6 glucuronide (**120**).

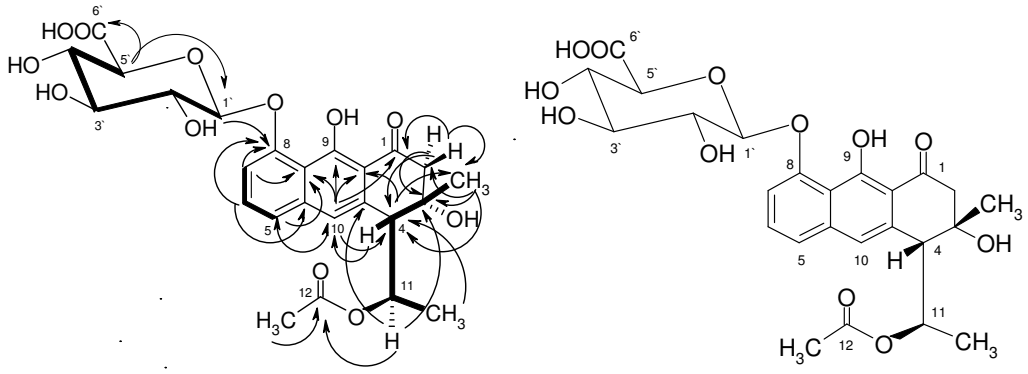


Figure 52: $^{1}H,^{1}H$ COSY (▬) and selected HMBC (→) correlations of Julichrome Q_6 glucuronide (**120**).

The structure of the aglycone was finally elucidated as julichrome Q_6 (**125a**). The structure of the sugar moiety was further investigated on the basis of 2D C-H, H-H NMR couplings and NOE data. Based on the coupling constants (7.6~9.7 Hz), the oxy-methine protons of the sugar residue were axially oriented. This was further confirmed by an NOE effect of H-1' with H-5' (Figure 53) and by the high similarity with compounds containing the same sugar moiety[177,178]. Glucuronic acid occurs usually in the D-form, and according to the Klyne rule,[179] D-sugars usually form ß-glycosides, and *vice versa.* Glucuronic acid is a widespread sugar moiety in nature[61,105].

The singlet 3-CH_3 showed an NOE with H-4, so that 3-CH_3 and 4-H must be cofacial as in all the other julichromes (Figure 53). The CD spectra of **120** (Figure 54) were very similar to those of julichrome $Q_{5\cdot6}$ and indicated an identical absolute configuration in ring C of both compounds[175]. As a result, the structure of **120** was confirmed, and assigned as julichrome Q_6 glucuronide, the first reported monomeric gylcosidic julichrome in nature.

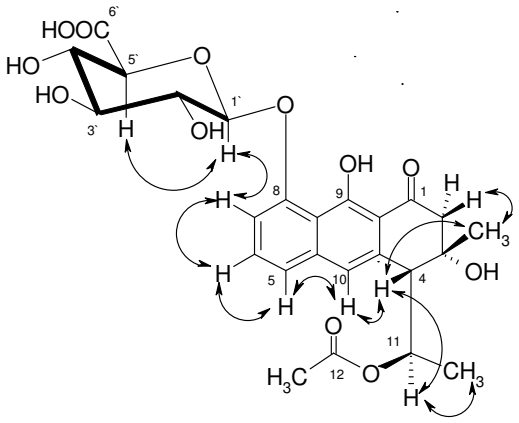


Figure 53: Selected NOESY assignments of Julichrome Q_6 glucuronide (**120**).

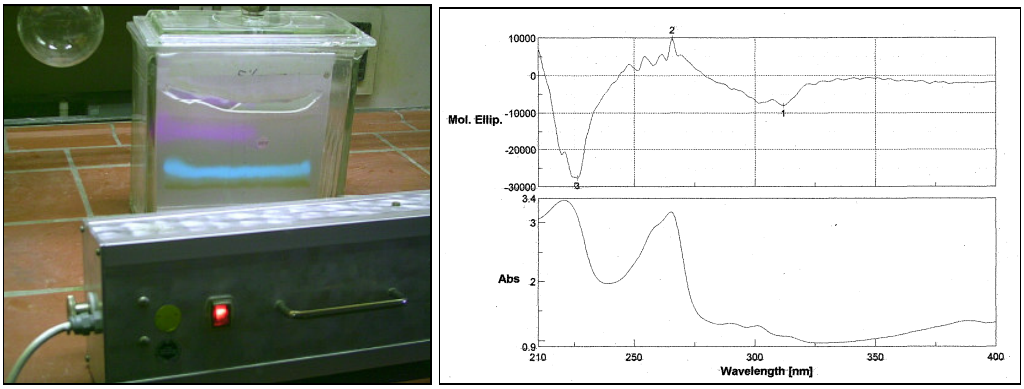


Figure 54: PTLC (366 nm) and CD spectra (MeOH) of Julichrome Q_6 glucuronide (**120**).

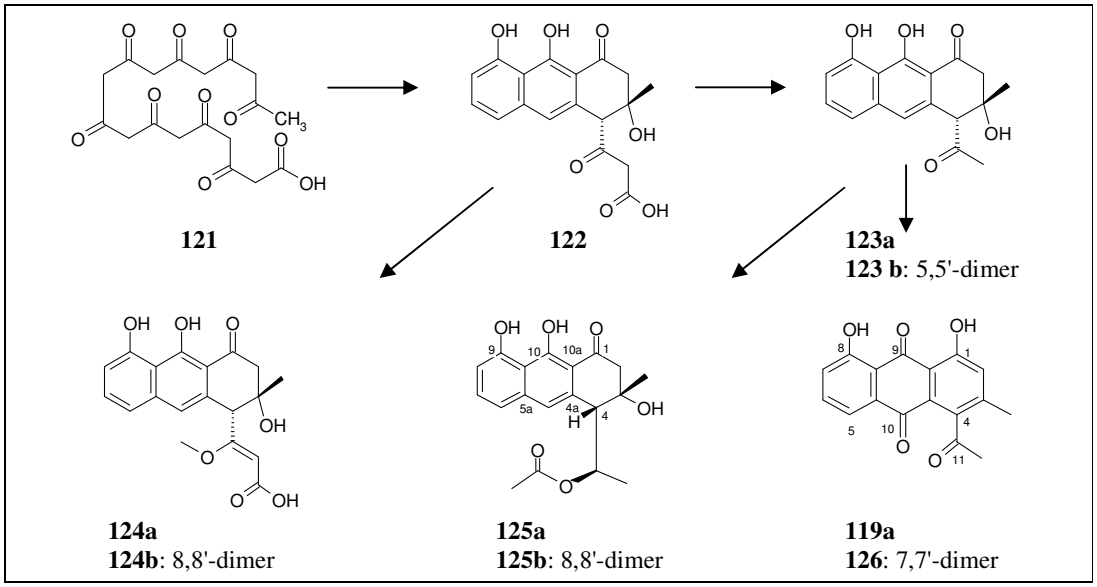
Table 12: ^{13}C (125 MHz) and ^{1}H NMR (600 MHz) spectroscopic data for Julichrome Q_6 glucuronide (**120**) in DMSO-d_6.

Position	Julichrome Q_6 glucuronide (**120**)		Position	Julichrome Q_6 glucuronide (**120**)	
	δ_C	δ_H, (*J* in [Hz])		δ_C	δ_H, (*J* in [Hz])
1	204.0	-	10	119.4	7.22 (s)
2	48.7	2.85 (d, 18.6, H_a), 2.60 (dd, 18.5, 1.0, H_b)	10a	138.7	-
3	68.8	-	11	67.6	5.62 (dq, 6.2, 1.8)
3-CH_3	29.9	1.16 (s)	11-CH_3	19.3	1.22 (d, 6.5)
4	54.8	2.88 (s)	12	169.1	-
4a	135.8	-	13	20.7	1.68 (s)
5	121.1	7.46 (d, 8.1)	1'	100.5	5.21 (d, 7.6)
6	131.2	7.59 (t, 8.0)	2'	73.2	3.48 (t, 8.6)
7	110.2	7.17 (d, 7.9)	3'	75.8	3.37 (t, 9.1)
8	156.6	-	4'	71.4	3.44 (t, 9.3)
8a	114.9	-	5'	75.2	3.91 (d, 9.7)
9	164.0	-	6'	170.2	-
9a	111.2	-	OH	-	14.79 (brs)

Most julichromes (or julimycins) exhibited a potent antibacterial activity against Gram-positive bacteria[180]. However, julichrome Q_6 glucuronide (**120**) displayed no activity against a number of bacteria, fungi, and micro algae tested. It showed, on other hand a moderate and rather unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 12.3 µM (mean IC_{70} = 20.4 μM). A similar biological profile was observed for the julichromes $Q_{1\cdot5}$ and $Q_{3\cdot5}$ (**117a**, **118**), whose cytotoxic activity was approximately 2-fold more pronounced.

The julichromes (or julimycins)[61,175,176,181] were firstly obtained from a strain of *Streptomyces shiodaensis*, which produced a group of 20 of these unique dimeric naphthalene derivatives. The parent compound is julichrome $Q_{6\cdot6}$ (**125b**), a member of the julimycin B-I complex. The other julichromes are formally derived from **121** by a stepwise hydroxylation, epoxidation, and/or oxidation. As the end product, the dimeric anthraquinone julichrome $Q_{5\cdot5}$ (**126**) is formed *via* symmetrical and unsymmetrical intermediates.

The julichrome biosynthesis has not been investigated. It seems plausibly, that a nonaketide precursor **121** firstly cyclizes to the ketoacid **122**, which would yield **123a** after decarboxylation and **125a** after further reduction and acetylation, or could be stabilized as the enol ether spectomycin A2 (**124a**)[182]. Oxidation of phenols **123a**–**125a** would result in the respective dimers, spectomycin B1 (**124b**)[182] and julichrome $Q_{6\cdot6}$ (**125b**). In a similar way, the 5,5'-dimeric naphthalenes A-39183-A (**123b**) and A-39183-B (setomimycin)[183] could be formed. While the monomer **124a** is known as a natural product, compounds **123a**, **125a**, and **119a** have not yet been described.

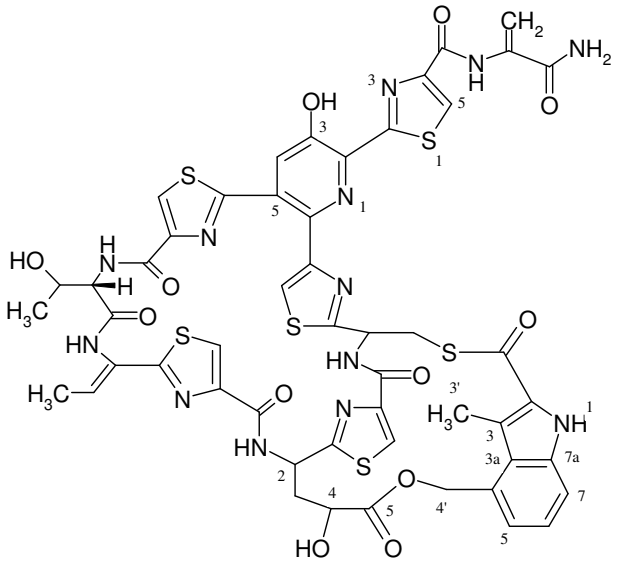


4.6.5 Nosiheptide

Compound **127** was isolated from the polar fraction V as yellow solid, exhibiting UV-absorbance and a green fluorescence during TLC. The green fluorescence property is beside others indicative of the presence of a thiazole residue in the structure of **127**. The compound displayed a blue colouration in the chlorine-tolidine reaction for amides, and turned violet with ninhydrin, as indicator of a peptide structure, containing a terminal amino group.

The molecular weight of **127** was established as 1221 Dalton *via* positive (1242.2 $[M+Na]^+$) and negative (1220.1 $[M-H]^-$) ESI MS. (+)-HRESI MS of **127** confirmed its molecular formula as $C_{51}H_{43}N_{13}O_{12}S_6$. The ^1H NMR spectrum (Table 13) of **127** showed three broad amide NH singlets (δ 11.19, 10.04 and 9.32). Between δ 8.65 ~ 7.12, it showed signals of 14 aromatic/olefinic protons. One 1H quartet (δ 6.46, ^3J = 6.4 Hz), along with 11 hydrogen signals of olefinic, oxygenated and/or α-amino acid protons were observed between δ 6.37 ~ 3.56. Additionally, an sp^2-attached methyl singlet (δ 2.63) was found together with two methylene multiplets at δ 2.44 (2H) and 1.80 (2H), and two doublet methyls at δ 1.72 and 0.95.

Based on the molecular formula, the ^1H NMR spectra, and a search in AntiBase, nosiheptide (**127**) was recognized as the only consistent structure. Nosiheptide (**127**) was further established by comparison of its spectroscopic data with authentic spectra and literature data (Table 13)[184].



127

Table 13: ^{1}H NMR (300 MHz) assignments of Nosiheptide (**127**) in DMSO-d_6 and comparison with literature data[184].

Assignments	Exp. (**127**)	Lit. (**127**)		Assignments	Exp. (**127**)	Lit. (**127**)	
	δ_H (ppm)	δ_H (ppm)	J = Hz		δ_H (ppm)	δ_H (ppm)	J = Hz
Thr-CH_3	0.95 (d)	0.95 (d)	6.4	Ind H5	7.12 (d)	7.12 (d)	7.0
but-CH_3	1.72 (d)	1.72 (d)	6.8	Ind H6	7.28 (dd)	7.28 (dd)	6.8, 8.2
Glu H3*R*	1.78 (m)	1.80 (ddd)	-	Thr OH	7.58 (br)	7.58 (br)	-
Glu H3*S*	2.44 (m)	2.44 (ddd)	-	Ind H7	7.60 (d)	7.60 (d)	8.3
Ind-CH_3	2.63 (s)	2.63 (s)	-	Thr NH	7.64 (d)	7.64 (d)	8.3
Cys H3	3.56 (dd)	3.56 (dd)	5.0, 13.9	Cys NH	7.72 (d)	7.72 (d)	9.6
Cys H3	3.86 (dd)	3.86 (dd)	3.0, 13.8	Pyr H4	7.80 (s)	7.82 (s)	-
Thr H3	4.00 (m)	4.00 (m)	-	Thz H5 (4)	7.88 (s)	7.88 (s)	-
Glu H4	4.09 (br)	4.09 (dd)	-	Glu OH	8.00 (br)	8.00 (br)	-
Thr H2	4.57 (dd)	4.57 (dd)	6.2, 8.4	Thz H5 (2)	8.16 (s)	8.16 (s)	-
Deala NH_2	5.00 (br)	5.00 (br)	-	Thz H5 (3)	8.30 (s)	8.30 (s)	-
Ind H4´	5.40 (d)	5.40 (d)	11.7	Glu NH	8.35 (d)	8.35 (d)	8.6
Ind H4´	5.59 (d)	5.59 (d)	11.8	Thz H5 (5)	8.55 (s)	8.55 (s)	-
Glu H2	5.60 (m)	5.63 (m)	-	Thz H5 (1)	8.65 (s)	8.65 (s)	-
Deala H3c	5.76 (s)	5.76 (s)	-	But NH	9.32 (s)	9.32 (s)	-
Cys H2	5.88 (m)	5.88 (ddd)	-	Deala NH	10.04 (s)	10.04 (s)	11.19 (br)
Deala H3t	6.37 (s)	6.37 (s)	-	Ind NH	11.19	11.19	-
But H3	6.46 (q)	6.46 (q)	6.9				

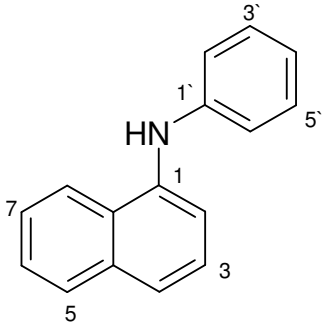
Nosiheptide (**127**) is a 26-membered polythiazole macrocyclic antibiotic produced by *Streptomyces actuosus*[185, 186]. Its structure and absolute configuration were elucidated 1977 by X-ray analysis[186]. It is related with multhiomycin[187], thiopeptin[188], thiostrepton [189] and taitomycin[190]. Nosiheptide (**127**)[191] and related structural analogues (e.g. thiostrepton, thiopeptin and the micrococcins[192] interfere

with protein synthesis in Gram-positive bacteria. The biosynthesis of **127** was studied by feeding experiments in 1988[184].

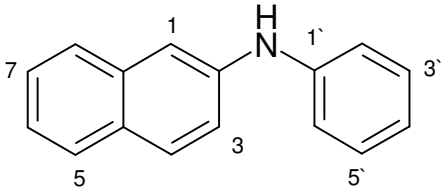
These types of peptides are characterised by their antibacterial activity, having similar physicochemical properties, e.g. they are mostly green fluorescent due to their thiazole residues. They are highly active against Gram-positive bacteria and show very low toxicity, while they are inactive against Gram-negative bacteria and yeasts. Therefore, the antibacterial activity of the crude extract of the terrestrial *Streptomyces* GW6225 was likely due to nosiheptide (**127**)[193].

4.6.6 N-Phenyl-β-naphthylamine

Compound **129** was isolated as colorless solid, having a molecular weight of 219 Dalton by EIMS. HREI-MS confirmed the molecular formula of **129** as $C_{16}H_{13}N$. Besides, the ^{1}H NMR spectrum displayed the presence of 12 aromatic protons and of a broad 1H singlet (δ 5.93) of an NH. The aromatic protons of the molecule were located in the region of δ 7.73-6.98, while the nitrogen atom was not incorporated in the aromatic nuclei. This pointed to an α- or β-naphthyl-aniline moiety (**128**, **129**). Intensive studies of the spectroscopic data, including 2D NMR (HMBC, and H, H COSY) confirmed the structure as N-phenyl-β-naphthylamine (**129**) but not N-phenyl-α-naphthylamine (**128**)[83] (Figure 55). As also **128** had been obtained from bacteria and as it was possible to reproduce the isolation of both isomers from their strains avoiding all contact with plastic materials, this definitely ruled out an artificial origin of **128** or **129**.



128



129

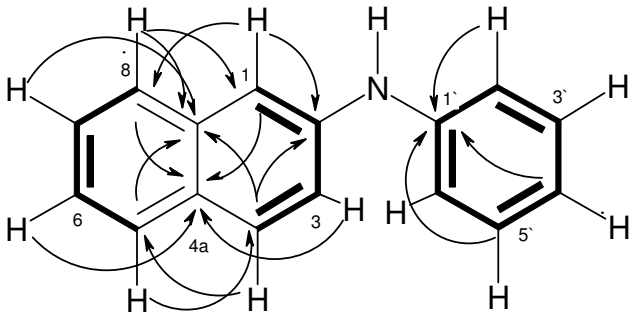


Figure 55: H,H COSY (▬) and HMBC NMR correlations (→) of N-Phenyl-β-naphthylamine (**129**).

4.7 Terrestrial *Streptomyces* sp. RSF18

According to the biological assays in our pre-screening, the terrestrial *Streptomyces* isolate RSF18 was highly active against Gram-positive bacteria and showed weak cytotoxic activity against brine shrimps (*Artemia salina*) (Table 56). The extract exhibited several UV absorbing bands, which stained yellow and/or dark blue on spraying with anisaldehyde/sulphuric acid. These bands were examined by HPLC MS/MS, affording two ion-peaks, with corresponding molecular weights of *m/z* 1131 and 1115, respectively, containing isotope signals characteristic for a sulphur content.

After usual work up of a 15 L culture using M_2 medium, three strong antibiotics were isolated; geninthiocin (**130**), the new thiopeptide Val-geninthiocin (**131**), and chalcomycin A (**132**) (Figure 56). As the whole work including the taxonomic description, chemical and biological studies of the three metabolites has already been published[194], we describe them her only briefly.

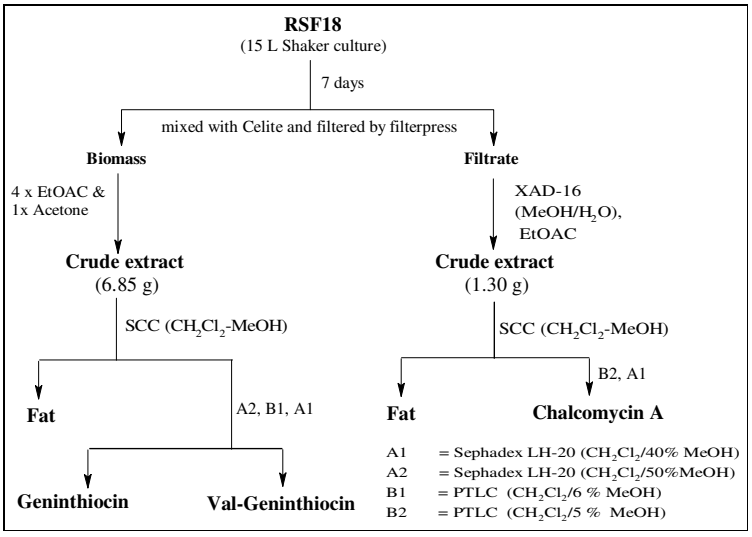


Figure 56: Working up scheme of the terrestrial *Streptomyces* sp. RSF18

4.7.1 Geninthiocin

Compound **130** was isolated as a UV absorbing white amorphous solid, exhibiting a violet colouration on spraying with ninhydrin and a blue one with the chlorine-tolidine reagent, as indicative for a peptide structure. The molecular weight of **130** was established by ESI MS as 1131 Dalton, and the corresponding molecular formula $C_{50}H_{49}N_{15}O_{15}S$ was deduced by HRESI MS.

According to the ^{1}H NMR spectrum, 13 of the 49 protons were D_2O exchangeable. In the aromatic, olefinic and heteroatom-connected sp^3 region (δ 4.30 ~ 10.9), numerous signals integrating for *ca*. 30 H were displayed. In the aliphatic region, five methyls were visible; three of them were singlets, and the remaining two appeared as doublets. The ^{13}C NMR spectrum displayed all expected 50 carbon signals. Most of them (42) appeared in the sp^2 region (δ 165~104), four signals of hetero-bound sp^3 carbon atoms (δ 72-65) and five methyl signals (δ 10~28) appeared in the aliphatic region.

A search in AntiBase[61] resulted in geninthiocin (**130**). The structure was further confirmed by comparison with literature data[195].

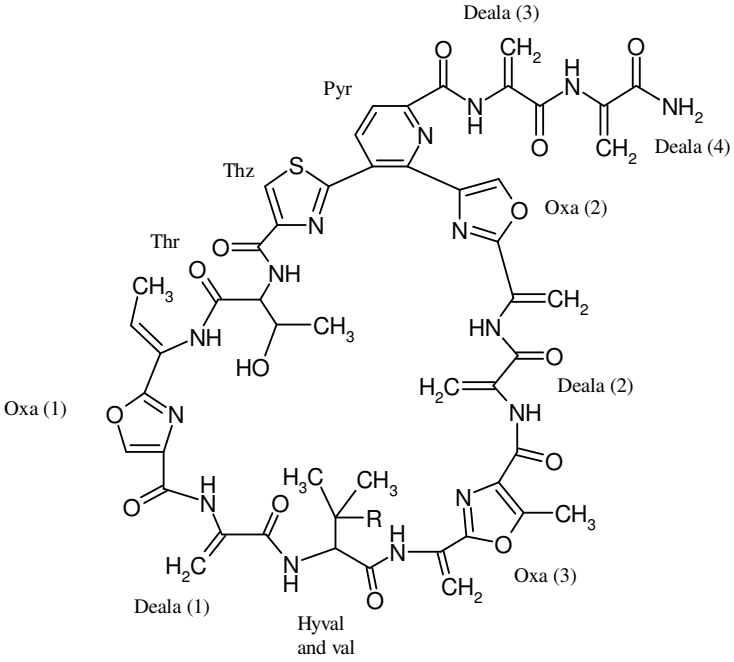
4.7.2 Val-Geninthiocin

Compound **131** was obtained as colourless solid as well, showing the same physico-chemcial and chromatographic properties as **130**. The molecular weight of

131 was determined by ESI MS as 1115 Dalton, and its (+)-HRESI MS confirmed the molecular formula as $C_{50}H_{49}N_{15}O_{14}S$ with one oxygen atom less than **130**.

The compound showed an NMR pattern similar to that of **130**, except that the oxy-carbon at δ 71.0 (βC of hydroxy-valine) in **130** was replaced by a non oxygenated one at δ 29.5 in **131**. Alternatively, the *gem*-dimethyls of the hydroxyvaline residue in geninthiocin (**130**) were changed into a 6H doublet, corresponding to an isopropyl system (δ0.97, J= 6.6 Hz) in **131**.

Therefore, compound **131** was identified as deoxy-derivative of geninthiocin (**130**), and named Val-geninthiocin.



130: R = OH, **131**: R = H

4.7.3 CID-MS/MS studies of Geninthiocin (130) and the new Val-Geninthiocin (131)

As part of our ongoing structure elucidation of *cyclo*peptides by MS methods, it was of interest to investigate the fragmentation behaviour of both metabolites in parallel. For this purpose, MS^2 and MS^3 experiments using a quadrupol ion trap were performed (Table 14), and additionally, high-resolution CID-MS/MS measurements

were carried out on a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer to determine the elemental composition of key fragments.

Tandem mass spectrometry is a very powerful method for sequence analysis of peptides and proteins. The fragmentation of linear peptides have been described comprehensively and detailed knowledge of the fragmentation mechanism have been obtained[196-197]. However, the structure analysis of cyclic peptides, which represent an important class of bioactive natural products, by mass spectrometry, remains a challenging task. Complex fragmentation patterns by two-bond cleavage at different ring positions, ring-opening reactions and uncommon rearrangement reactions complicate the interpretation of CID-MS/MS spectra[198-199]. Frequently, higher order MS^n investigations are required. Furthermore, the cyclic peptides produced as secondary metabolites from bacteria often contain uncommon amino acid residues leading to different fragmentation reactions.

In general, for sequencing linear peptides, fragmentation of the $[M+2H]^{2+}$ ions is used successfully, but in the case of geninthiocin (**130**) and his minor congener, fragmentation spectra of those ions were dominated by doubly charged fragment ions without significant sequence information. Furthermore, $[M+2H]^{2+}$ ions were observed with very low intensity under the conditions described. Also the MS^2 spectra of $[M+Na]^+$ and $[M-H]^-$ provided little sequence information. Therefore, CID-MS/MS studies were concentrated on the more intense $[M+H]^+$ species (Figure 57).

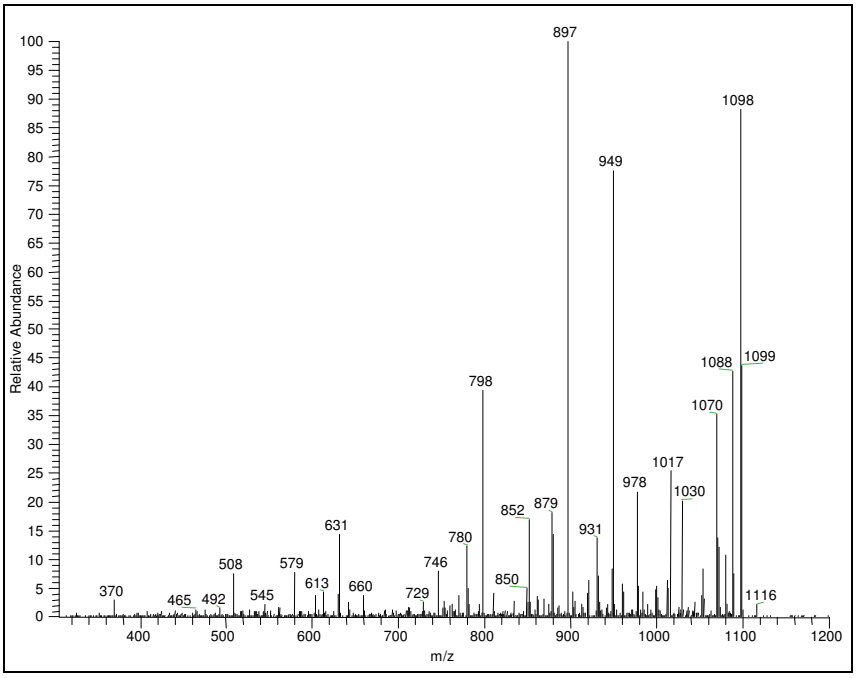


Figure 57: CID-MS/MS spectrum of Val-Geninthiocin (**131**,); fragmentation of $[M+H]^+$ measured on a quadrupol ion trap instrument.

Beside an unspecific loss of H_2O, CO, and NH_3, the following main fragmentation pathways were observed (Latin numbers correspond to the fragments indicated in Figure 58):

I Cleavage of a single amino acid residue unit from the peptide ring system is found basically for Hyval (-115 Dalton) and Val (-99 Dalton) in **130** and **131**, respectively (cleavage of two CO-NH peptide bonds). Therefore, the structural difference between both compounds could be allocated at this amino acid at a very early stage.

II Cleavage of a single amino acid residue unit from the peptide ring system by fragmentation of one CO-NH peptide bond and one CH_2=C-NH bond (H-Oxa-NH_2, H-Hyval/Val-NH_2, H-Deala-NH_2) was detected. Obviously, the -NH-C=CH_2- bond between Oxa(1)-Deala(1) and Oxa(3)-Deala(2) as well as between Deala(2)-Oxa(2) and Hyval/Val-Oxa(3) could be broken favourably leading to a second preferred fragmentation pathway beside peptide bond (-CO-NH-) cleavage.

III Cleavage of Oxa-Deala dipeptide units with high preference resulted in the prominent fragment ion peaks at *m/z* 913 (**130**) and 897 (**131**), respectively. Furthermore, fragmentations of Deala-Hyval/Val occured with lower intensity.

IV Cleavage of the tripeptide unit Hyval/Val-Oxa-Deala gave the key fragment at *m/z* 798. After cleavage of the tripeptide unit Hyval/Val-Oxa-Deala, a further dipeptide Oxa-Deala loss resulted in the fragment ion *m/z* 579 (IV+III).

V Fragmentation of the linear peptide unit predominantly occurred by cleavage of the NH-CO bond between Deala(4) and Deala(3) forming a b-fragment ion (V b_{10}). Furthermore, formation of a c-type fragment (V c_9) by cleavage of CH_2=C-NH bond between Deala(3) and Pyr induced by the dehydroalanine structure is observed.

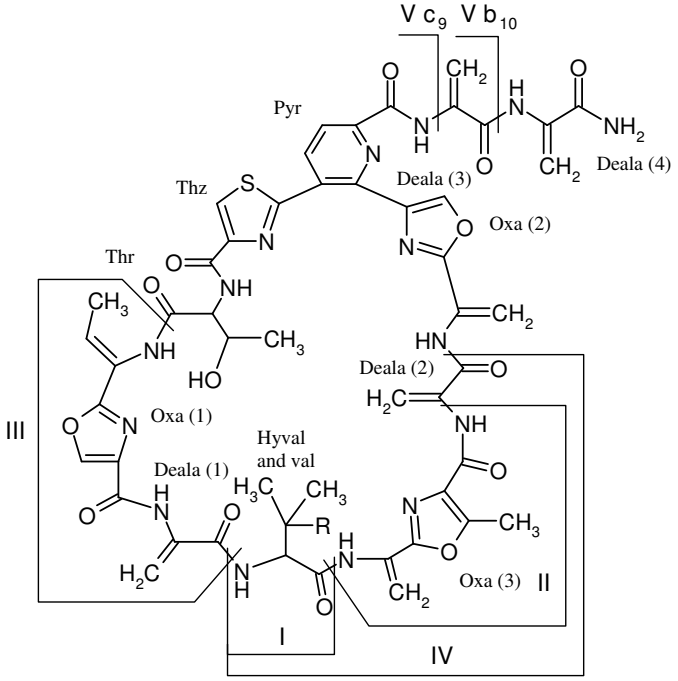


Figure 58: Selected CID-MS/MS key fragments of the $[M+H]^+$ precursor ions of Geninthiocin (**130**) and Val-Geninthiocin (**131**) obtained on a quadrupole ion trap mass spectrometer; Table 14.

The fragmentation pathways derived from collision activated dissociation using a quadrupole ion trap (Table 14) were confirmed by high resolution MS/MS in an FT-ICR mass spectrometer providing the elemental composition of key fragments. By comparison of MS^2 spectra, the position of valine in **131** was determined unambigu-

ously. The information obtained during such fragmentation pathways could be applied to further investigations of this class of substances in order to identify low amounts of derivatives.

Table 14: MS^2 and MS^3 product ions of the $[M+H]^+$ precursor ions of Geninthiocin (**130**) and Val-Geninthiocin (**131**) obtained on a quadrupole ion trap mass spectrometer

Geninthiocin (**130**)		Val-Geninthiocin (**131**)		
MS^2 fragment ion *m/z*	MS^3 fragment ions *m/z*	MS^2 fragment ion *m/z*	MS^3 fragment ions *m/z*	Proposed MS^2 fragment ions
1115		1099		$-NH_3$
1114		1098		$-H_2O$
1104		1088		-CO
1074	1056; 1046; 988; 936; 907; 855; 812; 798			$-C_3H_6O$ from Hyval
1046	1028; 1018; 985; 949; 879; 851	1030	1012; 1002	-(H-Deala-NH_2)
1017	1000; 999; 982; 931; 879; 862; 798; 579	1017	1000; 999; 931; 879; 798; 579; 493	-Hyval/Val
994	966; 936; 974; 370	978	960; 950; 370	$-C_6H_6N_2O_2$ from side chain
965	948; 947; 921; 907; 903; 879; 850; 827; 798; 631	949	931; 921; 905; 903; 863; 850; 811; 631	- (H-Oxa-NH_2)
947		931		-(H-Oxa-NH_2)-H_2O
913	896; 895; 877; 855; 852; 837; 798	897	879; 851; 833; 811; 798; 793; 579	-Oxa-Deala
907	889; 879; 863; 821; 769; 752; 631			$-C_{10}H_{15}N_3O_3$ -(H-Oxa-NH_2)-C_3H_6O
896		880		-H-Oxa-Deala-NH_2
895	878; 877; 851	879	861; 851	-Oxa-Deala-H_2O
889				$-C_{10}H_{17}N_3O_4$ -(H-Oxa-NH_2)-C_3H_6O-H_2O
		852		
850		850		-H-Hyval/Val-Oxa-NH_2
798	781; 780; 770; 754; 712; 660; 631; 579	798	781; 780; 770; 754; 712; 660; 631; 579	-Hyval/Val-Oxa-Deala
780		780		-Hyval/Val-Oxa-Deala-H_2O
746	729; 728; 660; 608; 579	746	729; 728; 660; (608; 579)	-Deala-Oxa-Hyval/Val-$COCCH_2$+2H
631	603; 545; 493	631	603; 545; 493	-H-Oxa-Deala-Hyval/Val - Oxa-NH_2
579	551; 535; 493; 441	579	561; 551; 535; 493; 441	-Oxa-Deala-Hyval/Val-Oxa-Deala
508	370	508	370	

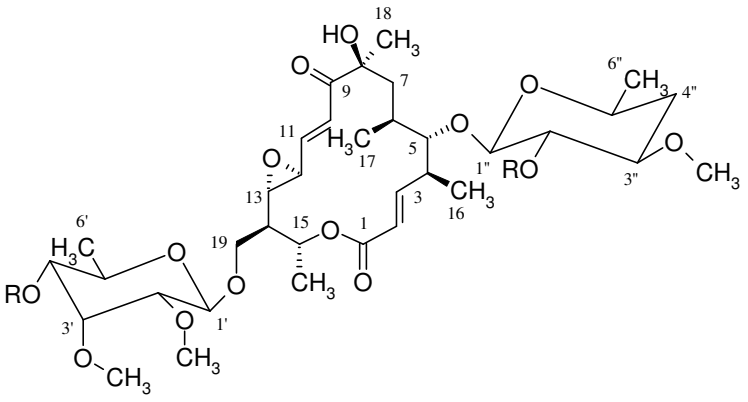
Val-Geninthiocin (**131**) is a new thiopeptide[194], having a very close similarity with geninthiocin (**130**)[195]. Both compounds are thiopeptide antibiotics[200,201], characterized by several common structural features, such as oxazole and thiazole units

and unusual amino acids; especially dehydroamino acids are typical[202,203]. Among this group are thioxamycin[204], berninamycin A[205], sulfomycin I[206-208] and A10255[209], possessing the thiazole-pyridine-oxazole substructure. Moreover, thiocillin I [210,211], micrococcin P [212] and GE2270 A [213] are thiopeptides characterized by a thiazole-pyridine-thiazole moiety. These peptides are used as antibacterial agents against Gram-positive bacteria and anaerobes, including pathogens resistant to antibiotics currently in use[214,215]. They have also potential activity as growth inhibitors of the human malaria parasite[216]. Recently, thiopeptides have been proved as effective growth promoters for domestic animals[201,217]. Most of the thiopeptide antibiotics inhibit protein synthesis in bacteria, and share a common mode of action[218]. Thiostrepton, whose antibiotic activity is best understood, acts by binding tightly to the prokaryotic ribosome, and thus inhibits translation[218,219]. Geninthiocin (**130**) is known as an activating agent for transcription of the *tip A* promoter in *Streptomyces*[195].

4.7.4 Chalcomycin A

Compound **132** was isolated as a white solid with a UV absorbing zone, which turned dark blue with anisaldehyde/sulphuric acid. According to ESI MS, the molecular weight was established as 700 Dalton, corresponding to the molecular formula $C_{35}H_{56}O_{14}$. The $^{1}H/^{13}C$ NMR spectra revealed the presence 35 carbon atoms, classified into a ketone and an ester carbonyl, four sp^2 methines, representing two double bonds conjugated with carbonyls, two acetals and sixteen oxygenated sp^3 carbons of methines and methyls. Finally, three non-hetrobound methines, two methylenes and six methyls were observed.

Based on these data, a search in AntiBase deduced the structure as chalcomycin A (**132**), a 16-membered macrolide, which previously reported from *Streptomyces bikiniensis*[220~221]. Compound **28**, a structural analogue, was recently reported by our research group together with **132** from the culture broth of the marine *Streptomycete* isolate B7064[52]. Chalcomycin A (**132**) has a strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, which is higher than that of erythromycin A (**4**). Chalcomycin A (**132**) was investigated here for the first time by ESI MS/MS, and the details are given in Figure 59.



132: R = H
28: R = $COCH_2CH_3$

Table 15: ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data of Chalcomycin A (**132**).

No.	δ_C	No.	δ_C	No.	δ_C	No.	δ_C
1	165.3	10	124.8	19	66.9	1''	100.8
2	120.7	11	146.4	20	18.2	2''	81.9
3	151.5	12	59.6	1'	103.1	2''-OCH_3	58.6
4	41.6	13	58.9	2'	75.0	3''	79.6
5	87.7	14	49.4	3'	80.4	3''-OCH_3	61.7
6	34.0	15	68.6	3'-OCH_3	56.6	4''	72.6
7	36.9	16	18.5	4'	36.7	5''	70.6
8	78.3	17	19.1	5'	67.7	6''	17.7
9	nd	18	27.7	6'	20.8	-	-

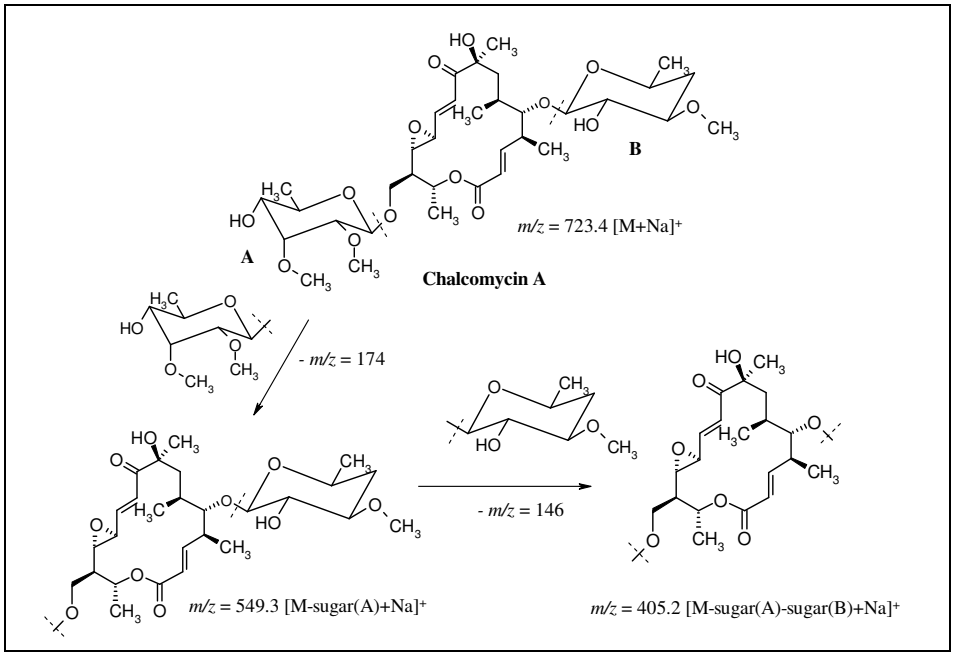


Figure 59: ESI MS/MS of Chalcomycin A (**132**).

4.7.5 Biological Activity

In comparison with chalcomycin A (**132**), both peptides geninthiocin (**130**) and Val-geninthiocin (**131**) exhibited lower antibacterial activities against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptomyces viridochromogenes*). The three compounds **130**-**132** showed weak antifungal activity against *Mucor miehei* (Tü 284) and *Candida albicans*. No activity was found against Gram-negative bacteria or micro-algae.

4.8 Terrestrial *Streptomyces* sp. ANK26

The crude extract of the *Streptomyces* sp. ANK26 drew our attention due to its high biological activity against a set of test organisms in the biological screening (Table 57). The crude extract showed a low and rather unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 112 µM (mean IC_{70} = 106 μM); Table 45.

In the chemical screening, *semi*-polar zones were present which gave unusual yellow, dark green, violet and black colourations with anisaldehyde/sulphuric acid spraying reagent. Additionally, HPLC MS/MS of the crude extract showed many interesting peaks in the positive mode with MS/MS of an sugar molecule with a mass of 158 Dalton (**A**), which resulted directly in picromycin derivatives by searching in AntiBase[61] as the only type of molecules containing this sugar moiety.

A 50 L shaker culture of *Streptomyces* sp. ANK26 was grown on M_2 medium at 28 °C for 7 days, giving a yellowish-brown culture broth, which was filtered over Celite and adsorbed on Amberlite XAD-16, while the mycelium was extracted with ethyl acetate and acetone. By column chromatography on silica gel, seven compounds were obtained: 10,11-dihydro-9,12-epoxy-8,9-anhydro-cromycin (**133**), cromycin (**138**), 10,11-dihydro-cromycin (**135**), *seco*-decarboxy-cromycin (**136**), (4*E*,8*E*)-4,8-dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**), beside bafilomycins B_1 (**110**) and B_2 (**111**) [222, 154]. The remaining more polar fraction VI contained picromycin analogues, 10,11-dihydro-9,12-epoxypicromycin-9-methylether (**134**), picromycin (**139**) and picromycin-C (**140**) (Figure 60 & Figure 61).

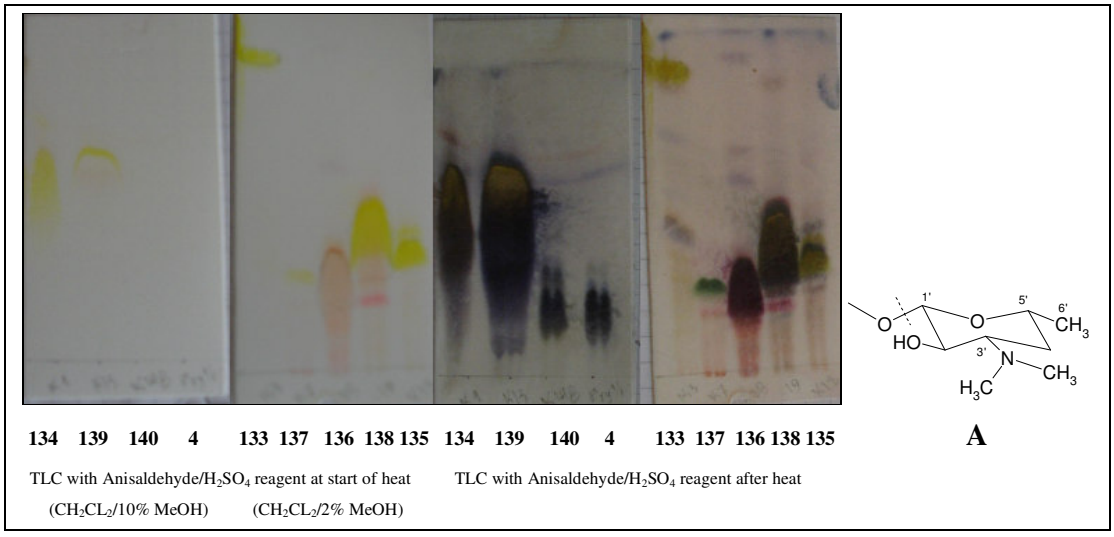


Figure 60: TLC of isolated compounds **133~140** from the terrestrial *Streptomyces* sp. ANK26 after spraying with anisaldehyde/H_2SO_4 reagent.

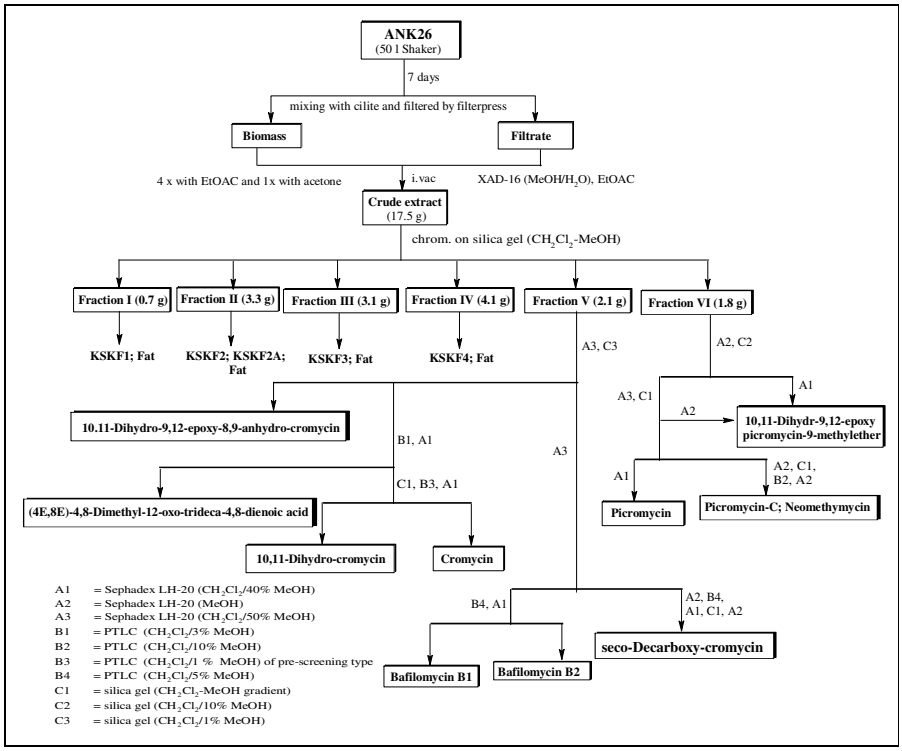


Figure 61: Work-up procedure of the terrestrial *Streptomyces* sp. ANK26

4.8.1 10,11-Dihydro-9,12-epoxy-8,9-anhydro-cromycin

Compound **133** was obtained as a UV absorbing colourless oil, which stained to yellow by exposing to anisaldehyde/sulphuric acid. The molecular weight was de-

duced by ESI MS in the positive mode as 334 Dalton, and six double bond equivalents were calculated for the corresponding molecular formula $C_{20}H_{30}O_4$.

The ^{1}H NMR spectrum displayed two 1H dd signals at δ 6.62 and 5.10, mostly likely of olefinic and oxymethine protons, respectively. Additionally, a quartet at δ 4.00 (1H) attached to a doublet methyl signal which appeared at δ 1.40 based on the H,H COSY was observed. Two methyl signals were seen at δ 1.80 (J ~1.1 Hz) and 1.67 (s), which was most likely attached to an sp^2 carbon of olefinic systems. Further three methyl signals were observed at δ 1.21 (s), 1.00 (d) and 0.92 (t); the latter showed an H,H COSY coupling with multiplet methylene protons (δ 1.62 and 1.48), confirming their attachment. Finally, a 1H multiplet was observed at δ 2.90 along with the signals of a methylene group at δ 2.76 (dd) and 1.68 (m). Two further methylene groups appeared as multiplets at δ 2.60 (2H), 2.23 (1H) and 1.68 (1H).

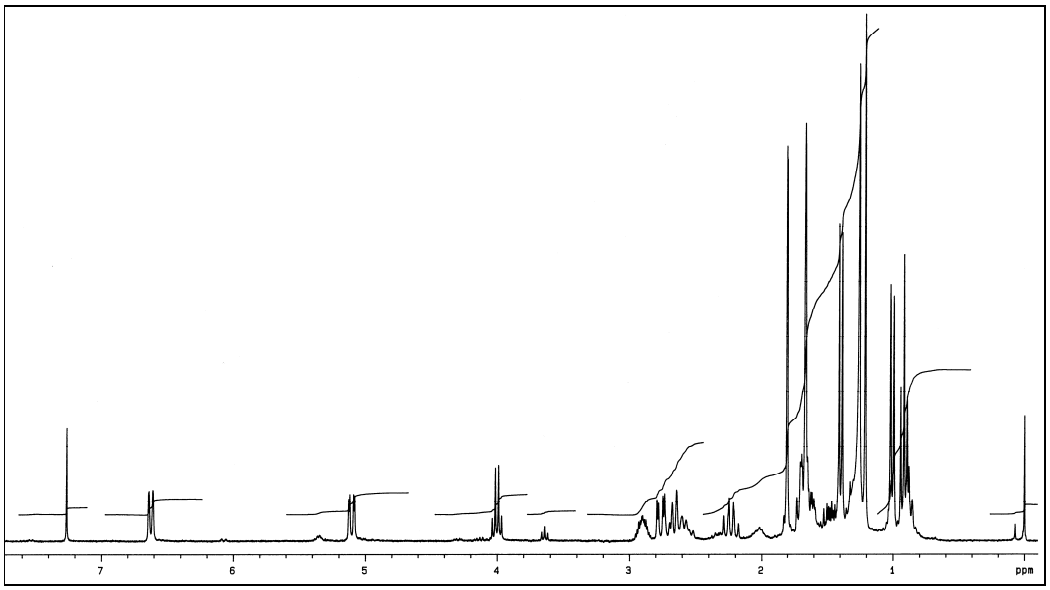


Figure 62: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 10,11-Dihydro-9,12-epoxy-8,9-anhydro-cromycin (**133**).

The ^{13}C NMR/HSQC spectra (Table 16) revealed the existence of 20 carbon signals, five of them were of quaternary carbon atoms (δ 196.3, 173.0, 151.5, 97.3 and 86.8), including the carbonyl of a ester or lactone (173.0) and a ketone in conjugation with a benzene ring or an olefinic double bond. Among the remaining 14 carbons, four were of methines, of which one at δ 148.1, corresponding to a β-methine conjugated with a carbonyl and three of sp^3 property (δ 76.8, 46.6 and 34.2), at

which the first of them was oxygenated. The other 10 carbons appeared between δ 35.1~ 10.3 and were due to six methyls and four methylene carbons.

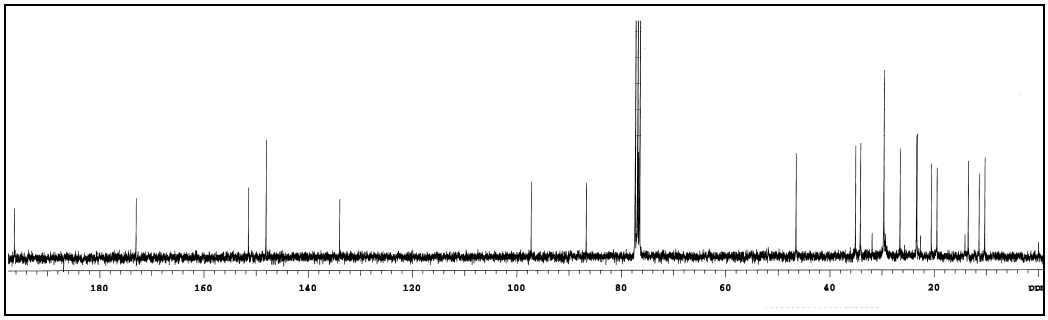


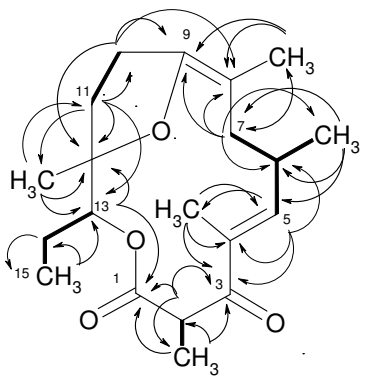
Figure 63: ^{13}C NMR spectrum ($CDCl_3$, 75.5 MHz) of 10,11-Dihydro-9,12-epoxy-8,9-anhydro-cromycin (**133**).

The methyl doublet at δ1.40 (δ_C 13.5) displayed two ^3J correlations in the HMBC spectrum (Figure 64) with the ester and ketone carbonyls (δ173.0, 196.3), confirming the position of the adjacent methine carbon (C-2, δ 46.6) in between. An olefinic methyl doublet (δ1.80) exhibited two important correlations with the carbons C-4 (134.0) and C-5 (148.1) of the olefinic double bond as well as a ^3J coupling with the ketone carbonyl (C-3, δ196.3), establishing an α-position with respect to this carbonyl (4-CH_3). Finally, the last β-methine proton (H-5, δ6.62) exhibited an HMBC coupling with the methylene protons at δ2.76 and 1.68 (C-7, δ_C 35.1) and the methyl doublet at δ1.00 (6-CH_3, δ_C 19.5), establishing a direct attachment of an CH_2-$CH(CH_3)$- fragment to the β-methine carbon (C-5) of the previous α,β-enone system, which was additionally proved by H,H COSY correlations (Figure 64).

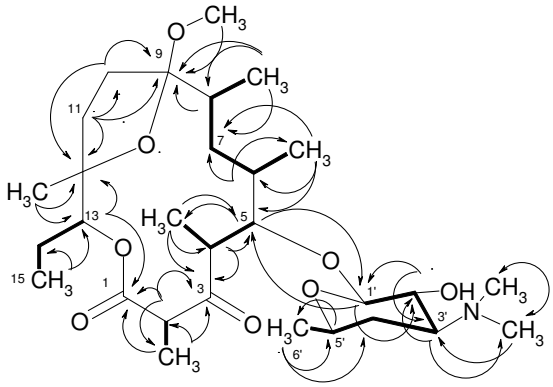
On other hand, the oxymethine proton at δ5.10 (H-13) displayed a ^3J coupling to the lactone carbonyl (C-1, δ173.0) confirming their connection *via* oxygen. With this methine proton, the methylene multiplets of H_2-14 at δ1.62 and 1.48 (δ_C 23.5) showed H,H COSY correlations. The latter methylene group was in turn linked with the methyl group giving a triplet at δ0.92 (C-15, δ_C 10.3), resulting in an oxypropane partial system. The methyl singlet of CH_3-12 (δ_H 1.21/δ_C 23.4) displayed a ^3J cross signal to the C-13 oxymethine (δ76.8) and the oxycarbon C-12 (δ86.8), confirming a direct connection between CH_3-12 and C-12, besides the attachment between C-12 and C-13. The remaining two methylene carbons C-11 (δ29.7) and C-

10 (δ26.6) were attached during the detected ^3J in H,H COSY between their corresponding protons. This showed in turn a significant HMBC correlation with the oxy-carbon (C-12), and ^3J versus the methyl carbon (12-CH_3) and C-13, indicating their attachment.

The singlet of CH_3-8 (δ 1.67) exhibited two HMBC correlations (^2J and ^3J) toward the quaternary olefinic carbons at δ97.3 (C-8) and 151.5 (C-9) as well as a ^3J coupling to the methylene carbon C-7 (δ35.1) and *vice versa*, confirming their attaching at the olefinic carbon (C-8). The deep field oxygenated olefinic carbon; C-9 (δ 151.5), to which the methylene group, C-10 (δ_H 2.60, δ_C 26.6) exhibited a cross section, proving their connection. Finally, the remaining two quaternary oxy-carbons, C-9 and C-12, were attached *via* a sole oxygen atom. This is fully matched with all aspects of molecular formula, double bond equivalents and 2D experiments (Figure 64), and this compound was elucidated as 10,11-dihydro-9,12-epoxy-8,9-anhydrocromycin (**133**). According to AntiBase, the Dictionary of Natural Products (DNP) and the Chemical Abstracts, this macrolide is new.



133



134

Figure 64: H,H COSY (▬) and HMBC (→) connectivities of macrolides **133** and **134**.

Table 16: ^{13}C and ^{1}H NMR data of 10,11-Dihydro-9,12-epoxy-8,9-anhydro-cromycin (**133**) and 10,11-Dihydro-9,12-epoxypicromycin 9-methyl ether (**134**).

Position	10,11-Dihydro-9,12-epoxy-8,9-anhydro-cromycin (**133**)		10,11-Dihydro-9,12-epoxypicromycin 9-methyl ether (**134**)	
	δ_C [a]	δ_H (J in [Hz])[b]	δ_C [c]	δ_H (J in [Hz])[b]
1	173.0	-	169.7	-
2	46.6	4.00 (q, 7.1)	53.6	3.87 (q, 7.1)
2-CH_3	13.5	1.40 (d, 7.1)	14.5	1.40 (d, 7.1)
3	196.3	-	207.6	-
4	134.0	-	47.5	3.18 (m)
4-CH_3	11.4	1.80 (d, 1.1)	15.7	1.31 (d, 6.9)
5	148.1	6.62 (dd, 9.2, 1.0)	82.5	3.78 (dd, 9.2, 2.1)
6	34.2	2.90 (m)	35.6	1.78 (m)
6-CH_3	19.5	1.00 (d, 7.2)	17.4	1.04 (d, 6.9)
7	35.1	1.68 (m), 2.76 (dd, 13.5, 3.5)	32.7	1.72 (m), 2.02 (m)
8	97.3	-	33.4	1.80 (m)
8-CH_3	20.6	1.67 (s)	15.2	0.85 (d, 6.8)
9	151.5	-	112.2	-
9-OCH_3	-	-	50.6	3.19 (s)
10	26.6	2.60 (m)	31.2	1.80 (m), 1.60 (m)
11	29.7	1.68 (m), 2.23 (m)	32.5	1.80 (m), 2.02 (m)
12	86.8	-	84.3	-
12-CH_3	23.4	1.21 (s)	23.5	1.31 (s)
13	76.8	5.10 (dd, 11.0, 2.6)	78.6	5.03 (dd, 9.9, 2.7)
14	23.5	1.48 (m), 1.62 (m)	23.9	1.30 (m), 1.60 (m)
15	10.3	0.92 (t, 7.3)	10.6	0.91 (t, 7.3)
1’	-	-	105.0	4.35 (d, 7.3)
2’	-	-	70.1	3.30 (dd, 10.1, 7.4)
3’	-	-	65.7	2.57 (m)
4’	-	-	28.5	1.21 (m), 1.66 (m)
5’	-	-	69.3	3.55 (m)
6’	-	-	21.0	1.24 (d, 6.1)
3’-$N(CH_3)_2$	-	-	40.1	2.33 (s)

[a] (75.4 MHz); [b] (300 MHz); [c] (125.7 MHz)

4.8.2 10,11-Dihydro-9,12-epoxy picromycin-9-methylether

Similarly, compound **134** was obtained as middle polar colourless oil, which on TLC turned to yellow, dark green, and finally violet by spraying with anisaldehyde/sulphuric acid and heating. Its molecular weight was established by (+)-ESI MS as 541 Dalton, and its corresponding molecular formula as $C_{29}H_{51}NO_8$, having five double bond equivalents.

The NMR pattern of **134** displayed a very close similarity with that of **133**, (Table 16). The ^{1}H NMR spectrum of **134** showed a methoxy singlet at δ 3.19 (δ_C 50.6), while the olefinic methine proton (H-5) detected at δ 6.62 in **133** had disappeared and was replaced in **134** by an oxymethine proton (δ_H 3.78, δ_C 82.5). The

quaternary carbon C-4 was converted into a methine multiplet (δ_H 3.18, δ_C 47.5). The latter showed a H,H COSY correlation (Figure 64) with a methyl doublet at δ 1.31 (δ_C 15.7), confirming alternatively, the absence of the double bond between C-4 and C-5 in **133** and pointing to a 5-oxypropionyl fragment in **134**. This explained the deepfield shift of the corresponding carbonyl (C-3) at δ 207.6 ($\Delta\delta$ ~ 11.3) for **134** higher than **133** (Table 16). Consequently, the methyl singlet at C-8 in **133** was replaced by doublet methyl group (δ_H 0.85, δ_C 15.2) coupling with the methine multiplet of C-8 (δ_H 1.80; δ_C 33.4), which was recognized *via* H,H COSY correlation. Also the double bond situated between C-8 (33.4) and C-9 (112.2) was absent, while the methoxy group at C-9 was shifted to δ 3.19 (δ_C 50.6); this partial structure was supported by ^3J cross-signal between OMe and C-9 in an HMBC experiment (Figure 64). The residual ^{1}H NMR pattern of the lactone system of **134** was similar to that in **133**, confirming **134** as dihydro-macrolactone analogue of **133**. The HMBC ^3J coupling directed from H-13 to the carbonyl (C-1, 169.7) of the lactone confirming the ring closure and excluded the epoxide between C-9 and C-13, but indicated an oxygen bridge between C-9 and C-12 as in **133**.

The NMR spectra indicated additionally a singlet at δ 2.33 (δ_C 40.1) corresponding to 2 CH_3 groups of magnetically equivalent methyl protons attached to nitrogen, together with the doublet of an anomeric proton at δ 4.35 (J~7.3, δ_C 105.0). Further three oxymethine (3.55, 3.30 and 2.57), two methylene protons (δ 1.66 & 1.21) and one doublet methyl signal at δ 1.24 (J ~6.1) were observed, confirming a sugar moiety. The N,N-dimethyl group (δ_H 2.33, δ_C 40.1) was placed at C-3′ (δ 65.7) of this sugar by ^3J correlation between both of them, while the methyl with doublet at δ 1.24 was connected to C-6′. This identified the sugar system as desosamine in the β-configuration due to the high coupling constant (J = 7.3 Hz) of the anomeric proton (δ 4.35). The sugar system was found by HMBC experiments to be at C-5 of the macrolactone due to the observed ^3J cross-signals between H-5 (δ 3.78) and the anomeric carbon (C-1′) at δ 105.0 and *vice versa*. Furthermore, on the bases of ESI-MS^2 spectra, four ion peaks were visible at *m/z* 542, 510, 353 and 158 which were attributed to loss of OCH_3 and desosamine ($C_8H_{14}NO_2$), respectively, from the parent structure (**134**) (Figure 65). This identified the final structure of **134** as 10,11-dihydro-9,12-epoxy picromycin-9-methylether, a new macrolide derivative.

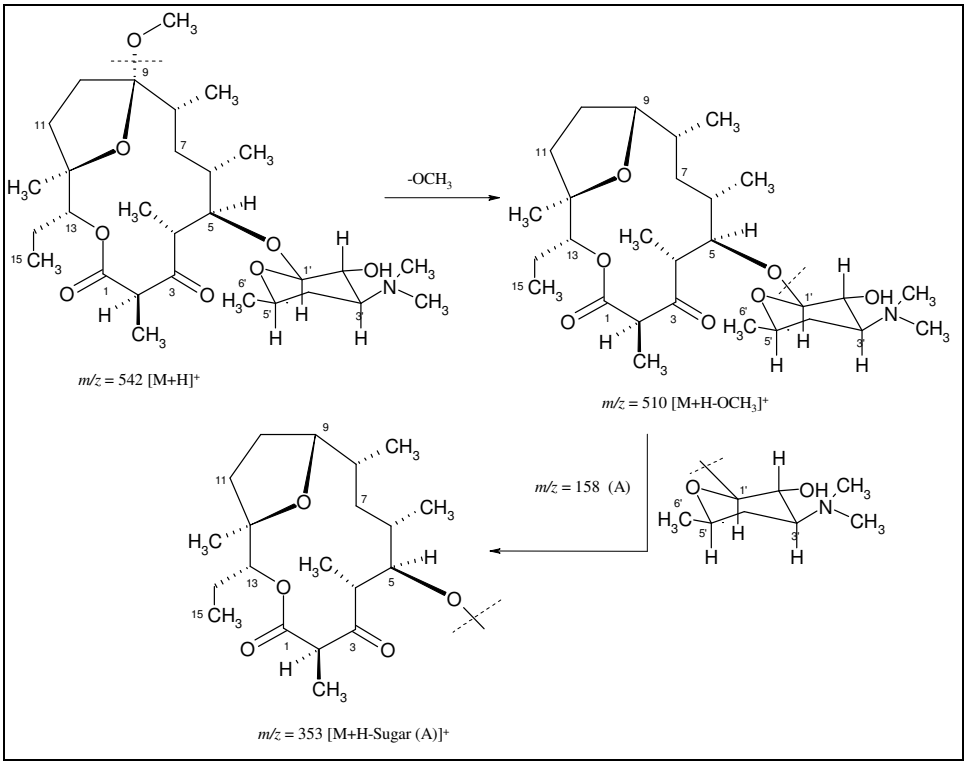
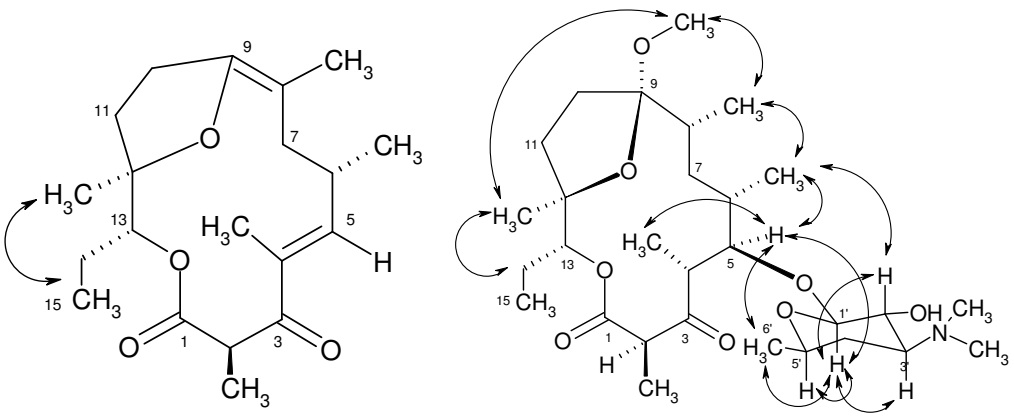


Figure 65: ESI MS^2 Fragmentation pattern of 10,11-Dihydro-9,12-epoxy picromycin-9-methyl ether (**134**).

Both macrolides, **133** and **134** displayed positive optical rotation values ($[\alpha]_D^{20}$ = +144 and +17, respectively). A nuclear Overhauser effect was observed between the oxymethine (H-13) and the terminal ethyl group (14-H_2 and 15-H_3) and the methyl singlet at 12-position. A further NOE was detected between 9-OCH_3 at δ3.19 and both the doublets of 8-CH_3 and 12-CH_3. This indicated that both compounds **133** and **134** kept the same configuration as all other reported (2*R*,6*S*,12*S*,13*R*) cromycin and (2*R*,4*R*,5*R*,6*S*,8*R*,9*S*,12*S*,13*R*) picromycin derivatives, respectively (Figure 66).



133 **134**

Figure 66: Selected Nuclear Overhauser effect (NOE) correlations for compounds **133** and **134**.

4.8.3 10,11-Dihydro-cromycin

Compound **135** was obtained as colourless and UV absorbing oil, which turned yellow by spraying with anisaldehyde/sulphuric acid. The molecular weight was established as 352 Dalton, using (+)-ESI MS, and the corresponding molecular formula as $C_{20}H_{32}O_5$, containing five double bond equivalents.

Based on the $^{1}H/^{13}C$ NMR and HSQC spectra (Table 17), compound **135** displayed a high structural similarity to **133**. It showed the same α,β-unsaturated carbonyl (C-3, δ 196.0), and the corresponding olefinic carbons (C-4, C-5) were at δ 137.7 and 147.3, respectively. The β-olefinic proton was at δ 6.36, and 4-CH_3 was at δ 1.86 (δ_C 12.2). In contrast, the olefinic bond between C-8 and C-9 of compound **133** vanished in **135** and was replaced by a methine multiplet for C-8 (as in **134**), which appeared at δ 2.40 (δ_C 41.8), and correlated by H,H COSY (Figure 68) with a methyl doublet at δ 1.03 (δ_C 15.5). On other hand, C-9 was downfield shifted to δ 215.5 in ^{13}C NMR, indicating an acetyl fragment. Hence the tetrahydrofuran moiety in **133** or **134** was absent in **135**, as the corresponding oxycarbon (C-12) was upfield shifted to 73.2 ($\Delta\delta$ ~13.6) with respect to the furan analogues **133** and **134**, pointing to a hydroxyl group at C-12. The remaining partial structures of **135** were similar to **133**, affording the structure of 10,11-dihydro-cromycin, which was fully deduced by HMBC and H,H COSY experiments (Figure 69).

Compound **135** exhibited a positive specific rotation ($[\alpha]_D^{20}$ = +33) which may be interpreted as a (2*R*,6*S*,8*R*,12*S*,13*R*) configuration as in other cromycin analogues. A search in different databases confirmed **135** as new macrolide.

4.8.4 *seco*-Decarboxy-cromycin

Compound **136** was isolated as colourless UV absorbing oil, which on spraying with anisaldehyde/sulphuric acid turned to violet. The molecular weight was established as 324 Dalton by ESI MS, and the corresponding molecular formula $C_{19}H_{32}O_4$, delivered four double bond equivalents.

The ^{1}H NMR spectrum exhibited two methyl triplet at δ 1.06, while the triplet methyl described in the macrolides above (~0.92) disappeared. The spectra displayed additionally two *trans* olefinic protons (*J*~15.5) at δ 6.48 and 6.40, while that of the β-proton (H-5) found in the macrolides **133** and **135)** was shifted to δ 6.24 (*J* ~10.2) confirming the existence of the same -CH=C(CH_3)-CO fragment. The shift of the conjugated carbonyl signal was however at δ 204.0, at a higher chemical shift ($\Delta\delta$ ~8 ppm) than those for **133** and **135**. With this C-3 carbonyl, a terminal ethyl group was connected based on the HMBC correlations, between the methyl triplet (δ_H 1.06, δ_C 8.5) and the ^2J coupling of the methylene carbon at δ 30.5 (δ_H 2.65) with CO-3. Additionally, a connection of a pentyl group with the β-olefinic carbon C-5 was recognized. In a similar way, a C_5 fragment from 6-Me *via* C-6,7,8 to 8-Me was derived from COSY and HMBC couplings; atom C-5 connects both parts to give the partial structure **A** (Figure 68).

The ^{13}C NMR spectra of **136** displayed no carbons at ~ 173, confirming the missing lactonized carbonyl. The *trans*-olefinic protons at δ 6.48 (δ_C 148.5) and 6.40 (δ_C 127.0) showed ^3J and ^2J correlations with a carbonyl which appeared at δ 202.1, establishing an additional α,β-conjugated enone system. The methyl triplet at δ 1.06 (δ_C 11.1) exhibited a ^3J correlation with a quaternary oxymethine carbon visible at δ 80.3, and the latter was stronger downfield shifted ($\Delta\delta$ ~ +4) than that in the lactones **133**, **134** and **135**. The methine proton H-13 (δ 3.36) displayed a ^3J coupling with the olefinic β-carbon (δ_C 148.5) and the methyl singlet of CH_3-12 (δ_C 24.5), confirming the attachment between the enone system and the residual partial struc-

ture (CH_3CH_2-CH(OH)-C(OH)(CH_3)-) through C-11 (δ 148.5) and C-12 (δ 76.5). This was further confirmed by two ^3J correlations from the methyl singlet (CH_3-12, δ 1.29) at the olefinic β-carbon (C-11, δ 148.5) and C-13 (δ 80.3), and hence the partial structure **B** was fixed (Figure 68).

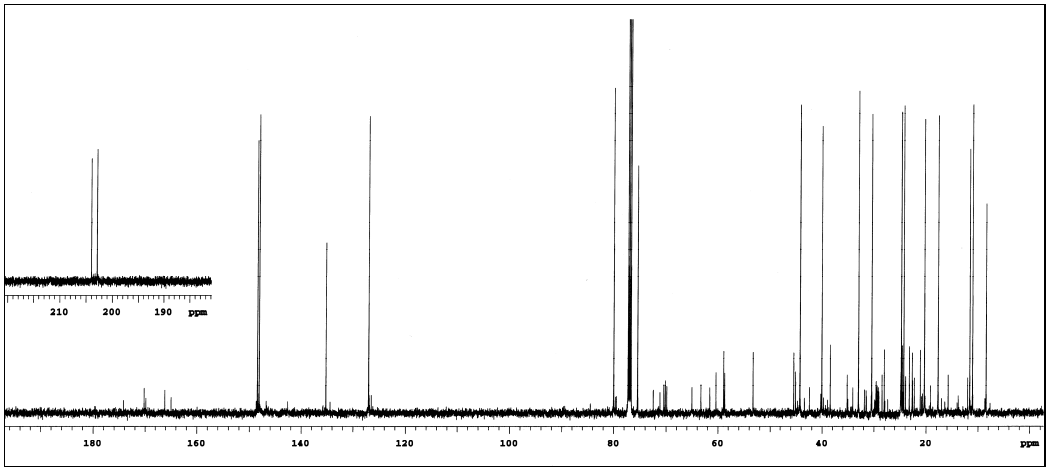
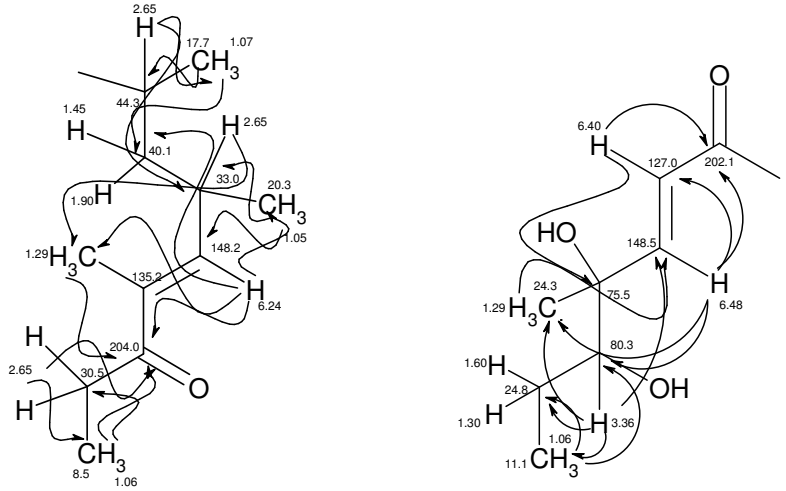


Figure 67: ^{13}C NMR spectrum ($CDCl_3$, 125.7 MHz) of *seco*-Decarboxy-cromycin (**136**).

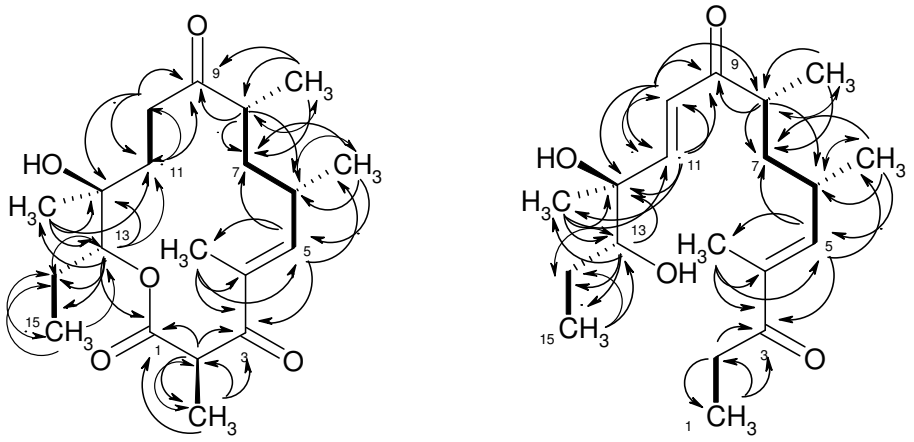


A **B**

Figure 68: Selected HMBC correlations of the constituent partial structures, **A** & **B** of compound **136**.

Both fragments, **A** and **B** were connected through C-8 and C-9 based on the following observations: the α-methine proton (H-10, 6.40) in fragment **B** displayed a ^3J correlation towards the methine carbon C-8 (44.3) in **A** besides the cross signal be-

tween the methine proton at δ2.65 (H-8 of **A**) and the carbonyl (C-9, 202.1) of **B** (Figure 69). This established compound **136** as *seco*-decarboxy-cromycin, which according to the search in different databases (AntiBase, DNP and Chemical Abstracts) it as new compound. It seems plausible to assume that the configuration is (6*S*,8*R*,12*S*,13*R*) as in the cromycin analogues.



135 **136**

Figure 69: H,H COSY (▬, ↔) and HMBC (→) correlations of **135** and **136**.

Table 17: ^{13}C and ^{1}H NMR assignments of 10,11-Dihydro-cromycin (**135**) and *seco*-Decarboxy-cromycin (**136**) in $CDCl_3$.

Position	10,11-Dihydro-cromycin (**135**)		*seco*-Decarboxy-cromycin (**136**)	
	δ_C [a]	δ_H (*J* in [Hz]) [b]	δ_C [c]	δ_H (*J* in [Hz]) [b]
1	171.9	-	8.5	1.06 (t, 7.4)
2	45.4	4.33 (q, 6.9)	30.5	2.65 (m)
2-CH_3	14.2	1.43 (d, 6.2)	-	-
3	196.0	-	204.0	-
4	137.7	-	135.2	-
4-CH_3	12.2	1.86 (d, 1.2)	11.6	1.75 (d, 1.3)
5	147.3	6.36 (dd, 9.8, 1.2)	148.2	6.24 (dd, 10.2, 1.2)
6	32.4	2.90 (m)	33.0	2.65 (m)
6-CH_3	20.7	1.08 (d, 6.5)	20.3	1.05 (d, 7.2)
7	40.6	1.55 (m), 1.41 (m)	40.1	1.90 (m), 1.45 (m)
8	41.8	2.40 (m)	44.3	2.65 (m)
8-CH_3	15.5	1.03 (d, 6.9)	17.7	1.07 (d, 7.3)
9	215.5	-	202.1	-
10	36.9	2.50 (m), 2.40 (m)	127.0	6.40 (d, 15.5)
11	32.0	1.45 (m), 1.80 (m)	148.5	6.78 (d, 15.5)
12	73.2	-	75.5	-
12-CH_3	22.9	1.19 (s)	24.3	1.29 (s)
13	81.9	4.80 (dd, 10.8, 2.3)	80.3	3.36 (dd, 10.7, 2.0)
14	22.5	1.46 (m), 1.68 (m)	24.8	1.60 (m), 1.30 (m)
15	10.8	0.92 (t, 7.4)	11.1	1.06 (t, 7.3)

[a] (75.4 MHz); [b](300 MHz); [c](125 MHz)

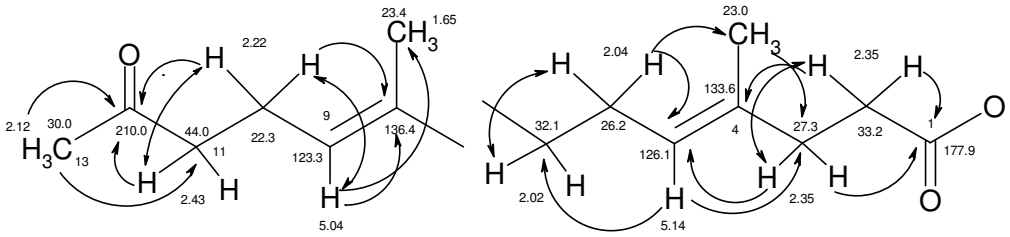
4.8.5 (4E,8E)-4,8-Dimethyl-12-oxo-trideca-4,8-dienoic acid

Compound **137** was isolated as a low polar colourless solid, which showed no UV absorbance on TLC, but was detected by spraying with anisaldehyde/sulphuric acid as yellow spot, which turned later to green. The molecular weight was deduced from (+)-ESI MS as 252 Dalton, and its molecular formula as $C_{15}H_{24}O_3$, containing four double bond equivalents.

The ^{1}H NMR/HMQC spectra (Table 18) displayed three methyl signals, one of them at δ 2.12 (δ_C 30.0) as singlet, most likely of an acetyl group, while the other two methyls were seen as doublets (J~ 1Hz) at δ 1.66 (δ_C 23.0) and 1.65 (δ_C 23.4), respectively, bound to sp^2 carbons of olefinic systems. Two olefinic methine signals at δ 5.14 (δ_C 126.1) and 5.04 (δ_C 123.3) were according to their triplet splitting (J~7 Hz) connected to methylene groups one side, while the neighbouring olefinic carbons could be attached to any of the above methyl groups. This resulted in two olefinic fragments of structure CH_2-CH=C(CH_3)-. Moreover, four methylene groups were displayed as multiplets; one of them with an intensity of 4H appeared at δ 2.35 (δ_C 33.2 and δ_C 27.3), and the residual signals were each of 2H at δ 2.04 (δ_C 26.2) and 2.02 (δ_C 32.1), respectively.

The ^{13}C NMR spectrum displayed four quaternary carbons: a ketone carbonyl at 210.0, and an ester or carboxylic acid signal at δ 177.9, while the remaining two carbons (136.4 and 133.6) were corresponding to two olefinic double bonds. In the HMBC spectrum, the methyl singlet at δ 2.12 showed a ^2J correlation with the carbonyl C-12 (210.0), which confirmed the acetyl group. The latter was additionally proven by the downfield shift of the connected methyl carbon (30.0) as well as the ^3J cross-signal with the methylene carbon (C-11) at δ 44.0, and *vice versa*. The latter methylene (CH_2-11, δ 44.0) showed in turn a ^3J coupling from the olefinic methine proton (H-9) at δ 5.04, and displayed two correlations (^2J and ^3J) at δ 123.3 and 136.4. The latter quaternary carbon must be attached to the methyl at δ 1.65 (23.4) due to the displayed ^2J coupling. The methylene group in between, (CH_2-10; δ_H 2.22, δ_C 22.3) was established by H,H COSY, constructing the hept-5-en-2-one partial structure **C** (Figure 70).

The downfield shift and the HMBC correlations of the carboxyl group (177.9) indicated its attachment to the methylene groups C-2 and C-3. Both methylene groups showed correlations with the two olefinic carbons, C-4 (δ 133.6) and C-5 (δ 126.1). The absent COSY correlation between the methine proton (H-5) at δ 5.14 and H-3 (δ 2.35) excluded a neighbouring of such methylene group. The remaining two methylene groups appeared at δ 2.04 and 2.02 were vicinal due to the ^3J coupling seen in the H,H COSY spectrum. The direct attachment of the methylene carbon C-6 (δ 26.2) to C-5 (δ 126.1) was derived from H,H COSY and the ^3J correlation with the methyl group 4-CH_3 (23.0, δ_H 1.66). The other methylene (H_2-7) showed a ^3J coupling with the olefinic methine carbon C-5 (126.1), confirming the second partial structure, **D** (Figure 70).



C **D**

Figure 70: Selected H,H COSY (↔) and HMBC(→) correlations of the constituent partial structures, **C** and **D** of compound **137**.

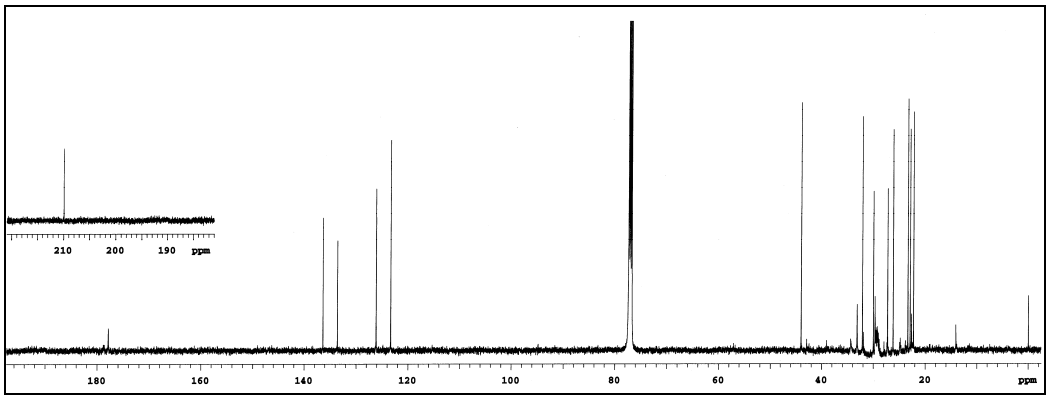


Figure 71: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of (4*E*,8*E*)-4,8-Dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**).

The only way to connect the partial structures, **C** and **D** is via the quaternary sp^2 carbon C-8 (δ 136.4) and the methylene one of C-7 (δ 32.1). This was confirmed by

the HMBC correlation between the methyl doublet of CH_3-8 (δ 1.65) with the methylene carbon C-7 (δ 32.1), and additionally by correlations from the methylene protons (H_2-7, δ 2.02) toward CH_3-8 and the two carbons (C-8 and C-9) of the olefinic bond (Figure 72). This resulted in 4,8-dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**), where the missing NOE couplings of the two methyls CH_3-4/-8 with their methine partners indicated the *trans* configuration of both double bonds, pointing to **137** as (4*E*,8*E*)-4,8-dimethyl-12-oxo-trideca-4,8-dienoic acid. A search for this acid in diverse databases proved its novelty.

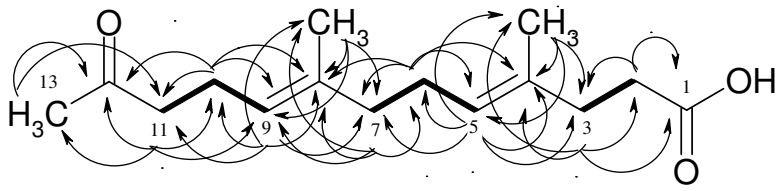


Figure 72: H,H COSY (▬) and HMBC (→) correlations of **137**.

Table 18: ^{13}C NMR and ^{1}H NMR of (4*E*,8*E*)-4,8-Dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**) in $CDCl_3$.

Position	(4*E*,8*E*)-4,8-Dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**)	
	δ_C(125.7 MHz)	δ_H (*J* in [Hz]) (599.7 MHz)
1	177.9	-
2	33.2	2.35 (m)
3	27.3	2.35 (m)
4	133.6	-
4-CH_3	23.0	1.66 (d, 0.9)
5	126.1	5.14 (t, 6.2)
6	26.2	2.04 (m)
7	32.1	2.02 (m)
8	136.4	-
8-CH_3	23.4	1.65 (d, 1.2)
9	123.3	5.04 (t, 7.1)
10	22.3	2.22 (q, 7.1)
11	44.0	2.43 (t, 7.4)
12	210.0	-
13	30.0	2.12 (s)

4.8.6 Cromycin, Picromycin and Picromycin-C

Compound **138** was isolated as white amorphous UV absorbing solid, which turned initially yellow and later to greenish-blue with anisaldehyde/sulphuric acid spraying reagent on TLC.

The molecular weight was established as 350 Dalton using ESI MS, and the corresponding molecular formula as $C_{20}H_{30}O_5$, delivering six double bond equivalents, that is one more than those of compound **135**.

The NMR spectrum of **138** displayed a very close similarity with that of **135**, (Table 19) with the replacement of 2 methylene groups by a double bond ($\delta_{H\alpha}$6.06, J = 16.6 Hz, $\delta_{c\alpha}$ 127.1 and $\delta_{H\beta}$ 6.73 d, J = 16.6 Hz, $\delta_{c\beta}$ 149.8). Searching in AntiBase using the above spectroscopic data resulted in cromycin (**138**).

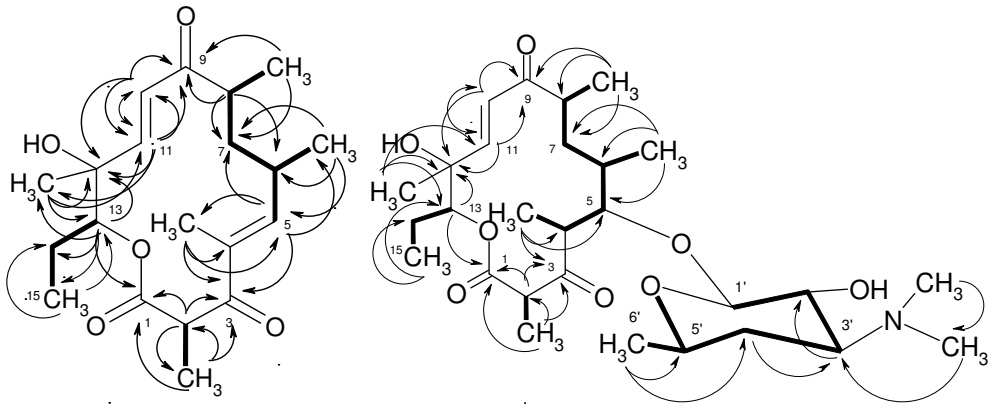
Likewise compound **139** was isolated as UV absorbing white amorphous solid, which turned initially to yellow and then to dark-blue with anisaldehyde/sulphuric acid. The molecular weight of **139** was determined as 525 Dalton using ESI MS, and the corresponding molecular formula was $C_{28}H_{47}NO_8$, delivering 7 double bond equivalents, with one more than in compound **138**.

The NMR pattern of **139** and **138** displayed a very close similarity; the difference in the molecular weight of Δm 158 ($C_8H_{17}NO_3$), was due to the presence of the sugar molecule **A** in the case of **139**, which was clear from the ^{1}H and ^{13}C NMR spectra. The structure of **139** was determined as picromycin (**139**) by searching in AntiBase using the above spectroscopic data and the relation to other compounds isolated from this strain.

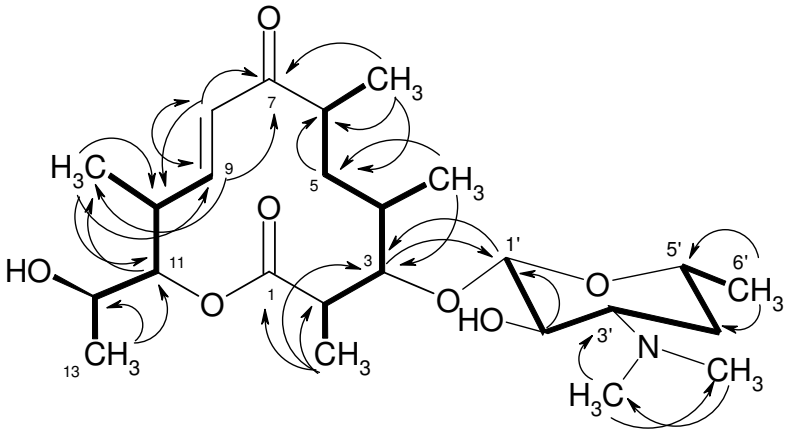
Similarly, compound **140** was obtained from the same fraction as compound **139** as UV absorbing white amorphous solid, which turned to dark-green with anisaldehyde/sulphuric on TLC.

Using the molecular weight or the corresponding molecular formula and NMR spectra for a search in AntiBase resulted in picromycin-C (**140**) as the only coincident structure.

The carbon assignments of the known 14-macrolactones cromycin (**138**) and picromycin (**139**)[223] were erroneously reported in literatures, as modern spectroscopic techniques were not available at this time. Therefore, the signal assignment of these macrolides along with picromycin-C (**140**)[224,225] was determined using 2 D NMR experiments (H,H COSY, HMQC and HMBC) (Figure 73 and Table 19).



138 **139**



140

Figure 73: H,H COSY (↔, ▬) and selected HMBC (→) correlations of Cromycin (**138**), Picromycin (**139**), and Picromycin-C (**140**).

At least 79 cromycin derivatives of polyketide origin have been reported from microorganisms so far[61]. Polyketides are the structurally most divers family of all reported classes of natural products[226], among them the macrolides. The latter are characterized by their wide range of potent activities against gram-positive bacteria such as *Staphylococcus aureus*. Most macrolides are produced by *Streptomycetes* sp., and contain 12- or 14-membered lactone rings[61]. Some of them, e.g. picromycin (**139**), erythromycins (A-E), kujimycin A[227], lankamycin[228], megalomicin[229], methymycin and neomethymycin are clinically used in human and veterinary medicine, and found application in agriculture and animal nutrition[230~232]. This encouraged the

search for related substances and provided demanding targets in total synthesis of natural products.

Table 19: ^{13}C and ^{1}H NMR assignments of Cromycin (**138**), Picromycin (**139**) and Picromycin-C (**140**) in $CDCl_3$.

Position	Cromycin (**138**)		Picromycin (**139**)		Picromycin-C (**140**)	
	δ_C [a]	δ_H (J in [Hz]) [b]	δ_C [c]	δ_H (J in [Hz]) [b]	δ_C [a]	δ_H (J in [Hz]) [b]
1	171.4	-	170.0	-	174.8	-
2	45.4	4.31 (q, 7.0)	53.4	3.88 (q, 7.4)	43.9	2.88 (m)
2-CH_3	13.7	1.41 (d, 6.0)	13.3	1.47 (d, 7.4)	15.9	1.41 (d, 7.2)
3	196.1	-	212.7	-	85.6	3.60 (d, 10.4)
4	138.0	-	46.5	3.20 (m)	33.4	1.25 (m)
4-CH_3	12.3	1.90 (d, 1.2)	14.9	1.31 (d, 6.5)	17.4	1.02 (d, 6.7)
5	146.9	6.33 (dd, 10.1, 1.3)	80.2	3.96 (s, br)	34.1	1.68 (m, H_A), 1.40 (m, H_B)
6	32.8	2.95 (m)	35.9	2.13 (m)	45.1	2.52 (m)
6-CH_3	21.0	1.08 (d, 6.5)	17.4	1.05 (d, 7.1)	17.6	1.19 (d, 7.0)
7	42.9	1.62 (m), 1.41 (m)	37.6	1.50 (m), 1.00 (m)	205.1	-
8	39.4	2.65 (m)	42.9	2.68 (m)	126.2	6.44 (dd, 15.7)
8-CH_3	14.8	1.09 (d, 6.5)	14.8	1.10 (d, 6.5)	-	-
9	204.1	-	203.7	-	147.1	6.76 (dd, 15.7, 5.5)
10	127.1	6.06 (d, 16.6)	129.1	6.31 (d, 15.7)	35.4	3.05 (m)
10-CH_3	-	-	-	-	9.8	1.16 (d, 6.8)
11	149.8	6.73 (d, 16.6)	145.5	6.64 (d, 15.7)	75.4	4.80 (dd, 9.0, 2.1)
12	73.6	-	75.0	-	66.4	3.89 (m)
12-CH_3	21.4	1.35 (s)	22.9	1.33 (s)	-	-
13	80.3	4.86 (dd, 10.9, 2.3)	83.4	5.02 (dd, 11.2, 2.4)	21.0	1.20 (d, 7.0)
14	21.7	1.46 (m), 1.80 (m)	23.2	1.75 (m), 1.48 (m)	-	-
15	10.3	0.92 (t, 7.2)	10.7	0.89 (t, 7.3)	-	-
1’	-	-	104.8	4.38 (d, 7.3)	105.0	4.25 (d, 7.3)
2’	-	-	69.8	3.29 (m)	70.2	3.23 (m)
3’	-	-	65.6	2.68 (m)	65.9	2.52 (m)
3’-$N(CH_3)_2$	-	-	40.0	2.27 (s)	40.2	2.33 (s)
4’	-	-	28.7	1.72 (m), 1.25 (m)	28.6	1.68 (m, H_A), 1.20 (m, H_B)
5’	-	-	69.3	3.59 (m)	69.3	3.48 (m)
6’	-	-	21.1	1.26 (d, 6.1)	21.1	1.23 (d, 6.1)

[a] (125.7 MHz); [b](300 MHz); [c](75.4 MHz)

5 Marine-derived *Streptomyces* sp.

The marine streptomycetes investigated here were obtained from different sources. Mei strains were obtained from the collection of Prof. Meiners (Emden) and were precultivated on M_2 medium in 100% artificial sea water with additional calcium carbonate at 28 °C. The other marine bacterial strains were mainly obtained from the collection of E. Helmke (Bremerhaven), and cultivated on the standard M_2^+ medium (= M_2 medium + 50% artificial sea water) or fermented as stated otherwise.

5.1 Marine-derived *Streptomyces* sp. Act8970

Based on chemical screening using TLC, the extract of the marine-derived *Streptomyces* sp. Act8970 showed a number of UV non-absorbing bands, which were stained blue on spraying with anisaldehyde/sulphuric acid. The strain exhibited moderate activity against the algae *Chlorella vulgaris* and *Chlorella sorokiniana*, and the Gram-negative bacteria *Escherichia coli*. The extract was weakly active against the Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, the fungus *Mucor miehei* (Tü284), and the yeast *Candida albicans*.

Act8970 was therefore upscaled using M_2^+ medium (+50% artificial sea water) for 11 days to isolate its bioactive secondary metabolites. After usual work up, a brown crude extract was obtained which in turn was applied to flash silica gel column chromatography. Three fractions were obtained by TLC-guided fractionation. Purification of the fast moving fraction **I** using Sephadex LH-20 followed by silica gel led to attiamycin A (**141**) and attiamycin B (**143a**) as colourless oils. Purification of the middle polar fraction **II** delivered homononactic acid (**145a**), homononactic acid methyl ester (**145b**) and dinactin (werramycin) (**148**) as colourless oils. Finally, working up of fraction **III** led to nonactic acid (**144a**) and dinactin (**148**) as further colourless oils (Figure 74).

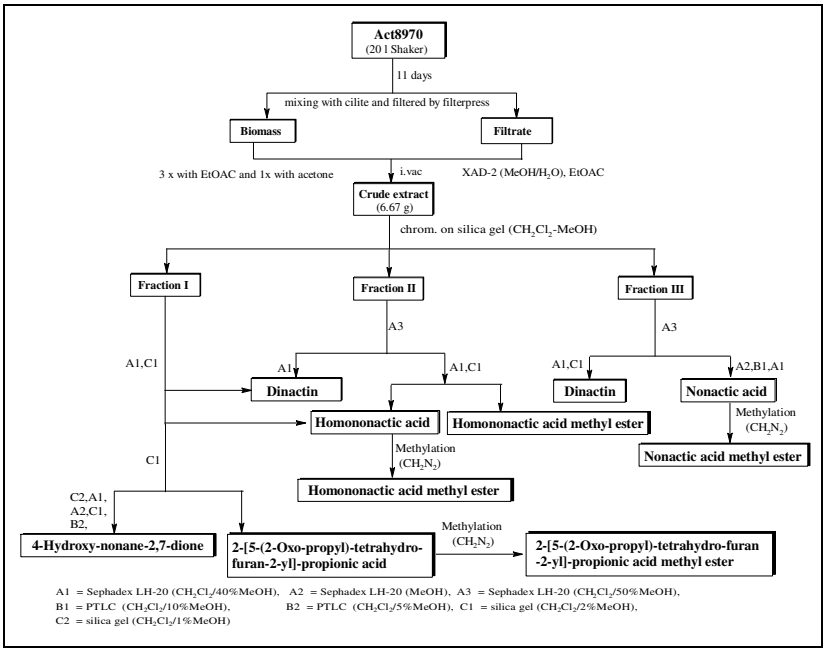


Figure 74: Work-up procedure of the marine-derived *Streptomyces* sp. Act8970.

5.1.1 Attiamycin A

Compound **141** was isolated as low polar colourless oil from fraction I, which was stained yellow and later to brown by anisaldehyde/sulphuric acid. The molecular weight of **141** was established as 172 Dalton using ESI and EI MS. ESI HRMS established the corresponding molecular formula as $C_9H_{16}O_3$, entailing two double bond equivalents.

The ^{1}H NMR spectrum displayed no aromatic or olefinic signals. Multiplet signals of an oxymethine (δ4.01) in addition to another three multiplets were observed. The latter three signals between 2.61~2.46 (6H) and 1.73 (2H) were due to four methylene groups, the first three (2.61~2.46) being of sp^2 systems. A methyl singlet (δ 2.18) was attached to a sp^2 carbon and indicated an acetyl moiety. The remaining signal was a methyl triplet (δ1.06) linked to a methylene group, which appeared as a quartet at δ2.46.

The ^{13}C/APT NMR spectra of **141** displayed 9 carbon signals, two among them were of ketone carbonyls (δ211.7 and 209.7). The residual carbon signals indicated one oxy-methine (δ66.8), four methylene carbons (δ50.0, 38.1, 36.0 and 29.9) along with two methyl carbons (δ30.6, 7.8).

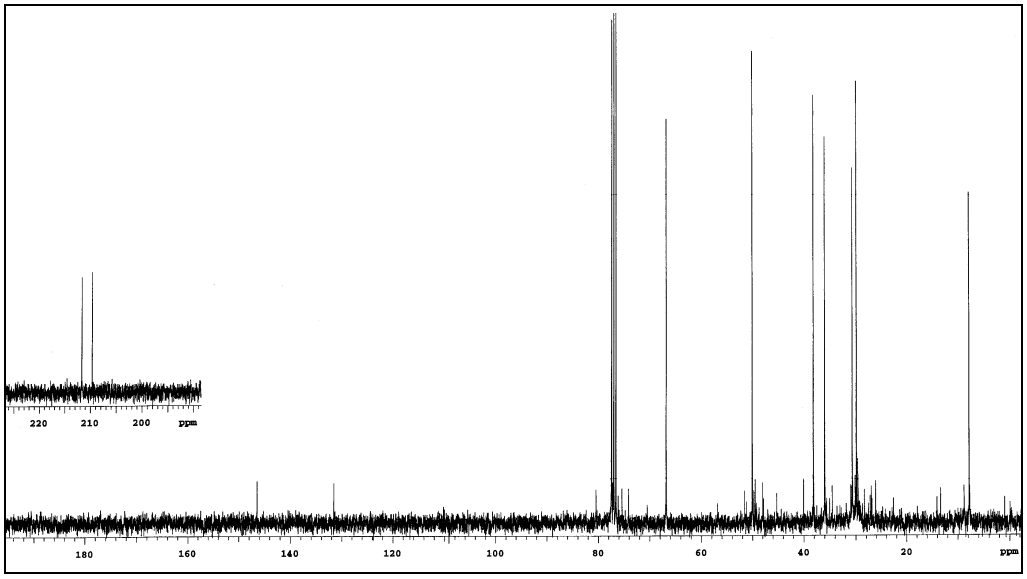


Figure 75: ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of 4-Hydroxy-nonane-2,7-dione (**141**).

Based on the HMBC correlations (Figure 76) of **141**, protons of the methyl triplet (C-9) at δ 1.06 exhibited a ^3J correlation towards the carbonyl (C-7) at δ 211.7, constructing a propionyl fragment (O=C-CH_2-CH_3). The methyl singlet (C-1, 2.18) showed a ^2J correlation to the carbonyl C-2 at 209.7, and hence an acetyl group was supposed. The two methylene groups at 2.61 (C-3, C-6) displayed two ^2J correlations to both carbonyls C-2 and C-7. The acetyl group (δ 2.18) showed a ^3J correlation to one of the methylene carbons (δ 50.0, C-3), confirming the direct attachment between C-2 and C-3. Moreover, the oxy-methine carbon C-4 showed ^2J and ^3J correlations with protons of the three methylene groups CH_2-3/CH_2-6 (δ 2.61), and CH_2-5 (δ 1.73), while the latter methylene group (CH_2-5) displayed three other relevant correlations with the two methylene carbons C-3 (δ 50.0, ^3J) and C-6 (δ 38.1, ^2J), and the oxy-methine carbon C-4 (δ 66.8, ^2J). This resulted in the -CH_2-CH(OH)-CH_2-CH_2- chain, which was further confirmed by the H,H COSY data. Hence, the final structure of **141** was deduced as 4-hydroxy-nonane-2,7-dione.

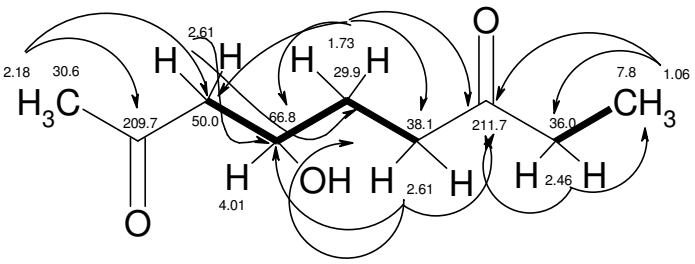
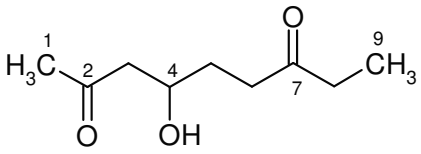
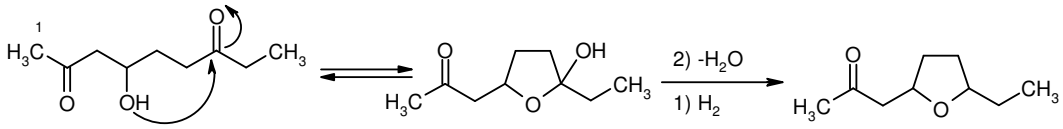


Figure 76: ^{1}H, ^{1}H COSY (▬) and HMBC (→) correlations of 4-Hydroxy-nonane-2,7-dione (**141**).

A search in the current databases (AntiBase, DNP and CA), pointed to the novelty of **141**. So, we have named it as attiamycin A. Attiamycin A (**141)** might be a precursor in the biosynthesis of decarboxy-oxo-nonactic acid (**142**) by ring closure, followed by an elimination of water and hydrogenation.



141



141 **142**

Figure 77: A biosynthetic approach to correlate Attaimycin A (**141**) and Decarboxy-oxo-nonactic acid (**142**).

5.1.2 Attiamycin B

Attiamycin B (**143a**) was obtained from fraction I as an additional low polar colourless oil. It was not UV absorbing and turned reddish-brown (later reddish violet) on spraying with anisaldehyde/sulphuric acid. The molecular weight of **143a** was deduced from ESI MS to be 200 Dalton. ESI and ESI HRMS confirmed the molecular formula as $C_{10}H_{16}O_4$.

The ^{1}H NMR spectrum displayed a similar signal pattern as **141**. An acidic broad singlet (δ 6.30) might be of an acidic hydroxyl group, and two multiplets of

oxy-methines (δ 4.32 and 4.08) were observed. In the region of δ 2.80~1.50 with integration of 7H, a series of multiplets was present, corresponding to three methylene groups and one methine proton (δ 2.54). The latter methine appeared as quartet, pointing to its direct connection with a methyl group, which gave a doublet at δ 1.18. As in **141**, compound **143a** showed a methyl singlet at δ 2.20, corresponding to an acetyl group. On esterfication of **143a** using diazomethane, component **143b** was obtained, showing the singlet of a methyl ester (δ 3.69) and a molecular weight 14 amu higher than **143a**. This indicated that **143a** was a free carboxylic acid.

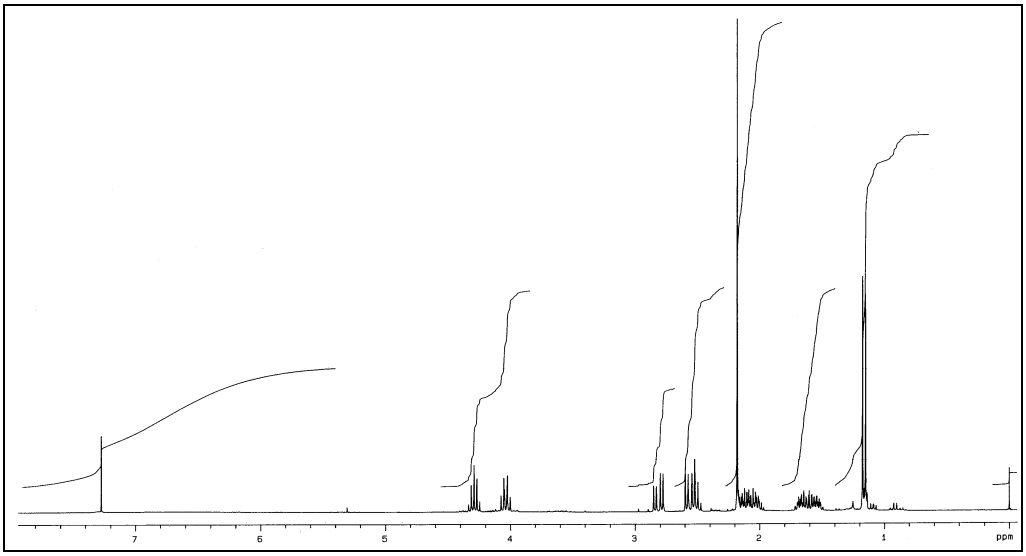


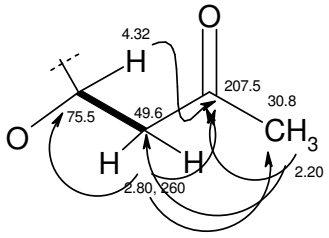
Figure 78: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Attiamycin B (**143a**).

The ^{13}C/APT NMR spectra of **143a** displayed 10 carbon signals, two of which at δ 207.5 and 179.7 were of ketone and carboxylic acid carbonyls, respectively. Additionally, two oxycarbons (δ 80.3 and 75.5), and three methylene carbons (δ 49.6, 30.9 and 28.4) were visible with the methylene carbon at δ 49.6 possibly attached to an sp^2 system. Finally, one methine signal (δ 45.1) and two methyl carbons (δ 30.8 and 13.2) were exhibited. The first of the two methyl carbons was directly attached to the ketone carbonyl δ 207.5 to afford the expected acetyl group.

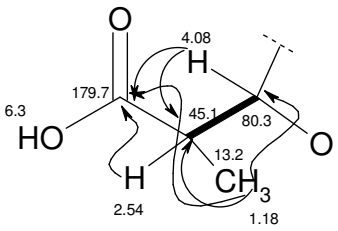
Based on the HMBC (Figure 79) correlations of **143**a, the acetyl group and the keto carbonyl (C-2', 207.5) were further confirmed through the ^{2}J cross-signal between H_3-1' (δ 2.20) and C-2' (δ 207.5). This acetyl group was directly attached to the methylene carbon C-3' (δ 49.6), as seen by the ^{3}J coupling from H_3-1', resulting

in a propanone partial structure. Towards the ketone carbonyl (C-2'), the oxy-methine H-5 (δ 4.32) showed a ^3J correlation, confirming the direct attachment between C-5 (δ 75.5) and C-3'. So, fragment A was deduced, which was further established by the H,H COSY correlation between H_2-3' and H-5.

The methine quartet of proton H-2" (δ 2.54) displayed a ^2J coupling with the carboxylic carbonyl C-1" (δ 179.7), and the latter showed in turn a ^3J cross-signal with the methyl doublet of H_3-3" (1.18). This indicated the direct linkage between the carboxylic acid and CH-CH_3. In the H,H COSY spectrum, a ^3J correlation was found between H-2" and the remaining oxy-methine H-2, confirming the direct attachment between C-2 (δ 80.3) and C-2" (δ 45.1). So, fragment B was concluded.

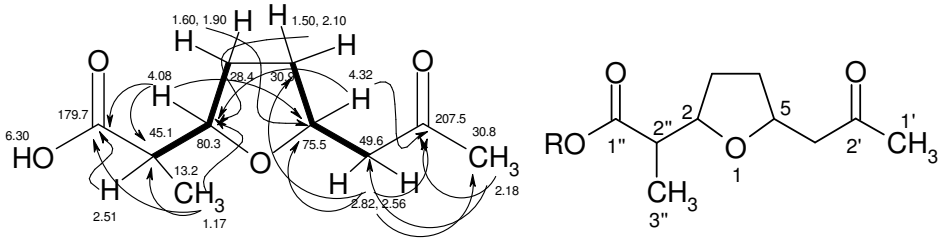


A



B

Based on H,H COSY, the remaining two methylene groups H_2-3 (1.90/1.60) and H_2-4 (2.10/1.50) were directly connected with each other. The methylene protons H_2-4 displayed a ^3J correlation to the oxy-carbon C-2 (δ 80.3), while H_2-3 displayed a ^3J to the other oxy-carbon C-5 (δ 75.5). This confirmed the direct linkage between both fragments A and B through the assigned ethandiyl group (C2-C3). Finally, the two oxy-carbons at δ 75.5 and 80.3 must be connected *via* oxygen creating a ring closure to obtain a tetrahydrofuran ring, containing the isopropanoic acid and propanone groups at 2- and 5-positions. As a result, 2-[5-(2-oxo-propyl)-tetrahydrofuran-2-yl]-propionic acid (143a), a new oxy derivative of nonactic acid was fixed, which we named as attiamycin B.



143a: R = H; **143b**: R = CH_3

Figure 79: H,H COSY (▬) and HMBC (→) correlations of Attiamycin B (**143a**).

The relative stereochemistry of compound **143a** was partially established on the basis of NOESY experiments (Figure 80). The proton at δ 4.32 (H-5) showed a coupling with one of the H_2-4 protons (δ 2.10) and the latter showed in turn a coupling with one of the H_2-3 protons (δ 1.90). The oxy-proton H-2 (δ 4.08) showed a coupling with the same H_2-3 proton (δ 1.90) besides another coupling with the methyl H_3-3" (δ 1.17) in addition to a small coupling with H-2" (δ 2.51). Further couplings from H_3-3" with H_2-3 proton (δ 1.90) and H-2" (δ 2.51) with H_2-3 proton (δ 1.60) were observed. So, the three chiral centres of **143a** were assigned to have a (2*S**,5*R**,2"*S**) configuration.

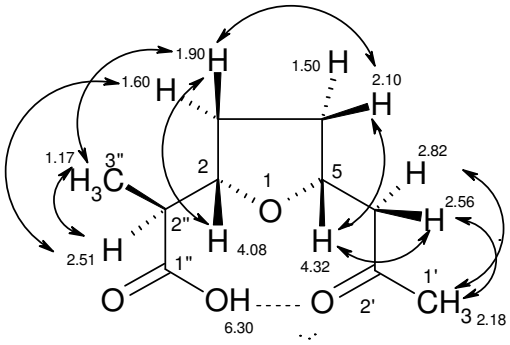


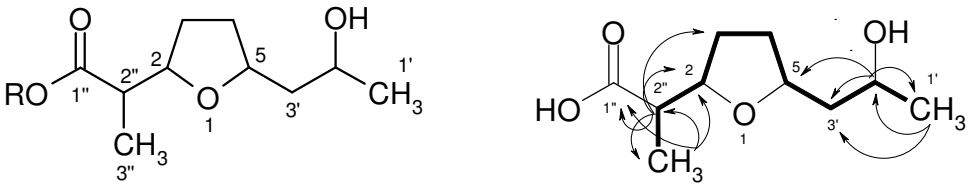
Figure 80: Diagnostic NOESY correlations of Attiamycin B (**143a**).

5.1.3 Nonactic acid

Compound **144a** was obtained as colourless oil, showing similar chromatographic properties as **143a**. The molecular weight (202 Dalton) and the corresponding formula ($C_{10}H_{18}O_4$) of **144a** obtained by ESI MS indicated two hydrogens more than in **143a**.

The $^{1}H/^{13}C$ NMR spectra revealed **144a** as a structural analogue of **143a**, the sole difference was attributed to the reduction of the acetyl carbonyl (207.5), affording an oxy-methine located at δ 66.1 (δ_H 3.91). The terminal carboxylic acid group was established by esterification, and the corresponding methyl ester singlet was found at δ 3.69.

Based on detailed spectroscopic data, compound **144a** was elucidated as a hydrogenated derivative of **143a**, i.e. nonactic acid (**144a**), which was as further confirmed by H,H COSY, HMQC and HMBC correlations (Figure 81) and literature data[233].



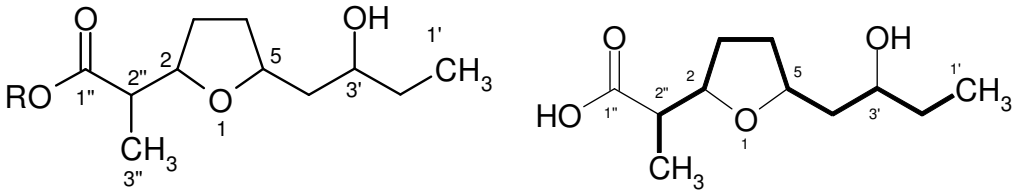
144a: R = H; **144b**: R = CH_3

Figure 81: H,H COSY (▬) H,H and HMBC (→) correlations of Nonactic acid (**144a**).

5.1.4 Homononactic acid

A further tetrahydrofuran derivative was was again obtained as colourless oil. The molecular weight of **145a** was deduced as 216 Dalton, and HRESI MS gave the corresponding molecular formula $C_{11}H_{20}O_4$, containing one methylene group more than **144a**. In the ^{1}H NMR spectrum, the compound exhibited a identical pattern as for **144a**, except that one of the methyl doublet present in the side chain of **144a** was replaced by an ethyl group, which was responsible for the methyl triplet at δ 0.91.

Based on the discussed MS and NMR data and a search in AntiBase, (±)-homononactic acid (**145a**) was found as the sole coincident structure. The compound was further confirmed by comparing the spectroscopic data with the literature[233] as well as by H,H COSY correlations (Figure 82).



145a: R = H; **145b**: R = CH_3

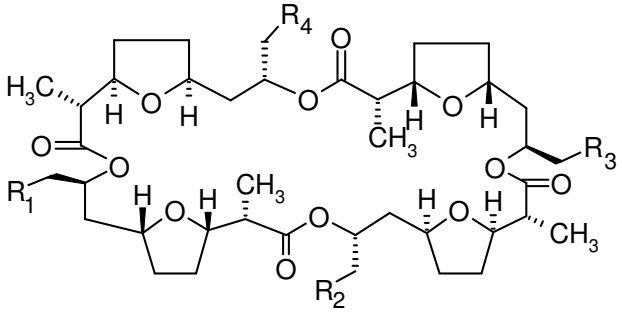
Figure 82: H,H COSY (—) correlations of Homononactic acid (**145a**).

5.1.5 Homononactic acid methyl ester

Homononactic acid methyl ester (**145b**) was obtained as a further colourless oil. Its molecular weight (230 Dalton), the H NMR pattern and the identity of the spectroscopic data with those of the synthetic one[233] confirmed the structure. Compound **145b** is reported here as a natural product for the first time.

5.1.6 Dinactin; Werramycin-B

The major product (>1.5 g) **148** was isolated from fractions I~III as colourless oil. It showed no UV absorbance or fluorescence during TLC, however, it was detected as brown zone after spraying with anisaldehyde/sulphuric acid and heating. Based on its spectroscopic data (MS, ^{1}H NMR, and ^{13}C NMR), and their comparison with literature, **148** was identified as dinactin (werramycin-B).



146:	R_1 = H	R_2 = H	R_3 = H	R_4 = H
147:	R_1 = CH_3	R_2 = H	R_3 = H	R_4 = H
148:	R_1 = CH_3	R_2 = H	R_3 = CH_3	R_4 = H
149:	R_1 = CH_3	R_2 = CH_3	R_3 = CH_3	R_4 = H
150:	R_1 = CH_3	R_2 = CH_3	R_3 = CH_3	R_4 = CH_3
151:	R_1 = CH_3	R_2 = CH_3	R_3 = H	R_4 = H
152:	R_1 = CH_3	R_2 = CH_3	R_3 = H	R_4 = CH_3

The family of macrotetrolide antibiotics is commonly produced by *Streptomyces* sp., and is named collectively as nactins. They comprise a series of homologues

based upon a parent 32-membered ring[234,235]. The lowest homologue, nonactin (**146**), is constructed from four nonactic acid subunits (**144a**), linked in alternating enantiomeric sequence (+ - + -), so that the overall macrocycle possesses a meso-configuration. Monactin (**147**), dinactin (**148**), trinactin (**149**), tetranactin (**150**), as well as isodinactin (**151**) and isotrinactin (**152**), are examples of further macro-tetrolide antibiotics.

Nonactic acids, the building blocks[236] of macrotetorlides[237], have been chemically synthesized[238-240]. They are currently discussed in the literature, however, were purified always as methyl esters[241]. Nonactic acid and homononactic acid were isolated as mixtures of pure (+)- and (-)-enantiomers, but also as racemates or with an excess of the (+)-enantiomers. 2-Epi-homononactic acid was described as the (-)-enantiomer[233].

It is important to mention that feigrisolides A-B (**153**-**154**) were first reported by Tang *et al.*[242], which might be building block of nonactic acid (**144a**) and homononactic acid (**145a**), respectively (Figure 83 and Figure 84) were recently excluded by synthesis. The total synthesis of the proposed structure of feigrisolide A (**153**) was performed by Alvarez-Bercedo *et al.*[243], however, the synthesized one was structurally not identical with the natural one, indicating that the wrong structure was previously published. Therefore, the reported **153** and **154** were actually **144a** and **145a**, respectively (i. e feigirisolide A = (+)-nonactic acid and feigrisolide B = (+)-homonanoactic acid).

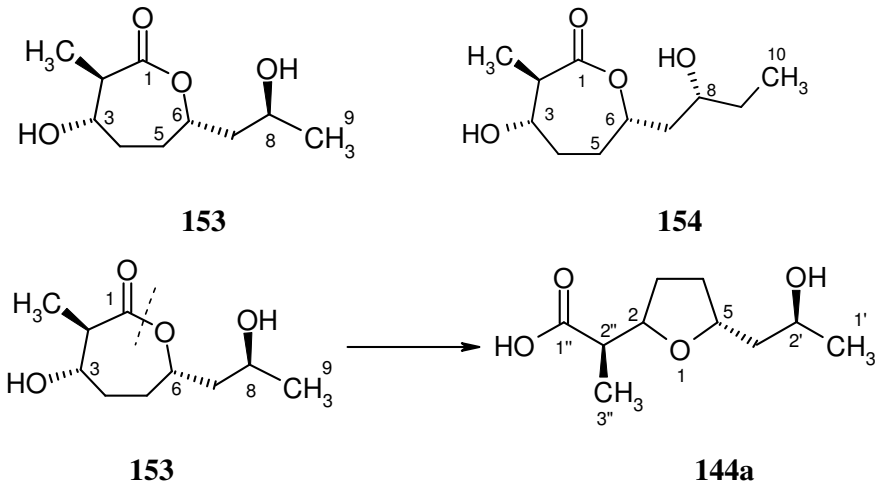
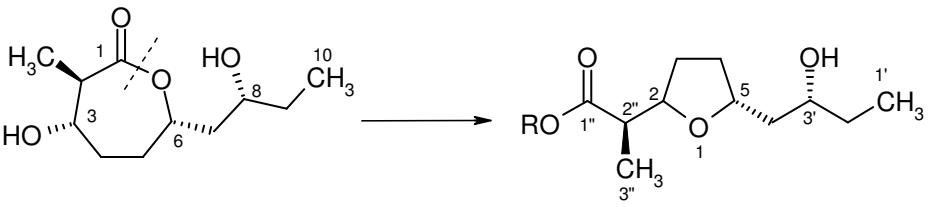


Figure 83: Correlation between Feigrisolide A (**153**) and Nonactic acid (**144a**).



154 **145a**

Figure 84: Correlation between Feigrisolide B (**154**) and Homononactic acid (**145a**).

Biosynthetically, homononactic acid (**145a**) and nonactic acid (**144a**) may be constructed from acetate, propionate or succinate. As a result, a hydroxylated carboxylic acid chain is obtained, which in turn is cyclized to deliver homononactic acid derivates (Figure 85).

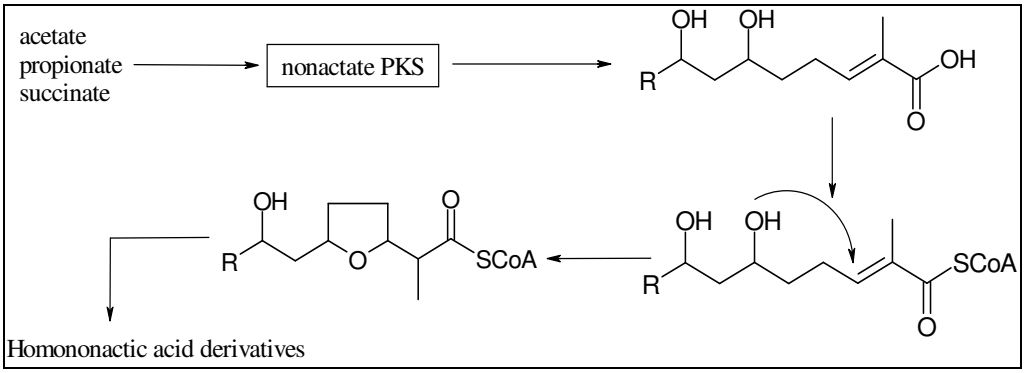


Figure 85: Biosynthesis of Nonactic and Homononactic acids (**144a-145a**).

5.1.7 Biological Activity

The cytotoxic activities of attiamycin A (**141**), attiamycin B (**143a**), nonactic acid (**144a**), homononactic acid (**145a**) and dinactin (**148**) are listed in Table 61. Dinactin (**148**), attiamycin B (**143a**) and nonactic acid (**144a**) showed a highly selective cytotoxicity against a range of human tumor cell lines with a mean IC_{50} of 0.002 μg/ml (mean IC_{70} = 0.011 μg/ml), IC_{50} of 1.160 μg/ml (mean IC_{70} = 3.423 μg/ml) and IC_{50} of 1.256 μg/ml (mean IC_{70} = 3.532 μg/ml), respectively.

5.2 Marine-derived *Streptomyces* sp. Mei37

The marine *Streptomyces* sp. isolate Mei37 had previously been investigated in our research group[244,245], due to the high biological activity of its extract. However,

the constituents were isolated in amounts insufficient for analysis. Therefore, the strain was re-cultivated as 30 l shaker culture, using the previously used cultivating conditions.

The dark-red crude extract was fractionated using Sephadex LH-20 and eluted with dichloromethane/methanol to give fractions I and II. Further chromatography of the main fraction II afforded four sub-fractions IIA, IIB, IIC and IID. Working up of the first two low polar sub-fractions IIa and IIb led to the isolation of albaflavenone (**167**), (4*S*)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (**233**), indole-3-acetic acid and indole-3-carboxylic acid. Re-purification of the middle polar fraction IIC using Sephadex LH-20 afforded uracil and 2,3-dihydroxy-1-(1*H*-indol-3-yl)-propanone-1-one. Finally, purification of the polar sub-fraction IID afforded the new isoquinolinequinone mansouramycin D (**156**) along with 2'-deoxy-thymidine and 2'-deoxy-uridin (Figure 86). Compound (**233**) was discussed in details with the other γ-butyrolactones for better comparison (see p. 194).

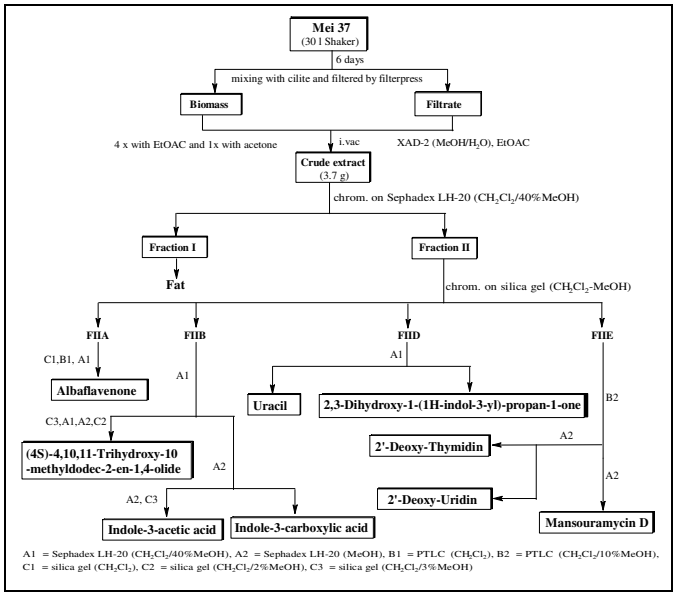


Figure 86: Work-up procedure of marine-derived *Streptomyces* sp. Mei37.

5.2.1 Mansouramycin D

Mansouramycin D (**156**) was obtained as a polar dark red solid, which turned to blue by spraying with anisaldehyde/sulphuric acid and heating. The molecular weight was established as 246 Dalton on the bases of EI and ESI MS. HRESI MS fixed the molecular formula as $C_{12}H_{10}N_2O_4$.

The ^{1}H NMR spectra of **156** showed three 1H singlets at δ9.36 (H-5), 8.71(H-8) and 5.90 (H-2). The downfield shifts of the first two protons were attributed to their location in a heterocyclic aromatic system e.g. a pyridine. Moreover, the compound showed a 1H multiplet at δ6.13 (NH) together with two methyl signal at δ4.07 and 2.99. The first methyl signal was of a methyl ether or ester, while the latter was the doublet of an NH-Me moiety. Based on the blue colour reaction with anisaldehyde/sulphuric acid and the UV data, this compound exhibited a close relation with previously isolated isoquinolinequinone, which we named mansouramycins.

The ^{13}C NMR spectrum displayed the O-CH_3 signal at δ53.4, along with two quinone carbonyl signals at δ180.6 (C-4) and 179.8 (C-1). In addition, four quaternary carbons (153.3~126.0), three methine carbons (δ 148.0, 120.7 and 101.5) and the N-methyl carbon at δ29.3 were observed.

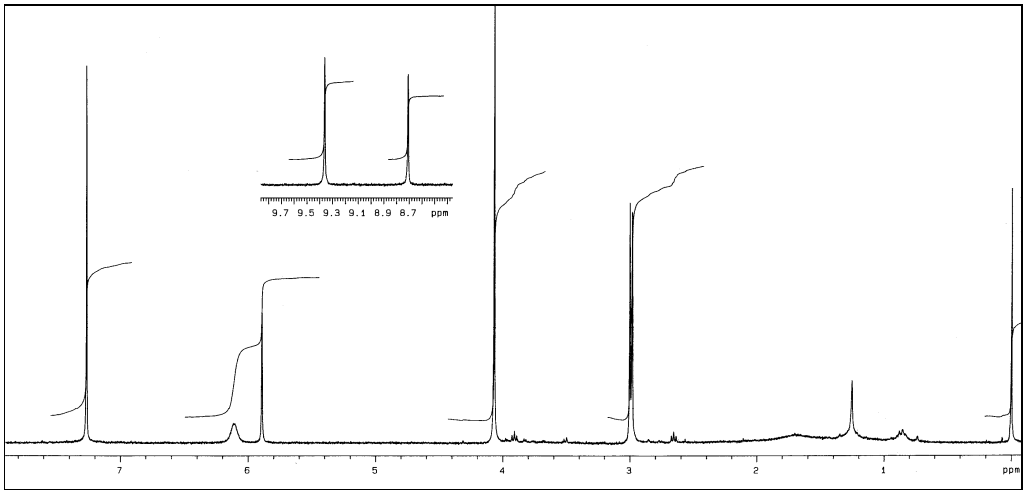
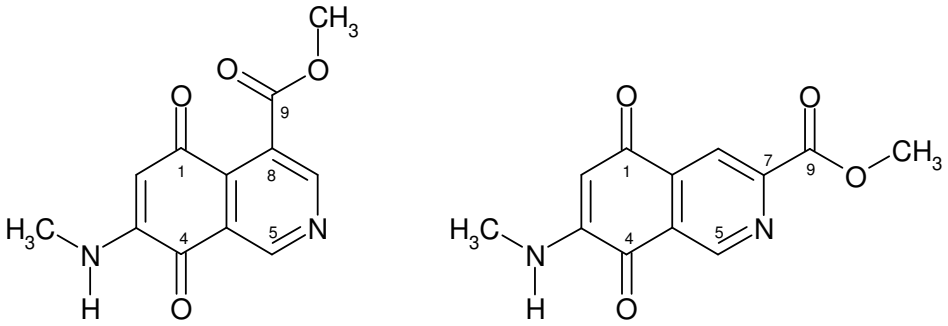


Figure 87: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Mansouramycin D (**156**).

In the HMBC spectrum (Figure 88), the methoxy group (δ4.05) exhibited a ^{3}J and ^{4}J couplings with the quaternary carbons at δ 164.3 and δ 153.3 respectively. The quaternary carbon δ153.3 was assigned as C-7 due to the following facts: it showed a ^{3}J coupling with 5-H (δ9.36) and a ^{2}J coupling from the 8-H (δ8.71). Additionally, H-8 displayed two ^{3}J correlations towards the quinone carbonyl C-1 (δ179.8) and the quaternary carbon C-4a (δ126.0). Accordingly, the carboxy methyl ester group was recognized at 7-position and not at 8-position as in **155**, and hence the compound's structure was confirmed as 7-methylamino-5,8-dioxo-5,8-dihydro-

isoquinoline-3-carboxylic acid methyl ester (**156**); it is a new member of the mansouramcins and was assigned with the letter D.



155 **156**

Besides **156** seven further new isoquinolinequinones had been isolated from the North Sea streptomycete Mei37 and another marine-derived *Streptomyces* sp. B3497[83, 244-246]. They were named as mansouramyins A (**157**), B (**158**), C (**159**), E (**162**), F (**163**), G (**164**), H (**165**), and I (**166**).

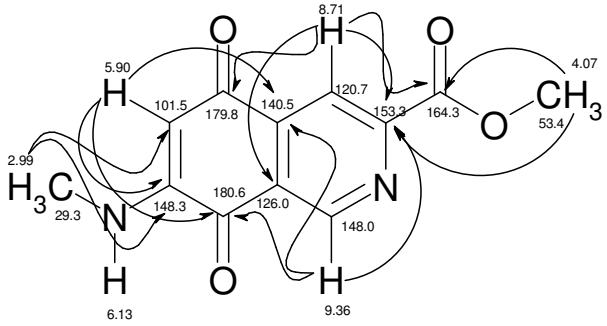
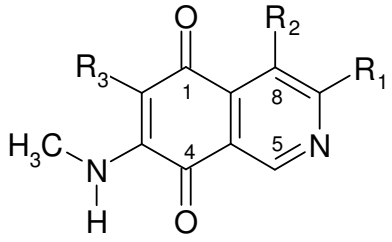
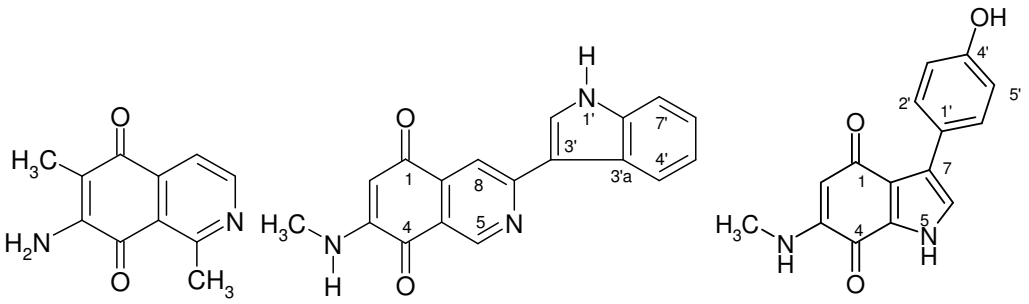


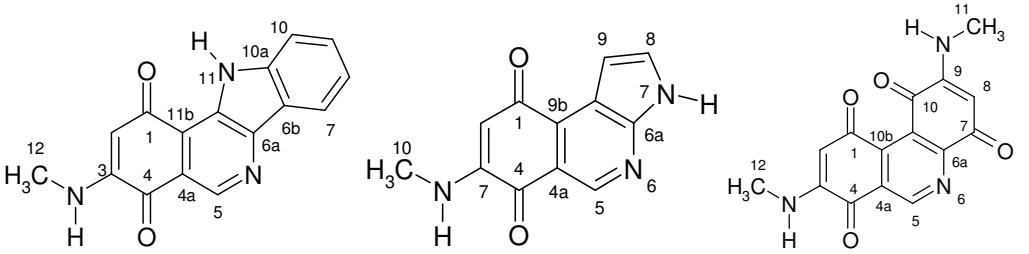
Figure 88: HMBC (→) correlations of Mansouramycin D (**156**).



156:	$R_1 = COOCH_3$	$R_2 = H$	$R_3 = H$
157:	$R_1 = CH_3$	$R_2 = CH_3$	$R_3 = H$
158:	$R_1 = CH_3$	$R_2 = H$	$R_3 = H$
159:	$R_1 = CH_3$	$R_2 = H$	$R_3 = Cl$
160:	$R_1 = H$	$R_2 = H$	$R_3 = H$



161 **162** **163**



164 **165** **166**

Only one isoquinolinequinone has previously been reported from bacteria, while a few others are known from porifera[247,248]. A number of isoquinoline quinones including the cribrostatins, renierone and *O*-demethylrenierone[249~252] have been isolated as natural products from the marine sponges, *Cribrochalina* sp.[253] and *Petrosia* sp. [254] and were found to be interest as cancer inhibitors. The related caulibugulones A (**160**~F were isolated from the marine bryozoan *Caulibugula intermis*[255]. The blue marine sponge *Cribrochalina* sp. delivered the cribrostatins (e.g. cribrostatin I, **161**)[251]; the renierones[249,252]were obtained from *Reniera*, *Petrosia*, and *Haliclona* spp. Isoquinoline quinones[256~258] have also been obtained from *Calothrix*[259] and an associated *Streptomyces lavendulae* strain[247,248], however, they were not reported from any other microbial sources. All these isoquinolinequinones showed strong antimicrobial activity[258,260] against *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* and pronounced cytotoxicity against L1210[12] and other cell lines with IC_{50} values as low as 30 ng/ml.[255] Some of these sponge metabolites were also found to be active against viruses and malaria. The isolation of sponge-related metabolites from the marine-derived streptomycetes Mei35 and B3497 further supports the idea that bacteria may be involved in the biosynthesis of sponge constituents.

5.2.2 Structure Activities Relationships of Mansouramycins

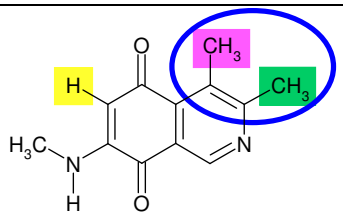
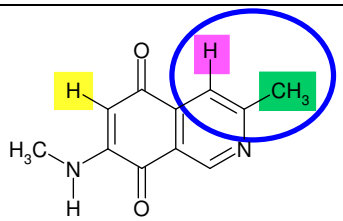
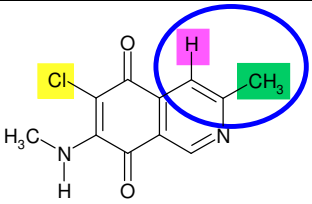
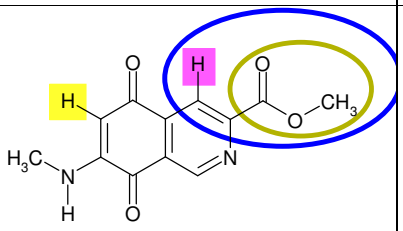
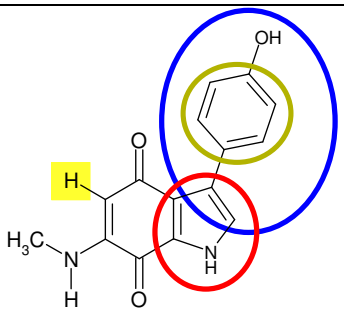
Cytotoxicity profiling of mansouramycins in a panel of up to 36 tumor cell lines indicated significant cytotoxic activity of several derivatives with a pronounced selectivity for non-small cell lung cancer, breast cancer, melanoma, and prostate cancer cells.

The cytotoxic effect of the mansouramycins A (**157**) ~ C (**159**), D (**156**), E (**162**) and F (**163**) was determined in a monolayer cell proliferation assay using a panel of up to 36 human tumor cell lines, comprising 14 different solid tumor types. Mansouramycin D (**156**) proved to be most active with an overall potency of 0.089 μM (mean IC_{50} value of 36 tumor cell lines tested). It was followed by mansouramycin C (**159**, mean IC_{50} = 2.7 μM) and mansouramycin B (**158**, mean IC_{50} = 3.49 μM) in decreasing order of cytotoxic potency (Table 63). Mansouramycin A (**157**) showed moderate concentration-dependent cytotoxicity with a mean IC_{50} value of 13.44 μM. Mansouramycin F (**163**) did not show pronounced cytotoxic activity in the test range up to 37.31 μM (Table 63). Mansouramycin D (**156**) displayed significant *in vitro* tumor cell selectivity towards ten of the 36 tested tumor cell lines (based on an individual IC_{50} value < 1/2 of the mean IC_{50} value as threshold). Above average activity was pronounced in tumor cell lines of bladder cancer (T-24), glioblastoma (SF-268), lung cancer (LXFA 629L), mammary cancer (MCF-7), melanoma (MEXF 276L, MEXF 514L, MEXF 520L), ovarian cancer (OVCAR-3), renal cancer (RXF 944L), and uterus cancer (UXF 1138L), with IC_{50} values ranging from 0.008 μM to 0.02 μM (Table 63). Cytotoxic selectivity of the other isoquinoline quinones was observed in 6/36 (**157**, **158**), 1/18 (**159**) or 0/36 (**163**) cell lines (Table 63). Thus, antitumor potency of the mansouramycin molecules differed according to their substitution pattern. The quinones **157** and **159** displayed comparable overall cytotoxicity against human cancer cell lines, indicating that the substitution at position C-2 of the isoquinoline quinone moiety is not crucial for their cytotoxic potency, apart from the chlorination of **159** which obviously influenced cytotoxic selectivity. By contrast, variation of the C-8 (**157**) or C-7 position (**156**) of the isoquinoline quinone, or the C-7 position of the indolequinone skeleton (**163**), respectively, strongly influenced cytotoxicity (Table 20). Due to the availability of material, further studies concentrated on synthetic[261] mansouramycin B (**158**), which showed selective cytotoxicity

towards six out of the 36 tested cell lines, i.e. T-24 (bladder cancer), HNXF 536L (head and neck cancer), MCF-7 (mammary cancer), MEXF 276L and MEXF 520L (melanoma), and DU-145 (prostate cancer), with IC_{50} values ranging from 0.24 μM to 1.11 μM (Table 63).

Inhibition of clonogenicity of tumor cells by mansouramycin B (**158**) was evaluated in additional tumor models using a clonogenic assay. The anti-proliferative activity was evaluated in cell suspensions prepared from human tumor xenografts of 8 different tumor types, which were cultured as solid tumors in serial passage on immune deficient nude mice. In addition, **158** was tested in two preparations of hematopoietic stem cells as a model system for non-malignant tissue. The overall potency of **158** in the clonogenic assay (mean IC_{50} = 2.94 μM) confirmed the results observed in cell lines. IC_{50} values ranged from 0.05 μM to 10.32 μM (Table 64). Cytotoxic selectivity of **158** for lung cancer (LXFA 629, IC_{50} = 1.63 µM) and melanomas (MEXF 276 and MEXF 514, IC_{50} = 0.33 μM and 0.05 μM, respectively) could be confirmed. Colony formation of hematopoietic stem cells derived from cord blood was inhibited by **158** with IC_{50} values of 4.58 μM and 4.53 μM, respectively (Table 64). Thus, based on IC_{50} values, the sensitive tumor models mentioned above were in average about 15-fold more sensitive than hematopoietic stem cells as representative model system for non-malignant tissue, confirming the tumor specific nature of activity.

Table 20: Structure activities relationship of Mansouramycins:

Compound	Structure	FA potency (mean IC_{50}, µM)	FA selectivity (n, %)
Mansouramycin A (**157**)		13,44	6/36 17
Mansouramycin B (**158**)		3,49	6/36 17
Mansouramycin C (**159**)		2,7	1/18 6
Mansouramycin D (**156**)		0,089	10/36 28
Mansouramycin F (**163**)		36,25	0/36 0

5.2.3 Albaflavenone

On purification of the fatty sub-fraction IIA using a series of chromatographic techniques (silica gel column, PTLC and Sephadex LH-20), compound **167** was obtained as a UV absorbing colourless oil, which turned to orange on spraying with anisaldehyde/sulphuric acid, and later to violet. The molecular weight of **167** was

established to be 218 Dalton according to EI and ESI mass spectra. HRESI MS of **167** displayed its molecular formula as $C_{15}H_{22}O$.

The ^{1}H NMR spectrum showed no protons in the sp^2 region. The aliphatic region delivered an ABX pattern of a CH_2 group at δ2.40 (J=7.3, 17.3, Ha) and 2.02 (J=13.2, 17.3, Hb), one methyl singlet (δ2.02) attached to an sp^2 carbon. Two other singlets and one methyl doublet were observed at δ1.15, 1.12 and 1.07, indicating their attachment to sp^3 carbons. Finally, a number of multiplet signals with integration of ten protons were observed in the region of δ2.20-1.20 (Figure 89).

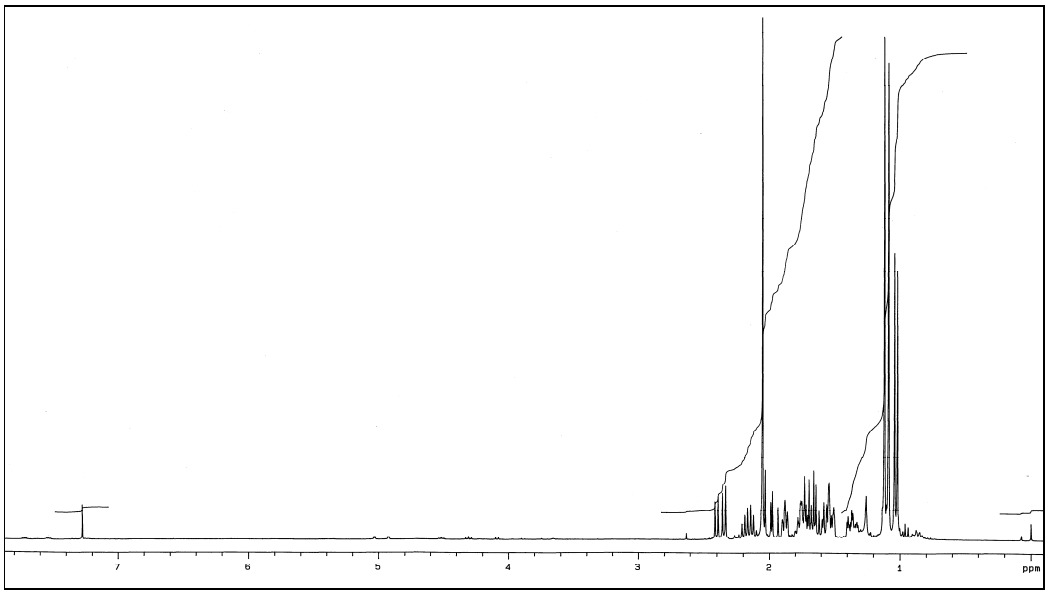


Figure 89: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Albaflavenone (**167**).

The ^{13}C/APT NMR and HMQC spectra established the presence of three sp^2 carbons, two saturated quaternary carbons atoms, four methylenes, two methines, and four methyls. This gave altogether 15 carbons, pointing to a sesquiterpenoid structure.

A search in AntiBase using the above spectroscopic data combined with the molecular formula resulted in albaflavenone (**167**) as the sole result. Further confirmation of the structure was achieved by comparison of its spectroscopic data with literature and 2D spectroscopy values (HMBC, Figure 90 and Table 21).

Albaflavenone (**167**), an unsaturated sesquiterpene ketone with a zizaene skeleton, was isolated from *S. albidoflavus* and reported as antibacterial agent with MIC of 8~10 µg/ml against *Bacillus subtilis*[262]. In our antitumor assay, albaflavenone

(**167**) showed a moderate and rather unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 5.017 µM (mean IC_{70} = 9.379 μM), Table 46.

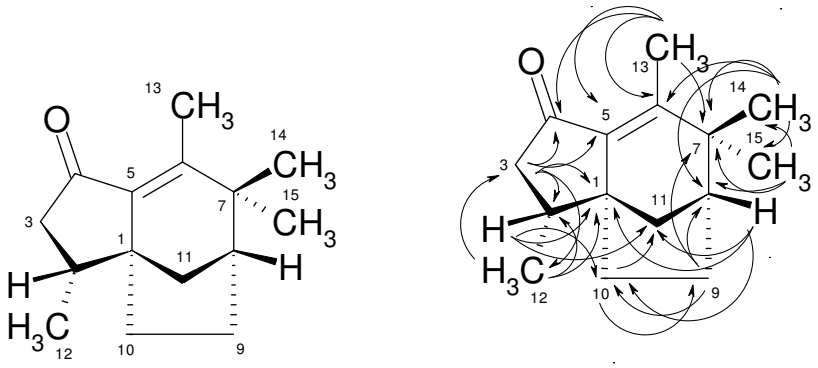


Figure 90: HMBC correlations (→) of Albaflavenone (**167**).

Table 21: ^{13}C and ^{1}H NMR data ($CDCl_3$) of Albaflavenone (**167**) in comparison with literature values[262].

Position	Literature data		Experimental	
	δ_C a)	δ_H b) (*J* in [Hz])	δ_C c)	δ_H b) (*J* in [Hz])
1	51.8	-	51.8	-
2	33.3	2.20 (*J*= 6.8, 13.2, 7.3)	33.2	2.20 (*J*= 6.8, 13.2, 7.3)
3	47.4	2.40 (*J*=7.3, 17.3, Ha), 2.02 (*J*=13.2, 17.3, Hb)	47.1	2.40 (*J*=7.3, 17.3, Ha), 2.02 (*J*=13.2, 17.3, Hb)
4	207.4	-	207.4	-
5	138.8	-	138.8	-
6	153.0	-	153.0	-
7	42.7	-	42.6	-
8	46.2	1.91 (*J*=6.8, 5.4)	46.2	1.91 (*J*=6.8, 5.4)
9	24.4	1.75 (*J*=3.5, 6.8, 11.4, 13.8, Ha), 1.79 (*J*=9.1, 3.6, 1.8, 13.8, Hb)	24.3	1.75 (*J*=3.5, 6.8, 11.4, 13.8, Ha), 1.79 (*J*=9.1, 3.6, 1.8, 13.8, Hb)
10	29.6	1.61 (*J*=6.3, 11.4, 11.3, Ha), 1.39 (*J*=2.3, 9.1, 11.3)	29.5	1.61 (*J*=6.3, 11.4, 11.3, Ha), 1.39 (*J*=2.3, 9.1, 11.3)
11	37.0	1.70 (*J*=5.4, 10.9, Ha), 1.56 (*J*=1.8, 10.9, 2.3, Hb)	36.9	1.70 (*J*=5.4, 10.9, Ha), 1.56 (*J*=1.8, 10.9, 2.3, Hb)
12	14.2	1.07 (*J*=6.8)	14.2	1.07 (*J*=6.8)
13	13.0	2.09 (s)	13.0	2.09 (s)
14	28.3	1.15 (s)	28.3	1.15 (s)
15	24.5	1.12 (s)	24.4	1.12 (s)

a) 150 MHz; b) 300 MHz; c) 75 MHz

5.3 Marine *Streptomyces* sp. B7874

The marine *Streptomyces* sp. B7874 was pre-cultivated to examine the biological and chemical properties. In the biological screening, the extract was antimicrobially inactive against a set of pathogenic microorganisms (

Table 65). However, the extract exhibited *in vitro* a potential antitumor activity against a number of cancer cell lines (Table 45). On TLC, the extract exhibited several yellow bands, which showed no colour change on spraying with anisaldehyde/sulphuric acid.

A 24 L culture of the strain, using M_2^+ medium, delivered after working up as usually two new compounds namely 7-(3-methyl-but-2-enyl)-isatin (**168**) and 5-methyl-5,10-dihydro-phenazine-1,6-dicarboxylic acid diamide (**178**) together with ferulic acid (**181**) (Figure 91).

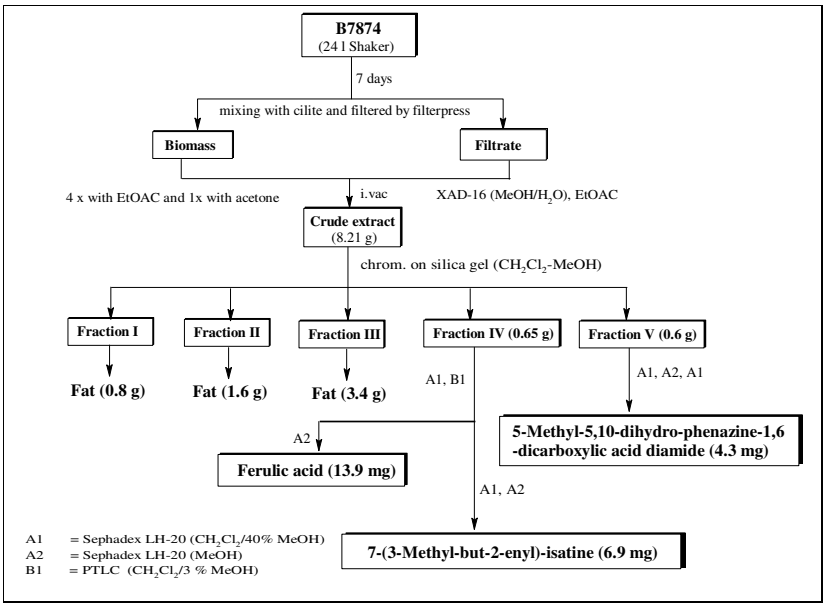


Figure 91: Work-up procedure of the marine *Streptomyces* sp. B7874

5.3.1 7-(3-Methyl-but-2-enyl)-isatin

Compound **168** was isolated from fraction IV as middle polar yellowish-orange UV-absorbing solid. The compound showed a colour change to light red on treatment with dilute sodium hydroxide, while it is not affected by treatment with sulphuric acid. The molecular weight of **168** was established as 215 Dalton on the bases of EI and ESI mass spectra. On EI MS, two ion peaks were displayed at *m/z* 187 and 172, as a result of successive expulsion of carbonyl and methyl groups from the parent molecular ion (*m/z* 215). HRESI-MS confirmed the molecular formula of **168** as $C_{13}H_{13}NO_2$, containing eight double bond equivalent.

The ^{1}H NMR spectrum of **168** displayed one broad 1H singlet (δ 8.59) together with three *ortho*-coupled aromatic protons (δ 7.47~7.05, *J*~7.3 Hz) of a 1,2,3-*tri*-substituted benzene system. Moreover, a 1H multiplet (δ 5.25) in addition to the AB part of an ABX system (δ 3.32, *J*~ 7.1 Hz) of a methylene group were seen, which was supported by the pronounced ^{3}J correlation in the H,H COSY experiment (Figure 92). The downfield shift of the methylene protons indicated its flanking by two sp^2 carbons. The spectra exhibited another two methyl doublets (δ 1.79 and 1.76) with a small allyl coupling (*J*~1 Hz), indicative of their neighbourhood to an olefinic proton as in a isopropenyl group. Hence, a C-prenylated system [-CH_2-CH=C(CH_3)$_2$] was recognized, responsible for the three characteristic fragments in EI-MS at *m/z* 187, 172 and 159. These fragments were attributed to the loss of methyl (*m/z* 15), isopropyl (*m/z* 43) and isobutenyl (*m/z* 55) groups.[83,263]

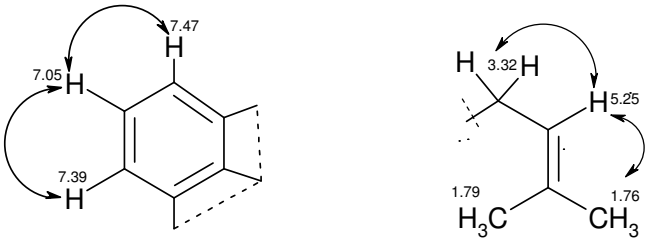


Figure 92: H,H COSY (↔) connectivities of the partial structures of 7-(3-Methyl-but-2-enyl)-isatin (**168**).

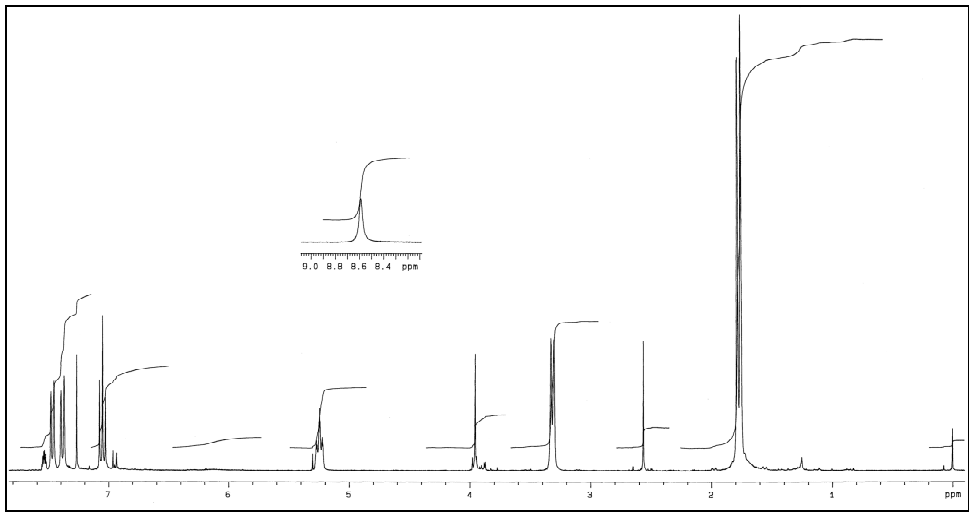
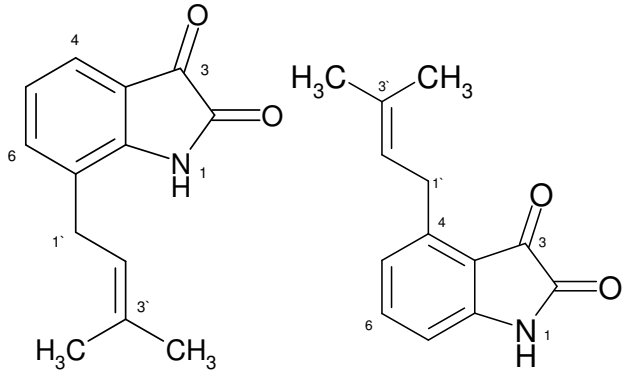


Figure 93: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 7-(3-Methyl-but-2-enyl)-isatin (**168**).

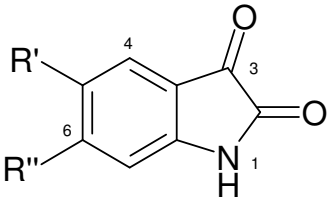
In the ^{13}C NMR/HMQC spectra, compound **168** displayed 13 carbon signals, which were classified into ten sp^2 aromatic/olefinic carbons; the remaining three carbons were due to two methyls (δ25.7 and 18.0) and one methylene (δ28.9). The three aromatic methines were at δ138.9, 123.8 and 123.3, while that of the olefinic one in the C-prenyl chain was at δ119.7. Three sp^2 quaternary carbons were downfield shifted at δ183.3, 159.7 and 147.7 indicating the first of them being of a conjugated ketone carbonyl.

By subtracting the prenyl chain (C_5H_9) and tri-substituted aromatic system (C_6H_3), respectively, from the molecular formula, the remaining C_2HNO_2 partial structure with the three double bond equivalents, must be of a five membered heterocyclic ring fused with the aromatic residue. The nitrogen atom in this five-memberd ring could be flanked by two carbonyls either symmetrically (forming an imide system) or unsymmetrically (forming an isatin system). The first case was excluded as the compound was an orange solid, and the ^{13}C shifts of the two carbonyls were not sililar (δ~169), but exhibited a large difference of $\Delta\delta$~24. This pointed to **168** an isatin skeleton, at which the two carbonyls, C-2 and C-3, are usually found at δ~159 and 183, respectively[120]. Based on the position of the prenyl side chain on the aromatic moiety, two alternatives were suggested: 7-(3-methyl-but-2-enyl)-isatin (**168**) and 4-(3-methyl-but-2-enyl)-isatin (**169**).



168

169



170: R' = H, R'' = OH
171: R' = OCH_3, R'' =OH
172: R' = OH, R'' = OH
173: R' =OH, R'' = OCH_3

The compound was therefore subjected to HMBC experiments. The proton H-4 (δ7.47) was confirmed to be in *peri*-position with respect to the carbony1 C-3

(δ 183.3) because of the pronounced ^3J coupling towards C-3. Similarly, the prenyl group was recognized at 7-position of the isatin system (Figure 94). So, the structure of this pigment was established as the new 7-(3-methyl-but-2-enyl)-isatin (**168**). Two structural analogues, 5-prenylisatin and 6-isopentensylisatin are naturally occurring in *Chaetomium globosum*[264] and *Streptomyces albus*[265], respectively.

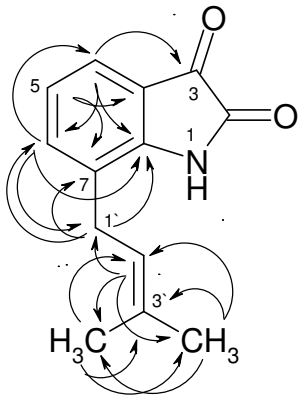


Figure 94: HMBC correlations (→) of 7-(3-Methyl-but-2-enyl)-isatin (**168**)

Table 22: ^{13}C and ^{1}H NMR spectroscopic data for Isatin **168** and Phenazine **178** in $CDCl_3$, (*J* in [Hz]).

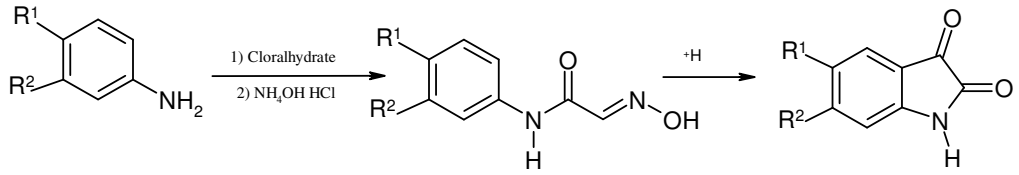
	7-(3-Methyl-but-2-enyl)-isatin (**168**)		Position	Phenazine **178**	
Position	δ_C (150 MHz)	δ_H (300 MHz)		δ_C (125 MHz)	δ_H (600 MHz)
1-NH	-	8.59 (brs)	1	111.7	-
2	159.7	-	1-CO	170.4	-
3	183.3	-	2	120.6	7.03 (dd, 8.1, 0.7)
3a	118.0	-	3	119.0	6.52 (t, 8.1)
4	123.3	7.47 (d, *J* = 7.2)	4	115.4	6.37 (d, 7.5)
5	123.8	7.05 (t, *J* = 7.6)	4a	138.1	-
6	138.9	7.39 (d, *J* = 7.7)	5-CH_3	39.1	2.95 (s)
7	125.2	-	5a	133.1	-
7a	147.7	-	6	123.8	-
1'	28.9	3.32 (d, *J* = 7.1)	6-CO	170.4	-
2'	119.7	5.25 (tq, *J* = 7.1, 1.4)	7	121.8	6.62 (dd, 7.7, 1.3)
3'	135.7	-	8	120.9	6.54 (t, 7.7)
3'-CH_3	18.0	1.76 (s)	9	112.9	6.36 (dd, 7.5, 1.3)
4'	25.7	1.79 (d, *J* = 1.0)	9a	137.3	-
-	-	-	10-NH	-	9.93 (s)
-	-	-	10a	139.9	-
-	-	-	$2NH_2$	-	7.25 (brs, 2H), 5.38 (brs, 2H)

Recently, three further new isatins derivatives, 6-hydroxyisatin (**170**), 6-hydroxy-5-methoxy-isatin (**171**) and 5-hydroxy-6-methoxy-isatin (**173**) were isolated[266] in our group from *Streptomyces* spp., however, the structures could not un-

equivocally be confirmed, due to the small amounts. This stimulated the synthesis of **173** and the positional isomer **177** from the methoxy-isatins **176a** and **176b** (Scheme 1). The synthesis of the latter was achieved by condensation of substituted anilines[267], **174a**-**b** with chloral hydrate to give the corresponding isonitrosoacetanilides, **175a**-**b** (Scheme 2).

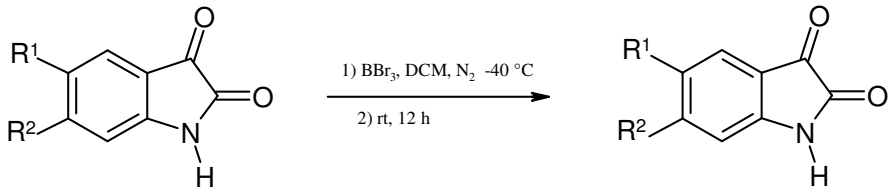
Compound **175a** exhibited two characteristic IR bands at ν 3400 and 1720 cm^{-1}, corresponding to amino and/or hydroxyl and carbonyl groups, respectively. The ^1H NMR data of **175a** confirmed the imino proton by a singlet at δ7.70, along with two singlets of the exchangeable oxime (=N-OH) and amide protons at δ 12.00 and δ10.00, respectively[268].

The isonitrosoacetanilides **175b** was cyclized by acid catalysis (Scheme 3)[267] to give the corresponding isatins, **176a** and **176b**, respectively. Hydrolysis of 5-methoxy isatin (**176a**) using boron-tribromide gave the hydroxyl analogue[269] (**177**), whereas the dimethoxyisatin **176b** gave mainly 5-hydroxy-6-methoxy-isatin (**173**).



174a: $R^1 = OCH_3$, $R^2 = H$ **175a:** $R^1 = OCH_3$, $R^2 = H$ **176a:** $R^1 = OCH_3$, $R^2 = H$

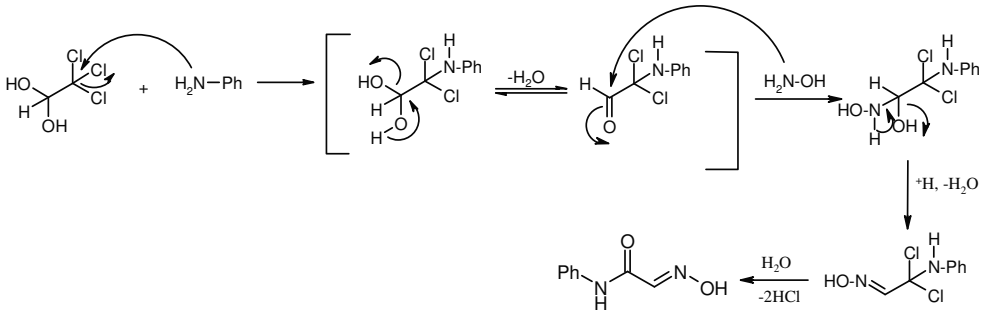
174b: $R^1 = OCH_3$, $R^2 = OCH_3$ **175b:** $R^1 = OCH_3$, $R^2 = OCH_3$ **176b:** $R^1 = OCH_3$, $R^2 = OCH_3$



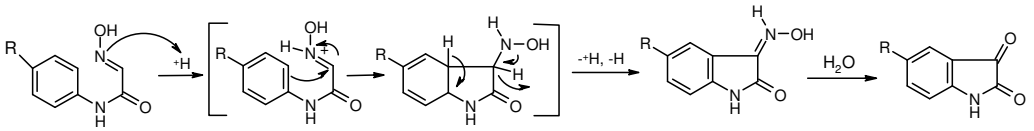
176a: $R^1 = OCH_3$, $R^2 = H$ **177:** $R^1 = OH$, $R^2 = H$

176b: $R^1 = OCH_3$, $R^2 = OCH_3$ **173**: $R^1 = OH$, $R^2 = OCH_3$

Scheme 1: Synthesis of the Isatins **173**, **176a**, **176b**, and **177**.



Scheme 2: Suggested mechanism for the formation of isonitrosoacetanilides from chloralhydrate.



Scheme 3: Proposed mechanism for the acidic cyclization of isonitrosoacetanilides to isatin derivatives[266,268].

Isatins are very rare in nature[105]. Only six isatin derivatives are known from microorganisms[61], while bromo-isatin is the only one which is known from higher organisms (mollusks)[270, 271]. Melosatins (A~D), a series of phenyl pentyl isatins, were produced as plant metabolites by *Melochia tomentosa*[272~274]. Isatin was reported as one the substances with a vital role as signalling substance between microorganisms for production of antibiotics. In addition, it is active as inhibitor of Xanthine-oxidase of milk[275] and monoamine oxidase[276]. Isatin is known as fungal pigment from a mutant of *Schizophyllum commune*[277]. Synthetically, it was obtained for first time during an oxidative degradation of indigo by nitric or chromic acid[278].

5.3.2 5-Methyl-5,10-dihydro-phenazine-1,6-dicarboxylic acid diamide

From fraction V, compound **178** was isolated as yellowish-orange solid, exhibiting similar chromatographic properties as **168**. The molecular weight of **178** was determined by EI and ESI mass spectra as 282 Dalton, and the corresponding molecular formula was established as $C_{15}H_{14}N_4O_2$ by HRESI MS.

The ^{1}H NMR spectra of **178** displayed a 1H singlet at δ 9.93, which was attributed to an NH group. In the aromatic region, six signals were located between δ 7.03~6.36, and classified into three doublets of doublets (δ 7.03, 6.62, 6.63), two

triplets (δ 6.54, 6.52), and one doublet (δ 6.37). This pointed to the existence of two 1,2,3-trisubstituted aromatic residues. Moreover, two broad 2H singlets were shown at δ 7.27 and 5.38, in addition to a methyl singlet at δ 2.95, most likely of an N-methyl group.

Based on ^{13}C NMR/HMQC spectra, compound **178** exhibited 15 carbon signals, among them two (δ 170.4) were most likely attributed to carbonyls of amide carbonyls, while the six aromatic methine carbons were located between δ 121.8~112.9. The 1,2,3-trisubstitution of two aromatic systems was fully assigned according to H,H COSY and HMQC experiments (Figure 95). The remaining carbon signals were belonging to six sp^2 quaternary carbons (δ 139.9~111.7) and one methyl carbon (δ 39.1) of an N-CH_3 group. Accordingly, the two aromatic residues must be fused *via* nitrogen atoms forming i.e. phenazine system, where the remaining blocked position in each of the two aromatic moieties must be attached identically to carboxamide groups. The NH singlet (δ 9.93) and the N-methyl group (δ 2.95) supported a 5,10-dihydrophenazine system. Moreover, the slightly different chemical shifts for each set of aromatic systems confirmed the transoid but not *syn*-periplanar positions of the two carboxamido groups with respect to each others.

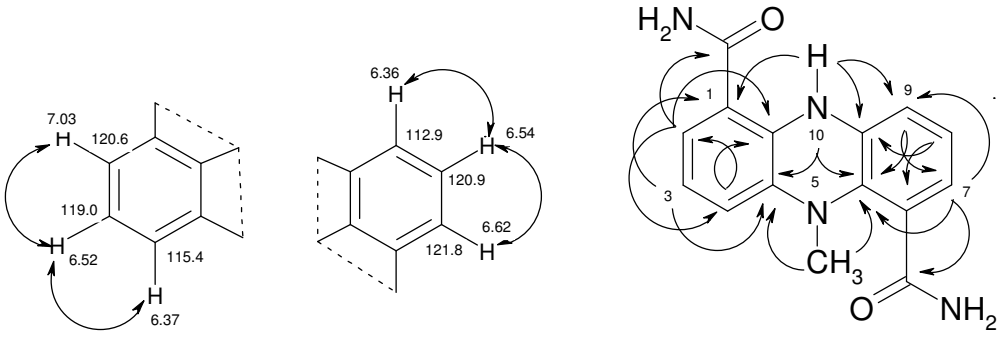
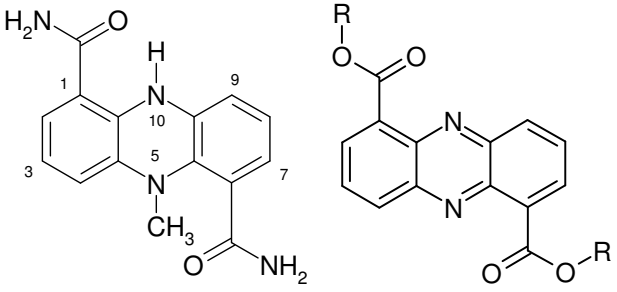


Figure 95: a) H,H COSY and HMQC connectivities of the aromatic residues as well as b) HMBC (→) correlations of 5-methyl-5,10-dihydro-phenazine-1,6-dicarboxylic acid diamide (**178**).

To deduce the final structure, HMBC experiments were performed. The down-field shift of the NH singlet (δ 9.93) could be attributed to its chelation with one of the amide carbonyls. This proton (δ 9.93) showed six cross signals; two of them were ^3J correlations with the *peri*-methine carbon C-9 (δ 137.3) and the quaternary C-1 (δ

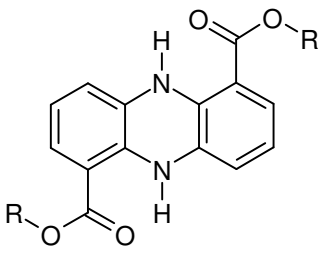
111.7). The remaining four correlations concerned the four nitrogen-connected carbons C-9a (δ 137.3), 10a (δ 139.9), C-4a (δ 138.1) and C-5a (δ 133.1) of the phenazine system (2[=C-N-C=]). Hence, one of the acid amide groups was positioned at C-1, as additionally shown by the ^3J couplings from H-2 (δ 7.03) to 1-CO (δ 170.4) and C-10a.

On other hand, the opposite N-methyl group (δ_H 2.95, δ_C 39.1) of the phenazine moiety indicated two relevant ^3J correlations to C-4a (δ 138.1) and C-5a (δ 133.1), confirming its position in between. As a result, the remaining quaternary carbon atom (δ 123.8) must be occupied by the other acid amide group (6-CO, δ 170.4). This conclusion was further supported by the observed ^3J coupling between H-7 (δ 6.62) and this carbonyl (Figure 95). Therefore, compound **178** was finally identified as 5-methyl-5,10-dihydro-phenazine-1,6-dicarboxylic acid diamide; it is a new compound.



178

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



180a: R = H
180b: R = CH_3

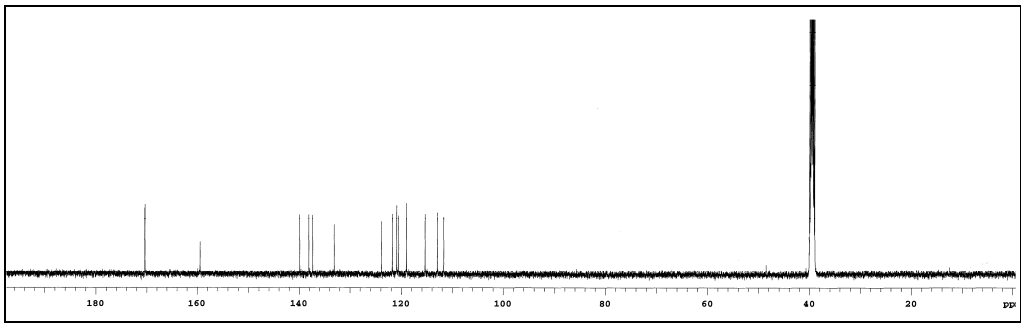
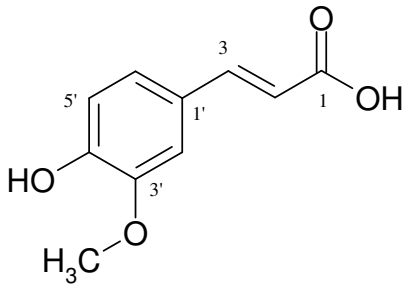


Figure 96: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of 5-methyl-5,10-dihydro-phenazine-1,6-dicarboxylic acid diamide (**178**).

Phenazine derivatives show antibiotic properties and are characterized by their inhibitory activities against xanthine oxidase[105,279]. Phencomycin (**179a**) and its

methyl ester (**179b**) were isolated from *Streptomyces* sp. as a new antibiotics with antitumor activity[83,280]. HIL Y-9031725. Dihydrophencomycin (**180a**) and its dimethyl ester **180b** were previously isolated in our group[139].

Ferulic acid (**181)** was obtained as colourless solid and identified by the molecular weight (*m/z* 194) and NMR data. It is a widespread component in plants[281-284] and also frequently found in microorganisms.



181

5.4 Marine *Streptomyces* sp. B8112

The crude extract obtained by fermentation of the marine *Streptomyces* sp. isolate B8112 was moderately active against both Gram-positive and Gram-negative bacteria as well as against yeast (*Candida albicans*), while it was strongly active against fungi (*Mucor miehei* Tü284) (Table 67). It exhibited also *in vitro* antitumor activity against cancer cells at very low concentrations (Table 45). Chemical examination of the extract exhibited numerous UV absorbing bands during TLC, two of the new components (**185**, **186**) were stained blue with anisaldehyde/sulphuric acid, one of them (**185**) turned later to dark green, while the third one (**188a**) turned to purple by staining with the same reagent. HPLC-MS of the extract combined with a search in AntiBase[61] revealed the presence of piericidins, e.g. piericidin-A (**182**). Additionally, a compound with a molecular weight of *m/z* 548.2 $[M+H]^+$ and a retention time of 15.8 min (MeOH-H_2O) was observed. A search in the databases for a piericidin derivative with the latter molecular weight gave not hits, suggesting its novelty.

Therefore, the *Streptomyces* isolate B8112 was cultivated on a large scale on M_2^+ medium: 140 of 1 l-Erlenmeyer flasks each containing 250 ml of M_2^+ containing calcium carbonate afforded a yellowish-brown culture broth which was extracted as usually. The crude extract delivered on Sephadex LH-20 two fractions. The first frac-

tion I was re-fractionated on flash silica gel and delivered five sub-fractions FIA~E. Further purification of the fast-moving sub-fraction FIA led to spatozoate (**186**). Purification of sub-fraction FIC gave 5-(2-methylphenyl)-4-pentenoic acid (**187a**) and monensin-B (**191**). From the middle polar sub-fraction FID, 5-oxo-5-o-tolyl-pentanoic acid (**188a**), piericidin-A (**182**), glucopiericidin A (**183**), phenylacetic acid (**189**) and 1,4-dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**) were isolated. The polar sub-fraction FIE gave glucopiericidin C (**185**), 2'-deoxy-uridin)[285] and 2'-deoxy-thymidin[286,287] after a series of chromatographic purification steps (Figure 97). From fraction II, indole-3-carboxylic acid and uracil were isolated.

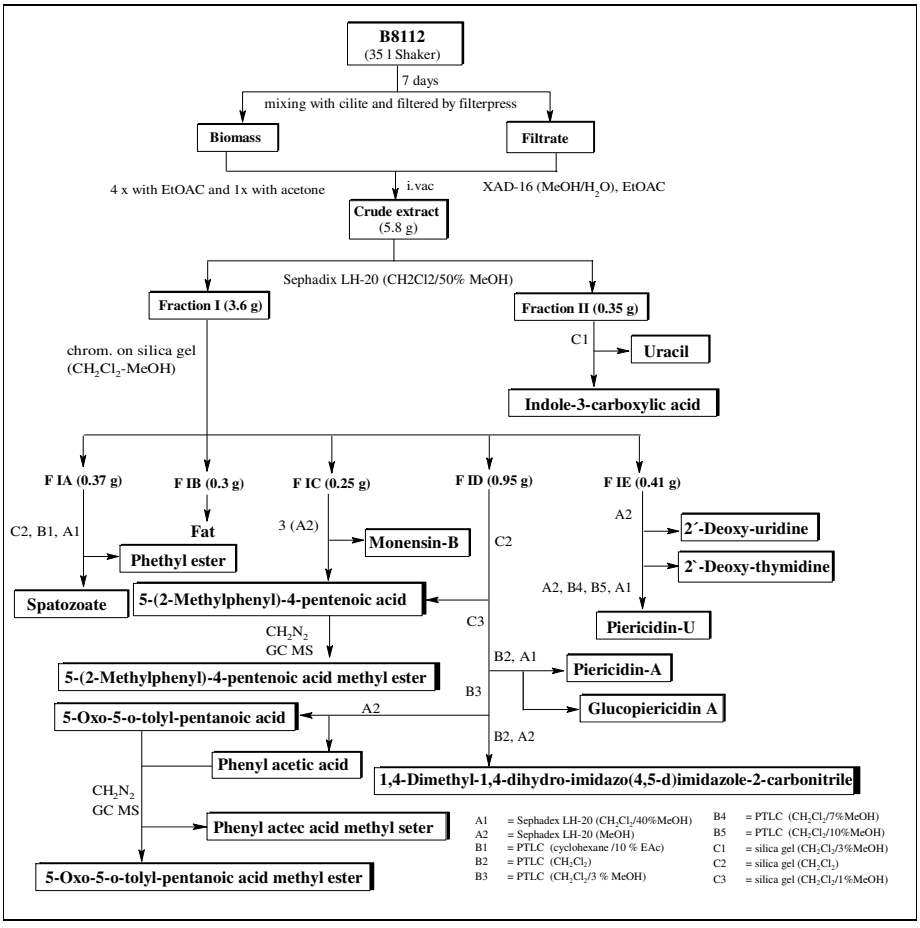


Figure 97: Work-up procedure of the marine *Streptomyces* sp. B8112

5.4.1 Piericidin-A

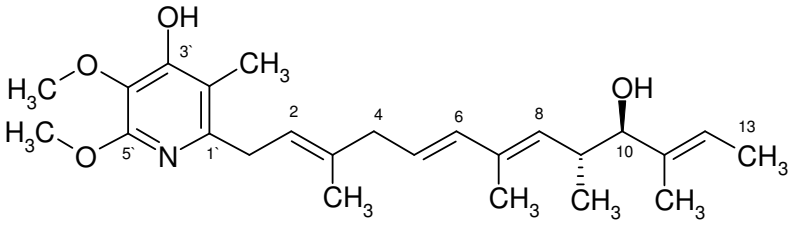
From sub-fraction FID, compound **182** was isolated as a low polar faint yellow oil, which showed an UV absorbance at 254 nm and stained to blue with anisalde-

hyde/sulphuric acid. The molecular weight was established from ESI MS in both modes as 415 g/mol.

The ^{1}H NMR spectrum of **182** showed a signal at δ6.10 (J ~15.5 Hz) for a *trans* olefinic proton, multiplet signals of 4H between δ 5.63~5.40 owing most likely to olefinic protons and a doublet of an oxymethine proton (δ 5.22, J = 9.8 Hz). Additionally there were two methoxy singlets at δ3.95 and 3.85, one methine doublet at δ 3.63, two CH_2 doublets at δ3.36 and 2.79, one multiplet methine at δ2.65 along with five methyl signals (δ2.10, 1.78, 1.73, 1.61 and 0.78). Two among the latter methyl signals (δ 1.61 and 0.78) were doublets. The others (δ 2.10, 1.78 and 1.73) were singlets of sp^2-bound methyls (Table 24).

The ^{13}C NMR spectra of compound **182** displayed 25 carbon signals: 13 of them were of sp^2 carbons, one sp^3 oxy-methine (δ82.8), two methoxy carbons (δ60.5 and 53.0), and two carbons at δ43.0 and 34.3 of sp^3 methylene groups linked to sp^2 systems. A carbon signal (δ36.8) of an sp^3 methine carbon in neighbourhood to an sp^2 moiety was seen. Finally, six methyl signals were visible in the region of δ17.3~10.4 (Table 24).

Based on the detailed spectroscopic data and searching in AntiBase, piericidin-A (**182**) was identified. This conclusion was further confirmed by comparison with the literature[288, 289].



182

Piericidin-A (**182**) displayed a high cytotoxicity against a range of human tumor cell lines with a mean IC_{50} of 0.6 μM (mean IC_{70} = 9.8 μM) and moderate selectivity (Table 69).

5.4.2 Glucopiericidin A

Compound **183** was isolated as amorphous powder from sub-fraction FID, exhibiting similar chromatographic properties on TLC as piericidin-A (**182**). This pointed to **183** a further piericidin analogue. The molecular weight was established by ESI MS in positive and negative modes to be 577 Dalton.

The ^{1}H NMR spectrum of **183** showed a very similar pattern as piericidin-A (**182**). It displayed a doublet signal at δ 6.10 of *J*~15.5 Hz, as indicative of a *trans*-coupled olefinic proton. Additionally, 4H multiplets of olefinic protons appeared in the range between δ 5.70-5.20. Moreover, a 1H doublet at δ 4.20, together with some oxy-methines and methylenes in the region of δ 3.10-3.80 were observed, pointing to the existence of a sugar moiety. This sugar moiety showed an ion peak at *m/z* 396 ($C_6H_{11}O_6$) on ESI MS^2 as a result of its splitting from the parent structure (**183**). Finally, two methoxy signals (δ 3.84 and 3.95) in addition to six other methyls were observed between δ 2.08-0.79.

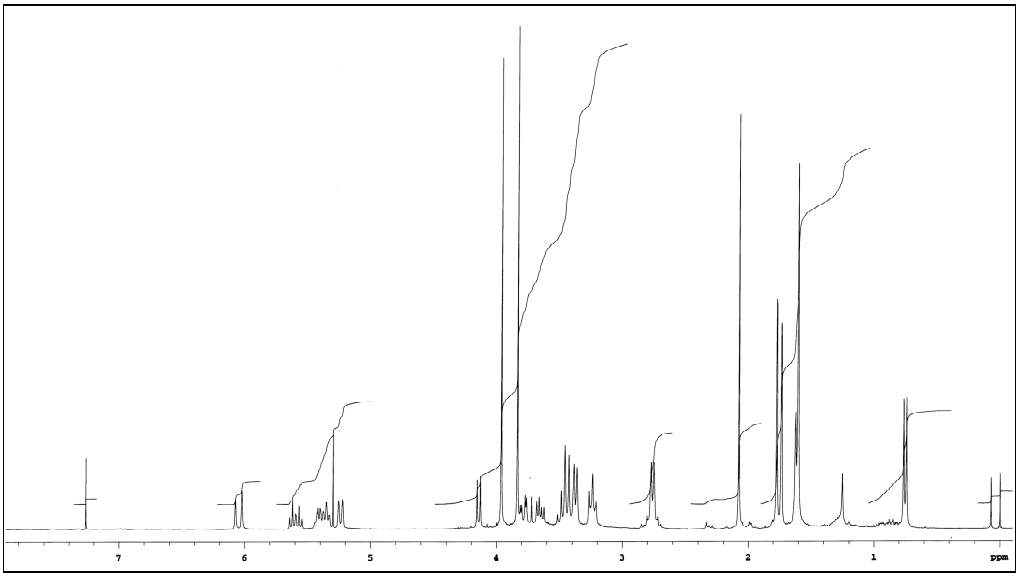


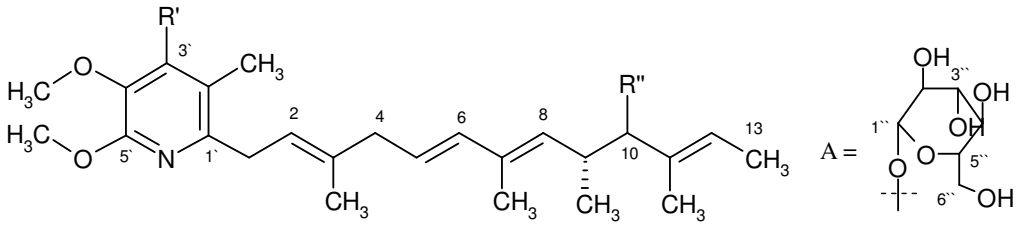
Figure 98: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Glucopiericidin A (**183**).

The ^{13}C NMR spectra of **183** displayed 31 carbon signals, among them 13 sp^2 methine carbons, one anomeric carbon (δ 103.7), four sp^3 oxymethines (δ 76.3~70.5), and one sp^3 oxymethylene (δ 62.7). The remaining carbons were two oxy-methyls (δ 60.6 and 53.6), two methylenes (δ 42.9 and 34.2) linked to sp^2 systems, one methine

(δ35.1) likely attached to an sp^2 moiety, besides six methyl signals in the region of δ 16.9~10.5.

According to the spectroscopic data, a search in AntiBase afforded two known glucopiericidin isomers, glucopiericidin A (**183**) and B (**184**). The anomeric proton of the sugar system in **184** was downfield shifted to δ4.80, due to its attachment to the aromatic residue. On the other hand, the corresponding anomeric proton in **183** is upfield shifted (δ4.20, literature: 4.13)[288,290] as it is attached to an sp^3 system (Table 23). As a result, our component was identified as piericidin-(A)-10-O-β-D-glucoside (**183**), and not piericidin-(A)-3'-O-D-glucoside (glucopiericidin A, **184**).

Glucopiericidin A (**183**) was found to inhibit antibody formation[288], and it was discovered to have antihypertensive properties. Furthermore, it exhibited cytotoxic activity against L5178Y cell cultures, and cytocidal activities against HeLa S_3 cells in *vitro.* In our biological activity examination, compound **183** displayed antibacterial activity against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative *Escherichia coli*. Additionally, it showed an activity against the micro algae *Chlorella vulgaris* (Table 68).



183: R' = OH R" = A
184: R' = A R" = OH

Table 23: ^{1}H NMR assignments of Glucopiericidin A (**183**) in comparison with literature data (in $CDCl_3$, coupling constant *J* in [Hz]).

Position	Glucopiericidin A (**183**) lit.[288,290]		Glucopiericidin A (**183**) Exp.	
	δ_C	δ_H	δ_C (125 MHz)	δ_H (300 MHz)
1	34.5	3.36 (d, 7.0)	34.2	3.36 (d, 7.0)
2	122.3	5.73 (m)	121.9	5.60 (m)
3	134.7	-	134.7	-
3-CH_3	16.6	1.73 (brs)	16.6	1.74 (s)
4	43.0	2.77 (d, 7.0)	42.9	2.77 (d, 7.0)
5	126.7	5.61 (td, 15.5, 7)	126.7	5.60 (td, 15.5, 7.0)
6	135.7	6.05 (d, 15.5)	135.5	6.05 (d, 15.5)
7	134.4	-	134.6	-
7-CH_3	13.0	1.78 (brs)	13.0	1.78 (s)
8	134.4	5.23 (d, 9.5)	134.4	5.24 (d, 9.5)
9	35.3	2.77 (m)	35.1	2.77 (m)
9-CH_3	17.0	0.75 (d, 7.0)	16.9	0.75 (d, 7.0)
10	94.2	3.45 (d, 4.0)	94.4	3.43 (d, 4.0)
11	135.4	-	135.3	-
11- CH_3	11.1	1.61 (s)	10.9	1.61 (s)
12	123.3	5.40 (m)	123.5	5.40 (m)
13	13.2	1.62 (d, 6.0)	13.2	1.62 (d, 6.0)
1'	150.8	-	150.6	-
2'	112.3	-	112.5	-
2'-CH_3	10.5	2.08 (s)	10.5	2.08 (s)
3'	154.2	-	153.6	-
4'	128.0	-	128.1	-
4'-OCH_3	60.5	3..85 (s)	60.6	3..85 (s)
5'	153.6	-	154.5	-
5'-OCH_3	53.1	3.94 (s)	53.6	3.95 (s)
1''	103.7	4.14 (d, 8.0)	103.7	4.15 (d, 8.0)
2''	74.4	3.23 (t, 8.0)	74.3	3.25 (t, 8.0)
3''	76.4	3.48 (t, 8.0)	76.3	3.47 (t, 8.0)
4''	70.8	3.42 (m)	70.7	3.43 (m)
5''	75.5	3.28 (m)	75.3	3.28 (m)
6''	62.5	3.64 (dd, 12.0, 6.0)	62.7	3.65 (dd, 12.0, 6.0)
		3.81 (brd, 12.0)		3.81 (dd, 12.0, 3.6)

5.4.3 Glucopiericidin C

Compound **185** was obtained as middle polar faint yellow oil, showing an UV absorbance and staining to blue and later to dark green with anisaldehyde/sulphuric acid. The molecular weight was established by ESI MS as 547 g/mol, which corresponds to the molecular formula $C_{30}H_{45}NO_8$, according to HRESI MS. This revealed the presence of nine double bond equivalents.

The ^{1}H NMR spectrum showed the same signal pattern as in **183** including the five olefinic protons. However, one of the methoxy signals in **183** was absent in **185** and replaced by a 1H singlet at δ 5.86. A doublet of an anomeric proton (δ 4.20, *J*

~8.0 Hz), along with several signals of oxy-methine and/or oxy-methylene protons were also visible in the region of δ 3.80-3.10, pointing to the presence of a same sugar moiety of glucopiericidin A **183** in **185** with β-configuration.

Additionally a methoxy singlet (δ 3.84) together with five methyl signals (δ 1.95, 1.72, 1.60, 1.60 and 0.81) were visible. Two among the latter were doublets (δ 1.60 and 0.81), while the remaining ones were singlet, and attached probably at sp^2 systems, except that at δ0.81, which was linked to an sp^3 carbon.

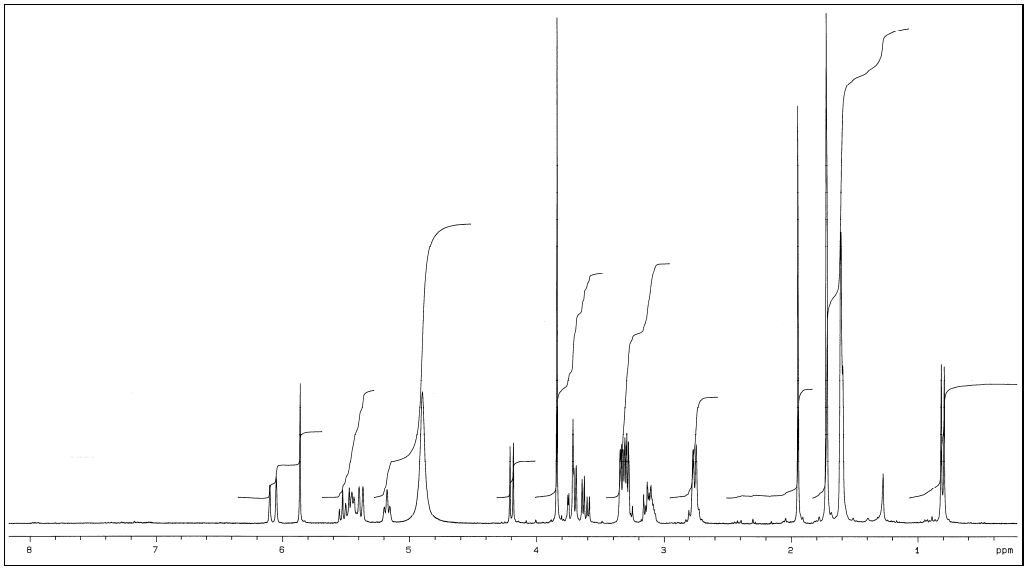
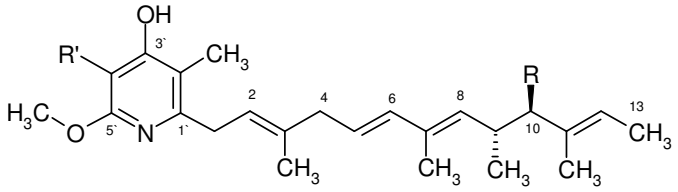
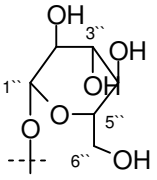


Figure 99: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of Glucopiericidin C (**185**).



A =



182: R = OH R' = OCH_3
183: R = A R' = OCH_3
185: R = A R' = H

Table 24: ^{13}C and ^{1}H NMR assignments of Glucopiericidin C (**185**) in comparison with Piericidin-A (**182**) and Glucopiericidin A (**183**), *J* in [Hz].

Position	Piericidin-A (**182**)[a]		Glucopiericidin A (**183**)[a]		Glucopiericidin C (**185**)[b]	
	δ_C[c]	δ_H (300 MHz)	δ_C[d]	δ_H (300 MHz)	δ_C[c]	δ_H (300 MHz)
1	34.3	3.36 (d, 6.9)	34.2	3.36 (d, 7.0)	32.2	3.55-3.28 (m)
2	122.1	5.63 (m)	121.9	5.60 (m)	121.0	5.20 (t, 7.0)
3	135.4	-	134.7	-	138.2	-
3-CH_3	13.1	1.73 (s)	16.6	1.73 (s)	16.7	1.72 (s)
4	43.0	2.79 (d, 7.0)	42.9	2.77 (d, 7.0)	43.8	2.76 (m)
5	126.7	5.61 (dt, 15.5, 7.0)	126.7	5.61 (dt, 15.5, 7.0)	125.9	5.53 (m)
6	136.0	6.10 (d, 15.5)	135.5	6.05 (d, 15.5)	137.8	6.10 (d, 15.5)
7	134.7	-	134.6	-	134.5	-
7-CH_3	16.6	1.78 (s)	13.0	1.78 (s)	13.2	1.72 (s)
8	133.0	5.22 (d, 9.8)	134.4	5.23 (d, 9.5)	136.2	5.48 (d, 9.1)
9	36.8	2.65 (m)	35.1	2.77 (m)	36.7	2.76 (m)
9-CH_3	17.3	0.78 (d, 6.7)	16.9	0.75 (d, 7.0)	17.7	0.81 (d, 6.8)
10	82.8	3.63 (d, 9.1)	94.4	3.45 (d, 4.0)	93.7	3.71 (d, 4.0)
11	135.6	-	135.3	-	136.9	-
11- CH_3	10.5	1.61 (s)	10.9	1.61 (s)	11.8	1.60 (s)
12	123.5	5.40 (m)	123.5	5.40 (m)	124.3	5.50-5.43 (m)
13	13.1	1.62 (d, 6.0)	13.2	1.62 (d, 6.0)	13.1	1.60 (brs)
1'	150.8	-	150.6	-	149.3	-
2'	112.1	-	112.5	-	117.6	-
2'-CH_3	10.4	2.08 (s)	10.5	2.08 (s)	10.4	1.95 (s)
3'	154.0	-	153.6	-	149.3	-
4'	127.8	-	128.1	-	92.8	5.86 (s)
4'-OCH_3	53.0	3..85 (s)	60.6	3..85 (s)	-	-
5'	153.6	-	154.5	-	161.7	-
5'-OCH_3	60.5	3.95 (s)	53.6	3.94 (s)	56.1	3.84 (s)
1"	-	-	103.7	4.14 (d, 8.0)	104.1	4.20 (d, 7.8)
2"	-	-	74.3	3.23 (t, 8.0)	75.7	3.16-3.10 (m)
3"	-	-	76.3	3.48 (t, 8.0)	78.2	3.35-3.28 (m)
4"	-	-	70.7	3.42 (m)	71.5	3.35-3.28 (m)
5"	-	-	75.3	3.28 (m)	77.7	3.16-3.10 (m)
6"	-	-	62.7	3.64 (dd, 12.0, 6) 3.81 (brd, 12.0)	62.7	3.76-3.59 (dd, 2.6, 11.7)

[a]($CDCl_3$); [b](CD_3OD); [c](75 MHz); [d](125 MHz)

The ^{13}C /HMQC NMR spectra displayed 31 carbon signals (Table 24). Comparison of the spectral data with those glucopiericidin A (**183**) exhibited a high similarity, except that the methoxy group at 4'-position in **183** was replaced by a singlet at δ 5.86 (δ_C 92.8) in case of **185** (Table 24). The structure of **185** was further deduced according to HMBC and H,H COSY correlations. Accordingly, two partial structures were obtained; the piericidin aglycone (**A**), and the glycoside system (**B**). The latter conclusion was confirmed by ESI MS^2 spectra, which delivered an ion peak at *m/z* 368 owing to loss of the sugar moiety **B** ($C_6H_{11}O_6$) from parent compound **185** (Figure 100). The sugar system might be connected either at position C-3'

of the pyridine system or at position C-10 of the side chain of the aglycone **A**. The HMBC experiments showed a characteristic cross-signal (^3J) between the anomeric proton H-1" (δ 4.20) and C-10 (δ 93.7) besides the reversed ^3J coupling from H-10 (δ 3.71) toward to the anomeric carbon C-1" (δ 104.1), confirming the connection of the sugar moiety at C-10. The relative configuration of sugar was derived from the coupling constants (δ 7.6~9.7 Hz), indicating the existence of all ring protons in axial positions as in glucopiericidin A (**183**). According to the Klyne rule [179], D-sugars are usually forming β-glycosides, and *vice versa*. This resulted finally in a β-D-configuration for the glucoside moiety in **185**.

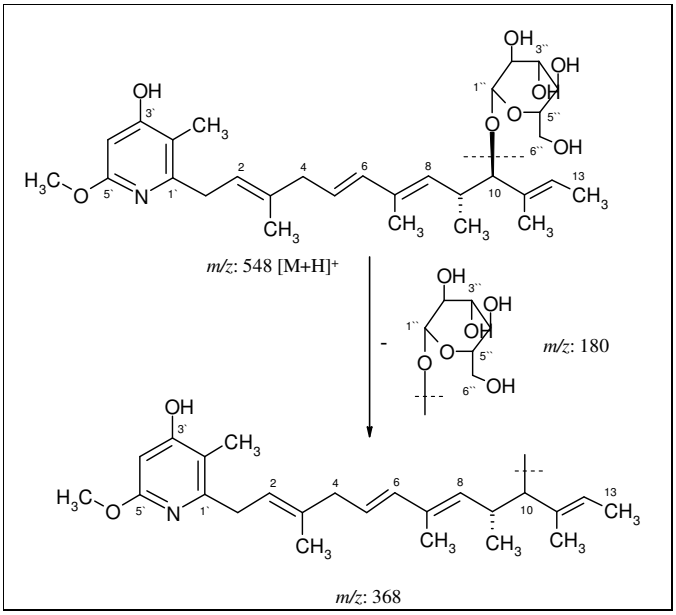


Figure 100: ESI MS^2 Fragmentation pattern of Glucopiericidin C (**185**).

Accordingly, compound **185** was identified as a new piericidin derivative, which was named glucopiericidin C. Glucopiericidin C (**185**) displayed a similar antibacterial activity as glucopiericidin A (**183**) against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative *Escherichia coli.* It showed an additional activity against the micro algae *Chlorella vulgaris* (Table 68). Moreover, compound **185** displayed selective and moderate cytotoxicity against a range of human tumor cell lines with a mean IC_{50} of 2.0 μM (mean IC_{70} = 4.2 μM), (Table 69).

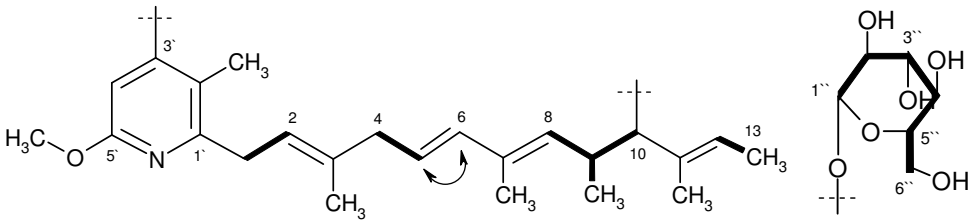


Figure 101: H,H COSY correlations (▬, ↔) of Glucopiericidin C (**185**).

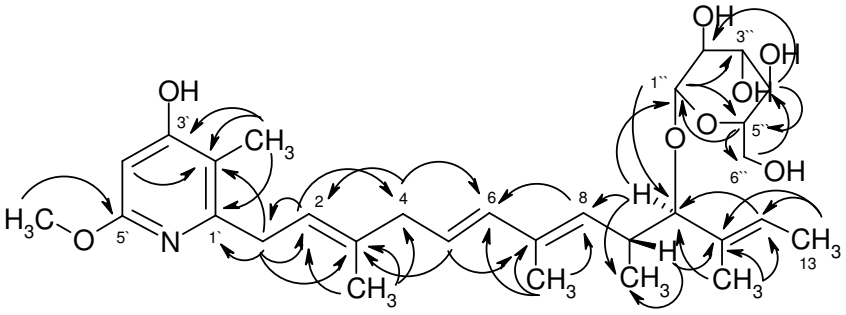


Figure 102: Selected HMBC (→) connectivities of Glucopiericidin C (**185**).

More than 32 naturally occurring piericidin antibiotics were reported in the literature[61,105]. Some of these antibiotics, for example glucopiericidins A (**183**) and B (**184**), showed antimicrobial activity and *in vitro* inhibitory activity against antibody formation[288]. In addition, glucopiericidins A (**183**) and B (**184**) are known for their high antimicrobial activities in comparison with piericidin A (**182**) and other derivatives e.g. 3'-rhamnopiericidin A1 (SN-198-C)[291], 3'-deoxytalopiericidin A1; (DTPA) [292], 7-demethylpiericidin A1 and 7-demethyt-3'-rhamnopiericidin A1 [293]. Acute toxicities of these substances in mice were, in contrast, lower than those of piericidin A (**182**), indicating that the existence of D-glucose in glucopiericidin molecules modulates their physiological activities[288]. Glucopiericidinols A1 and A2 were also reported for their cytocidal activities against HeLa S3 cells *in vitro*[290].

5.4.4 Spatozoate

Compound **186** is a colourless UV absorbing oil of middle polarity and molecular weight of 312 Dalton (EI MS), which turned to blue with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum displayed nine aromatic protons between δ 7.76-7.30, owing to the presence of two phenyl residues. The first phenyl was 1,2-disubistituted, while the latter was of a benzoyl system, which was established by an ion peak at *m/z* 206.1 on EI MS as a result of the loss of a PhCO fragment from the parent structure. The ^{1}H NMR spectra exhibited a 2H singlet at δ 5.36 attributed to an oxy-methylene group flanked by two strongly electron-withdrawing systems. Additionally, three methylene signals were shown at δ 4.20, 1.65 and 1.39, the first of them as a triplet of an oxy-methylene, while the last two were multiplets. Finally, a methyl triplet of a terminal ethyl group was visible at δ0.92.

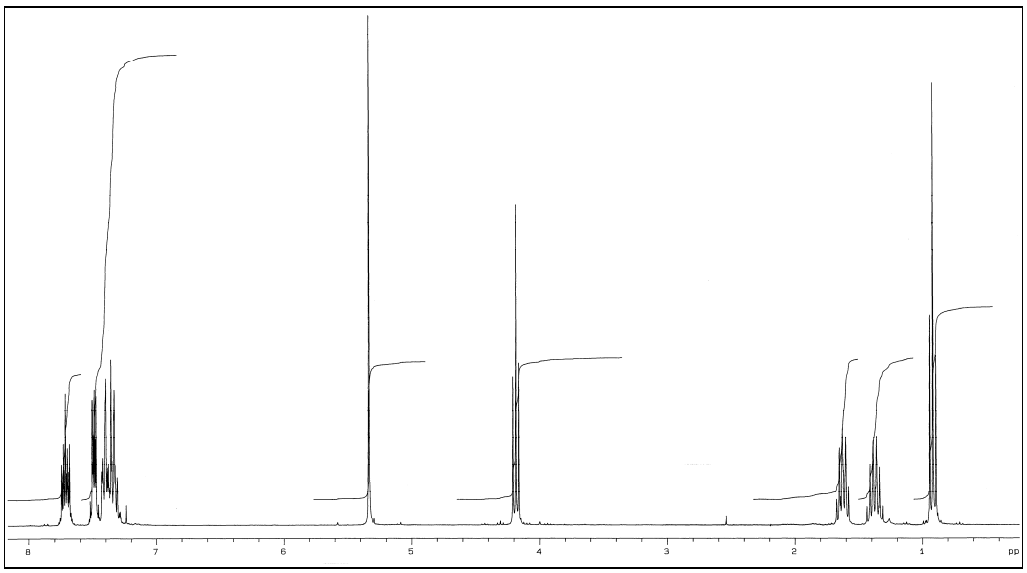
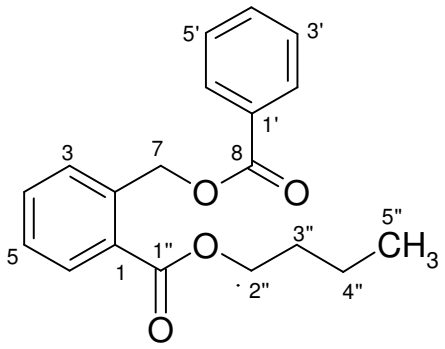


Figure 103: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Spatozoate (**186**).

The ^{13}C NMR spectrum of **186** displayed 17 carbon signals, which integrated for 19 carbons. Two of them were ester carbonyls (δ 167.5 and 167.2), nine due to sp^2 methines (δ 131.0~128.2), three belonged to quaternary sp^2 carbons (δ 135.3, 132.3 and 131.6). Additionally, two oxy-methylene carbons were at δ67.2, 65.4 as well as two methylenes (30.3 and 19.0) and one carbon (δ 13.6) of a terminal methyl group were shown.

Searching in AntiBase[61] with these data delivered structure **186** of spatozoate, which was further confirmed by comparison with literature date[294]. Spatozoate (**186**) was isolated here for first time here as a bacterial metabolite. The compound

was previously isolated from the brown algae *Spatoglossum variabile*[294], but might be formed by associated bacteria.



186

5.4.5 5-(2-Methylphenyl)-4-pentenoic acid

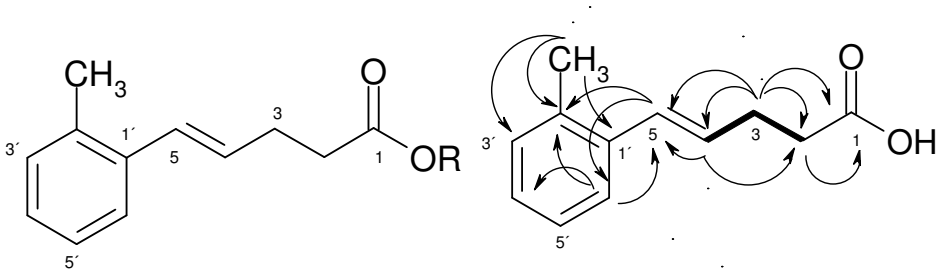
Compound **187a** was isolated as middle polar semisolid material, which stained purple by spraying with anisaldehyde/sulphuric acid. Its molecular weight was established by EI MS as 190 Dalton, with the formula of $C_{12}H_{14}O_2$ according to its HRESI MS.

The ^{1}H NMR spectrum showed multiplets of 4H for an *ortho*-disubstituted aromatic ring. Additionally, two olefinic proton signals were observed at δ 6.65 and 6.08 of an (*E*)-disubstituted double bond (J ~ 15.7 Hz). In the aliphatic region, a methyl singlet (δ 2.32) attached to an aromatic ring together with a broad singlet (δ 2.58) of two methylene groups were observed.

The APT /^{13}C NMR spectrum showed 12 carbon signals, among them one sp^2 carbon (δ 179.2) corresponding to the carbonyl of an acid or ester, beside to further 8 sp^2 carbons, two methylenes and one methyl signals were displayed. A free carboxylic acid was inferred as no methoxy signal was observed in the NMR spectra. Treatment of **187**a with diazomethane afforded the corresponding ester **187b**, which showed the methyl ester signal at δ 3.69 in the ^{1}H NMR spectrum.

Based on the revealed spectroscopic data and searching in AntiBase[61], 5-(o-tolyl)-4-pentenoic acid (**187a**) was deduced, which was further confirmed by comparison with the literature[295] and finally confirmed using 2D experiments (HMBC and COSY correlations) (Figure 104). 5-(2-Methylphenyl)-4-pentenoic acid (**187a**)

was recently isolated from bacteria as first time by Mukku in our research group and confirmed by synthesis. Compound **187a** was subjected to antitumor activity test, and showed a selective moderate cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 7.8 μM (mean IC_{70} = 9.3 μM), Table 53.



187a: R = H; **187b:** R = CH_3 **187a**

Figure 104: Selected H,H COSY (▬) and HMBC (→) connectivities of 5-(2-methylphenyl)-4-pentenoic acid (**187a**).

5.4.6 5-Oxo-5-o-tolyl-pentanoic acid

Compound **188a** was isolated as a middle polar colourless semi-solid, which showed a purple colour on spraying with anisaldehyde/sulphuric acid and heating. The molecular weight of **188a** was established by DCI MS as 206 Dalton.

The ^{1}H NMR and H,H COSY spectra displayed a multiplet signal with integration of 4H between δ 7.39~7.30, characteristic of an 1,2-disubstituted aromatic system. Four additional signals were shown in the sp^3 region; three of them (δ 2.95, 2.44 and 2.01) represented three methylene groups. The first two methylenes (δ 2.95, 2.44) gave triplet and were bound to sp^2 systems. All three methylene groups showed correlation to each other in the H,H COSY spectrum, indicating a 1,3-disubstituted propanediyl system (-CH_2-CH_2-CH_2-). The fourth signal was a singlet at δ 2.47 of a aromatic bound methyl group.

The ^{13}C NMR spectrum displayed 12 carbon signals, including two carbonyls at δ 203.4 and 179.6 for an aromatic ketone and a carboxylic acid, respectively. The latter group was proven by methylation with diazomethane to afford the corresponding methyl ester **188b** with a methoxy signal at δ 3.70 and a mass 14 amu higher than of the parent structure **188a**. Additionally, compound **188a** exhibited four aromatic methines between δ 132.0~125.7, along with two signals of quaternary carbons at δ

138.1 and 137.1. In the sp^3 region, four signals corresponding to three methylenes (δ 39.9, 33.0 and 18.9) and one methyl (δ 21.0) were visible.

The compound was finally deduced as 5-oxo-5-o-tolyl-pentanoic acid (**188a**) using HMBC correlations (Figure 105). A directed coupling (^3J) from the aromatic proton, H-6' (δ 7.61) towards the benzenoid carbonyl (203.4) confirmed the direct attachment between the aromatic residue and this carbonyl. Also, the methyl singlet was assigned to C-2' of the aromatic moiety. Compound **188a** is closely related to 5-(2-methylphenyl)-4-pentenoic acid (**187a**), except in the replacement of the double bond between 4,5-positions in (**187a**) by a -CO-CH_2- group in **188a** (Figure 105 and, Table 25).

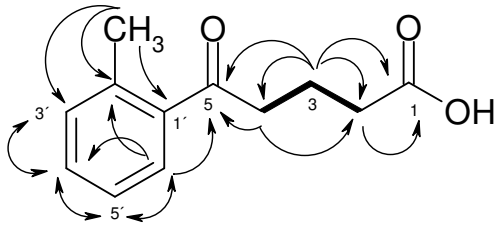
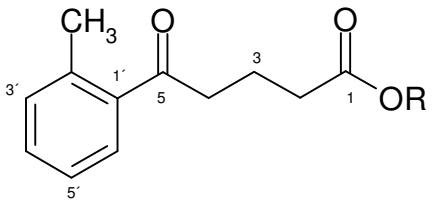
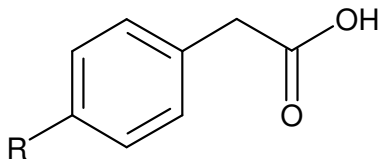


Figure 105: H,H COSY (↔, ▬) and selected HMBC (→) connectivities of **188a**.

Table 25: ^{13}C&^{1}H NMR of 5-(2-Methylphenyl)-4-pentenoic acid (**187a**) and 5-Oxo-5-o-tolyl-pentanoic acid (**188a**) (in $CDCl_3$, coupling constants *J* in [Hz]).

Position	5-(2-Methylphenyl)-4-pentenoic acid (**187a**)		5-Oxo-5-*o*-tolyl-pentanoic acid (**188a**)	
	δ_C (50.3 MHz)	δ_H (300 MHz)	δ_C (50.3 MHz)	δ_H (300 MHz)
1	179.2	-	179.6	-
1-OH	-	-	-	10.54 (brs)
2	28.2	2.58 (m)	33.0	2.44 (t, 7.1)
3	33.9	2.58 (m)	18.9	2.01(m)
4	129.2	6.08 (dm, 15.7)	39.9	2.95 (t, 7.2)
5	139.3	6.65 (d, 15.8)	203.4	-
1'	135.1	-	137.1	-
2'	136.4	-	138.1	-
2'-CH_3	19.8	2.32 (s)	21.0	2.47 (s)
3'	130.2	7.13 (m)	132.0	7.30 (m)
4'	127.1	7.13 (m)	131.4	7.30 (m)
5'	126.0	7.13 (m)	125.7	7.30 (m)
6'	125.5	7.39 (t, 4.5)	128.5	7.61 (dd, 8.3, 1.2)





188a: R = H; **188b:** R = CH_3 **189:** R = H; **190:** R = OH

In the cytotoxicity test, compound **188a** displayed a moderate but unselective cytotoxicity against a range of human tumor cell lines with a mean IC_{50} of >10 µM (mean IC_{70} = >10 µM, Table 53).

5.4.7 Monensin-B

Purification of sub-fraction FIC on Sephadex LH-20 resulted in compound **191**. It was obtained as a white amorphous not UV absorbing solid of middle polarity, which was detected as orange-red zone on spraying with anisaldehyde/sulphuric acid. (+)-ESI MS of compound **191** showed signals at *m/z* 1357 and 680, representing $[M+Na]^+$ and $[2M+Na]^+$ ions, respectively. (-)-ESI MS showed a signal at *m/z* 655 of $[M-H]^-$. Accordingly, the molecular weight was deduced as 657 Dalton.

The ^1H NMR spectrum (Figure 106) exhibited signals with integration of ~ 60 protons, all localised in the aliphatic region. Between δ4.50 and 3.20, several oxymethine/methylene protons were found with one methoxy at δ3.38. Moreover, eight methyl signals were seen, two of them as singlets, while the remaining six were doublets. Finally, a number of multiplets appeared in the range of δ2.60~1.00 corresponding to sp^3 protons of methine and/or methylene groups. This pointed to a polyether structure.

In the ^{13}C NMR spectra of compound **191**, 35 carbon signals were displayed: one carbonyl of ester, acid or amide (δ181.1), two acetal carbons (δ106.8 and 98.1), and eleven oxy-carbons in the region of 86.3~53.3. Between δ44.9~10.4, for 21 carbon signals were visible.

Based on the above spectroscopic data, compound **191** was preliminarily identified as a monensin derivative and finally confirmed as monensin B by comparison with literature data [296~298].

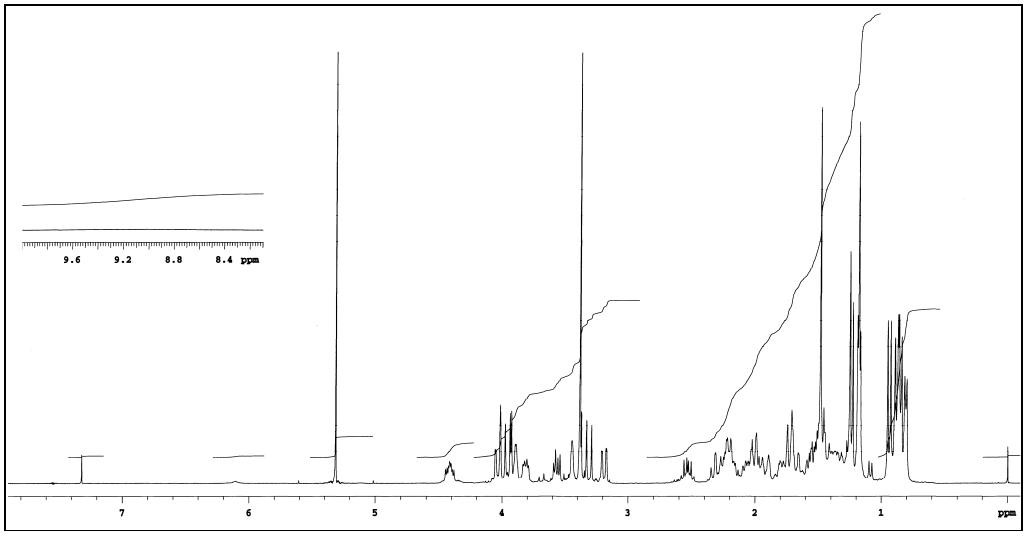
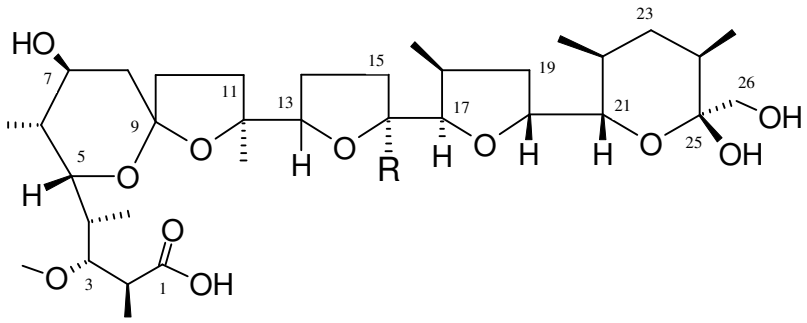


Figure 106: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Monensin-B (**191**).



191: R = CH_3
192: R = CH_2CH_3

The full assignment of several polyethers was reported, however, this of monensin B (**191**) had been not yet been published. Therefore, the NMR assignments for monensin B (**191**) is carried out here for the first time using 2D measurements (COSY, HMQC and HMBC), Figure 107 and Table 26.

Monensin A (**192**) and B (**191**) are polyether antibiotics[299], firstly isolated from *Streptomyces cinnamonensis* [300,301]. They are successfully used to control coccidiosis in poultry and to promote the growth of cattle. Monensin B (**191**) differs from **192** in the size of the side chain attached to C-16 only.

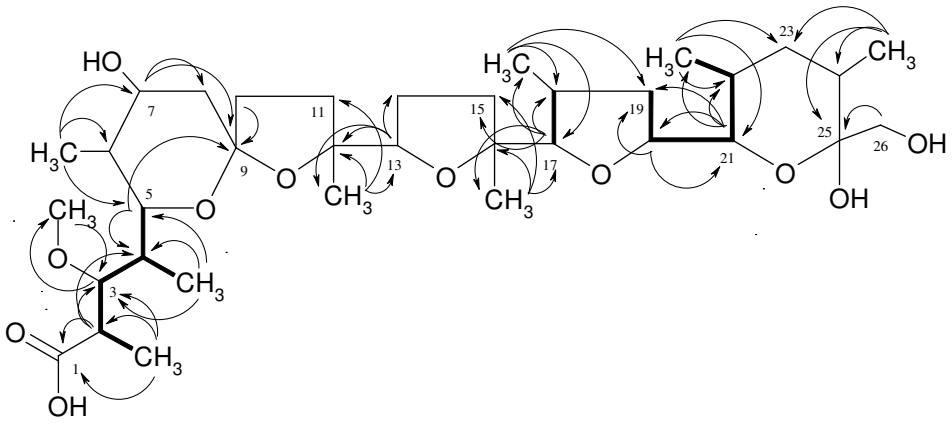


Figure 107: Selected H,H COSY (▬) and HMBC (→) connectivities of Monensin B (**191**).

Table 26: ^{13}C and ^{1}H NMR assignments of Monensin B (**191**) in $CDCl_3$, *J* in [Hz].

Position	δ_C[a]	δ_H (300 MHz)	Position	δ_C[a]	δ_H (300 MHz)
1	181.3	-	14	27.3	1.80 (m), 1.55 (m)
2	44.9	2.56 (m)	15	30.3	2.31 (m), 1.48 (m)
2-CH_3	16.6	1.24 (d, 6.7)	16	83.8	-
3	82.9	3.20 (dd, 10.3, 1.6)	16-CH_3	23.9	1.18 (s)
3-OCH_3	57.9	3.38 (s)	17	86.4	3.94 (d, 3.3)
4	37.4	2.06 (m)	18	34.5	2.20 (m)
4-CH_3	11.0	1.18 (d, 6.7)	18-CH_3	14.1	0.88 (d, 7.0)
5	68.2	4.03 (dd, 11.1, 2.1)	19	33.5	1.94(m), 1.71 (m)
6	34.8	2.20 (m)	20	76.6	4.41 (m)
6-CH_3	10.4	0.94 (d, 7.1)	21	74.5	3.81 (m)
7	70.4	3.90 (brd, 2.6)	22	32.9	1.56 (m)
8	39.3	2.00 (m), 1.70 (m)	22-CH_3	16.7	0.81 (d, 5.4)
9	106.9	-	23	31.8	1.34 (m)
10	36.4	1.46 (m)	24	35.7	1.34 (m)
11	33.1	2.00 (m), 2.20 (m)	24-CH_3	16.0	0.85 (d, 6.1)
12	85.2	-	25	98.1	-
12-CH_3	27.6	1.49 (s)	26	64.8	4.00 (d, 11.9), 3.31 (d, 11.9)
13	81.5	3.56 (brm)	-	-	-

[a] 125 MHz

5.5 Marine *Streptomyces* sp. B8108

In the agar diffusion assay, the extract of the marine *Streptomyces* sp. isolate B8108 exhibited a moderate bioactivity against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) and a rather high activity against the fungus *Mucor miehei* (Tü284) (Table 70). *In vitro*, the extract showed antitumor activity against a number of human cancer cells (IC_{70} 11.565 μg/ml), in addition to 100% cytotoxicity at concentration of 100 μg/ml (Table 45).

In the TLC screening, the extract revealed one band, which did not absorb UV light and was stained initially blue and later to pink on spraying with anisaldehyde/sulphuric acid. In addition, UV absorbing bands were observed, some of which showed a blue UV fluorescence and turned yellow on spraying with anisaldehyde/sulphuric acid.

The strain was fermented as 24 L shaker culture using M_2^+ medium. After fermentation for 6 days, the brown culture broth was applied to the usual working up giving two separate extracts from mycelial cake and filtrate. As TLC evaluation of both extracts confirmed their identity, they were combined and concentrated *in vacuo* to afford 3.52 g of brown crude extract.

The extract was subjected to flash chromatography on silica gel and eluted with dichloromethane-methanol gradient to give six fractions. Purification of the middle polar fraction **III**, using PTLC, silica gel column and then Sephadex, lead to 1,4-dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**), 5-(2-methylphenyl)-4-pentenoic acid (**187a**) and piericidin-A (**182**). Fraction **IV** delivered eight compounds: 4,9-dihydroxy-9-methyl-decan-4-olide (**237**), 2-acetamino-benzoic acid (**194a**), indole-3-carboxylic acid, indole-3-acetic acid, anthranilic acid, 3-(hydroxyacetyl)-indole, tyrosol (**229**) and homononactic acid (**145a**). Additionally, from the polar fractions **V** and **VI**, another seven compounds were isolated: 2'-deoxy-uridine, 2'-deoxy-adenosine[152], 5'-methyl-thioadenosine[152], uracil, staurosporine (**198a**), nonactic acid (**144a**), glucopiericidin A (**183**) together with **194a,** (Figure 108). Compound **237** was discussed in details with the other γ-butyrolactones for better comparison (see p. 197).

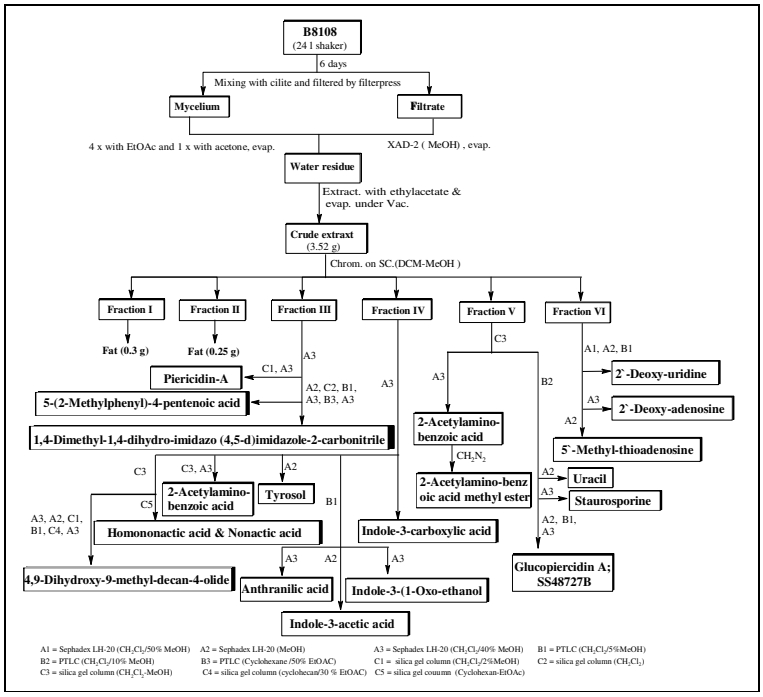


Figure 108: Work-up scheme of marine *Streptomyces* sp. B8108.

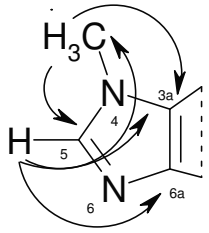
5.5.1 1,4-Dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile

Sub-fraction III delivered compound **193** as a colourless solid with medium polarity. It was UV absorbing and exhibited no colour reaction with anisaldehyde/sulphuric acid. The molecular weight of **193** was established from ESI and EI MS as 161 Dalton. Compound **193** was confirmed (HREI-MS) to have the molecular formula $C_7H_7N_5$, with 7 double bond equivalents.

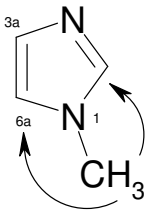
The ^{1}H NMR spectra of **193** displayed 3 singlets, which integrated for 7 protons. One of them corresponded to 1H at δ 7.71, and the others to 2 methyl groups at δ 3.81 and δ 3.44, which were attached to nitrogen, as oxygen was absent in the molecular formula.

Corresponding to the molecular formula, the ^{13}C NMR spectra displayed 7 carbon signals, five of them were observed in the sp^2 region: one was classified as a methine carbon (δ 140.1) and four as quaternary carbons (δ 148.8, 112.4, 111.1 and 90.8). The remaining two carbons (δ 37.9 and 33.5) corresponded to N-methyl groups.

In the HMBC spectra, the singlet at δ_H 7.71 exhibited three ^3J connectivities to the quaternary carbons C-3a (δ90.8) and C-6a (δ148.8) and the N-methyl carbon at δ33.5. This established the imidazole system (**A**). In addition, the N-methyl (δ3.44) showed two significant ^3J couplings to the quaternary carbons, C-6a (δ148.8) and C-2 (δ111.5). This confirmed an additional imidazole moiety (**B**) fused with the first one (**A**) *via* the carbons, C-6a and C-3a.



A



B

The two fused N-methyl-imidazoles **A** and **B** afforded the formula $C_6H_7N_4$, which suggested a CN group for the missing atoms and the remaining two double bond equivalents, and this was supported by EI-MS, which showed an ion fragment peak at *m/z* 135.1 ($[M\text{-}CN]^+$) and by the IR spectrum with a strong nitrile band at ν 2235 cm^{-1}. This left C-2 as the only vacant position in the molecule, that could contain the nitrile group, thereby establishing the final structure as 1,4-dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**). It is the first time that a compound with an imidazo-imidazole skeleton has been isolated from nature. The compound showed an unselective cytotoxic activity with IC_{50} of 7.146 μg/ml (Table 46).

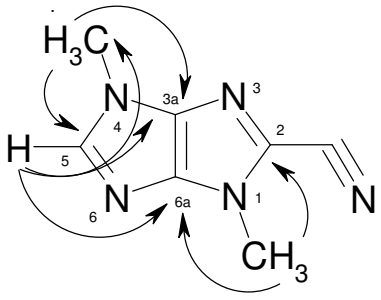


Figure 109: Structure and HMBC (→) correlations of 1,4-Dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**).

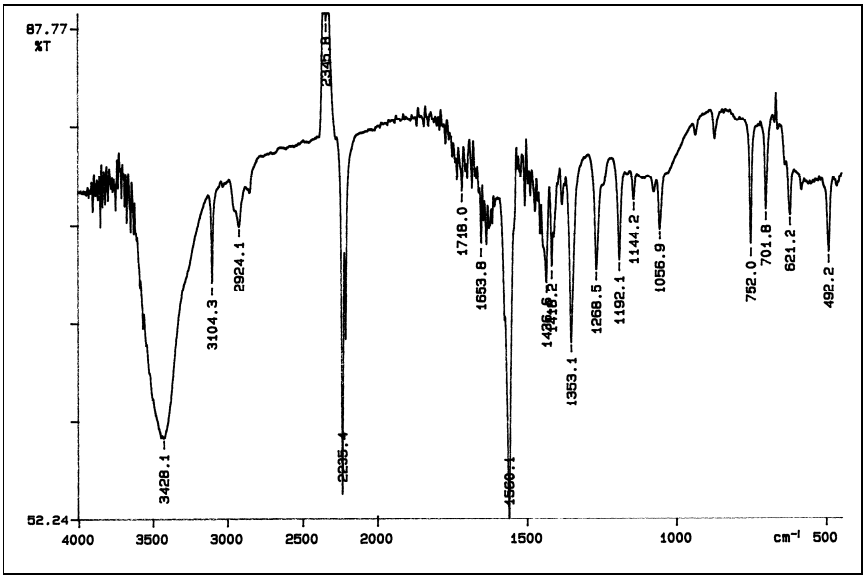


Figure 110: IR (KBr) of 1,4-Dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**).

Table 27: ^{13}C and ^{1}H NMR data (acetone-d_6/$CDCl_3$) of 1,4-Dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**).

1,4-Dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**)				
Position	δ_C (Acetone-d_6)[a]	δ_C ($CDCl_3$)[a]	δ_H (Acetone-d_6)[b]	δ_H ($CDCl_3$)[b]
1-NCH_3	37.9	37.5	3.44	3.48
2	112.4	111.5	-	-
3a	90.8	90.7	-	-
4-NCH_3	33.5	33.4	3.81 (s)	3.76 (s)
5	140.1	138.1	7.71 (s)	7.37 (s)
6a	148.8	148.2	-	-
7	111.1	110.2	-	-

[a](150 MHz), [b] (300 MHz)

5.5.2 2-Acetylaminobenzoic acid

Sub-fractions IV_A-V_A delivered compound **194a** as a white UV absorbing and blue fluorescent solid, which turned pale yellow on spraying with anisaldehyde/sulphuric acid. EI-MS of **194a** established a molecular weight of 179 g/mol, indicating an odd number of nitrogen atoms.

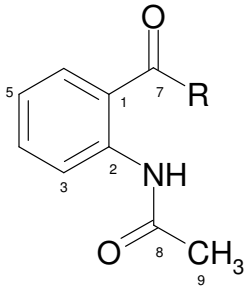
The ^{1}H NMR spectrum of compound **194a** showed four 1H signals of a 1,2-disubstitued aromatic system. Two among these signals were doublets at δ 8.50 and

8.05, while the remaining two were td at δ 7.50 and δ7.10. Additionally, an sp^2 bound or a heteroatom-connected methyl group was detected at δ2.18.

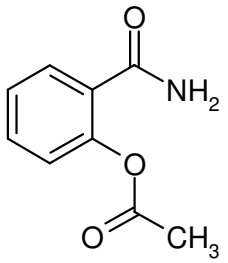
^{13}C/APT NMR spectra showed two carbon signals at δ171.5 and 171.4 of ester, acid and/or amide carbonyls. Six sp^2 carbons were detected, two of them were quaternary (δ 142.2 and 118.0), while the remaining were four aromatic methines (δ134.9~121.3) of the previously discussed 1,2-disubstituted aromatic residue. In addition, there was a methyl signal at δ25.0.

Based on the spectroscopic data, four possibilities **194a**-**197** were taken into consideration. The acetophenone carbonyls in **196** and **197** are normally located at δ ~203, and these structures were therefore excluded. Methylation with diazomethane afforded a molecular ion peak at *m/z* 193, 14 amu higher than the precursor, pointing to a phenol or a free carboxylic acid. Correspondingly, another methoxy singlet (δ 3.93) was observed in the ^{1}H NMR spectrum. Therefore, the structure of the natural product was deduced as 2-acetylaminobenzoic acid (**194a**) and not 2-acetyloxy-benzamide (**195**).

Structure of **194a** was further verified using HMBC correlations (Table 42), where H-6 (δ8.05) displayed a ^3J cross-signal to the carbonyl C-7 (δ171.5) and C-2 (δ142.2). The latter showed in turn a cross signal (^3J) with the triplet of H-4 (δ7.10). The high chemical shift of C-2 (δ 142.2) was indicative for its linkage to nitrogen[120]. The methyl singlet (δ2.18) was of an acetamide group, and it showed the expected cross-signal (^2J) to the amide carbonyl (δ171.4). Compound **194a** is new as microbial product, however, it is known from synthesis[302].



194a: R = OH,
194b: R = OCH_3



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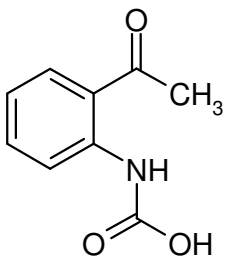

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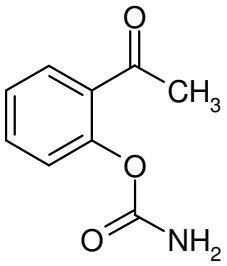
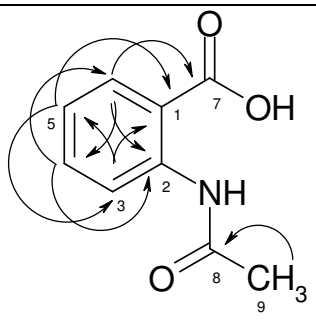


197

Table 28: HMBC (→), ^{13}C and ^{1}H NMR spectroscopic data of 2-Acetylamino-benzoic acid (**194a**) in CD_3OD, (*J* in [Hz]).

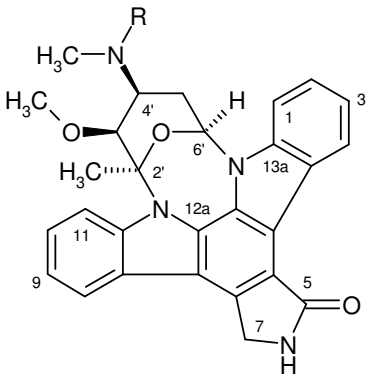
2-Acetylamino-benzoic acid (**194a**)		
Position	δ_C (125 MHz)	δ_H (300 MHz)
1	118.0	-
2	142.2	-
3	121.3	8.50 (dd, 8.4, 0.7)
4	134.9	7.10 (td, 8.0, 1.1)
5	123.9	7.50 (td, 7.4, 1.6)
6	132.5	8.05 (dd, 8.0, 1.6)
7	171.5	-
8	171.4	-
9	25.0	2.18 (s)



5.5.3 Staurosporine

Compound **198a** was isolated as white powder from fraction V using column chromatography on silica gel, followed by PTLC and finally purified on Sephadex LH-20. The compound showed an UV absorbance at 254 nm and a blue fluorescence at 366 nm during TLC analysis. This band turned reddish-brown on spraying with anisaldehyde/sulphuric acid. The molecular weight of **198a** was established as 466 Dalton by (+)-ESI-MS, and HRESI-MS confirmed the molecular formula as $C_{28}H_{26}N_4O_3$.

Only one compound with this formula has been obtained from microorganisms so far: staurosporine (**198a**)[303]. This assumption was confirmed indeed by the ^{1}H and ^{13}C NMR data. The proton spectrum exhibited two methyl singlets (δ 1.54 and 2.35), two 1H multiplets (δ 2.40 and 2.74) of a methylene group, a quartet of methine (δ 3.34), and one singlet of a methoxy group (δ 3.41). Furthermore, one doublet oxy-methine (δ 3.87), and singlet of heteroatom-bounded methylene (δ 5.01) were observed. Additionally, two signals of singlet (δ_H 6.57) and doublet (δ_H 6.55) methines were detected in the aromatic area. Furthermore, a complex multiplet signal of 5H protons was observed between δ 7.20-7.50, together with three doublets each of 1H at δ 7.88, 7.93 and 9.43. Comparison with reference spectra of authentical staurosporine confirmed the identity.



198a: R = H, **198b:** R = $CONH_2$

Staurosporine (**198a**) is an indolo[2,3-a]carbazole alkaloid and was first isolated from *Streptomyces staurosporeus* Awaya (AM-2282)[304] and subsequently from several other actinomycetes. Biosynthetically, staurosporine (**198a**) is derived from tryptophane and connected stereospecifically during unusual double N-glycosidic linkage with the amino sugar[305]. Staurosporine (**198a**) possesses inhibitory activity against fungi and yeast but has no significant activity against bacteria[305]. It shows strong antihypertensive activity and pronounced *in vitro* activity against a number of experimental tumours[306]. It is a potent inhibitor of protein kinase C and platelet aggregation[307]. About 50 derivatives have been found in nature. Recently, N-carboxamido-staurosporine (**198b**) was isolated from the marine *Streptomyces* sp. QD518 by Fotso *et al.*[97] in our research group.

5.6 Marine-derived *Streptomyces* sp. Mei02-8,1

The marine derived *Streptomyces* sp. Mei02-8,1 showed in the biological screening antimicrobial activity against all our test microorganisms (Table 71). Moreover, on TLC of the extract, several UV absorbing bands were visible, which changed to greenish-blue on spray with anisaldehyde/sulphuric acid. Additionally, one blue fluorescence band appeared which stained to reddish-brown and a low polar UV absorbing band was shown, which turned violet-blue with the same spraying reagent.

For the isolation of these metabolites, 80 of 1 L Erlenmeyer flasks (each containing 250 ml of M_2 100% artificial sea water + $CaCO_3$) were inoculated and cultivated for 7 days at 28 °C. After usual working up, the oily crude extract was fractionated and purified following the scheme shown below (Figure 111). As a result,

six compounds were obtained: 4-hydroxy-10-methyl-11-oxododec-2-en-1,4-olide (**236**), 1,N^6-dimethyladenosine (**199**), staurosporine (**198**), 2'-deoxy-uridine, 2'-deoxy-thymidine and 2'-deoxy-adenosine[152] . For the discussion of compound **236**, with the other γ-butyrolactones for a better comparison, see p. 196.

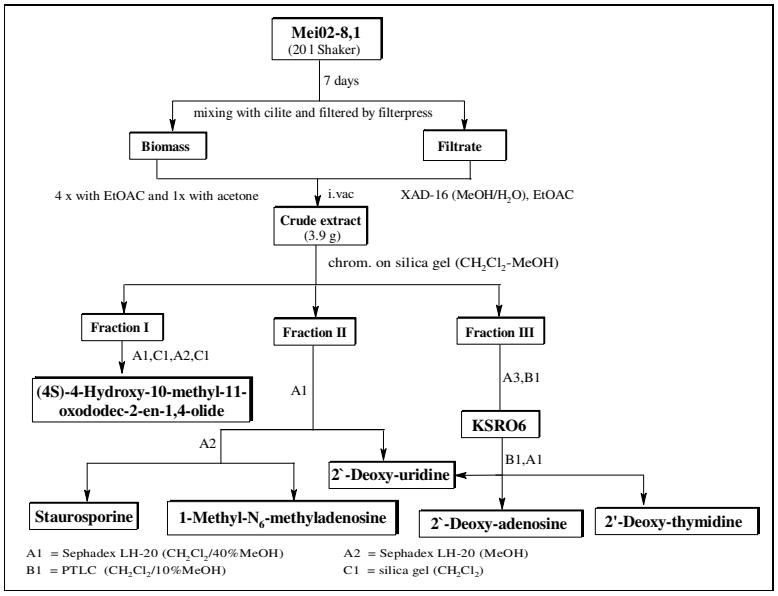


Figure 111: Work-up procedure of the marine *Streptomyces* sp. Mei02-8,1

5.6.1 1,N^6-dimethyladenosine

Compound **199** was obtained as a colourless solid from the sub-fraction IIA. It is UV absorbing, turned to intensive greenish-blue with anisaldehyde/sulphuric acid after heating. The molecular weight of **199** was established as 295 Dalton by (+)-ESI and EI mass spectra. (+)-HRESI MS deduced the molecular formula as $C_{12}H_{17}N_5O_4$, containing seven double bond equivalents.

The ^{1}H NMR spectrum showed two downfield shifted 1H singlets (δ 8.42, 8.29), similar as in adenine. In the sugar region, a 1H doublet at δ 6.03, probably of a hemi-acetal (-OCH-O) or hemi-aminal (N-CH-O) proton was visible, together with three oxymethine signals at δ 4.62, 4.31 and 4.16. Two ABX signals (δ_{AB} 3.88 and 3.77) of an oxymethylene group along with a broad 6H singlet (δ 3.40-3.50) for two methyls were visible. One of the two methyl signals gave a very broad signal (δ 3.61) even at 600 MHz. The signal was higher at 300 MHz and sharpened by measuring the sample at higher temperature (50 °C) at 300 MHz (Figure 112), which is obviously due

to a coalescence phenomenon: It seems that the respective methyl group flips between two orientations at room temperature nearly with the spectrometer frequency.

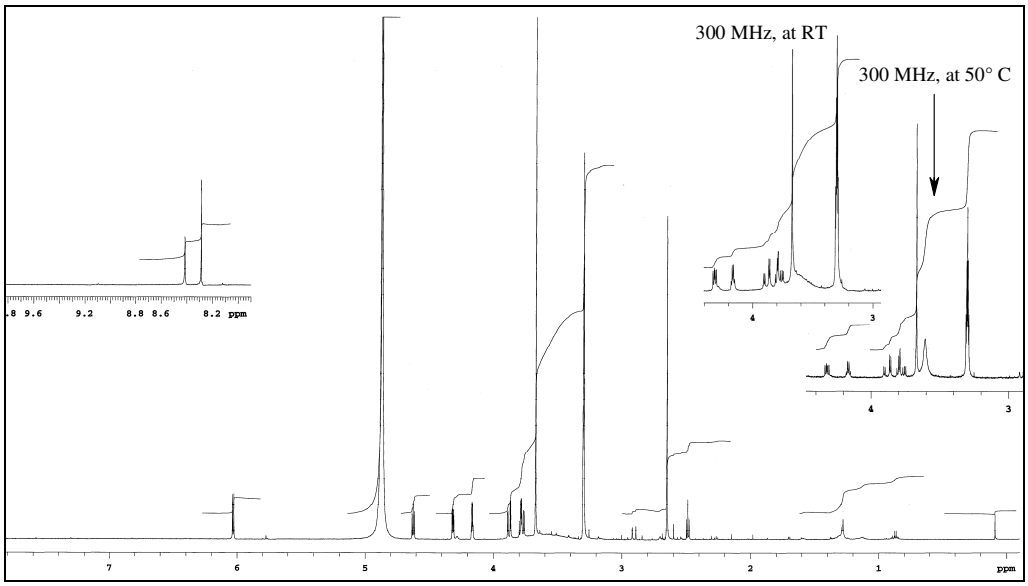
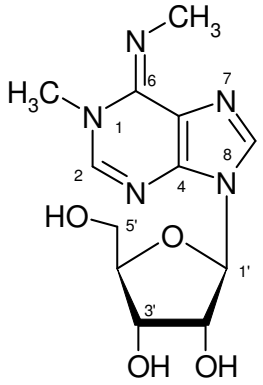


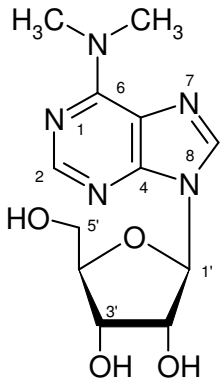
Figure 112: ^{1}H NMR spectrum (600 MHz, CD_3OD) of 1,N^6-Dimethyladenosine (**199**).

The ^{13}C NMR spectrum of compound **199** showed five aromatic carbon signals, three of which were quaternary (δ 149.5, 141.2, 121.6). The remaining two sp^2 methine carbons exhibited identical chemical shifts (δ 141.2). In the sugar region, a further set of five sp^3 carbon signals was observed; four of them were oxygenated methines (δ 91.0~72.3), and the other an oxymethylene (δ 63.0). Finally, two nitrogen-bound methyls appeared at δ 40.4 and 38.3.

A search in AntiBase[61] resulted in two possible adenosine analogues; 1,N^6-dimethyladenosine (**199**) and N,N-dimethyladenosine (**200**). The experimental data are in better agreement with **199**[308, 309], however, a final decision should be made best on the basis of synthetic material: 1,N^6-Dimethyladenosine (**199**) was first identified by Hammargren *et al.*[309] as a novel nucleoside in the urine of a lung cancer patient. Synthetically, **199** was obtained by methylation of adenosine using methyl iodide in dimethyl sulphoxide, catalyzed by potassium carbonate. The compound was also synthesized using methyl sulphate[310].



199



200

5.7 Marine-derived *Streptomyces* sp. Mei4-1,23

The crude extract of the marine *Streptomyces* sp. Mei4-1,23 exhibited high potent antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus, Streptomyces viridochromogenes* (Tü 57), *Escherichia coli, Candida albicans*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus* (Table 72). TLC of the extract exhibited one visible yellow band in daylight, which turned to brown by treatment with conc. sulphuric acid or by spraying with $PdCl_2$ reagent. Moreover, TLC displayed several UV absorbing spots, along with one under UV light blue fluorescence band. Most of these bands showed either greenish-blue or reddish brown colours by spraying with anisaldehyde/sulphuric acid. Additionally, further not UV absorbing compounds were detected as violet zones by spraying with anisaldehyde/sulphuric acid.

A 40 L culture in M_2 medium using artificial seawater and extraction as usually afforded a yellow oily crude extract. Fractionation and purification using different chromatographic techniques yielded nine constituents, namely, lumichrome (**201**), holomycin (**208**), homononactic acid (**145a**), nonactic acid (**144a**), dinactin (**148**), *p*-hydroxy-phenylacetic acid (**190**), uracil, 2'-deoxy-uridine, and 2'-deoxy-thymidine (Figure 113).

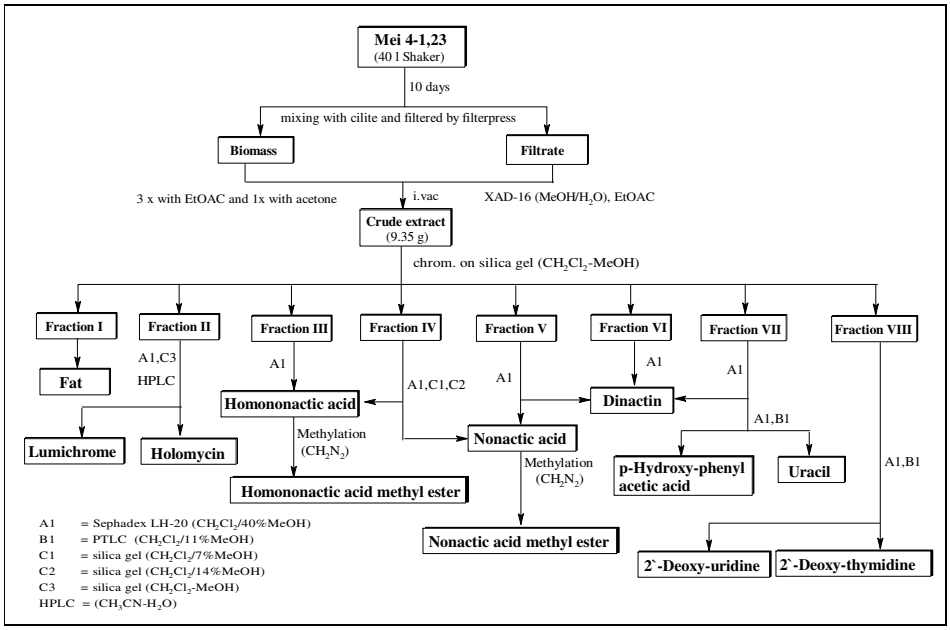


Figure 113: Work-up procedure of the marine *Streptomyces* sp. Mei4-1,23

5.7.1 Lumichrome

Compound **201**, a pale yellow UV absorbing solid, was obtained from fraction II using Sephadex LH-20 followed by HPLC (Figure 114). On TLC, the zone of **201** turned to yellow after spraying with anisaldehyde/sulphuric acid. The molecular weight was determined as 242 Dalton by ESI and EI mass spectra.

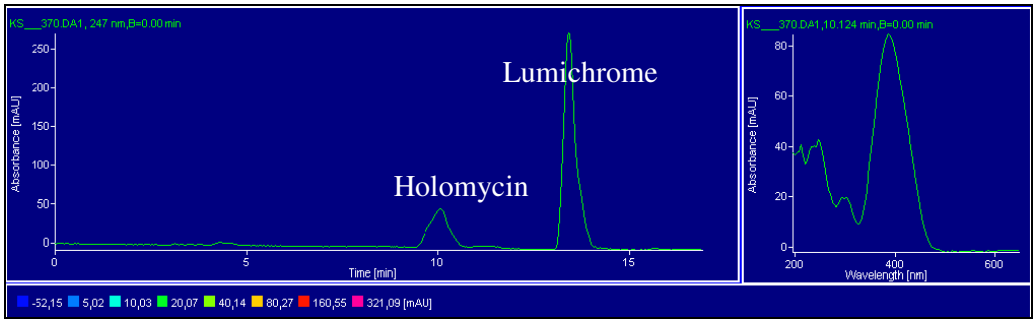


Figure 114: HPLC (CH_3CN-H_2O) chromatogram of Lumichrome (**201**) and Holomycin (**208**).

The ^{1}H NMR spectrum of **201** exhibited only two 1H singlets (δ7.70 and 7.90) in the aromatic region along with one broad 2H singlet of acidic groups (δ11.65). In the aliphatic region two overlapping signals with integration of 6H (δ2.49 and 2.46)

were visible. These two signals could be corresponding to two aromatic bound methyls and/ or N-CH_3 groups, similar as in **199/200**.

The APT/^{13}C NMR spectra displayed 12 carbon signals, including eight quaternary sp^2 carbons (δ 160.3~129.9) and two methines (δ 128.5 and 125.7). The downfield shifting the quaternary carbons pointed to their location at or between hetero atoms e.g. N and O. The remaining two carbon signals (δ 20.0, and 19.3) were indicative of aromatic bound methyls.

A substructure search in AntiBase for 2 C_q-CH_3 and 2 CH combined with the molecular weight (*m/z* 242) (Figure 115) lead to lumichrome (**201**) [311-313] as the sole coincident structure to the spectroscopic and chromatographic data. As there were no published spectroscopic data available for lumichrome (**201**) so far, the compound was further investigated using 2D experiments (HMQC & HMBC) (Figure 116). In the HMBC spectrum, ^3J correlations were seen from H-6 to C-9a, C-8 and 7-CH_3, from H-9 to C-5a, C-7 and 8-CH_3, from 7-CH_3 to C-6, C-8 and from 8-CH_3 to C-7 and C-9, confirming the benzene ring in **201**. Further correlations, especially those from the NH groups to the carbon atoms in ring **A**, were not seen.

Lumichromes are structural analogues of alloxazines (**202**), where they are present in an equilibrium with isoalloxazines (**203**)[314]; the same may happen with **201**. Lumichrome (**201**)[315], 1-methyllumichrome (**204**) [316] and the recently reported 1-(*α*-Ribofuranosyl)-lumichrome (**205**) were isolated from the *Micromonospora* sp. Tü 6368[312,313]. Lumichrome (**201**), a metabolite of riboflavin (**206**), competed with ^{3}H-labeled tetrachlorodibenzodioxin (**207**) for binding to the cytosolic TCDD receptor. Neither riboflavin nor riboflavin 5-phosphate competed for binding to the TCDD receptor in rat liver cytosol. Lumichrome was reported as the first endogenous chemical in the rat exhibiting affinity for the TCDD receptor[317].

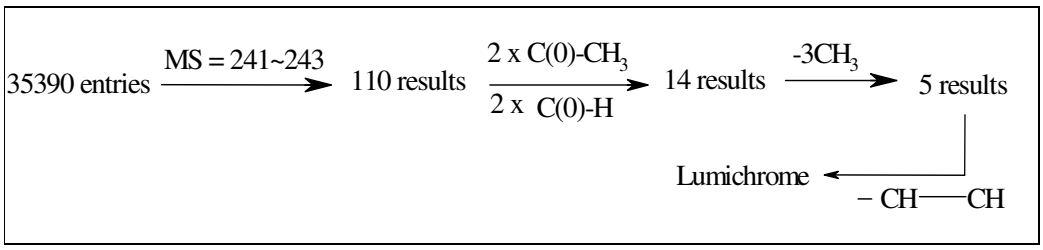


Figure 115: AntiBase search of Lumichrome (**201**).

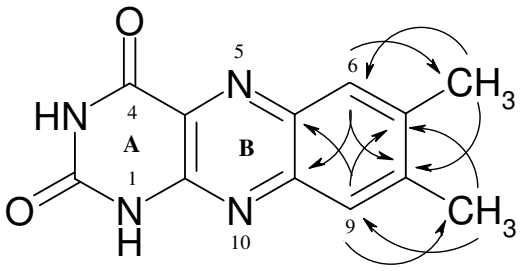
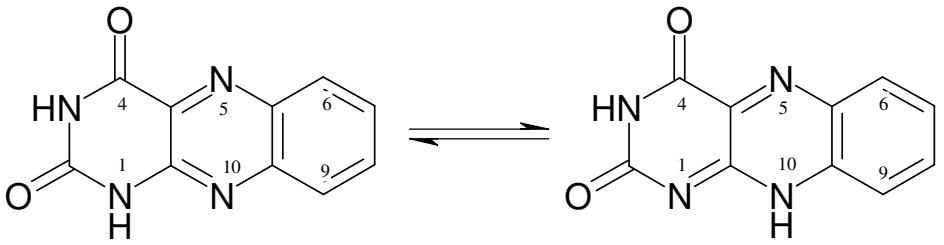


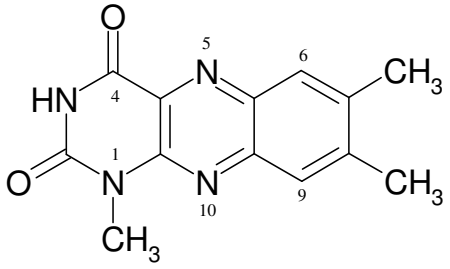
Figure 116: HMBC (→) connectivities of Lumichrome (**201**).

Table 29: ^{1}H and ^{13}C NMR assignments of Lumichrome (**201**) in DMSO-d_6.

Position	δ_C (75 MHz)	δ_H (300 MHz)	Position	δ_C (75 MHz)	δ_H (300 MHz)
1	-	11.65 (s br)	7	138.6	-
2	149.8	-	7-CH_3	19.3	2.46 (s)
3	-	11.65 (s br)	8	144.4	-
4	160.3	-	8-CH_3	20.0	2.49 (s)
4a	129.9	-	9	125.7	7.70 (s)
5a	138.2	-	9a	141.5	-
6	128.5	7.90 (s)	10a	146.2	-



202 **203**



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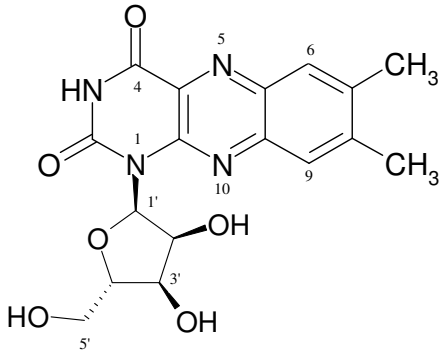

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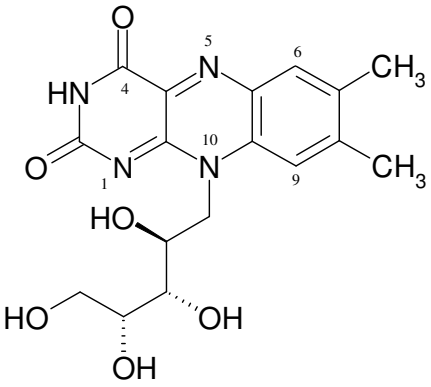

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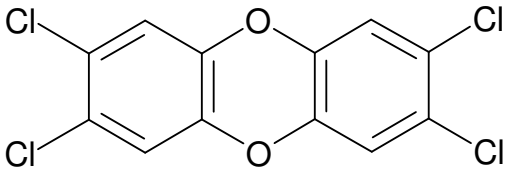
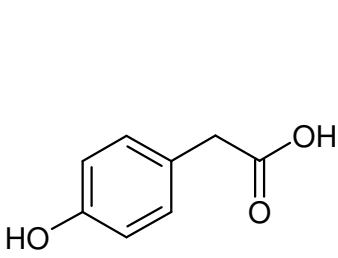


207

5.7.2 Holomycin

Holomycin (**208**) had already previously been isolated from the marine-derived *Streptomyces* sp. M095[152,318] in our group. It was identified on the basis of the same physical and chemical properties and the close similarities in the spectra (NMR, MS).

Holomycin (**208**) is a member of the pyrrothine group and was originally described as antibiotic from *Streptomyces griseus*[319] and *Streptomyces clavuliderus*. More recently, Kirby has discussed the genetic control of the holomycin biosynthesis by *S. clavuligerus*[319]. Due to the pronounced activities of holomycin (**208**) and its analogues thiolutin (**209**), aureothricin (**210**) and isobutyropyrrothine (**211**) against a variety of Gram-positive and Gram-negative bacteria, amoeboid parasites and fungi, many trials to synthesize this type of compounds have been reported[320,321].



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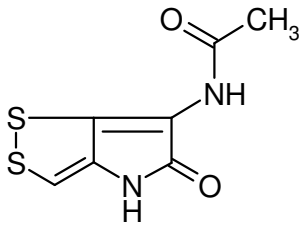
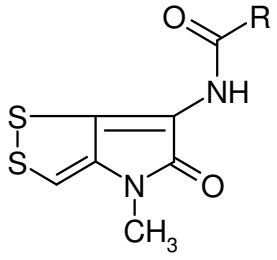


208



209: R = CH_3
210: R = CH_2CH_3
211: R = $CH(CH_3)_2$

5.8 Marine *Streptomyces* sp. B8300

The marine *Streptomyces* sp. B8300 had previously been investigated by Biabani in our group and afforded several metabolites with high bioactivities[322]. However, further compounds were isolated with insufficient amounts for analysis. Hence, the strain was re-cultivate to isolate a further new bioactive components.

TLC of the crude extract obtained from a 20 L shaker culture using M_2^+ medium exhibited a number of yellow, UV absorbing bands. Three further UV absorbing bands turned blue or reddish-brown on spraying with anisaldehyde/sulphuric acid. In the antimicrobial assay, the extract showed a strong activity against *Bacillus subtilis*, *Escherichia coli, Streptomyces viridochromogenes* (Tü 57) and *Candida albicans,* moderate activity against *Chlorella vulgaris* and *Chlorella sorokiniana*, while it exhibited no activity against either *Mucor miehei* (Tü 284) or *Staphylococcus aureus* (Table 73).

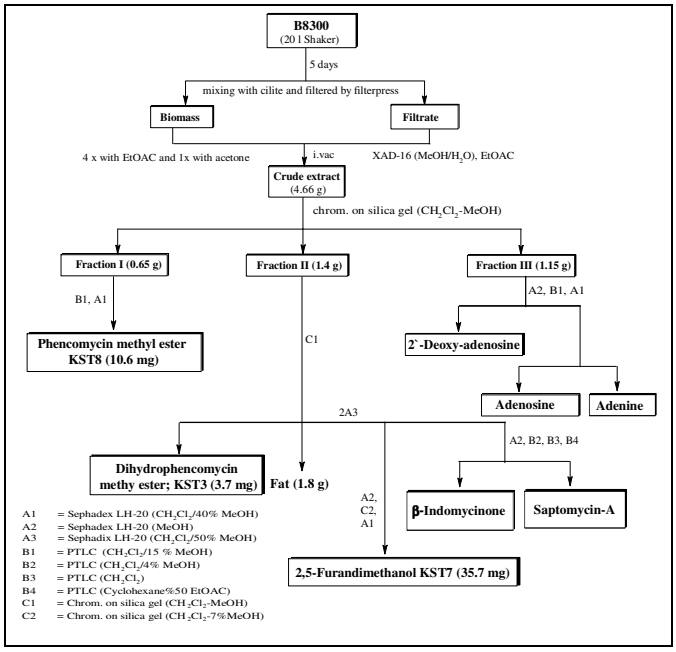


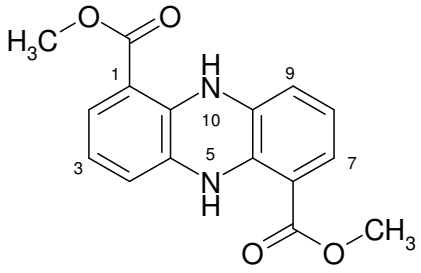
Figure 117: work-up procedure of marine *Streptomyces* sp. B8300

Purification of the extract was carried out by silica gel column chromatography and PTLC followed by size exclusion chromatography (Figure 117). As a result, eight compounds were isolated: 2,5-furandimethanol, dihydrophencomycin methyl ester (**212**), phencomycin methyl ester (**215**), *β*-indomycinone (**219**), saptomycin A (**220**), adenine[152], adenosine[152] and 2'-deoxy-adenosine[152].

5.8.1 Dihydrophencomycin methyl ester

Compound **212** was obtained as a low polar reddish-brown solid which exhibited no colour change on treatment with diluted solution of sodium hydroxide, excluding *peri*-hydroxyquinones. The EI mass spectrum exhibited a molecular weight of 298 Dalton. The molecular ion showed a loss of methanol and CO to give the fragment ions at *m/z* 266 and *m/z* 238.

The ^{1}H NMR spectrum showed one broad singlet of an acidic proton (δ 8.74), three downfield 1H signals (δ 6.98~6.13) belonging to a 1,2,3-trisubstitued aromatic ring, and the singlet methoxy group at δ 3.82. ^{13}C NMR & HMQC spectra of compound **212** displayed 8 carbon signals, of which three sp^2 methines, three quaternary carbon signals and a CO signal of an acid derivative (δ 168.4) in addition to the methoxy signal at δ 51.7 was found. These NMR patterns were indicative for a dihydrophenazine skeleton, consisting of two symmetrical 1,2,3-trisubstituted aromatic residues, each containing a carboxymethyl group. So, two possible structures were suggested: 5,10-Dihydrophenazine-1,6-dicarboxylic acid dimethyl ester (**212**) and 5,10-dihydro-phenazine-1,9-dicarboxylic acid dimethyl ester (**213**).



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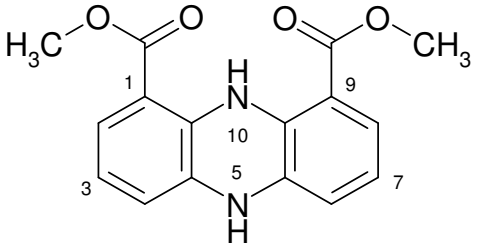


213

The structure of 5,10-dihydrophencomycin methyl ester (**212**) was finally confirmed by direct comparison with authentic spectra from our collection[139]. 5,10-Dihydrophencomycin methyl ester (**212**) was isolated previously in our research group from an unidentified marine *Streptomyces* sp. The compound had weak antibacterial activity against *E. coli* and *B. subtilis.*

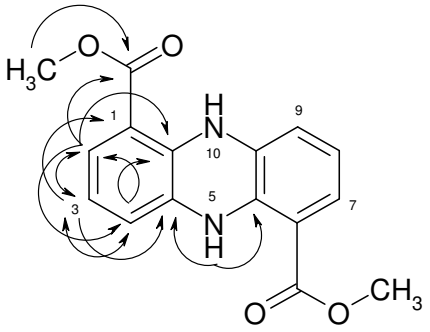


Figure 118: H,H COSY (↔) and HMBC (→) couplings of Dihydrophencomycin methyl ester (**212**).

Table 30: ^{13}C and ^{1}H NMR spectroscopic data for compounds **212** and **215** in $CDCl_3$ (*J* in [Hz]).

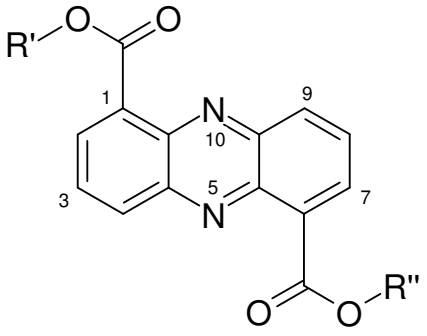
Position	Dihydrophencomycin methyl ester (**212**)		Phencomycin methyl ester (**215**)	
	δ_C (125 MHz)	δ_H (300 MHz)	δ_C (75 MHz)	δ_H (300 MHz)
1, 6	108.7	-	131.1	-
1, 6-CO	168.4	-	166.8	-
OCH_3	51.7	3.82 (s)	52.7	4.11 (s)
2, 7	122.5	6.98 (dd, 8.3, 1.4)	132.9	8.29 (dd, 6.9, 1.4)
3, 8	119.3	6.29 (t, 8.3)	129.5	7.89 (dd, 8.8, 6.9)
4, 9	134.3	6.13 (dd, 8.3, 1.3)	134.3	8.48 (dd, 8.8, 1.4)
4a, 9a	132.6	-	142.9	-
5, 10	-	8.74 (brs)	-	-
5a, 10a	138.4	-	140.9	-

5.8.2 Phencomycin methyl ester

Compound **215** was isolated as low polar yellow crystals from fraction I using PTLC and purified on Sephadex LH-20. Compound **215** showed also no colour colour change on treatment with sodium hydroxide, excluding *peri*-hydroxyquinones as in **212**. The molecular weight of **215** was determined by EI MS as 296 Dalton, 2 amu less than compound **212**. The molecular ion showed an expulsion of a methoxy group, affording a fragment ion at *m/z* 265. The latter fragment displayed a further splitting of carbonyl group to give a peak at *m/z* 238 as in **212**, as indicative of the presence of phenazine skeleton in **215**. HRESI MS confirmed the molecular formula as $C_{16}H_{12}N_2O_4$.

The ^{1}H NMR spectrum of **215** showed the same spectral pattern as **212** except that the acidic NH protons were missing. So, a 1,2,3-trisubstitued aromatic ring, an aromatic bound methoxy group (δ 4.11) were fixed.

The ^{13}C NMR spectrum of **215** showed three sp^2 methines (δ 134.3, 132.9, 129.5), two signals of quaternary sp^2 carbons (δ 142.9, 140.9), and one CO signal (δ 166.8) of an acid derivative, besides those of the methoxy signal (δ 52.7). This pointed to phencomycin methyl ester, and the identity was indeed confirmed by comparison with data of an authentic sample[139]. This is the first time that the methyl ester **215** was isolated as a natural product.



214: R' = CH_3, R" = H
215: R' = R" = CH_3
216: R' = R" = H

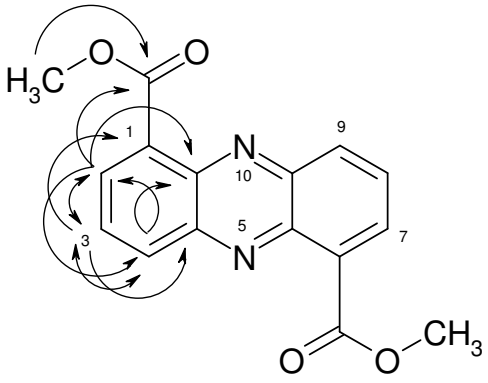
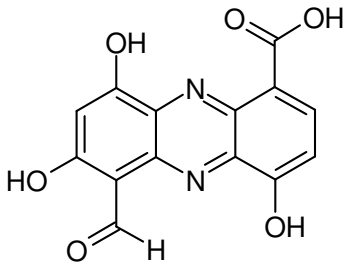


Figure 119: H, H COSY (↔) and HMBC (→) couplings of Phencomycin methyl ester (**215**).

To exclude the possibility that **212** and **215** were formed as artefacts during isolation, the extracts were worked up carefully avoiding any methanol as solvent. Even under these conditions, both esters were isolated again, indicating that **212** and **215** are natural products indeed.

Phenazine-1,6-dicarboxylic acid (**216**) inhibits xanthine oxidase, shows antibiotic properties, and is an intermediate in the biosynthesis of lomofungin (**217**) pro-

duced by *Pseudomonas* and *Streptomyces* sp.[105,279]. Phencomycin (**214**) was isolated from *Streptomyces* sp. HIL Y-9031725[280].



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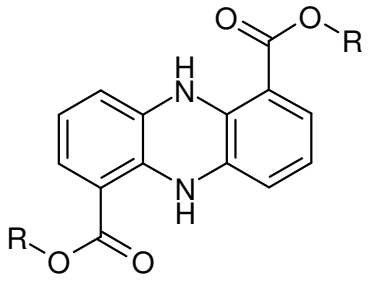


218: R = H, **212**: R = CH_3

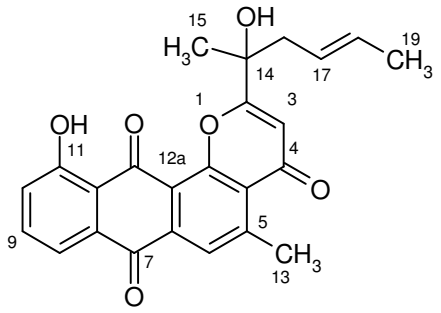
Due to the interesting biological activities of naturally occurring phenazines[139, 323-326], the synthesis of poly-substituted phenazines was developed by Holliman and co-workers[327] from the reductive cyclization of *o*-nitrodiphenylamines. However, the yield was poor due to the competitive cyclization. *N*-arylation has become more accessible owing to the advent of a new methodology developed by Hartwig and Buchwald[328]. The synthesis of phenazines were developed using sequential aniline arylation (palladium(II)-catalyzed intramolecular amination)[329].

5.8.3 β-Indomycinone

From sub-fraction II, compound **219** was isolated as a yellow solid. It was UV absorbing at 254 nm and showed a red fluorescence at 366 nm, and turned to red-violet by treating with 2N NaOH as indication of a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of **219** revealed singlets at δ 12.85 (OH) and δ 8.08, three *ortho*-coupled aromatic protons at δ 7.83, 7.70 and 7.38, a 1H singlet at δ 6.56. In addition, two multiplets of an olefinic double bond were detected (δ 5.74 and 5.38), and a methyl singlet at δ 3.02. Two methylene protons of an ABX system were observed (δ 2.91 and 2.78), together with two methyl (1 s, 1 d) linked to sp^2 or oxygenated carbons (δ 1.72 and 1.64).

The ESI mass spectrum resulted in a molecular weight of **219** as 404 Dalton. A search in AntiBase resulted in β-Indomycinone (**219**). β-Indomycinone (**219**) belongs to the pluramycins, a group of structurally highly diverse reactive agents, possessing antimicrobial and anticancer activity[330].



219

5.8.4 Saptomycin-A

A second *peri*-hydroxyquinone was obtained as yellow solid from sub-fraction II by PTLC followed by Sephadex LH-20. It showed similar physiochemical properties as **219**. The ESI mass spectrum fixed the molecular weight of compound **220** as 404 Dalton as an isomer of ß-indomycinone (**219**).

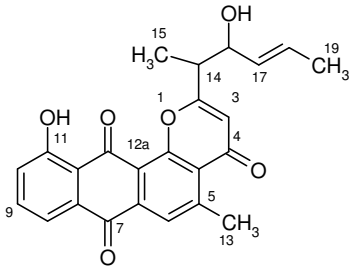
The ^{1}H NMR spectrum showed nearly the same signal pattern as *β*-Indomycinone (**219**). A singlet of a *peri*-hydroxy group at δ 12.64 was exhibited. In the aromatic region, a 1H singlet at δ 8.09, three aromatic protons (δ 7.83, 7.69 and 7.36) of an 1,2,3-trisubstituted aromatic ring, and a singlet of H-3 (δ 6.28) were shown. In addition, a singlet of a *peri*-methyl group (δ 3.02) among with a multiplet of an olefinic double bond (2 H, δ 5.40) was observed. Moreover, two methine protons were displayed at δ 5.40 and 2.98. The first methine group was oxygenated, while the second methine was flanked probably two sp^2 carbons. Finally, two methyl doublets were detected (δ 1.71 and 1.45), whereof the first was attached to an sp^2 carbon.

The corresponding molecular formula of compound **220** was determined as $C_{24}H_{20}O_6$, according to the Rule of 13[331]. Accordingly, compound **220** exhibited close structural similarity to the hydroxyquinone mentioned above. Based on the spectroscopic data, a search in AntiBase, as well as by comparison with authentic spectra and the literature, compound **220** was established as saptomycin A[332].

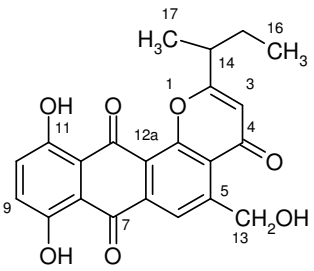
Pluramycins are metabolites containing the *4H*-anthra[1,2-*b*]pyran-4,7,12-trione nucleus to which amino sugars typically are attached at C-8 and C-10 positions. They are frequently isolated from terrestrial *Streptomycetes* sp.[322,333,334]. Pluramycins are

used as neuronal cell protecting substances, and able to suppress the toxicity of L-glutamate in N18-RE105 cells with an EC_{50} value 40 nM[335].

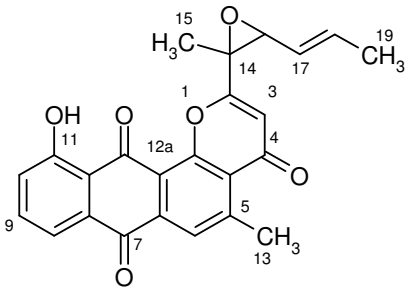
Several pluramycins analogues were isolated in our research group. For example espicufolin B (**221**), ε-indomycinone (**221**), SS 43405E (**223**), espicufolin (**224**), α-indomycinone (**225**), β-indomycinone (**219**), saptomycin-A (**220**), γ-indomycinone (**226**) and saptomycin-F (**221**) were isolated from the culture broths of the marine (B5543) and terrestrial *Streptomyces* sp. (GW3/1130)[**83**,**90**,336].



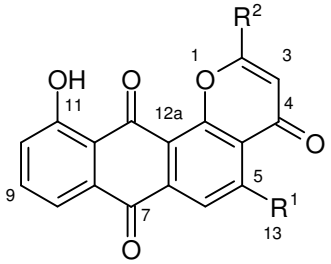
220



221



222



223:	$R^1 = CH_3$	$R^2 = CH(CH_3)CH_2CH_3$
224:	$R^1 = CH_2OH$	$R^2 = CH(CH_3)CH_2CH_3$
225:	$R^1 = CH_3$,	$R^2 = C(CH_3)=CH=CH(CH_3)$
226:	$R^1 = CH_3$,	$R^2 = C(OH)CH_3)CH_2CH_3$
227:	$R^1 = CH_3$,	$R^2 = C(CH_3)=CH(CH_3)$

5.9 Marine *Streptomyces* sp. B7828

The chemical screening of the marine *Streptomyces* sp. B7828[364] extract on TLC showed an UV absorbing spot at 254 nm which stained to blue by spraying with anisaldehyde/sulphuric acid; a middle polar blue fluorescent band and some zones without UV activity stained to blue by anisaldehyde/sulphuric acid, in addition to a middle polar blue fluorescent band which stained to yellow by anisaldehyde/sulphuric acid.

The antimicrobial assay of the extract showed weak activity against *Streptomyces viridochromogenes* (Tü 57) and *Staphylococcus aureus,* while it exhibited no activity against *Mucor miehei* (Tü 284), *Bacillus subtilis*, *Escherichia coli* as well as the algae *Chlorella vulgaris*, *Chlorella sorokiniana, Scenedesmus subspicatus*, and no activity against *Candida albicans*, (Table 74).

On cultivation on M_2^+ medium at 28 °C, the marine isolate B7828 formed a brown-yellow culture broth, which was filtered over Celite and adsorbed on Amberlite XAD-2, while the mycelium was extracted with acetone affording a brown crude extract.

TLC-directed work-up of the water extract by silica gel column chromatography and size exclusion chromatography resulted in 4-hydroxy-2,5,6-trimethyl-octan-4-olide (**230**), 4,5-dihydroxy-2,5,6-trimethyl-octan-4-olide (**231**), and 3,4-dihydroxy-2,5,6-trimethyl-octan-4-olide (**232**) as colourless oily substances. The mycelial extract delivered the known compounds, 1-acetyl-β-carboline (**228**)[337], 2'-deoxyuridine, tyrosol (**229**), and 1-hydroxy-4-methoxy-2-naphthoic acid (**71**) (Figure 120).

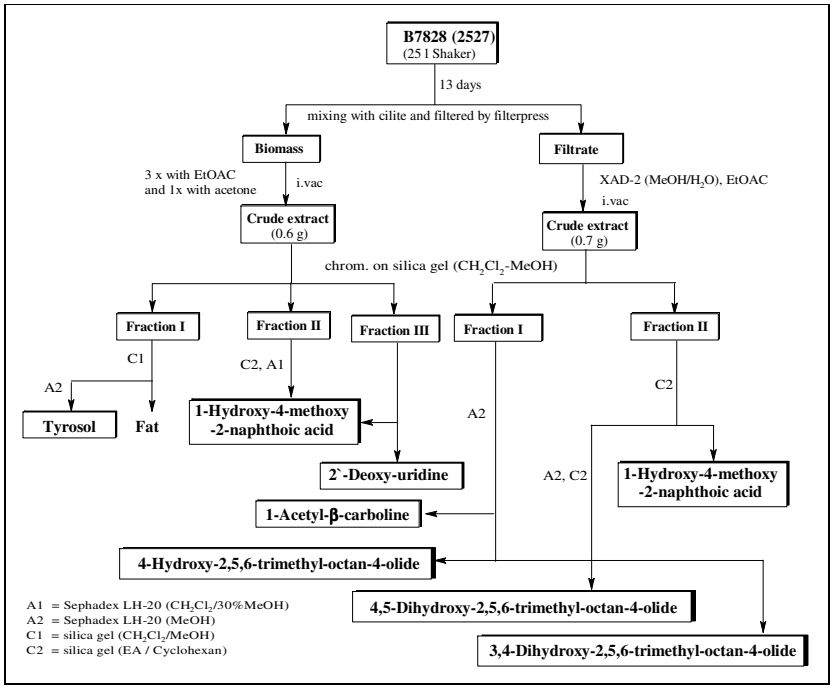
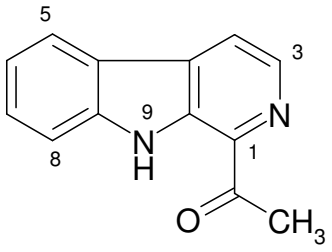
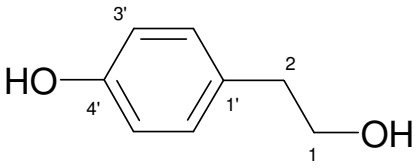


Figure 120: Work-up procedure of marine *Streptomyces* sp. B7828



228



229

5.9.1 4-Hydroxy-2,5,6-trimethyl-octan-4-olide

Compound **230** was obtained as a middle polar colourless oil, which was not UV absorbing and turned to blue with anisaldehyde/sulphuric acid. The molecular weight was determined by ESI mass spectra as *m/z* 184. EI HRMS established the molecular formula as $C_{11}H_{20}O_2$.

The proton NMR spectrum showed neither aromatic nor olefinic protons. One oxy-methine proton (δ 3.95, dd), three methyl doublets (δ 1.30, 0.90 and 0.86, J ~7 Hz) and one triplet (δ 0.90) were detected. Additionally, three methine multiplets were observed at δ 2.61 and 2.14, indicative for carbons next to sp^2 carbons or oxygenated groups; the third one absorbed at δ 1.26~1.11. Finally, two methylene multiplets were observed in the regions of δ 1.98-1.83 and 1.74-1.57.

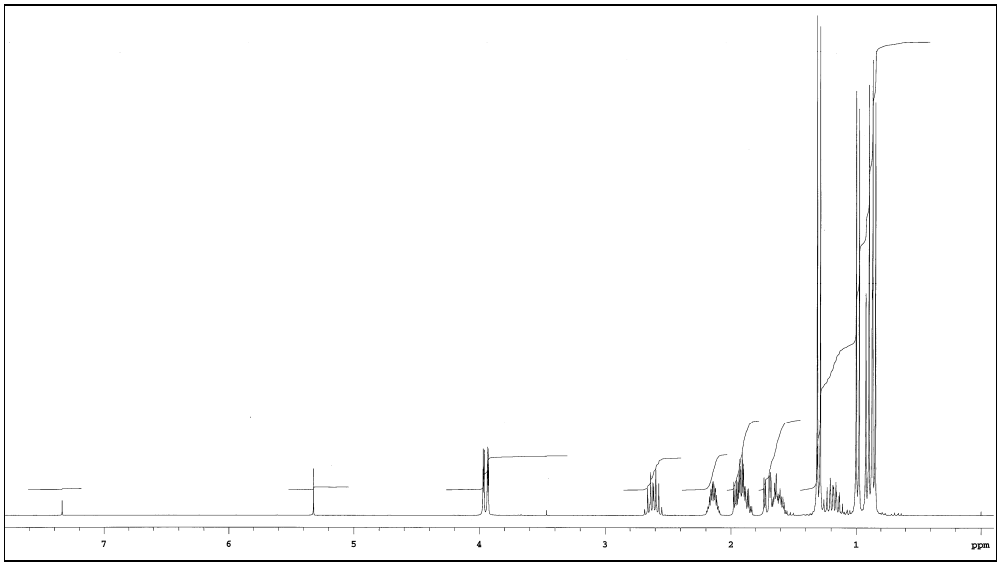


Figure 121: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 4-Hydroxy-2,5,6-trimethyl-octan-4-olide (**230**).

The ^{13}C NMR and HMQC spectra of **230** displayed 10 carbon signals, one carbonyl (δ 174.0) of an ester or amide, one oxygenated carbon (C-4, 86.8), four methine carbons (δ 36.0, 35.8, 30.8, 27.4); further on, two methylene carbons (δ 36.0 and 24.6) along with three carbon signals of four methyl carbons (δ 17.4, 13.4 and 10.0 [$2CH_3$]) were detected (Table 31). One of the two double bond equivalents of **230** was due to a carbonyl, the other one came from a lactone ring.

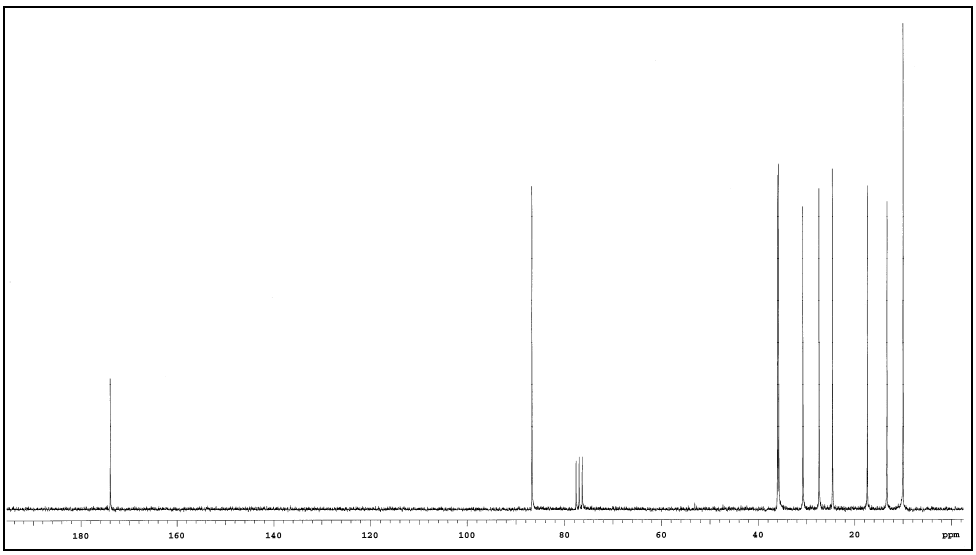


Figure 122: ^{13}C NMR spectrum ($CDCl_3$, 50 MHz) of 4-Hydroxy-2,5,6-trimethyl-octan-4-olide (**230**).

The structure was fully confirmed by 2D NMR correlations (Figure 123). The methine, methylene and methyl protons of **230** showed cross signals with each other, confirming the partial structure, [(-X-CH(CH_3)-CH_2-CH(O)-CH(CH_3)-CH(CH_3)-CH_2-CH_3)]. The methyl doublet (δ 1.30) displayed a ^3J coupling to the carbonyl (δ 174.0) confirming their attachment *via* the methine carbon C-2 (δ 30.8). Though no ^3J coupling directing from the oxymethine (δ_H 3.95, δ_C 86.8) to the carbonyl carbon (δ 174.0) was detected, the sole option for ring closure is lactonization. The absent direct correlation from the oxymethine to the carbonyl was also observed in many other butanolides, some of which were previously obtained by our group[97,345] or will be discussed below. This established the compound as 4-hydroxy-2,5,6-trimethyl-octan-4-olide (**230**), which is a new natural product.

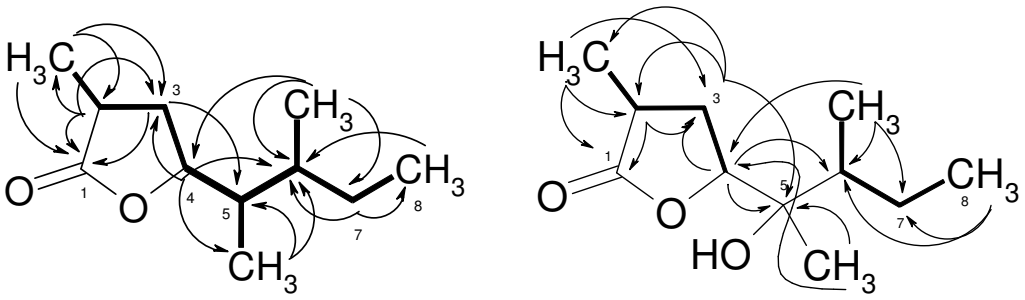


Figure 123: H,H COSY (▬) and HMBC (→) correlations of γ-Butyrolactones (**230**) and (**231**).

The relative stereochemistry of γ-butyrolactone **230** was partially established on the basis of NOESY experiments. One of the methylene protons at C-3 showed a coupling with the C-2 methyl group and additionally with H-4, resulting in a (2*S**, 4*R**) configuration. The configuration of the flexible side chain remains open.

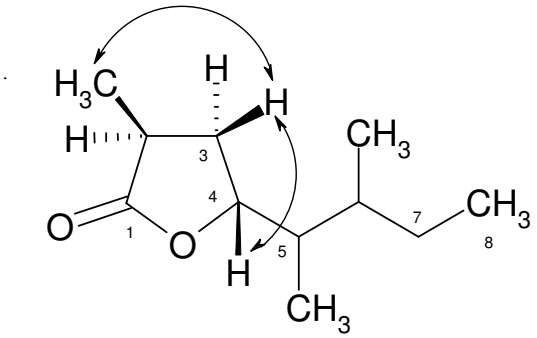


Figure 124: Diagnostic NOESY correlations of γ-Butyrolactone **230**.

5.9.2 4,5-Dihydroxy-2,5,6-trimethyl-octan-4-olide

Compound **231** was obtained as colourless oil from fraction II, showing the same color reaction on TLC as **230**, but with a higher polarity. The molecular weight of **231** was deduced by CIMS as *m/z* 200 with 16 au more than for **230**. HRMS of **231** confirmed its molecular formula as $C_{11}H_{20}O_3$, i.e. with one oxygen more. The formula indicated 2 double bond equivalents as in **230**.

The ^{1}H NMR spectrum of **231** exhibited the same pattern as **230**, except that one of the three methyl doublets in **230** was substituted by a methyl singlet at δ 1.40. Additionally, a hydroxy group was present due to the existence of a broad singlet at δ 2.25.

In the ^{13}C NMR/HMQC spectra, a carbonyl at δ 180.2 and the lactone oxymethine (δ 79.9) pointed again to a butanolide. A quaternary carbon (δ 87.5) was observed, while the methine carbon C-5 (δ 27.4) of **230** was not present (Table 31). Based on the 2D correlations, the methyl singlet at δ 1.40 was confirmed to be at C-5 (δ 87.5) due to its direct coupling with C-5 (δ 87.5) and the oxymethine C-4 (δ 79.9).

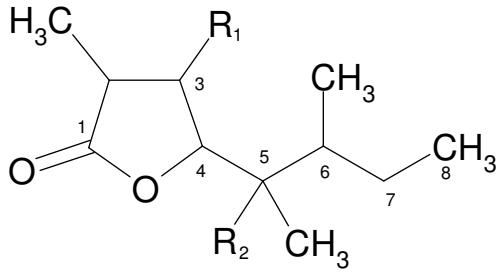
The structure determination of **231** by 2D couplings is shown in Figure 123. From these data, compound **231** was confirmed to be 4,5-dihydroxy-2,5,6-trimethyl-octan-4-olide, which is a new γ-butyrolactone.

5.9.3 3,4-Dihydroxy-2,5,6-trimethyl-octan-4-olide

Compound **232** was obtained as colourless oil from a slightly higher polar third fraction, having the same colour reaction on TLC as γ-butyrolactones **230** and **231**. The molecular weight of **232** was deduced by CIMS to be the same as for **231** (200 Dalton), that is 16 amu more than **230**. HRMS of **232** confirmed the same molecular formula as of **231** ($C_{11}H_{20}O_3$). This pointed to a structural isomer of **231**.

The ^1H NMR data of **232** indicated similarly the existence of a terminal ethyl group due to the methyl triplet at δ 0.92, along with the presence of three methyl doublets (δ 1.33, 0.90, 0.89) as in **230**. In addition to the oxymethine dd of the lactone moiety (H-4, δ 3.95), another dd oxymethine proton was observed at δ 3.75. The sole position to insert this new hydroxy-methine is in the lactone ring by replacing the methylene group (CH_2-3) present in **230** and **231** (Table 7).

This established compound **232** as 3,4-dihydroxy-2,5,6-trimethyl-octan-4-olide. A search in databases indicated **232** as a new compound.



230: R_1 = H R_2 = H
231: R_1 = H R_2 = OH
232: R_1 = OH R_2 = H

Table 31: ^{1}H and ^{13}C NMR assignments of γ-Butyrolactones **230-232**.

Position	γ-Butyrolactone (**230**)[a]		γ-Butyrolactone **231**[a]		γ-Butyrolactone **232**[b]
	δ_C[c]	δ_H (*J* in Hz)[d]	δ_C[c]	δ_H (*J* in Hz)[d]	δ_H (*J* in Hz)[d]
1	174.0	-	180.2	-	-
2	30.8	2.61 (m)	36.2	2.96-2.78 (m)	2.43-2.33 (m)
2-CH_3	17.4	1.30 (d, 7.1)	17.3	1.28 (d, 7.1)	1.33 (d, 7.1)
3	36.0	1.98-1.83 (m, H_a) 1.74-1.57 (m, H_b)	38.3	2.96-2.78 (m, H_a) 1.66-1.45 (m, H_b)	3.75 (dd, 10.4, 4.3)
4	86.8	3.95 (dd, 10.4, 2.4)	79.9	3.53 (d, 4.4,)	3.95 (dd, 10.3, 2.1)
5	27.4	2.14 (m)	87.5	-	1.91-1.78 (m)
5-CH_3	10.0	0.99 (d, 7.1)	25.0	1.40 (s)	0.90 (d, 7.1)
5-OH	-	-	-	2.25 (brs)	-
6	35.8	1.74-1.57 (m)	36.3	1.66-1.45 (m)	2.27-2.18 (m)
6-CH_3	13.4	0.86 (d, 6.8)	17.1	1.01 (d, 6.9)	0.89 (d, 6.8)
7	24.6	1.98-1.83 (m, H_a) 1.26-1.11 (m, H_b)	23.4	1.66-1.45 (m, H_a) 1.34-1.19 (m, H_b)	1.72-1.62 (m, H_a) 1.28-1.12 (m, H_b)
8	10.0	0.90 (t, 7.5)	11.6	0.91 (t, 7.5)	0.92 (t, 7.5)

[a]$CDCl_3$; [b]CD_3OD; [c] (50 MHz); [d](300 MHz)

The structures **230**-**232** are very simple and butanolides in general are rather common natural products, however, simple 2,3- or 2,4-dialkylated butanolides have not often been described from bacteria, and also from fungi, very few related butanolides are known. The various protolichesterinic acids, phaseolinic acid,[338] nephrosteranic acid[339] and a few other long-chain butanolides from lichens and some fungi, are having the closest similarity with **230**-**232**; they carry, however, an additional carboxy group at C3. Among the less than 20 related plant products, some are showing a surprising similarity with **230**-**232**: in the murrayacoumarins or the 6,7-dihydroclauslactones, butanolides of type **230**-**232** are connected with a coumarine system *via* an ether bond [340,341]. It is also surprising, that the opposite substituent pattern – methyl at C-4 and an alkyl residue at C-2 as in the virginiamycin inducing factors NFX-2 - NFX-4 has been described from bacteria more than ten times in the past [405,342, 343].

All three butanolides were inactive in the agar diffusion test at 40 μg/disk against selected bacteria, fungi, and microalgae[344]. They had also no influence on the spore formation of *Streptomyces viridochromogenes* (Tü 57) or of the producing strain. However, in the cytotoxcicity test, compound **230** displayed an unselective cytotoxicity against a range of human tumor cell lines with a mean IC_{50} of 22.09 μM (mean $IC_{70} > 10$ μM); Table 75.

5.10 Further γ-Butyrolactones from Different Marine Streptomycetes

α,β-Unsaturated γ-butyrolactones are low polar UV absorbing colourless oils. They are mostly detected on spraying with anisaldehyde/sulphuric acid as violet spots, which changed later to blue. Saturated γ-butyrolactones exhibited the same colour reaction on TLC of their α,β-unsaturated analogues, except that they are not UV absorbing.

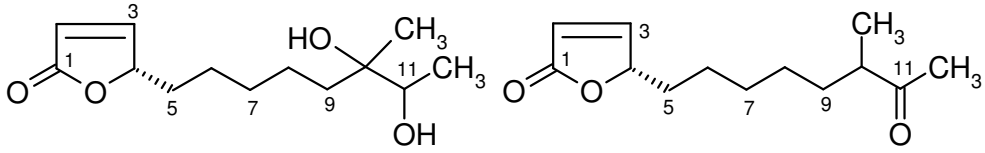
As part of a project to discover new secondary metabolites from bacteria, we examined the crude extracts obtained from the fermentation of several marine derived *Streptomyces* sp. In accordance, from the marine-derived *Streptomyces* sp. Mei37 and Mei02-8,1, the α,β-unsaturated γ-butyrolactones and (4*S*)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (**233**) and 4-hydroxy-10-methyl-11-oxododec-2-en-1,4-olide (**236**) were obtained, respectively, while the marine *Streptomyces* sp. B8108 yielded 4,9-dihydroxy-9-methyl-decan-4-olide (**237**). On the other hand, the marine-derived *Actinomyces* sp. Act8015 exhibited high productivity affording virginiae butanolide E (**242**), Graefe's Factors I (**249**) and III (**250**) in addition to the new butanolides 4,10-dihydroxy-10-methyl-dodecan-4-olide (**251**) along with its respected acid 4,10-dihydroxy-10-methyl-dodecanoic acid (**252**).

5.10.1 (4S)-4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide

Purification of the low polar sub-fraction IIB on silica gel followed by Sephadex LH-20 delivered compound **233** (see p. 133). It was a colourless UV absorbing oil, which turned violet on spraying with anisaldehyde/sulphuric acid and later changed to blue. The (+)-ESI mass spectra of **233** exhibited two *quasi*-molecular ions at *m/z* 507 ($[2M+Na]^+$) and 265 ($[M+Na]^+$), indicating the molecular weight as 242 Dalton.

The ^{1}H NMR spectrum showed two 1H dd signals each at δ7.44 and 6.10 (J~5.6, 1.9 Hz) in the sp^2 region. The small splitting of the olefinic protons established their location in a five membered heterocyclic system (e.g. furan). Two other 1H multiplets were observed at δ4.98 and 3.58, corresponding to two oxy-methines. Moreover, a methyl doublet at δ 1.14 in addition to another singlet at δ 1.09 were observed. Finally, a broad multiplet with an intensity of 10H was observed in the range δ1.78-1.20, as indicative of a long chain containing 5 methylene groups.

The ^{13}C and APT NMR spectra indicated thirteen carbon signals, which were classified into five methylenes (δ 38.7~22.8), four methines, two among them as olefinic (δ 156.6, 121.2), while the remaining two were oxygenated (83.4 and 72.6). In addition, two methyl groups (δ 22.8, 20.2), two quaternary carbons corresponding to a carbonyl of acid or ester (δ 173.3), and of an sp^3-oxy carbon (δ 74.7) were observed.



233 **234**

Based on the spectroscopic data, a substructure search in AntiBase delivered (4*S*)-4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**234**), which had been previously isolated in our group by Mukku[345]. The difference in the molecular weights of Δm = 18 between **234** and our component (**233)** was attributed to the substitution of the C-10 methine proton in **234** by a hydroxyl group, while the acetyl group (C-11) was reduced to give the corresponding secondary alcohol in **233**. The structure was further confirmed on the bases of 2D experiments (H,H COSY and HMBC, Figure 125). The absolute stereochemistry at C-4 was confirmed by CD spectra of compound **233** as *S*-configuration by comparison with the CD spectrum of the related butenolid **234**. Therefore, compound **233** was identified as (4*S*)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide, a new natural product. The compound was also isolated in parallel from the marine-derived *Streptomyces* sp. Mei35 in our research group together with 4,10-dihydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**235**)[97].

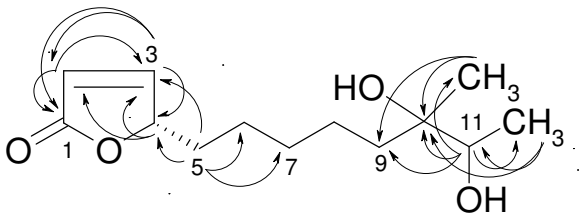
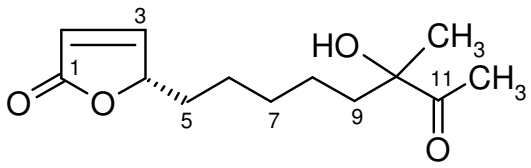


Figure 125: Selected HMBC (→) correlations of (4*S*)-4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide (**233**).

(4*S*)-4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide (233) showed a moderate and rather selective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 9.759 µM (mean IC_{70} = 10.265 µM), Table 62.



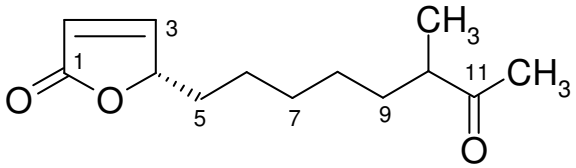
235

5.10.2 4-Hydroxy-10-methyl-11-oxododec-2-en-1,4-olide

Compound **236** was obtained as colourless oil from fraction I, showing a weak UV-absorbance (see p. 174). On spraying with anisaldehyde/sulphuric acid, it turned to violet and later changed to blue. ESI MS indicated a molecular weight of 224 Dalton.

The ^{1}H NMR spectrum showed two 1H dd signals of a double bond (δ7.48, 6.12), and the coupling constant pointed to a heterocyclic five memberd ring (e.g. a furan). At δ5.06 an additional signal (m) of an oxymethine proton was observed. In the aliphatic region, a methine multiplet (δ2.52) and doublet methyl (δ1.10) were detected, corresponding to $CHCH_3$. Furthermore, a methyl singlet at δ2.13 of an acyl group and a multiplet of ten protons between (δ1.80-1.20) of a long chain of methylene were visible.

A search in AntiBase using the NMR data and the molecular weight pointed to 4-hydroxy-10-methyl-11-oxododec-2-en-1,4-olide (**236**). This compound was previously isolated by Mukku in our research group[345]; identity was shown by direct comparison.



236

Butenolides (mainly but-2-enolides) are widespread in fungi[346], bacteria[347] and higher forms of life, e.g. gorgonians[348]. Their saturated analogues act as signal-

ling substances in bacteria[349] and enhance the spore formation in streptomycetes or induce metabolite production[13]. They were inactive in antibacterial tests.

5.10.3 4,9-Dihydroxy-9-methyl-decan-4-olide

Compound **237** was isolated as low polar colourless oil, showing no UV absorbance (see p. 167). On spraying with anisaldehyde/sulphuric acid, the compound turned blue and later changed to pink. The molecular weight of **237** was established as 200 Dalton by (+)-ESI-MS. ESIHR-MS confirmed the molecular formula of **237** as $C_{11}H_{20}O_3$, bearing two double bond equivalents.

The ^{1}H NMR spectrum established the existence of a multiplet of an oxymethine proton at δ 4.27. In addition two other multiplets were observed, the first of them was of 2 H at δ 2.65, while a further 11H multiplet was in the region of δ 2.00-1.40, which belonged to 5 methylene groups and 1 acidic hydroxyl proton. Furthermore, two singlets of methyl groups were detected at δ 1.23 and δ 1.22.

The ^{13}C NMR/HMQC spectra of **237** displayed 11 carbon signals, one ester carbonyl at δ 175.8 and two oxy-carbons (δ 80.7 and 70.5), the first of them corresponded to an oxymethine. In addition, 6 methylene (δ 39.2-23.0) and two methyl carbons (δ 29.7 and 28.9) were detected.

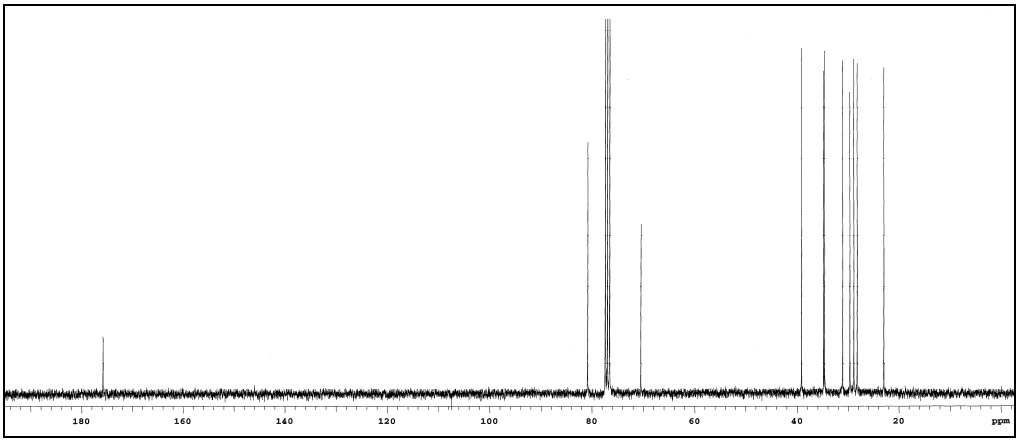


Figure 126: ^{1}H NMR spectrum ($CDCl_3$, 75 MHz) of 4,9-Dihydroxy-9-methyl-decan-4-olide (**237**).

Based on the HMBC correlations of **237** (Figure 127), the methyl singlets (δ 1.23, 1.22) were linked to the oxy-carbon at δ 70.5 *via* ^2J coupling. Also, the two

methyls directed ^3J cross-signals to the methylene carbon at δ 39.2 establishing the isobutyloxy fragment **A**.

Protons of the methylene multiplet at δ 2.65 (H_2-2) displayed two ^2J correlations to the ester carbonyl C-1 (δ 175.8) and the methylene carbon C-3 (δ 23.0), indicating their direct neighbourhood. This conclusion was supported by H,H COSY data, which further established the linkage between the latter methylene (H_2-3) and the oxymethine H-4 (δ 4.27). In addition, a ^4J cross-signal was directed from H_2-2 (δ 2.65) to the methylene carbon at δ 34.7 (C-5), and an H,H COSY correlation between H_2-5 and H-4 (δ 4.27) was observed, which confirmed their direct linkage. One of the two double equivalents of compound **237** was attributed to a carbonyl moiety, while the other one could be due to a ring closure between the hydroxy methine (H-4) and the carbonyl (C-1), constructing a γ-lactone moiety. This was established in spite of the missing ^3J coupling between the oxymethine proton (δ 4.27) and the ester carbonyl (δ 175.8) [97,345], and hence fragment **B** was obtained.

Partial structures **A** and **B** were joined *via* the two methylenes H_2-6 and H_2-7, resulting in 4,9-dihydroxy-9-methyl-decan-4-olide (**237**), a new γ-butyrolactone derivative. The chemical shifts of the methylene groups were assigned on the basis of ACD calculations.

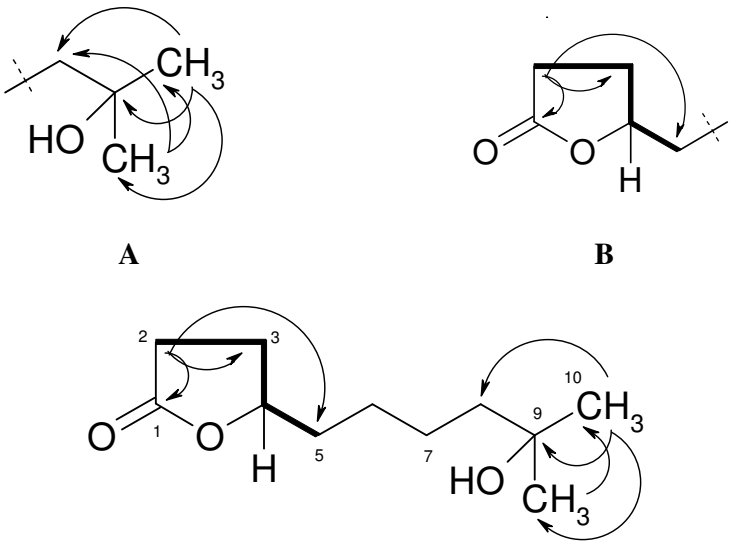


Figure 127: Selected ^{1}H,^{1}H COSY (▬) and HMBC (→) correlations of 4,9-Dihydroxy-9-methyl-decan-4-olide (**237**)

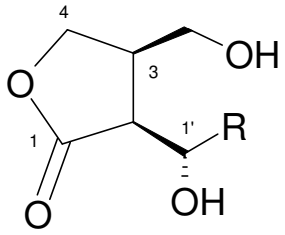
5.10.4 Virginiae butanolide E

Compound **242** was obtained as colourless oil during the purification of fraction II on silica gel followed by Sephadex LH-20 column chromatography (see p. 206). It was not UV absorbing and initially turned violet and later became greenish-blue by spraying with anisaldehyde/sulphuric acid. The molecular weight was determined by ESI-MS. Two *quasi*-molecular ion peaks in positive (*m/z* 239.0 $[M+Na]^+$ and 455.0 $[2M+Na]^+$) and one negative mode (*m/z* 261.0 $[M+HCOO]^-$) confirmed the molecular weight of **242** as 216 Dalton. (+)-HRESI-MS of **242** delivered the molecular formula $C_{11}H_{20}O_4$, entailing two double bond equivalents.

The ^1H NMR spectrum of **242** showed signals for 20 protons, all were located in the aliphatic region; one of the signals (δ2.40) represented two exchangeable protons, a doublet of two equivalent methyls (δ0.88) represented an isopropyl group, two signals at δ3.70 (2 H) and 4.03 were indicative for oxymethylene and a methine proton, respectively. Furthermore, a dd methine signal (δ2.54) besides two protons of another oxy-methylene (δ4.40 t, 4.08 dd), were visible. The remaining six protons located in the region of δ1.50~1.20 as three multiplets were of 3 further methylene groups.

The ^{13}C NMR/HMQC spectra of **242** displayed 11 carbon signals, among them one quaternary carbon (δ178.7) for an ester or acid, an oxy-methine (δ71.1) and two oxy-methylenes (δ 69.5, 63.4). Additionally, three methine signals (δ48.1, 38.1, 27.9), two methylenes (δ34.9 and 32.7) as well as two methyls (δ22.6, and 22.5) appeared.

Based on the spectroscopic data as well as the molecular formula, searching in AntiBase deduced the compound as virginiae butanolide E[13, 350-352]. Virginiae butanolide E (**242**) was fully assigned here for the first time using 2D experiments (H,H COSY and HMBC) (Table 32, Figure 128). Virginiae butanolides A~E (**238~242**), virginiamycin-inducing factors, were previously isolated from *Streptomyces virginiae*. They have 2,3-disubstituted butanolide as basic skeleton[353], bearing two hydroxyl groups in the side chains. They are known as initiators for the production of virginiamycin with minimum concentrations ~1 ng/ml.



238: R = $CH_2CH_2CH_2CH(CH_3)_2$
239: R = $CH_2CH_2CH(CH_3)CH_2CH_3$
240: R = $CH_2CH_2CH_2CH_2CH_3$
241: R = $CH_2CH_2CH_2CH_2CH_2CH_3$
242: R = $CH_2CH_2CH(CH_3)_2$

Table 32: ^{1}H and ^{13}C NMR assignments of Virginiae butanolide E (**242**).

Position	δ_C (75 MHz)	δ_H (300 MHz, *J* in [Hz])
1	178.7	-
2	48.1	2.54 (dd, 7.2, 3.7)
3	38.1	2.81 (m)
3-CH_2	63.4	3.70 (m)
4	69.5	4.40 (t, 8.8, H_b)
		4.08 (dd, 9.0, 6.5, H_a)
1’	71.1	4.03 (m)
2’	32.7	1.57(m)
3’	34.9	1.17-1.35 (m)
4’	27.9	1.57(m)
4’-CH_3	22.6	0.88 (d, 6.6)
5’	22.5	0.88 (d, 6.6)
OH	-	2.40 (brs)

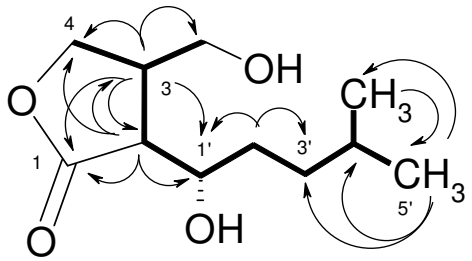
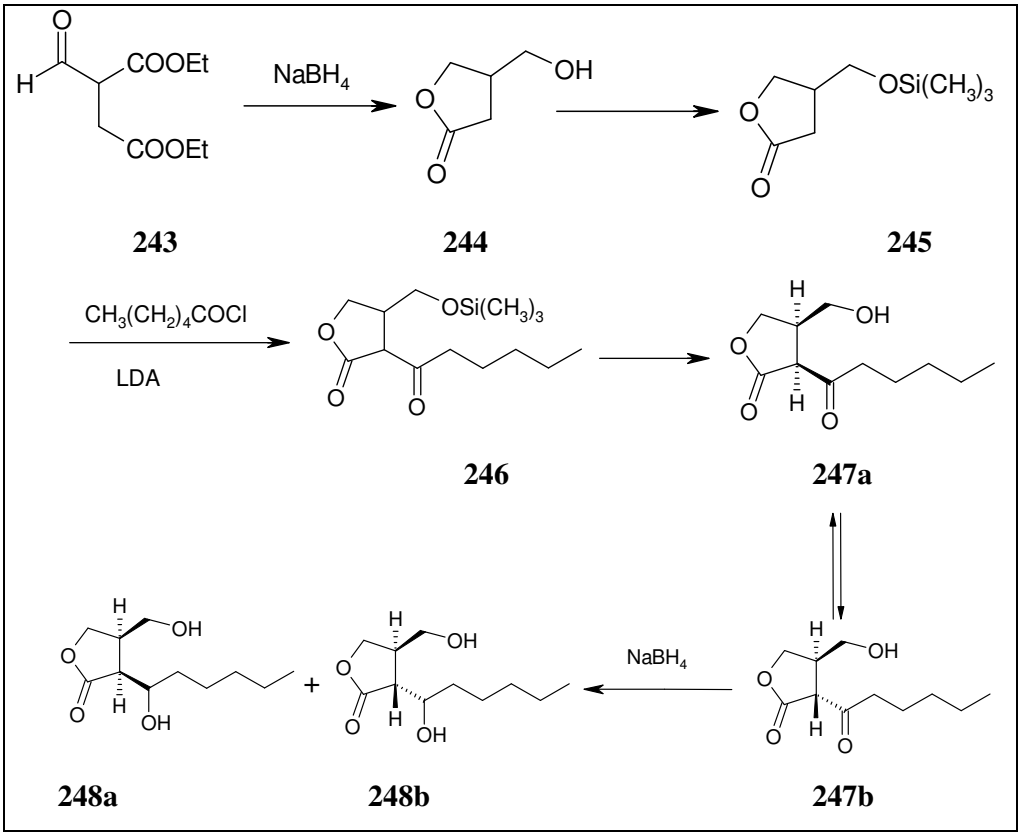


Figure 128: H,H COSY (▬) and selected HMBC (→) coupling in Virginiae butanolide E (**242**).

Virginiae butanolides were synthesized by treatment of diethyl formylsuccinate (**243**) with sodium borohydride to give 3-hydroxymethyl-butanolide (**244**). The hydroxymethyl group of **244** was protected and acylated by LDA and hexanoyl chloride**.** After deprotection, A-factor type compounds (**247a-b**) were obtained as racemic mixture, which afforded after reduction with $NaBH_4$, two hydroxy isomers (**248a** and **248b**) (Scheme 4)[351].

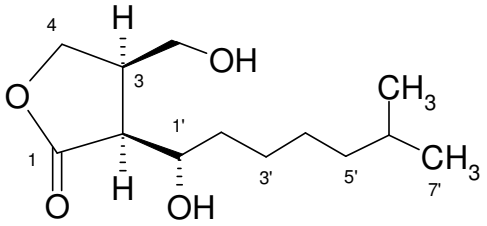


Scheme 4: A general synthetic rout for racemic Virginiae butanolides

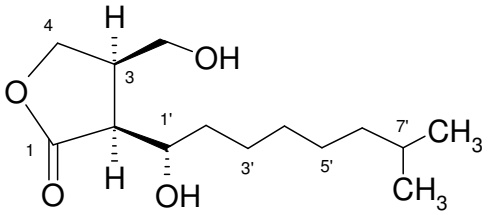
5.10.5 Graefe's Factors I and III

Compounds **249** and **250** were obtained in a mixture (see p. 206) as a colourless not UV absorbing oil, which became blue-violet after spraying with anisaldehyde/sulphuric acid reagent. The (+)-ESI MS spectra afforded their molecular weights as 244 and 258 Dalton.

The ^{1}H NMR pattern of **249** and **250** was closely related to that of **242**. Two methyl doublets (δ0.88) typical for an isopropyl group appeared along with three multiplets of oxygenated methine and methylene protons located at δ4.39, 4.08 and 3.70. The remaining proton signals with integration of 22H located between δ2.81-1.17 were most likely due to a chain of 11 methylene groups. Based on this description, a search in AntiBase and comparison with the literature[13,353354] identified these compounds as Graefe's Factors I and III (**249** and **250**), respectively.



249



250

From streptomycetes, signalling molecules with regulatory activity on secondary metabolite production or cytodifferentiation have been isolated.[351, 355-357,] Most of them are acyl homoserinlactones or larger peptides, but a few are simple mono- or disubstituted γ-butyrolactones, such as the virginiae butanolides or Graefe's factors[13]. γ-Butyrolactones play vital roles in antibiotic production and sporulation in *Streptomyces* species [358,359], and ~60% of *Streptomyces* species appear to produce γ-butyrolactones[360] as extracellular signalling molecules. For example, the A-factor (2-isocapryloyl-3*R*-hydroxymethyl-γ-butyrolactone) is known as essential signalling molecule for streptomycin production and sporulation in *Streptomyces griseus* [361,362]. The virginiae butanolides (**238~242**) have also been shown to induce antibiotic biosynthesis of *Streptomyces virginiae*[363], others promote pigment or spore formation, and some are cytotoxic or enzyme inhibitors; for other butanolides, no biological activity has been described so far.

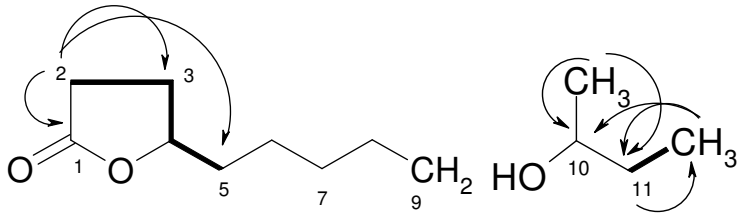
5.10.6 4,10-Dihydroxy-10-methyl-dodecan-4-olide

A third colourless oil, compound **251** was isolated from sub-fraction II (see p. 206), exhibiting the same colour reaction on TLC as virginiae butanolide E (**242**). The molecular weight of **251** was determined by (+)-ESI MS as 228 Dalton. (+)-HRESI-MS of **251** delivered the molecular formula $C_{13}H_{24}O_3$, containing two double bond equivalents.

The ^{1}H-NMR spectrum of **251** indicated signals for 24 protons, all localised in the aliphatic region, two of which were of methyl groups at δ0.85 (t) and 1.11 (s). Multiplets of fifteen protons (δ2.60 and 1.89-1.36) probably of a polymethylene chain were visible, as well as an oxymethine signal at δ4.23 (m). The ^{13}C/HSQC spectra indicated the presence of 13 signals, one of them was the carbonyl of an carboxylic acid derivative (δ175.9), two were of an oxymethine (δ72.8) and an quater-

nary sp^3 carbon (δ80.5), respectively. The residual carbon signals corresponded eight methylenes (δ40.9-19.9) and two methyls (δ26.3 and 8.2).

In the HMBC experiment, the observed ^2J coupling from the methylene protons of CH_2-2 (δ2.60) with the carbonyl C-1 (δ175.9) and CH_2-3 (δ23.0) in addition to its high chemical shift (δ2.60, 34.9) confirmed its position in between. The stability against diazomethane excluded a free carboxylic acid. Protons of the β-methylene group CH_2-3 (δ1.89, 1.62-1.36) exhibited H,H COSY correlations with the oxymethine H-4 (δ4.23) and the α-methylene CH_2-2 (δ2.60). In spite of the absent ^3J coupling from H-4 to C-1, a γ-byturolactone moiety was constructed[,364]. A direct attachment to the residual four methylene groups [CH_2(5)-CH_2-CH_2-CH_2-CH_2(9)] was recognized based on the MS and chemical shift. The first methylene CH_2-5 (δ_H 1.89, 1.62-1.36; δ_C 28.3) was linked to the oxymethine C-4 (δ 80.5) based on the H,H COSY correlation. This was confirmed by the ^4J HMBC coupling between H_2-2 and C-5. So, the partial structure **A** resulted (Figure 129).



A B

Figure 129: Selected HMBC (→) and H,H COSY (▬) correlationsfor the partial structures (**A** & **B**) of **251**.

The remaining partial formula $C_4H_9O_4$ was found by H,H COSY couplings to bear two methyls, one of them giving a triplet (δ_H 0.85, δ_C 8.2) for a terminal ethyl group, linked to methylene (δ_H 1.70 and 1.62-1.36 (δ_C 34.3). The methyl singlet (δ1.11) showed a ^2J coupling to the quaternary oxycarbon C-10 (δ72.8) and a ^3J coupling to the methylene C-11 (δ34.3) of the terminal ethyl. Hence, an isobutyl alcohol (**B**) as additional partial structure was constructed (Figure 129). Based on the observed ^3J couplings (in HMBC) from CH_2-8 (δ1.62-1.36) to C-10 and between the methyl singlet and CH_2-9 (δ 40.9), the two partial structures were connected be-

tween C_9 and C_{10} (Figure 130). Hence, structure of **251** was deduced as 4,10-dihydroxy-10-methyl-dodecan-4-olide, a new member of the butanolide group.

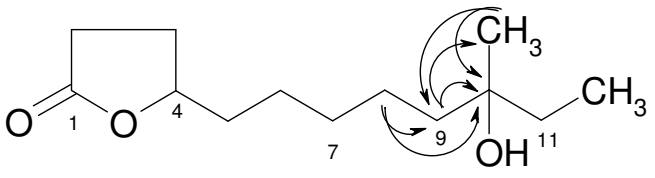


Figure 130: Selected HMBC (→) couplings in 4,10-Dihydroxy-10-methyl-dodecan-4-olide (**251**).

Table 33: ^{1}H and ^{13}C NMR assignments for compounds **251** and **252**, (*J* in [Hz]).

No.	Butanolide **251**		Compound **252**	
	δ_C (150 MHz)	δ_H (300 MHz)	δ_C (75 MHz)	δ_H (300 MHz)[b]
1	175.9	-	178.0	-
2	34.9	2.60 (m)	38.3	2.20 (t, 7.0)
3	23.0	1.89 (m, H_a) 1.62-1.36 (m, H_b)	27.4	1.60 (m)
4	80.5	4.23 (m)	72.3	3.55 (br, m)
5	28.3	1.89 (m, H_a) 1.62-1.36 (m, H_b)	26.8	1.65-1.30 (m)
6	34.6	1.62-1.36 (m)	38.3	1.65-1.30 (m)
7	36.9	1.62-1.36 (m)	39.0	1.65-1.30 (m)
8	19.9	1.62-1.36 (m)	21.2	1.65-1.30 (m)
9	40.9	1.62-1.36 (m)	42.3	1.65-1.30 (m)
10	72.8	-	73.5	-
10-CH_3	26.3	1.11 (s)	26.3	1.11 (s)
11	34.3	1.70 (m, Ha) 1.62-1.36 (m, H_b)	34.9	1.65-1.30 (m)
12	8.2	0.85 (t, 7.4)	8.5	0.85 (t, 7.4)

5.10.7 4,10-Dihydroxy-10-methyl-dodecanoic acid

Compound **252**, an additional colourless oil, was isolated from sub-fraction II (see p. 206), exhibiting the same color reactions on TLC as **251**. Its molecular weight was established by ESI MS as 246 Dalton, and the corresponding molecular formula ($C_{13}H_{26}O_4$) was fixed by (+)-HRESI MS, i.e. it contained one molecule of H_2O more than the lactone **251** and one double bond equivalent less.

This was interpreted as a ring opening of the γ-butyrolactone **251** by hydrolysis to afford the free acid **252**. This conclusion was supported by methylation of compound **18**, giving the corresponding acid ester with a molecular weight of 260 Dalton, and the singlet methyl ester appearing at δ 3.65 (**253**) in the ^{1}H NMR spectrum.

Accordingly, the oxy-methine multiplet of H-4 (δ4.23) and the methylene protons H_2-2 (δ2.60) in **251** were shifted upfield in **252** to δ3.55 and 2.20 (t), respectively.

The remaining NMR signals were approximately the same as in **251**, which was established on the bases of ^{13}C NMR/ HMQC spectra (Table 33). Finally, structure **252** was confirmed by H,H COSY and HMBC experiments, exhibiting the same connectivities as in **251** (Figure 131). Hence, compound **252** was deduced as 4,10-dihydroxy-10-methyl-dodecanoic acid (**252**), a further new compound.

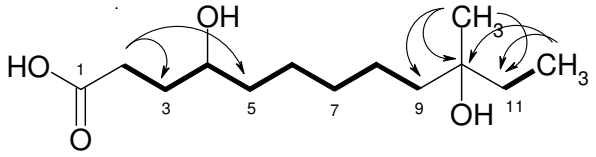
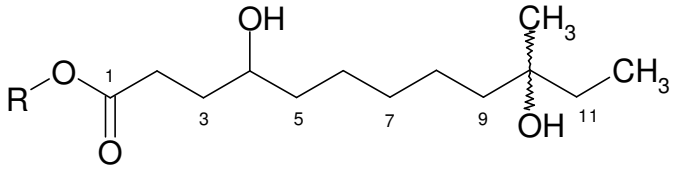


Figure 131: H,H COSY (—) and selected HMBC correlation in 4,10-Dihydroxy-10-methyl-dodecanoic acid (**252**).



252: R = H;

253: R = CH_3

5.11 Marine-derived *Streptomyces* sp. Act8015

In the agar diffusion assay, extracts of the marine-derived *Streptomyces* sp. Act8015 were inactive against all tested pathogenic microorganisms except *Staphylococcus aureus* and *Candida albicans* (Table 76). However, it exhibited a potent *in vitro* antitumor activity against cancer cells (Table 45).

TLC of the crude extract displayed several UV absorbing spots (254 nm), which became yellowish-brown or reddish brown on spraying with anisaldehyde/sulphuric acid. Further bands without UV absorption became violet-blue and later greenish-blue after spraying with the same reagent.

A large scale fermentation in M_2^+ medium at 28 °C for 5 days delivered 12 compounds, namely staurosporine (**198**), adenine[152], indole-3-carboxylic acid[152], ferulic acid (**181**), tryptophol[152], piperazimycin A (**254**), piperazimycin B (**255**), vir-

giniae butanolide E (**242**), Graefe's Factors I (**249**) and III (**250**) in addition to the new butanolides 4,10-dihydroxy-10-methyl-dodecan-4-olide (**251**) and 4,10-dihydroxy-10-methyl-dodecanoic acid (**252**) (Figure 132). Compounds **242**, **249**-**252** were discussed in details with the other γ-butyrolactones for better comparison (see pp. 199-205). The following results have already been published[365].

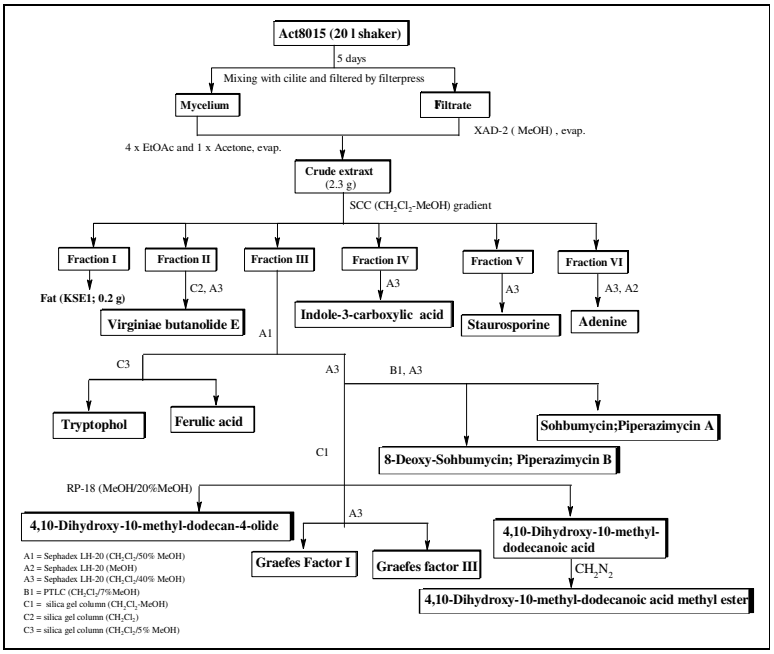


Figure 132: Work-up procedure of the marine-derived *Streptomyces* sp. Act8015

5.11.1 Piperazimycin A

Compound **254** was isolated as white crystals from sub-fraction IIB during a PTLC purification followed by chromatography on Sephadex LH-20. A blue colour reaction of **254** with chlorine/tolidine reagent indicated its peptide property, however with no terminal amino group as its negative ninhydrin reaction revealed. Based on the two *quasi*-molecular ion peaks observed at *m/z* 749 $[M+Na]^+$ and 725 $[M-H]^-$, in ESI MS of positive and negative modes, respectively, the molecular weight of **254** was determined as 726 Dalton. Two [M+2] ions in the ratio 3:1 with respect to the main ions were a hint for chlorine, which was confirmed by the corresponding molecular formula ($C_{31}H_{47}N_8O_{10}Cl$) of **254**.

The ^1H NMR spectrum exhibited numerous signals; six among them were deduced by HMQC spectra as exchangeable protons. Four methine protons were lo-

cated between δ 5.98~5.40 and were characteristic for olefinic protons (J~ 14~11 Hz). In addition, a further four α-methines of amino acids (δ 5.83~4.96) and their corresponding carbons appeared at δ ~50. Between δ 5.52~2.0, several signals of ~24 protons were visible, so of which could be assigned to oxymethine/methylene protons or protons at other electron-withdrawing systems. Finally, one methyl singlet at δ 1.60 along with a doublet signal at δ 1.00 (6H) of an isopropyl group were observed.

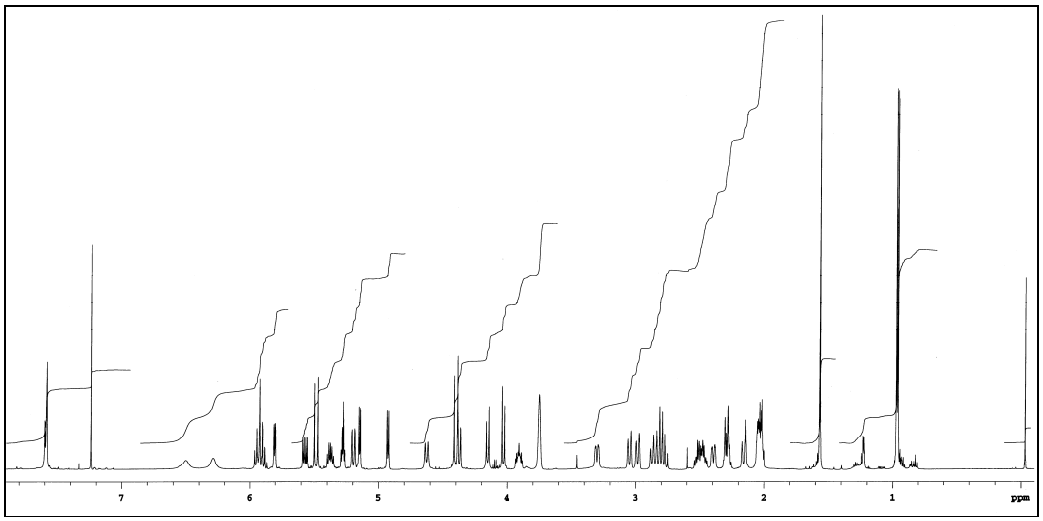
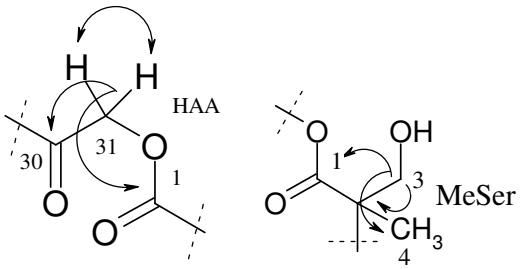


Figure 133: ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of Piperazimycin A (**254**).

The ^{13}C NMR and HMQC spectra depicted the presence of 31 carbon signals, which were classified into the following categories: six amide and/or ester carbonyl carbons (δ 174.3-169.1), four olefinic methine carbons (δ 141.5, 134.3, 126.7 and 124.7), two oxymethylenes (δ 64.2, 63.0), two oxymethines (58.8, 58.5), five α-carbons of amino acids, four of which were methines (δ 53.9- 50.4) and one quaternary (δ 63.0). The remaining signals were of three other methylenes (δ 35.7, 36.2 and 28.5) and three methyls (δ 22.2, 22.1 and 18.3). The negative reaction of **254** with ninhydrin established it as cyclodepsipeptide or cyclopeptide.

At the time when this compound was isolated, a substructure search in all databases delivered no hits, indicating the novelty of **254**. However, it was found that a compound of undetermined structure named sohbumycin[366] had the same mass; the published ^{1}H NMR diagram was very similar to that of **254**.

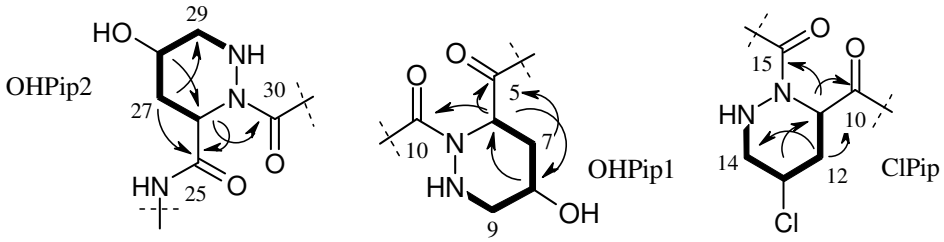
Subsequent analysis of HSQC, COSY, and HMBC NMR spectroscopic data indicated that **254** consisted of five amino acids and one hydroxy acetic acid. The specific assignments (Table 7), which were deduced from the interpretation of 1D and 2D NMR spectra of **254** were as follows: A hydroxyacetic acid (HAA) residue was assigned as the pair of oxygenated methylene signals (H_2-31, δ5.52, 4.43) exhibited exclusively a geminal coupling (d, J = 15.5 Hz), in addition to their HMBC correlations to carbonyl C-1 (δ173.6) and C-30 (δ169.1) delivering the partial structure **A**.



A **B** **C**

For the methylserine residue, the oxymethylene signals (H_2-3, δ 4.06/4.18; δ_C 64.2) displayed a ^3J correlation with the carbonyl C-1 (δ 173.6). In addition, couplings from H_2-3 and NH-1 protons to a methyl singlet (C-4, δ 18.3) were found. Conversely, protons of the methyl (H_3-4, δ1.60) exhibited three correlations towards C-1 (^3J), the quaternary carbon C-2 (δ63.0, ^2J) and the hydroxy-methyl C-3 (δ64.2, ^3J) confirming the methyl-serine fragment **B**.

Based on COSY correlations between the two methyl doublets at 1.00 (δ_C 22.2) and the methine proton H-22 (δ2.32), an isopropyl group was established. Moreover, the butadiene carbons (C_{21}-C_{18}) showed H,H COSY correlation *via* H-21 towards H-22, while H-18 showed a correlation at the methylene protons of C-17 (δ2.57, 2.48). In the same way, the methylene (H_2-17) was in turn attached to the α-methine carbon (C-16, δ50.4). Furthermore, the amide proton (NH-4, δ 7.60) showed ^2J couplings towards the directly attached C-16 (δ50.4), and the amide carbonyls (C-15, δ 172.2) and (C-25, δ169.5, ^3J), establishing the AMNA residue, fragment **C**.



D **E** **F**

The combined spectroscopic analyses allowed three substituted piperazic acids in **254** to be determined as γ-hydroxypiperazic acid (γOHPip2, fragment **D**), γ-hydroxypiperazic acid (γOHPip1, fragment **E**), and γ-chloropiperazic acid (γClPip, fragment **F**) residues. The obtained fragments were further confirmed using the TOCSY experiment (Figure 135 and Figure 136), delivering all amino acids contained in compound **254**.

The sequencing attachment between the amino acids of **254** was initially determined by interpretation of key correlations observed in the HMBC connectivities. So, the structure was completed as **254**.

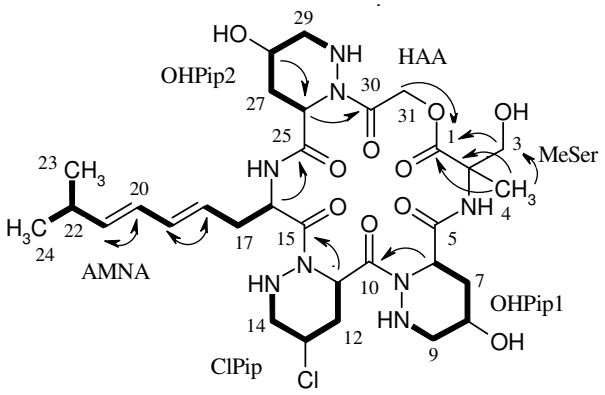
The ^{13}C and ^{1}H NMR data of **254** were identical with published values of sohbumycin within the experimental error[366]. As the 2D NMR data were also in agreement with the recently published structure of piperazimycin A (**254**)[367], it can be stated that the previously unidentified antibiotic sohbumycin is identical with piperazimycin A (**254**), a cyclodepsipeptide containing the rare acids γ-hydroxypiperazic acid, γ-chloropiperazic acid, 2-amino-8-methyl-4,6-nonadienoic acid, 2-amino-8-methyl-4,6-decadienoic acid, and hydroxyacetic acid. As all shifts and also the optical rotation were identical, it can be assumed that also the absolute configurations of the amino acids are identical.

The antibiotic was reported to possess a potent cytocidal activity against HeLa S3 cells and antimicrobial activities against Gram-positive bacteria with MIC ~ 0.3-0.6 μg/L in *in vitro* studies. This agrees well with our own results on a panel of 36 human tumor cell lines. Nevertheless, **254** was not effective against Gram-negative bacteria, yeast or fungi[366].

Table 34: ^{1}H and ^{13}C NMR assignments for Piperazimycin A (**254**) in $CDCl_3$.

Unit	No.	Piperazimycin A (**254**)		Unit	No.	Piperazimycin A (**254**)	
		δ_C[a], APT	δ_H (J in [Hz])[b]			δ_C[a], APT	δ_H (J in [Hz])[b]
αMeSer				**AMNA**			
CO	1	173.6 (C_q)	-	CO	15	172.2 (C_q)	-
α	2	63.0 (C_q)	-	α	16	50.4 (CH)	5.31 (q, 5.9)
β	3a	64.2 (CH_2)	4.06 (d, 11.8)	β	17a	36.2 (CH_2)	2.48 (m)
	3b		4.18 (d, 11.8)		17b		2.57 (m)
α-CH_3	4	18.3 (CH_3)	1.60 (s)	γ	18	124.7 (CH)	5.40 (m)
NH-1		-	7.61 (s)	δ	19	141.5 (CH)	5.98 (t, 10.9)
γOHPip1				ε	20	134.3 (CH)	5.94 (t, 12.9)
CO	5	172.3 (C_q)	-	Z	21	126.7 (CH)	5.60 (dd, 14.5, 6.6)
α	6	51.1 (CH)	5.18 (d, 6.7 Hz)	H	22	30.9 (CH)	2.32 (m)
β	7a	28.4 (CH_2)	2.10 (m)	Θ	23	22.2 (CH_3)	1.00 (d, 6.7)
	7b		2.32 (m)				
γ	8	58.8 (CH)	3.78 (brs)	H-CH_3	24	22.2 (CH_3)	1.00 (d, 6.7)
δ	9a	53.7 (CH_2)	2.91 (m)	NH-4		-	7.63 (d, 6.4)
	9b		3.08 (d, 14.2)				
NH–2		-	4.64 (d, 13.0)	**γOHPip2**			
OH–2		-	6.34 (brs)	CO	25	169.5 (C_q)	-
γClPip				α	26	50.9 (CH)	4.96 (d, 7.5)
CO	10	174.3 (C_q)	-	β	27a	28.4 (CH_2)	2.19 (d, 15.3)
					27b		2.32 (d, 14.1)
α	11	50.9 (CH)	5.83 (d, 6.1)	γ	28	58.5 (CH)	3.78 (brs)
β	12a	35.7 (CH_2)	2.05 (m)	δ	29a	53.9 (CH_2)	2.91 (m)
	12b		2.43 (brd, 13.5)		29b		3.01 (d, 13.2)
γ	13	51.3 (CH)	3.94 (m)	NH-5		-	4.40 (d, 12.8)
δ	14a	53.9 (CH_2)	2.78 (td, 13.5, 10.5)	**HAAC**			
	14b		3.33 (brd, 13.5)				
NH–3		-	5.22 (d, 12.1)	CO	30	169.1 (C_q)	-
					31a	63.0 (CH_2)	4.43 (d, 15.8)
					31b		5.52 (d, 15.8)

[a] 125 MHz; [b]600 MHz



254

Figure 134: H,H COSY (▬, ↔) and selected (→) HMBC coupling in Piperazimycin A (**254**)

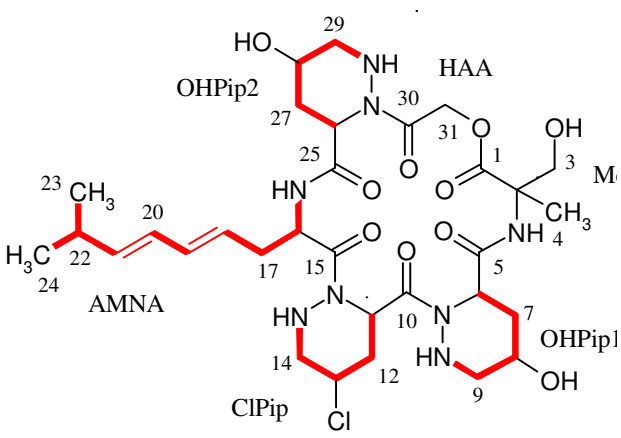


Figure 135: TCOSY (—, =) coupling in Piperazimycin A (**254**).

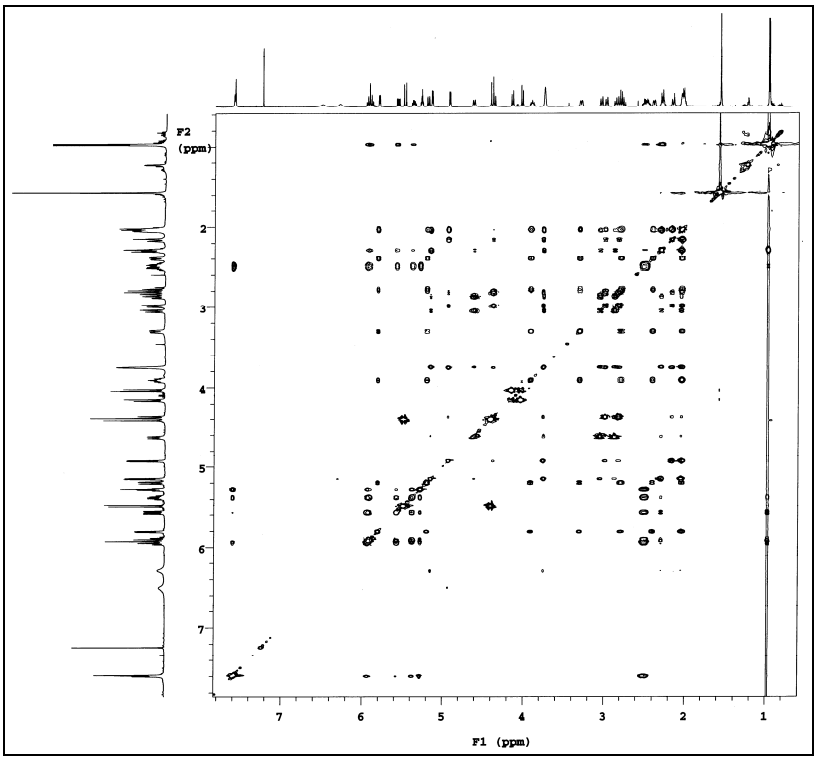
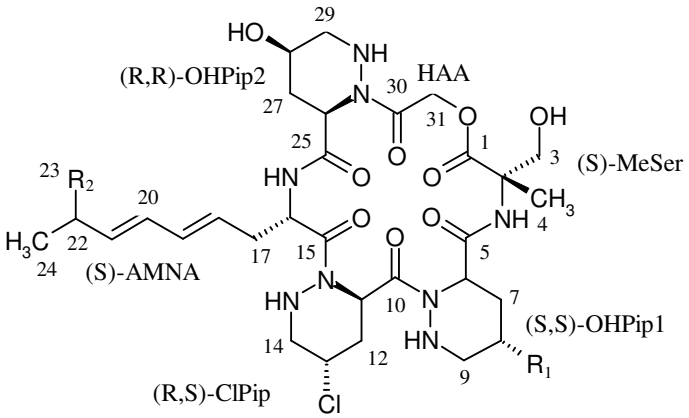


Figure 136: TOCSY spectrum ($CDCl_3$, 600 MHz) of Piperazimycin A (**254**).



254: $R_1 = OH$ $R_2 = CH_3$
255: $R_1 = H$ $R_2 = CH_3$
256: $R_1 = OH$ $R_2 = CH_2CH_3$

5.11.2 Piperazimycin B

Compound **255** was isolated from the same fraction as piperazimycin A (**254**) as a white solid, showing the same colour reaction on TLC, indicating a closely related cyclic peptide. The (+)- and (-)-ESI mass spectra showed *quasi*-molecular ion peaks at *m/z* 733.4 $[M+Na]^+$ and 709.2 $[M-H]^-$, respectively, fixing the molecular weight as 710 Dalton. The corresponding molecular formula $C_{31}H_{47}N_8O_9Cl$ was established by HRESIMS and indicated that one of the amino acids was deoxygenated. The ^1H NMR spectra of **255** confirmed a high structural similarity with **254**. The most notable difference between both peptides appeared at the γ-hydroxy methine (H-8) in **254**, which had been replaced by a methylene group (H_2-8) in **255**. So, a deoxypiperazic acid (Pip) unit in **255** was assumed. As the exact position of the latter could not be confirmed by NMR alone, further MS/MS analyses were performed for piperazimycin B (**255**) with piperazimycins A (**254**) as reference compound; results will be reported below.

Unfortunately, piperazimycins A (**254**) and B (**255**) were recently reported[367], along with piperazimycin C (**256**). The structure of **254** was solved by X-ray. Sohbumycin was reported as a potent cytotoxic agent against multiple tumor cell lines in the nanomolar range[366]. Recently, also the piperazimycins A and B (**254/ 255**) were found to be highly potent cytotoxins[367]. This agrees well with our own results, determined in a monolayer cell proliferation assay using a panel of 36 human tumor cell lines, comprising 14 different solid tumor types.

Cyclic peptides, containing piperazic acid, were reported to have high biological activities, e.g. antitumor[368-370], tuberculostatics[371], anti-inflammatory[372] and anti-HIV agents[373,374]. Monamycin, a series of antibiotics[16], containing piperazic acids have been isolated from the culture broth of numerous *Streptomyces* spp. [375-379] and strains of the genus *Actinomadura*.[369,380]. Biosynthetically, piperazic acids are derived from glutamine followed by reduction of the δ-carbonyl and conversion of either the α- or γ-amino group to a hydroxylamine, and intramolecular cyclization (Figure 137)[381].

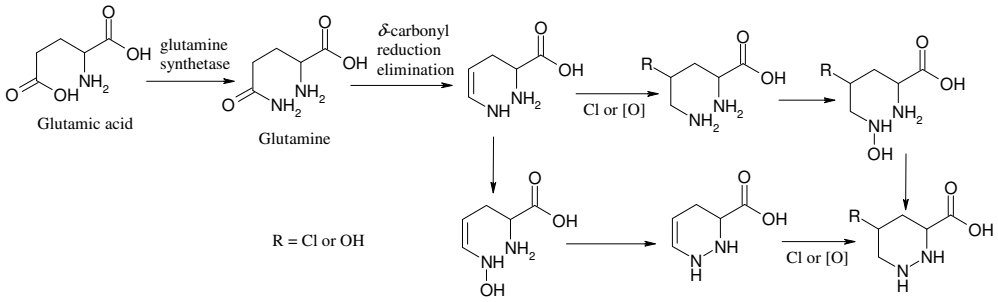


Figure 137: Hypothetical biosynthetic pathways to γ-substituted Piperazic acids.

5.11.2.1 CID- MS/MS Analysis of Piperazimycins A and B

In addition to their matching NMR and mass spectra with those reported the in literature[366,367], the structure assignments of piperazimycins A (**254**) and B (**255**) were further confirmed by tandem mass spectrometry (MS/MS) combined with HRCIDMS/MS, especially with the intention to confirm the position of γ-hydroxypiperazic acid in **255**[382]. MS/MS of piperazimycin A (**254**), which was analyzed as a standard, showed a sequential loss of amino acids from the ($[M+Na]^+$) ion at *m/z* 749, with six predominant peaks at *m/z* 648, 620, 602, 520, 492 and 328. According to their fragment compositions (Table 35), the ions at *m/z* 648 and 520 were assigned to a loss of Me-Ser and OHPip, respectively; while the ions of *m/z* 620 and 492 were due to the expulsion of CO from the precursor ions at *m/z* 648 and 520, respectively. The fragment ion at *m/z* 328 resulted from the loss of ClPip from *m/z* 492, followed by dehydration.

On other hand, a fragmentation of the *pseudo*molecular ion at *m/z* 727 $[M+H]^+$ led mainly to four fragment ions at *m/z* 709, 559, 541 and 376. Tilvi and co-workers proposed[383] that the first dissociation step for protonated cyclic depsipeptides can

result in either a cleavage of one of the amide bonds or the lactone bond. In the case case of **254** however, the OHPip and HAA residues were initially removed to afford *m/z* 559 ([M-OHPiP-HAA]+H), suggesting a ring opening at the lactone C-O. The fragment at *m/z* 559 showed a further expulsion of a water molecule (*m/z* 541), followed by splitting of the AMNA residue (*m/z* 376), which corresponded to the sequence of piperazimycin A (**254**) (Figure 138).

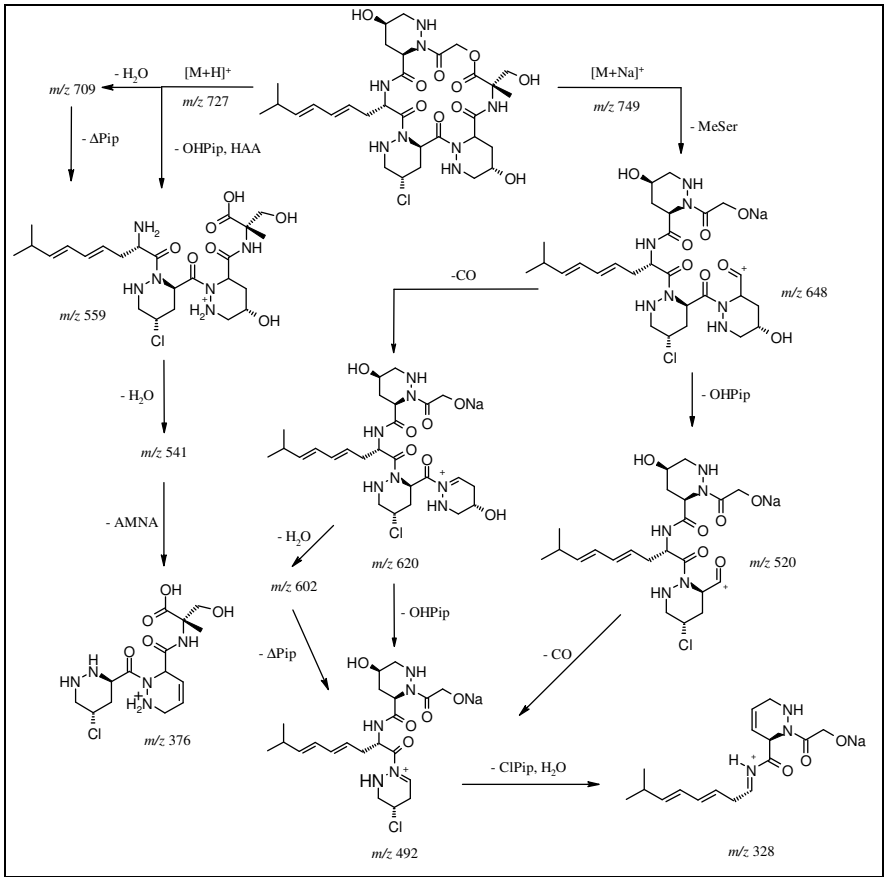


Figure 138: Fragmentation of Piperazimycins A (**254**) by CID-MS^2 sequencing of the *pseudo*-molecular ions $[M+H]^+$ and $[M+Na]^+$

Similarly, MS/MS of the molecular ion peak at *m/z* 733.3 $[M+Na]^+$ of piperazimycin B (**255**) was subsequently fragmented to deliver six major ion peaks at *m/z* 705, 632, 604, 519, 492 and 328. As in piperazimycin A (**254**), the fragment at *m/z* 632 was attributed to delactonization and splitting of Me-Ser, followed by a loss of CO (*m/z* 604). The fragment ion at *m/z* 492 (same as **254**) was due to a splitting of 112 amu (for Pip) from the ion peak at *m/z* 604 corresponding to a different amino

acid residue in **255** with respect to **254**. As in **254**, the last fragment at *m/z* 328 was indicative for the ClPip splitting from the ion at *m/z* 492 (Figure 138).

Table 35: HRCIDMS^2 of the key fragments from the pseudomolecular molecular $[M+H]^+$ and $[M+Na]^+$ ions of Piperazimycin A (**254**).

Mass	Molecular Formula	Amino Acid Sequence
328.16346	$C_{16}H_{23}N_3O_3Na$	HAA-OHPip-AMNA$^-$ H_2O
492.19934	$C_{21}H_{32}N_5O_5Cl$ Na	HAA-OHPip-AMNA-ClPip$^-$ CO + Na^+
520.19424	$C_{22}H_{32}N_5O_6Cl$ Na	HAA-OHPip-AMNA-ClPip + Na^+
602.24822	$C_{26}H_{38}N_7O_6Cl$ Na	HAA-OHPip-AMNA-ClPip-OH-Pip – CO, – H_2O + Na^+
620.25882	$C_{26}H_{40}N_7O_7Cl$ Na	HAA-OHPip-AMNA-ClPip-OH-Pip – CO + Na^+
648.25376	$C_{27}H_{40}N_7O_8Cl$ Na	HAA-OHPip-AMNA-ClPip-OH-Pip + Na^+
749.30262	$C_{31}H_{47}N_8O_{10}Cl$ Na	*cyclo*[HAA-OHPip-AMNA-ClPip-OH-Pip-MeSer] + Na^+
376.13892	$C_{14}H_{23}ClN_5O_5$	ClPip-OH-Pip-MeSer– H_2O + H^+
498.21242	$C_{22}H_{33}N_5O_6Cl$	HAA-OHPip-AMNA-ClPip + H^+
541.25498	$C_{24}H_{38}ClN_6O_6$	AMNA-ClPip-OH-Pip-MeSer - H_2O + H^+
559.26558	$C_{24}H_{40}ClN_6O_7$	AMNA-ClPip-OH-Pip-MeSer + H^+
709.30961	$C_{31}H_{46}N_8O_9Cl$	*cyclo*[HAA-OHPip-AMNA-ClPip-OH-Pip-MeSer - H_2O] + H^+
727.31763	$C_{31}H_{48}N_8O_{10}Cl$	*cyclo*[HAA-OHPip-AMNA-ClPip-OH-Pip-MeSer] + H^+

5.11.3 Biological Activity

The strong antibiotic and cytotoxic activity of the crude extract was in part due to staurosporine (**198**). Two further highly cytotoxic antibiotics were the chlorine-containing peptides, piperazimycins A (**254**) and B (**255**).

Sohbumycin was reported as a potent cytotoxic agent against multiple tumor cell lines in the nanomolar range[366]. Recently, also the piperazimycins A and B (**254/255**) were found to be highly potent cytotoxins[367]. This agrees well with our own results, determined in a monolayer cell proliferation assay using a panel of 36 human tumor cell lines, comprising 14 different solid tumor types. Piperazimycin A (**254**) showed cytotoxic activity against a range of human tumor cell lines with an overall potency of 0.13 μg/ml (mean IC_{50} value of 36 tumor cell lines tested), and a mean IC_{70} of 0.21 μg/ml. Cytotoxic activity was rather unselective with a steep concentration response between 0.03 μg/ml and 0.3 μg/ml. IC_{50} values determined in 32 out of 36 tumor cell lines ranged between 0.09 μg/ml and 0.13 μg/ml (Table 78). Three tumor cell lines were clearly less sensitive (head and neck cancer HNXF 635L,

ovary cancer OVXF 899L, and renal cancer RXF 486L) with IC_{50} values between 0.82 μg/ml and 1.13 μg/ml (Table 78).

In the brine shrimp microwell assay, piperazimycin A (**254**) showed a mortality rate of 20 % at 10 μg/ml. The antimicrobial activity of all compounds isolated from *Streptomyces* sp. Act8015 were determined using the agar diffusion method. At a concentration of 40 μg/disc, compounds **254** and **255** exhibited substantial activities against *Bacillus subtilis*, *Mucor miehei*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Candida albicans*, and *Mucor miehei* (Table 77). However, all butenolides isolated here were antibiotically inactive and did not stimulate spore formation in *Streptomyces viridochromogenes* or in the producing strain Act8015.

5.12 Marine *Streptomyces* sp. B7729

The marine *Streptomyces* sp. B7729[384] showed white colonies on incubation on agar using M_2^+ medium. In the biological screening, the extract exhibited potent antimicrobial activity against Gram-positive (*Bacillus subtilis, Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*) (Table 79). Also, it exhibited *in vitro* antitumor activity against numerous of cancer cell-lines at very low concentrations (<10 μg/ml) (Table 45). In the chemical screening, the strain extract exhibited two UV absorbing bands (254 nm) from TLC. Moreover, it showed one yellow band which changed to brown by treatment with sulphuric acid.

For these reasons, the strain B7729 was up-scaled (24 l) on a shaker using the same medium (M_2^+) for 7 days. After the usual working up, the obtained crude extract was purified using various chromatographic techniques. This afforded five compounds; enterocin (**257**), cinnamic acid (**260**[385], polypropylenglycol (**261**), lajollamycin (**262**) and the new 4',5'-dihydro-lajollamycin (**263**); Figure 139.

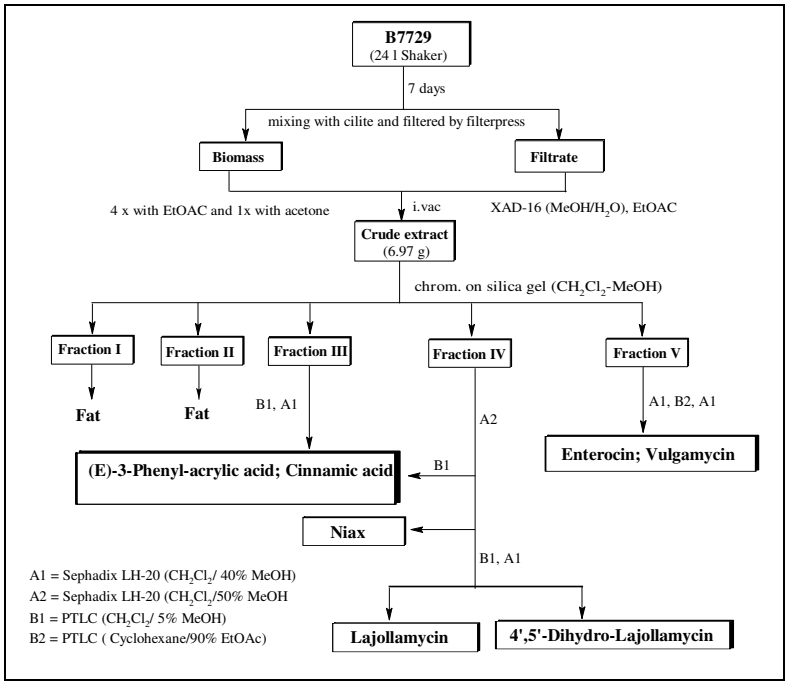


Figure 139: Work-up procedure of the marine *Streptomyces* sp. B7729

5.12.1 Enterocin

Compound **257**, an UV-absorbing colourless solid, was isolated from the polar fraction V. On spraying with anisaldehyde/ sulphuric acid during TLC, the band of **257** turned to yellowish-green. Positive and negative modes of ESI-MS established the molecular weight of compound **257** as 444 Dalton, and its corresponding molecular formula was recognized by HRESI-MS as $C_{22}H_{20}O_{10}$.

The ^{1}H NMR spectrum showed signals of five aromatic protons (δ 7.92~7.46, J~ 7.4 Hz), indicative of a phenyl residue. The downfield shift of the phenyl protons pointed to its attachment to an electron withdrawing group, e.g. benzoyl system. Moreover, two *m*-coupled protons (J ~ 2.0 Hz) were visible at δ6.39 and 5.62. In the aliphatic region, a methoxy signal (δ3.86), four methine signals (δ4.79~4.65), as well as two broad doublets each of 1H (δ2.63 and 1.86) were observed.

The ^{13}C NMR spectra displayed ~22 carbon signals, 13 of them were of sp^2 characteristic. The remaining nine carbons were sp^3, and classified into three oxygenated quaternary carbons (δ80.7, 77.5, 77.3), four methines (δ79.9, 71.0, 57.0 and 54.5), one methoxy (δ56.5) and one methylene (δ36.5). Three of the sp^2 carbons were low

field shifted (δ 197.7, 175.6 and 173.4), are most likely of a ketonic and two ester carbonyls, respectively. The remaining seven sp^2 carbons were corresponding to those of the phenyl residue and the two *m*-methines. Based on the molecular formula and the NMR data, compound **257** was easily identified as enterocin by means of AntiBase. The structure was further confirmed by comparison with authentic spectra and the literature.[388~387]. Enterocin (**257**) was reported firstly previously from a marine derived actinomycete[388] and an ascidian *Didemnum* sp.[389]; it has been repeatedly isolated in our group.

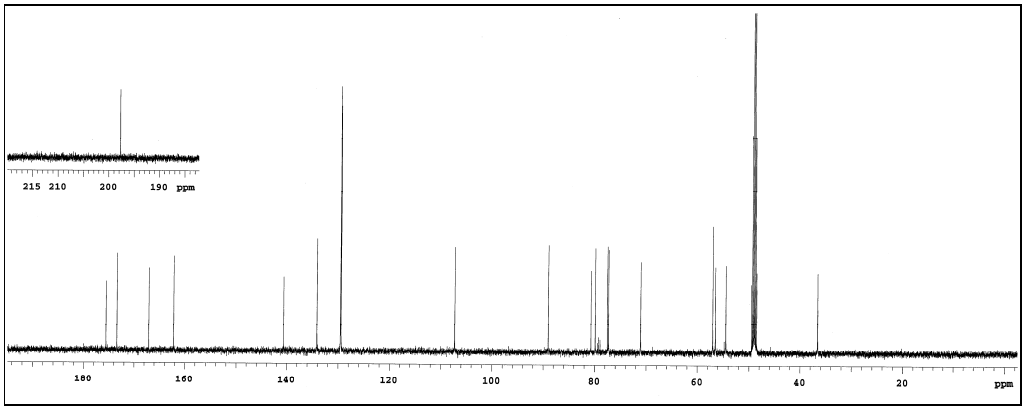
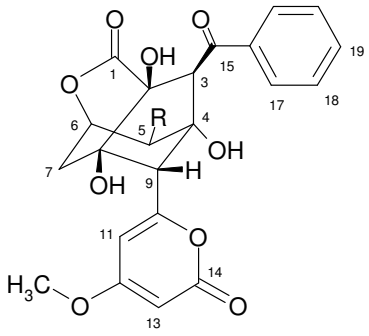
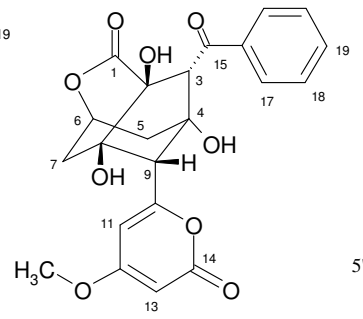
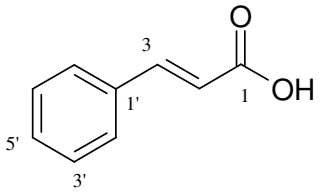


Figure 140: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of Enterocin (**257**).







257: R= OH, **258**: R = H **259** **260**

Biosynthetically, enterocin (**257**) is derived from an unusual benzoate starter unit and seven malonate molecules which undergoes a rare Favorskii-like carbon rearrangement, followed by cyclization (Figure 141)[390]. Several structural analogous of enterocin (**257**) were reviewed and characterized by their specific α-pyrone e.g. 5-Deoxyenterocin (**258**) and 3-*epi*-5-deoxyenterocin (**259**), as well as the three α-pyrone constituents, wailupemycins A-C [389,391,392].

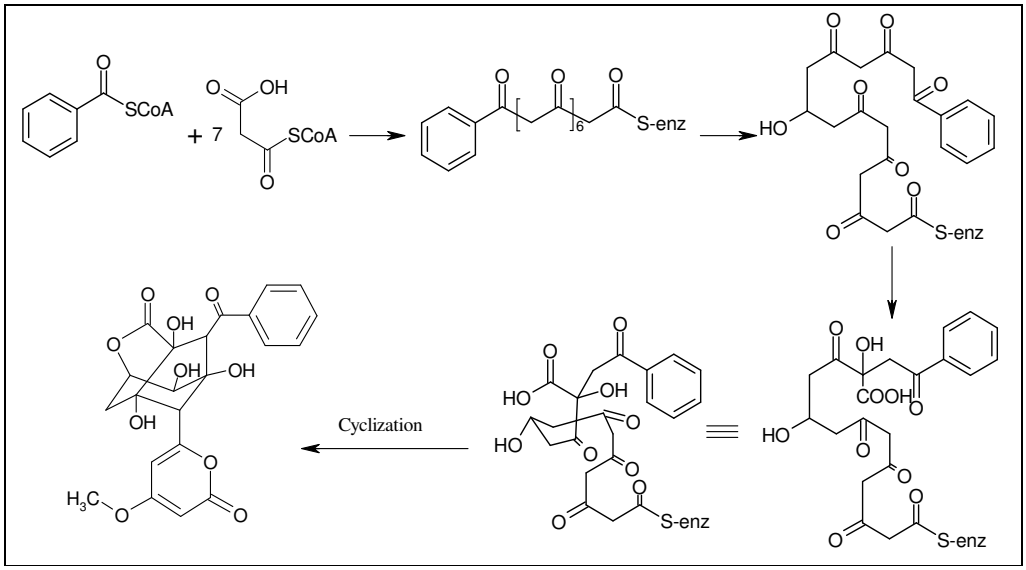
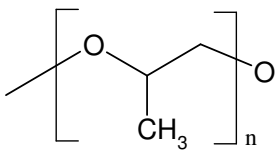


Figure 141: Hypothetic biosynthesis of Enterocin (257).

5.12.2 Polyisopropylenglycol

Compound **261** was obtained as colourless oil from sub-fraction IV using Sephadex LH-20. It is not UV absorbing and gave a white spot on a pink background after spraying with anisaldehyde/sulphuric acid or Orcin reagent. The NMR spectra showed a multiplet of an oxymethine proton (δ_H 3.37, δ_C ~75), an oxymethylene group (δ_H 3.52, δ_C ~73) and a methyl (δ_H 1.10, δ_C ~17), representing a propan-2-ol [O-CH(CH_3)-CH_2-O] partial structure. Based on the revealed data, a search in Anti-Base pointed to polyisopropylenglycol (niax, **261**), which was further established by comparison with an authentic spectrum and literature. Niax (**261**) is often added during the fermentation process in jar-fermentors to avoid the formation of foam. However, in this case the fermentation was carried out on a shaker without any antifoaming agents, which proves the natural origin of **261**; it has also been isolated from other strains by us and others[393].



261

5.12.3 Lajollamycin and 4', 5'-Dihydro-lajollamycin

Further purification of fraction IV by PTLC and Sephadex LH-20 led to the isolation of a mixture of **262** and **263** as bright yellow solid. On TLC it was UV absorbing and turned brown by treatment with conc. sulfuric acid, which may be indicative of a polyene system. The structural similarity of both components (**262**, **263**) made their separation from each other difficult.

The molecular weights of **262** and **263** were established by ESI-MS as 687 and 689 Daltons, respectively. Their molecular formulas were determined by HRESI MS as $C_{36}H_{53}N_3O_{10}$ and $C_{36}H_{55}N_3O_{10}$, which delivered 12 and 11 double bond equivalents.

The ^{1}H NMR spectra of **262** and **263** displayed numerous multiplets of olefinic protons (δ6.80~5.60) and oxy-methines (δ4.20~3.20), along with four NH/OH proton signals (δ6.36~2.36); the latter exchangeable protons were established by HMQC experiments. Additionally, two singlets of oxygen or nitrogen bound methyls (δ3.40 and 2.89) were found, together with five methyl singlets at δ2.29~1.09 and one doublet at δ1.78. Moreover, several signals of methine and methylene protons were located in the range of δ2.50~0.90.

In the ^{13}C NMR spectra, both compounds delivered similar patterns of ~36 overlapping carbon signals. Among these signals three sp^2 carbons were carbonyls of acid derivatives (δ177.9, 175.0 and 169.6). Additionally, twelve and ten olefinic carbons (δ146~126) were detected for **262** and **263**, respectively. The remaining 19/21 carbons were in the sp^3 region, among them seven oxy-carbons (δ86~76), one methoxy (57.3) and one N-methyl (δ26.6), along with further six methyl carbons located between δ26.0 and 15.2. Finally, a signal of a methylene carbon (δ33.1) was present for **262**, while two methylenes were present (δ41.2 and 33.0) for **263**.

According to the ^{13}C NMR spectra, all carbon signals were similar for both compounds, except that the double bond carbons at δ141.0 and 124.6 (C-4'/5') in compound **262** were replaced by a methine carbon at δ44.9 and its partner methylene carbon (C-5', δ41.2) in **263**. This was attributed to the addition of hydrogen to a double bond in compound **262**.

A search in AntiBase[61] using the above spectral data of compound **262** resulted in lajollamycin[394] as the sole known structure (Table 36). The identity with lajolamycin (**262**) was further established by 2D NMR experiments (HMQC and HMBC, Figure 143). A natural product with the composition of **263** was unknown.

The dihydro-position of compound **263** was elucidated on the basis of HMBC and MS/MS analyses. The methyl doublet of 4'-CH_3 showed ^2J and ^3J correlations to the methine C-4' (δ 44.9) and the C-5' methylene (δ 41.2), and *vice versa* (Figure 143). The ESI-MS/MS analysis of compound **263** showed a fragment ion peak at *m/z* 641 as a result of the expulsion of the nitro group. Subsequently, this ion peak (*m/z* 641) showed a further splitting at the amide bond (O=C-1'-NH-C-12) of the side chain, giving two corresponding fragments at *m/z* 395 and 246 (Figure 142).

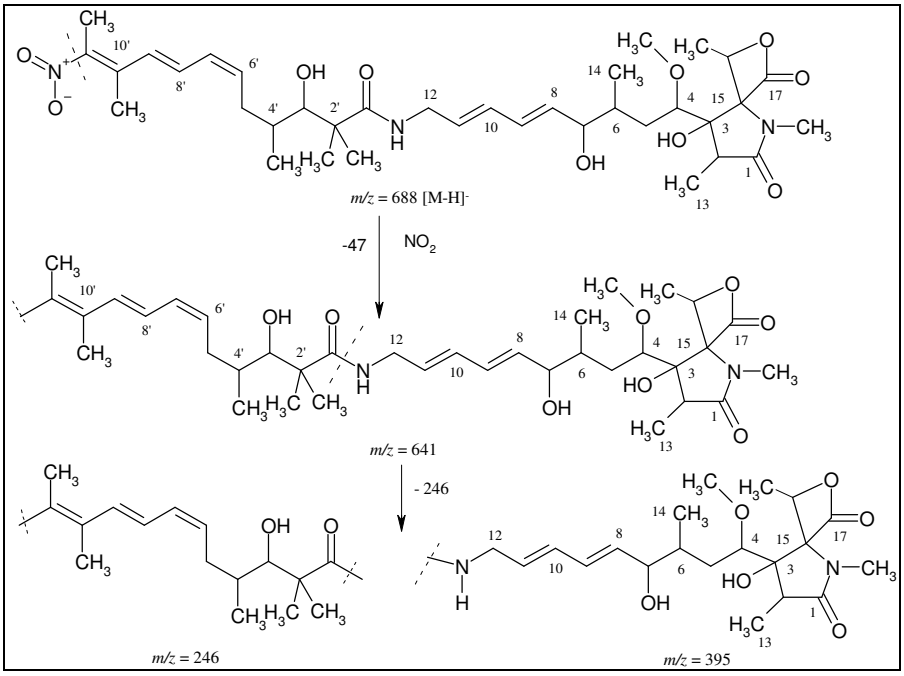
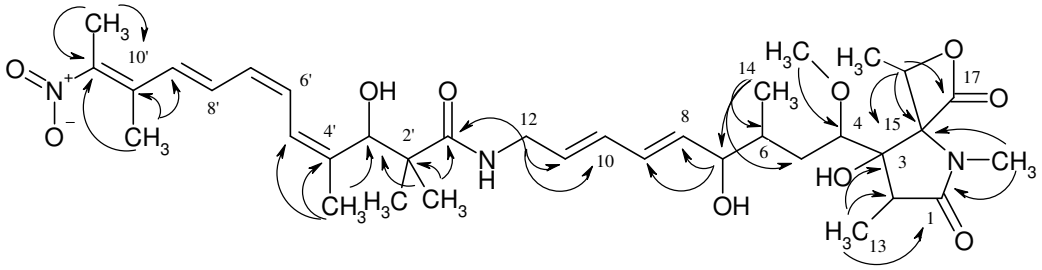


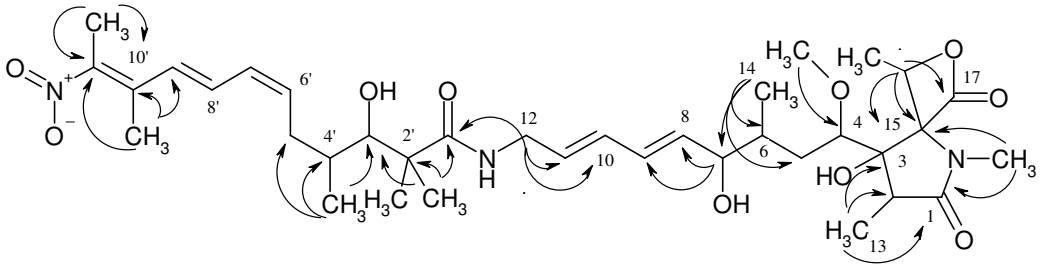
Figure 142: ESI MS/MS of 4', 5'-Dihydro-lajollamycin (**263**).

Lajollamycin (**262**) and 4',5'-dihydrolajollamycin (**263**), are a nitrotetraene spiro-β-lactone-γ-lactam and nitro-triene-spiro-β-lactone-γ-lactam antibiotics, respectively. Several triene spiro-β-lactone-γ-lactam antibiotics e.g. oxazolomycin (**264**)[395] 16-methyloxazolomycin[396] (**265**), and triedimycin B6 (**266**) were previously isolated from bacteria. Lajollamycin (**262**) was isolated from the marine actinomycete *Streptomyces nodosus* (NPS007994); it is a potent antimicrobial agent

against both against drug-sensitive and -resistant Gram-positive bacteria, as well as an *in vitro* inhibitor of B16-F10 tumor cells[394].

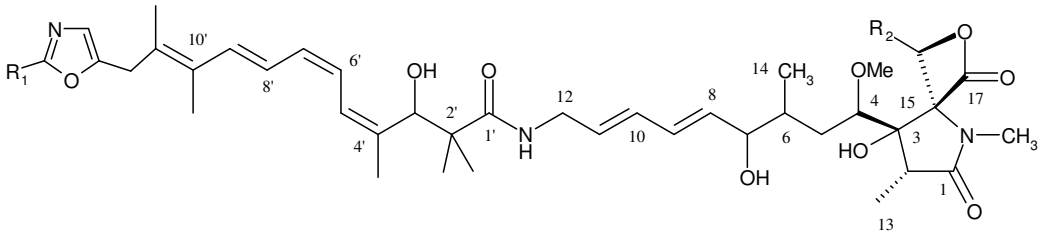


262



263

Figure 143: Selected HMBC correlations (→) of Lajollamycin (**262**) and 4',5'-Dihydrolajollamycin (**263**).



264: $R_1 = H$ $R_2 = H$
265: $R_1 = H$ $R_2 = CH_3$
266: $R_1 = CH_3$ $R_2 = CH_3$

Table 36: The NMR assignments for Lajollamycin (**262**) and 4',5'-Dihydro-lajollamycin (**263**) compared with the reported data of **262** in $CDCl_3$.

Position	Lajollamycin (**262**) Lit.[394]		Lajollamycin (**262**) Exp.		4', 5'-Dihydro-lajollamycin (**263**)	
	δ_C [a]	δ_H (*J* in Hz)[b]	δ_C[c]	δ_H(*J* in Hz)[d]	δ_C[c]	δ_H(*J* in Hz)[d]
1	175.0	-	175.1	-	175.1	-
2	43.0	2.43, 1H, q, 7.5	43.0	2.43, 1H, m	43.0	2.43, 1H, m
3	81.5	-	81.5	-	82.0	-
4	82.0	3.67, 1H, t, 4.5	82.0	3.67, 1H, m	82.0	3.67, 1H, m
5	33.1	1.37, m, 1H	33.0	1.37, m, 1H	33.0	1.37, 1H, m
		2.02, m, 1H		2.02, m, 1H		2.02, 1H, m
6	37.1	1.85, 1H, m	37.1	1.85, 1H, m	37.1	1.85, 1H, m
7	76.9	3.94, 1H, br t, 6.5	76.6	3.94, 1H, br t, 6.5	76.6	3.94, 1H, t, 6.5
8	134.2	5.67, 1H, m	134.2	5.67, 1H, m	134.2	5.67, 1H, m
9	131.7[a]	6.21, 1H, m	131.6	6.21, 1H, m	131.7	6.21, 1H, m
10	131.1[a]	6.16, 1H, m	131.1	6.16, 1H, m	131.1	6.16, 1H, m
11	129.5[b]	5.67, 1H, m	129.5	5.67, 1H, m	129.7	5.67, 1H, br m
12	41.2	3.90, 2H, m	41.2	3.90, 2H, m	42.0	3.88, 2H, m
13	10.0	1.18, 3H, d, 7.5	10.0	1.18, 3H, d, 7.5	10.0	1.17, 3H, d, 7.4
14	17.7	0.96, 3H, d, 6.5	17.6	0.96, 3H, d, 6.5	17.5	0.95, 1H, d, 6.7
15	84.3	-	84.2	-	84.2	-
16	77.5	4.84, 1H, q, 7.0	77.5	4.84, 1H, q, 7.0	77.5	4.84, 1H, q, 7.0
16-Me	16.8	1.78, 3H, d, 7.0	16.8	1.78, 3H, d, 7.0	16.8	1.78, 3H, d, 6.6
17	169.6	-	169.7	-	169.7	-
1'	177.9	-	177.7	-	177.7	-
2'	44.7	-	44.8	-	44.9	-
2'-Me_a	21.5	1.09, 3H, s	21.4	1.09, 3H, s	21.5	1.09, 3H, s
2'-Me_b	26.0	1.33, 3H, s	26.1	1.33, 3H, s	26.6	1.33, 3H, s
3'	75.6	4.64, 1H, d, 6.0	76.6	4.00, 1H, br	83.8	4.00, 1H, br
4'	141.2	-	141.0	-	44.9	2.42, 1H, m
4'-Me	19.8	1.83, 3H, br s	21.0	1.79, 3H, br s	21.5	1.09, 3H, d, 7.0
5'	124.6	6.49, 1H, d, 12.0	nd	6.49, 1H, m	41.2	3.90, 2H, brm
6'	127.5	6.38, 1H, m	127.5	6.38, 1H, m	137.0	6.70, 1H, m
7'	128.2	6.07, 1H, t, 11.5	128.3	6.07, 1H, t, 11.5	128.0	6.21, 1H, m
8'	131.8[a]	7.08, 1H, dd, 11.5, 15	127.5	7.08, 1H, m	131.8	6.60, 1H, m
9'	129.6[b]	6.41, 1H, d, 15	129.6	5.75, 1H, m	129.6	6.00, 1H, m
10'	133.3	-	133.0	-	133.8	-
10'-Me	15.2	2.06, 3H, br s	15.1	2.06, 3H, s	15.2	2.00, 3H, s
11'	146.0	-	144.5	-	145.5	-
11'-Me	15.8	2.29, 3H, br s	15.9	2.28, 3H, s	15.8	2.26, 3H, s
N-Me	26.6	2.89, 3H, s	26.6	2.89, 3H, s	26.6	2.88, 3H, s
4-OMe	57.3	3.40, 3H, s	57.2	3.40, 3H, s	57.2	3.39, 3H, s
NH	-	6.36, 1H, m	-	-	-	-
3-OH	-	3.18, 1H, s	-	3.24, 1H, s	-	3.26, 1H, s
3'-OH	-	4.52, d, 6.0	-	-	-	-
7-OH	-	2.36, br d, 2.5	-	-	-	-

[a] Assignments interchangeable; [b] Assignments interchangeable; [c] 125.7 MHz; [d] 600 MHz

5.13 Marine *Streptomyces* sp. B9054

The chemical screening of the marine *Streptomyces* sp. B9054[397] showed several red and orange-red coloured spots during TLC. These bands gave a blue colour reaction on treatment with sodium hydroxide, indicative for *peri*-hydroxyquinones. The crude extract exhibited activities against *Bacillus subtilis, Escherichia coli, Scenedesmus subspicatus,* and *Chlorella sorokiniana*, (Table 80).

A large-scale cultivation was performed using 100 of 1-liter-Erlenmeyer flasks, each containing 200 ml of M_2^+ medium. The flasks were incubated with 110 rpm at 28 °C for 5 days. After the usual working up, the crude extract was subjected to flash silica gel column chromatography and eluted with dichromethane-methanol gradient. Based on TLC monitoring, six fractions were afforded. Purification of the less polar fractions I and II, using silica gel and PTLC followed by Sephadex LH-20, yielded η-pyrromycinone (**267**) and ζ-pyrromycinone (**269**). In the same way, the middle polar fractions III~VI delivered the cinerubins A (**275**), B (**273**), X (**272**), Y (**271**); among them the new cinerubin K (**276**) was obtained from fraction IV (Figure 144).

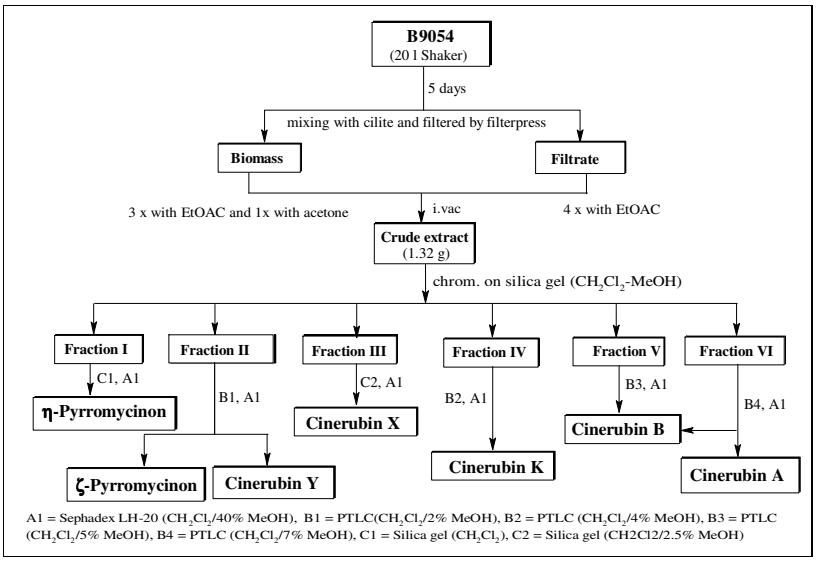


Figure 144: Work-up procedure of the marine *Streptomyces* sp. B9054

5.13.1 *η*-Pyrromycinone

Compound **267**, was isolated as a red solid from fraction **I** after purification on silica gel column chromatography followed by Sephadex LH-20. The ESI mass spectrum established the molecular weight of **267** as 392 Dalton.

The ^{1}H NMR spectrum of **267** displayed three *peri*-OH groups (δ 13.84, 13.10 and 12.31), in addition to two ortho-coupled aromatic protons at δ 8.52 and 7.60 and another 2H AB spin at δ 7.28. Additionally, two singlets of an sp^2 bound proton were observed at δ 8.25 and of a methoxy group at δ 4.10. A quartet at δ 2.81 and a triplet at 1.32 indicated an aromatic bound ethyl group.

A search in AntiBase (Figure 145) using the above detailed spectral data delivered the two structural isomers, η-pyrromycinone (**267**) and bis-anhydro-ε-rhodomycinone (**268**). A comparison of the spectroscopic data with authentic spectra in our collection confirmed the compound as η-pyrromycinone (**267**). The first members of the pyrromycinone family were isolated by Brockmann[398] from several *Streptomyces* sp. Some of them showed antibiotic activity and anti-tumour effects on sarcoma 180S cells[399].

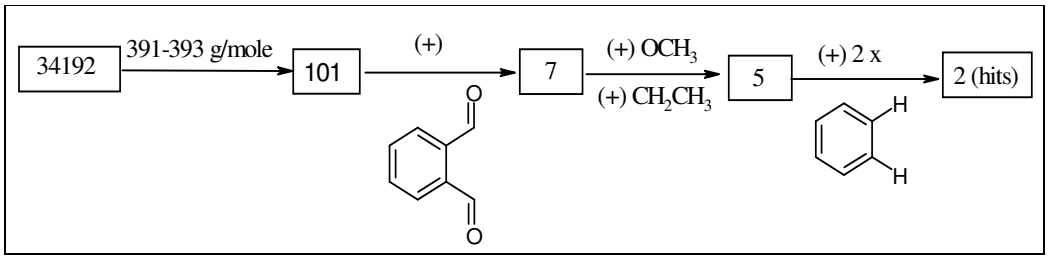
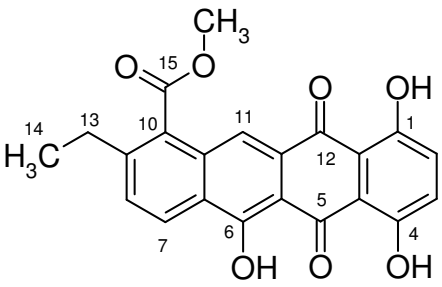
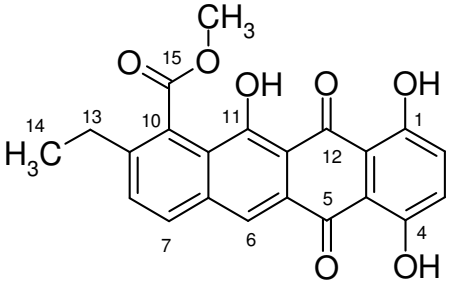


Figure 145: Searching scheme in AntiBase using spectroscopic data of **267**.



267



268

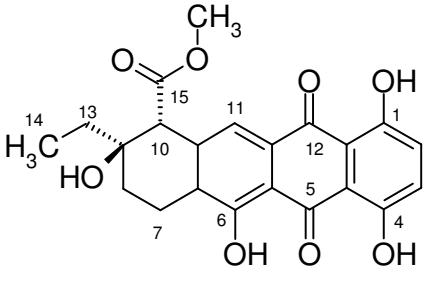
5.13.2 ξ-Pyrromycinone

Compound **269** was isolated from sub-fraction II using Sephadex LH-20 as an orange-red solid, showing an orange fluorescent band on TLC. With dilute sodium hydroxide, it turned blue, indicative of an additional *peri*-hydroxy quinone. Based on the ESI mass spectra in both modes, the molecular weight of **269** was determined as 412 g/mol.

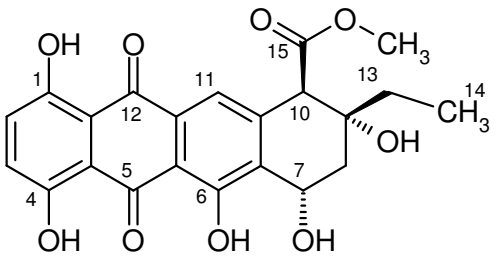
The ^{1}H NMR spectrum of **269** revealed the presence of three chelated OH groups at δ 13.05, 12.60 and 12.23. In the aromatic region, one singlet at δ 7.70 (1H) together with an AB signal at δ 7.29 (2H) were observed, pointing to a quinone

chromophore. Additionally, one oxy-methine proton (δ 3.96) and a methoxy group (δ 3.74) were detected. Subsequently, four multiplets each of 1H were present between δ 3.08~1.94, which might be attributed to two methylenes included in a ring system. Finally, a further multiplet of 2H at δ 1.65 as well as a methyl triplet at δ 1.10 were shown.

Based on the above data and searching in AntiBase, structure of **269** was fixed as ξ-pyrromycinone, which was further confirmed by comparison with the authentic sample and spectra. ξ-Pyrromycinone (**269**), an anthracycline antibiotic, was firstly described by K. Eckardt[400].



269



270

5.13.3 Cinerubin Y

From sub-fraction II, compound **271** was isolated as an orange-red solid during silica gel column chromatography followed by Sephadex LH-20. This compound gave also a colour change to blue on treatment with sodium hydroxide, but had a higher polarity than the previously described quinones (**267**, **269**). The ESI mass spectra (both modes) confirmed the molecular weight of **271** as 782 g/mol.

The ^{1}H NMR spectrum of **271** showed three signals of chelated hydroxy groups (δ 12.97, 12.80, 12.25), the singlet of an aromatic methine (δ 7.73) together with an AB signal (2H) at δ 7.32 and 7.34. Additionally, a broad methine doublet (δ 5.20), a 1H singlet (δ 4.13), a methoxy signal (δ 3.70), a methylene multiplet (δ 2.48, 2.32) and an ethyl group (δ 1.70 CH_2, 1.09 CH_3) were assignable to the ξ-pyrromycinone chromophor **269**. In addition, three oxy-methines appeared at δ 5.42, 5.32 and 5.05, characteristic of three anomeric protons in α-configuration. They were together with

a number of multiplets located between δ 4.80~1.25, indicating that compound **271** contained three sugar units.

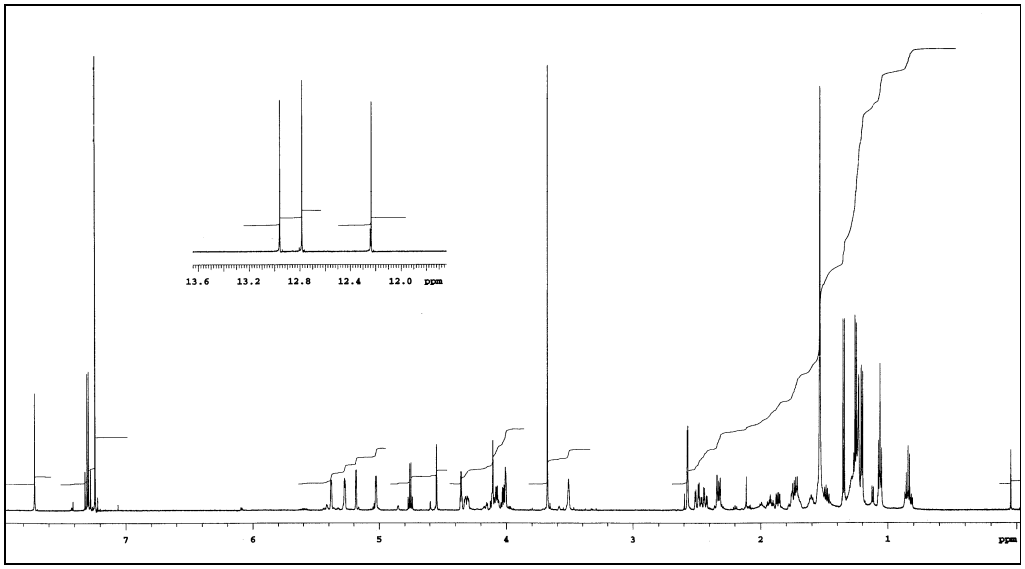
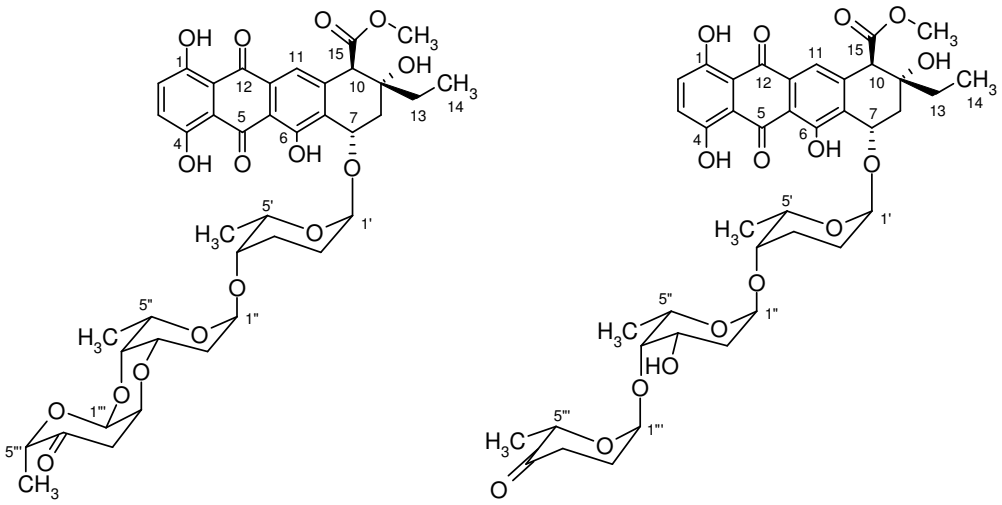


Figure 146: ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of Cinerubin Y (**271**).

The ^{13}C NMR spectrum of compound **271** showed two quinone-carbonyls, one ester carbonyl (δ 171.3), and the aromatic carbon signals of the pyrromycinone moiety. Moreover, one keto carbonyl (δ 207.3) was observed together with three anomeric methine carbons (δ 101.3, 99.9 and 91.4), nine methines (δ 75~62.9) and one oxygenated quaternary carbon signal (δ 71.6). Furthermore, five signals of methylene carbons (δ 39.6, 26.9, 24.3 and 24.2) were visible along with four of methyl carbons (δ 17.2, 16.17, 16.1 and 6.7). The last methyl is characteristic for C-14 in the pyrromycinone skeleton, while the other methyl carbons were located in the sugar residues. The ESI MS^2 fragmentation patterns of **271** afforded a base ion peak at *m/z* 433 as a result of expulsion of the linked trisaccharide followed by elimination of a water molecule to give the aromatized form; η-pyrromycinone (**267**) (Figure 147).

Based on the discussed spectroscopic data and searching in AntiBase, the structure of **271** was deduced as cinerubin Y. This determination was further confirmed by comparison with the corresponding literature[401].



271 **272**

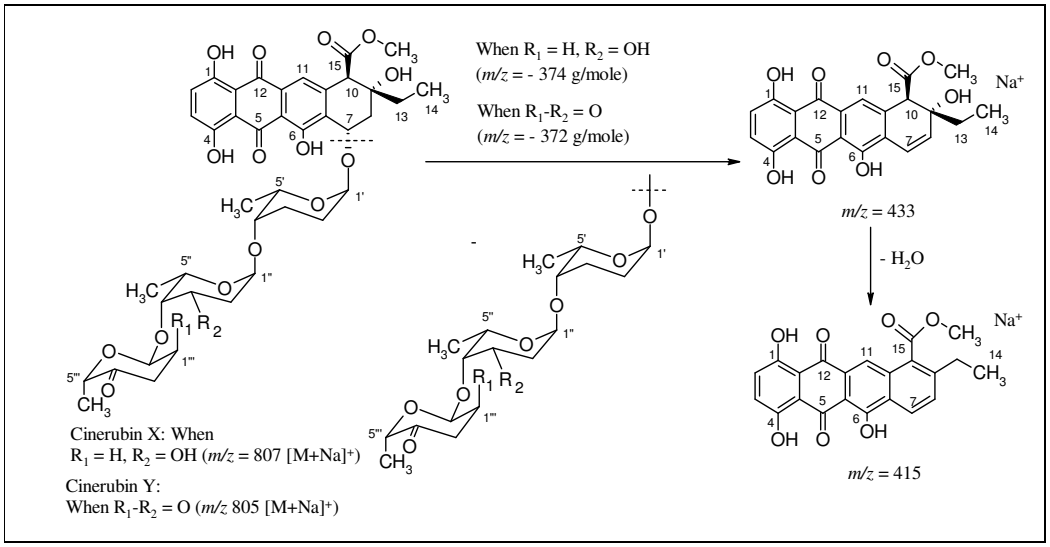


Figure 147: Fragmentation of Cinerubins Y (**271**) and X (**272**) in ESI-daughter ion MS.

5.13.4 Cinerubin X

An additional red-orange solid, compound **272** was obtained as major component from fraction III. It had a higher polarity than **271** and exhibited an orange UV fluorescence band during TLC, which turned violet by treatment with sodium hydroxide, pointing again to a *peri*-hydroxyquinone. According (+)- and (-)-ESI MS, the molecular weight of compound **272** was established as 784, 2 amu higher than **271**.

The ^{1}H NMR spectrum of **272** revealed the existence of the same pyrromycinone moiety as in **271** with a substitution at position 7. In the sugar region, there were four oxymethines (δ5.48 ~5.13 br), among them one proton at δ5.28, which was assignable to 7-H in ε-pyrromycinone (**270**)[402]. The remaining three protons were assigned to anomeric centres, pointing to three sugar units. In addition, three methyl doublets were observed between δ1.37~ 1.25, as well as seven hydroxymethines between δ4.81~3.50. On ESI-MS/MS of **272**, three characteristic fragment ions appeared at *m/z* 586, 433 and 415, attributed to the sequential loss of cinerulose A + 2-deoxyfucose (*m/z* 586), rhodosamine + H_2O (*m/z* 433) followed by a complete aromatization to give the parent chromophor of η-pyrromycinone (**267**, *m/z* 415) (Figure 147).

The ^{13}C NMR spectra of compound **272** displayed 40 carbon signals which were classified into several categories i.e. one keto carbonyl at δ210.0, as indicative of cinerulose A or B, two quinone carbonyls (δ190.3, 185.5) and one ester (δ171.2). Ten oxygenated sp^3 methine carbons (δ100.0~65.3) were found, out of which three were assigned as anomeric carbons (δ 101.3, 100.2 and 100.1). Hence, the terminal sugar unit of the trisaccharide was cinerulose A, not cinerulose B, as here a value of δ~91 is expected. The three sugar methyls were observed at δ17.1, 17.0 and 14.7.

In accordance, L-cinerulosyl-L-2-deoxyfucosyl-L-rhodinosyl-ε-pyrromycinone (cinerubin X, **272**) was deduced. The structure was further established by comparison of its spectroscopic data with an authentic sample, and with those reported by Nakagawa *et al.*[403].

Table 37: The ^{13}C NMR assignments of Cinerubins X (**272**)[403] and Y (**271**)[401] in comparison with literature data.

Position	Lit. (**272**)	Exp. (**272**)	Lit. (**271**)	Exp. (**271**)	Position	Lit. (**272**)	Exp. (**272**)	Lit. (**271**)	Exp. (**271**)
	δ_C	δ_C	δ_C	δ_C		δ_C	δ_C	δ_C	δ_C
1	157.9	158.3	158.0	158.4	15	170.8	171.2	170.9	171.3
2	130.0	130.0	129.8	130.1	15-OCH_3	52.6	52.5	52.6	52.5
3	129.4	129.6	129.4	129.7	1’	101.2	101.3	101.1	101.3
4	157.4	157.6	157.5	157.9	2’	24.6	24.7	24.6	24.3
4a	112.1	112.1	112.2	112.4	3’	24.6	24.3	24.6	24.2
5	190.0	190.3	190.1	190.7	4’	74.9	74.8	75.1	75.1
5a	114.5	114.5	114.6	114.8	5’	66.9	66.8	66.6	66.6
6	161.8	162.1	161.8	162.3	6’	17.4	17.1	17.4	17.2
6a	132.4	132.5	132.6	132.8	1’’	100.0	100.1	99.8	99.9
7	70.6	70.6	70.7	70.7	2’’	34.5	34.2	27.2	26.9
8	33.7	33.6	33.9	33.7	3’’	65.4	65.3	67.3	67.2
9	71.8	71.7	71.7	71.6	4’’	82.3	82.3	67.2	67.2
10	57.3	57.1	57.3	57.2	5’’	67.4	67.3	65.6	65.5
10a	142.2	142.4	142.2	142.5	6’’	17.3	17.0	16.4	16.2
11	120.2	120.3	120.2	120.5	1’’’	100.0	100.1	91.3	91.4
11a	131.3	131.5	131.3	131.5	2’’’	27.8	27.5	63.0	62.9
12	185.2	185.4	185.3	185.9	3’’’	34.0	33.6	39.8	39.6
12a	112.3	112.3	112.3	112.6	4’’’	209.2	210.0	207.3	207.3
13	32.3	32.0	32.4	32.1	5’’’	71.6	71.6	77.9	77.9
14	7.0	6.6	7.0	6.7	6’’’	15.0	14.7	16.5	16.2

5.13.5 Cinerubin B

Compound **273** was isolated as orange-red solid from fraction V. It exhibited also a colour change to violet with sodium hydroxide, and had a similar polarity as **271** and **272**. The molecular weight was determined as 825 Dalton using ESI mass spectra, i.e. containing an odd number of nitrogen atoms.

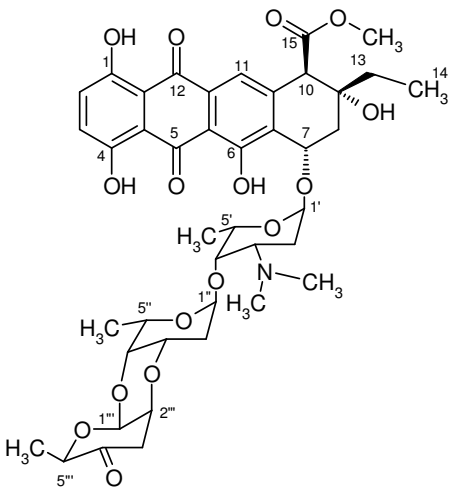
The ^1H and ^{13}C NMR spectra of **273** confirmed its structural similarity to **271** and **272**, containing the same pyrromycinone moiety, substituted at position 7. In the ^1H NMR spectrum, the compound exhibited three anomeric protons at δ 5.51 (s br), 5.19 (d) and 5.13 (m), along with the characteristic multiplet (δ 5.28) for 7-H in pyrromycinone. In addition, three methyl doublets (δ 1.37, 1.30 and 1.25) were observed together with a 6H singlet (δ 2.16) corresponding to an N-$(CH_3)_2$ group. Furthermore, seven hydroxymethines were displayed between δ 4.81~3.72 beside a sp^2-bound methylene doublet (δ 2.60). Finally, two methylene multiplets were visible at δ 2.09 (2"-H_2) and 1.87-1.60.

The ^{13}C NMR spectrum of compound **273** showed the same characteristic keto carbonyl (δ 208.2) of either cinerulose A or B. However, the anomeric carbon of cinerulose A was absent (δ 100.0) while the characteristic one for Cinerulose B (δ 91.5) was visible. The remaining two anomeric carbons were at δ 101.5 and 99.0.

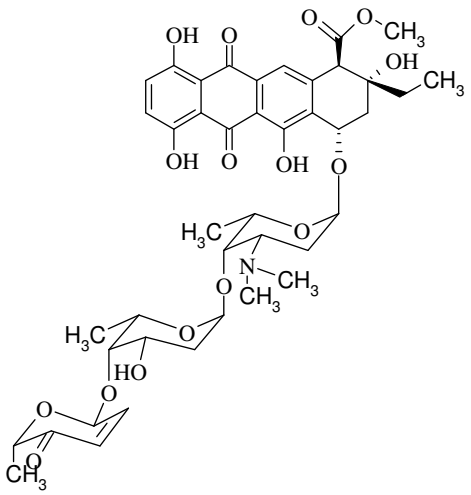
Also, a signal at δ 42.9 of double intensity, corresponding to ($N(CH_3)_2$) was observed together with the methyls of three sugar units at δ 17.9, 16.17, 16.0. Additionally based on the ESI-MS/MS fragmentation pattern, compound **273** was established to bear three sugar residues. This was attributed to the presence of a characteristic fragment at *m/z* 586 resulting from the expulsion of cinerulose B + 2-deoxyfucose from the parent molecule. An additional peak at *m/z* 393 corresponded to the complete loss of the sugar units [M–(cinerulose B+2-deoxyfucose)-rhodosamine+H]$^+$ from the molecule followed by aromatization (Figure 148).

Based on the above discussed spectroscopic data, two structures were found in AntiBase: cinerubin B (**273**) and pyrraculomycin (**274**). Pyrraculomycin (**274**) was, however, excluded as there were no signals of olefinic double bonds conjugated with a carbonyl in the sugar system present. Therefore, cinerubin B (**273**) was plausible, showing a close agreement with values of an authentic sample and the literature data[404].

Cinerubin B (**273**) showed antibacterial, antifungal and cytotoxic activity[405]. The MIC values of **273** against *Staphylococcus aureus* and *Candida albicans* were 3.13 and 6.25 μg/ml, respectively. Pyrraculomycin (**274**) showed higher activity against Gram-positive bacteria than cinerubin B (**273**)[406].



273



274

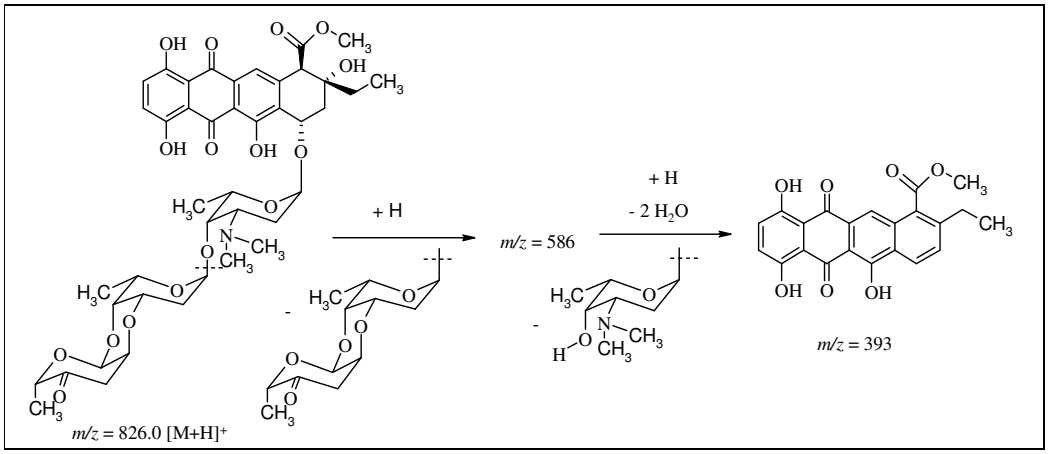


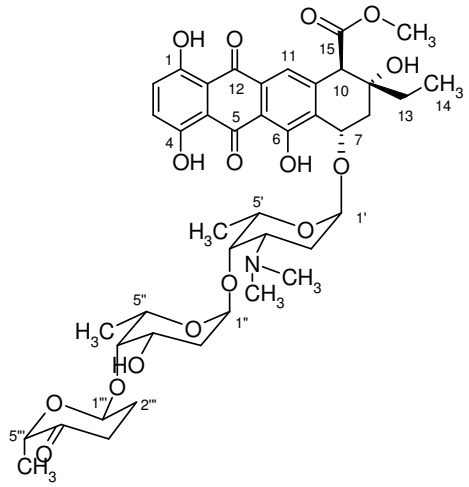
Figure 148: Fragmentation of Cinerubin B (**273**) in ESI-daughter ions MS.

5.13.6 Cinerubin A

PTLC purification of fraction IV led to the isolation of **275**, an orange red solid, exhibiting similar colour reactions as cinerubin B (**273**), but with a higher polarity. In accordance, compound **275** has very similar structure as **273**, with a molecular weight (827 Dalton) 2 amu higher than the latter.

This assumption was supported by the ^{1}H NMR spectrum, which indicated the same pyrromycinone skeleton, substituted at 7-position. Both compounds showed also a high similarity in the sugar region of the ^{1}H NMR spectrum. However, the four signals of 1'''-H (δ5.08), 1''-H (δ5.05), 5'''-H (δ4.54), and 5'-H at δ4.51 in **275** were overlapping, giving two multiplets, while they were highly resolved in case of **273**.

A search in AntiBase with the molecular weight and the ^{1}H NMR spectrum referred to cinerubin A. The structure was further confirmed by comparison of the ^{1}H NMR spectrum with the authentic one as well as literature data[404]. Cinerubin A (**275**) belongs to the class of anthracycline antibiotics isolated from several *Streptomyces* sp.[407]. Cinerubin A (**275**) was found to be much more active than cinerubin B (**273**) against *Candida albicans*, *Aspergillus fumigatus* and *Cladosporium* spp. The MIC values for *Cladosporium* spp., *A. fumigatus* and *C. albicans* were 0.4, 0.6 and 0.2 μg/ml, respectively. Cinerubin A (**275**) was inactive against *Citrobacter* spp., *Escherichia coli*, and multiple drug-resistant *Staphylococcus aureus*[404,408].



275

5.13.7 Cinerubin K

Compound **276** was isolated as red powder from fraction IV. On TLC, it showed an orange UV fluorescence and turned violet on treatment with a diluted sodium hydroxide, pointing to a further *peri*-hydroxyquinone. The molecular weight of compound **276** was determined by ESI MS as 770 Dalton. ESI high resolution of the molecular signal established the molecular formula of **276** as $C_{40}H_{50}O_{15}$.

The proton NMR spectrum of compound **276** established again the pattern of ε-pyrromycinone (**270**) substituted at 7-position as in the previously discussed four anthracyclines. Between δ 5.28-1.08 several signals typical for sugar units were observed, among them three methyl doublets were at δ 1.21, 1.15 and 1.08.

The ^{13}C NMR/APT spectra of **276** established the pyrromycinone moiety (**269**), containing three carbonyls of the quinone system (δ 190.7 and 185.9) and the ester (δ 171.4). In addition to the signals of the pyrromycinone chromophore, three signals of anomeric protons (δ 101.4, 99.5 and 99.4), together with 6 methylene and three methyl signals were observed.

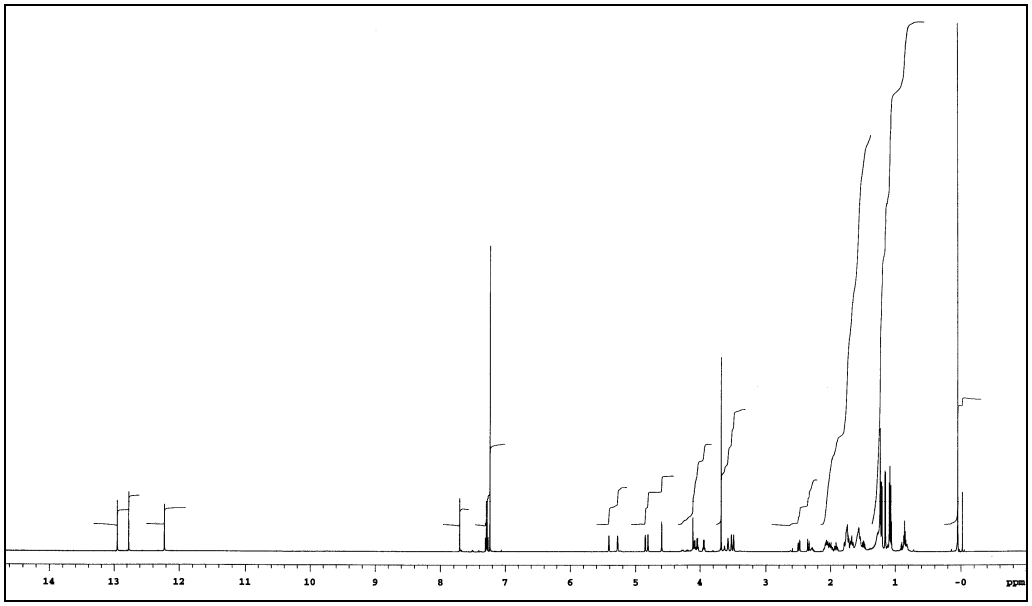


Figure 149: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of Cinerubin K (**276**).

On ESI-MS^2, an ion peak at *m/z* 383 was afforded as a result of the expulsion of 3 rhodinose residues + Na^+, in addition to a peak at *m/z* 415 corresponding to η-pyrromycinone + Na^+ after dehydration and aromatization of ε-pyrromycinone (**270**).

Analysis of the COSY and C,H7 correlations supported that compound **276** is an ε-pyrromycinone (**270**) derivative containing three identical rhodinose units. Their configurations at the anomeric centres were determined as α, as they have small coupling constants ranging between *J* ~1-3 Hz. The three sugar units were confirmed to be attached 7-position of **270** as the shown cross-signal between 7-H (δ_H 5.3) of the aglycone and the anomeric carbon of L-rhodinose (δ_C 101.4), and *vice versa*. Additionally, the residual two L-rhodinose moieties showed clear connectivities between the anomeric protons H-1'' (δ_H 4.81) and H-1''' (δ_H 4.85) and the corresponding carbons of the adjacent sugar, C-4' (δ_C 74.2) and C-4" (δ_C 74.9), respectively (Figure 151).

Cinerubin K (**276**) is an isomer of cytorhodin J (**277**)[409]. In the majority of all anthracyclines, the sugar is rhodinose in its L-configuration. That this is also the case for **276**, can be derived from the small coupling constant between H-4'/5' of the sugar moieties, but should be confirmed later. According to database searches, it is a new compound, which was named cinerubin K.

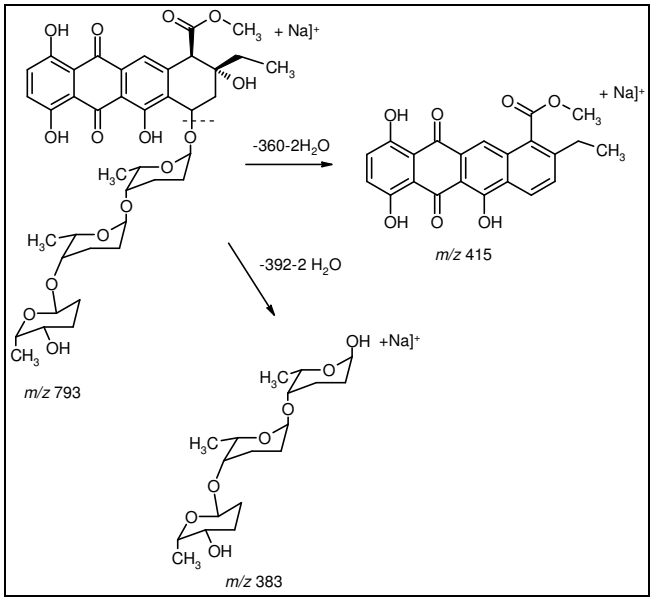
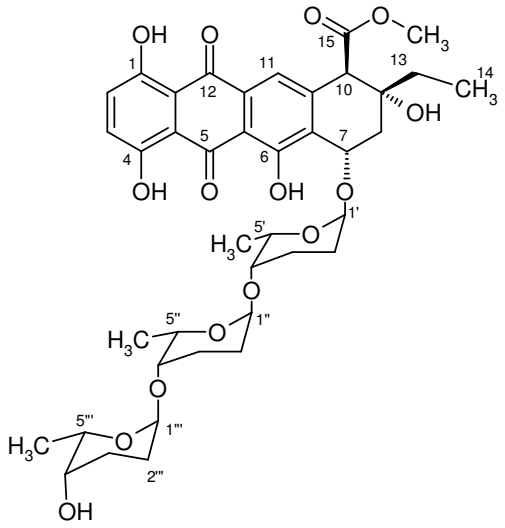
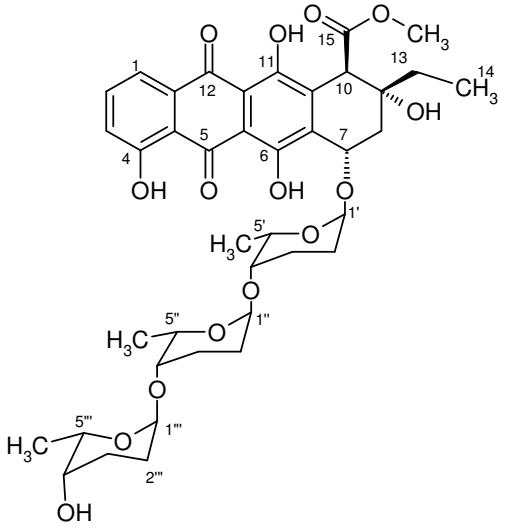


Figure 150: ESI MS^2 Fragmentation pattern of Cinerubin K (**276**).

About 500 naturally occurring Anthracycline antibiotics were reported in the literature[61,105,410], containing at least one sugar moiety. They are characterized by their antibacterial and antitumor activities, e.g cinerubins[411], ciclamycins[412], musettamycin[413], pyrromycin[398]. Antibacterial and antifungal activities of the isolated compounds (**267**, **269**, **271~273**, **275** and **276**) were *semi*-quantitatively determined using the agar diffusion method with 9 mm paper discs and 20 µg /disk. Cinerubin A (**275**) exhibited activity against all the tested microorganisms, while cinerubin B (**273**) showed only activity against *Staphylococcus aureus* (Table 81).



276



277

Table 38: ^{13}C and ^{1}H NMR assignments of Cinerubin K (**276**) in $CDCl_3$.

Position	δ_C [a]	δ_H (J in Hz) [b]	Position	δ_C [a]	δ_H (J in Hz) [b]
1	158.4	-	13	32.1	1.73 (m), 1.49 (m)
1-OH	-	13.00 (s)	14	6.7	1.11 (t, 7.3)
2	130.1	7.35 (d, 9.3)	15	171.4	-
3	129.7	7.32 (d, 9.4)	15-OCH_3	52.5	3.72 (s)
4	157.8	-	1’	101.4	5.46 (d, 3.3)
4-OH	-	12.27 (s)	2’	24.7	2.04 (m), 1.67 (m)
4a	112.4	-	3’	24.5	1.74 (m)
5	190.7	-	4’	74.2	3.57 (s)
5a	114.8	-	5’	67.5	4.10 (m)
6	162.3	-	6’	17.2	1.13 (d, 6.7)
6a	132.8	-	1’’	99.6	4.85 (d, 3.5)
6-OH	-	12.82	2’’	24.4	1.75 (m), 1.54 (m)
7	70.6	5.32 (d, 2.4)	3’’	23.6	1.67 (m)
8	33.7	2.55 (dd, 4.29, 15.0), 2.33 (d, 15.1)	4’’	74.9	3.53 (s)
9	71.7	-	5’’	67.0	3.99 (m)
9-OH	-	4.64	6’’	17.2	1.25 (d, 6.5)
10	57.2	4.16 (s)	1’’’	99.4	4.89 (d, 2.7)
10a	142.5	-	2’’’	26.0	2.05 (m), 1.73 (m)
11	120.5	7.76 (s)	3’’’	29.7	1.25 (m)
11a	131.6	-	4’’’	67.4	3.62 (s)
12	185.9	-	5’’’	66.7	3.99 (m)
12a	112.6	-	6’’’	17.1	1.20 (d, 6.6)

[a] 300 MHz, [b] 150 MHz

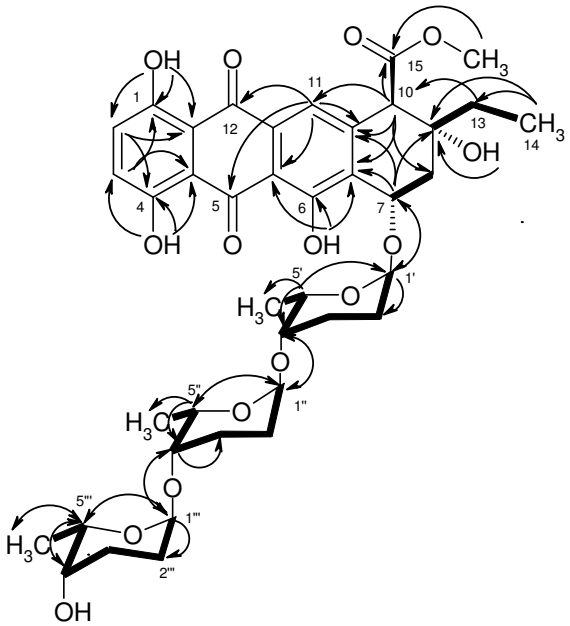


Figure 151: H,H COSY (▬) and HMBC (↔, →) correlation of Cinerubin K (**276**).

5.14 Marine-derived *Streptomyces* sp. Mei6-1,2

During our antimicrobial assay, the crude extract from the marine *Streptomyces* sp. Mei6-1,2 was potently active against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Candida albicans* and *Mucor miehei* (Table 82). HPLC-MS analysis of the extract displayed constituents with masses between 296~719 g/mol. TLC screening of the crude extract showed several yellow bands, which changed to red on treatment with diluted sodium hydroxide, as indication of *peri*-hydroxyquinones. Additional UV absorbing bands were observed, which turned reddish-brown by spraying with anisaldehyde/sulphuric acid. Therefore, fermentation of the strain was upscaled (20 L) using M_2^+ medium containing 100% artificial sea water. After usual working up, a yellow oily crude extract was obtained. Chromatography (Figure 152) afforded four new anthrayclinones, the boshracins A~D (**278**, **280**, **282**, **284**), together with bafilomycins B_1 (**110**) and B_2 (**111**)[152, 414,415], and bafilomycin C_1-amide (**116**).

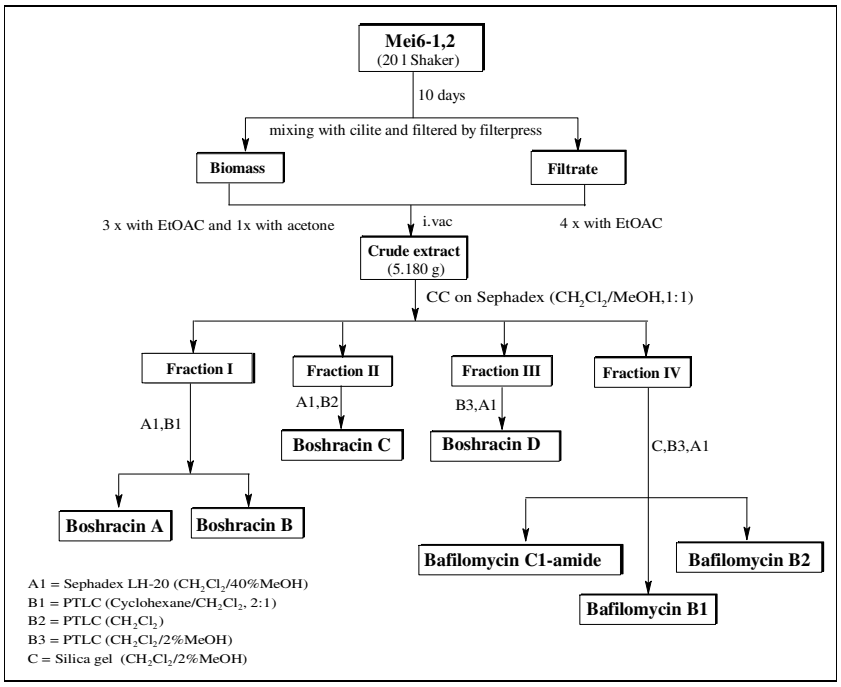


Figure 152: Work-up procedure of the marine *Streptomyces* sp. Mei6-2,1

5.14.1 Boshracin A

Compound **278** was isolated from fraction I by a series of chromatographic purification steps using Sephadex LH-20, silica gel and PTLC as a yellow solid with an

orange UV fluorescence on TLC. The orange colour changed to red on treatment with diluted sodium hydroxide, which is indicative of *peri*-hydroxyquinones. The molecular mass was determined by EI MS as 304 Dalton, and the corresponding high resolution delivered its molecular formula as $C_{19}H_{12}O_4$.

The ^{1}H NMR spectra of **278** exhibited two singlets of *peri*-hydroxy groups (δ 13.76 and 12.30), together with seven aromatic proton signals. Three of them showed the *o*-couplings of a 1,2,3-trisubstituted system; a second set was of a 1,2,4-trisubstituted system. The final signals appeared as a 1H singlet at δ 8.22; its downfield shift pointed to its location in *peri*-position to a carbonyl group. Finally, a methyl singlet was displayed at δ 2.57, which was most likely attached to an sp^2 carbon of the aromatic system.

The ^{13}C/APT NMR spectra of **278** showed 19 carbon signals, which were classified into two carbonyls of a quinone system (δ 191.8 and 181.9); their high shift difference of $\Delta\delta$ ~10 pointed to a chelation of only one of them. The remaining 17 carbon signals were assigned as 9 quaternary sp^2 atoms (among them two at δ 163.7, 162.6 were hydroxycarbons), 7 sp^2 methine carbons, and one sp^3 carbon (δ 22.0) of an aromatic bound methyl.

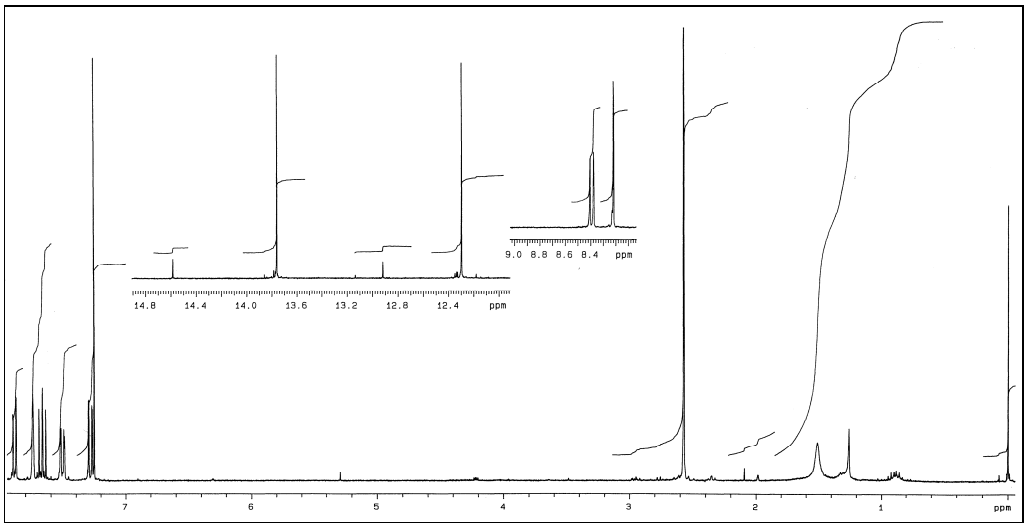


Figure 153: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Boshracin A (**278**).

Hence, three aromatic residues, a benzoquinone and a methyl group were concluded (Figure 154). So, two alternative tetracenequinones were proposed: 1,11-

dihydroxy-8-methyl-naphthacene-5,12-dione (**278**) and 1,11-dihydroxy-9-methyl-naphthacene-5,12-dione (**279**).

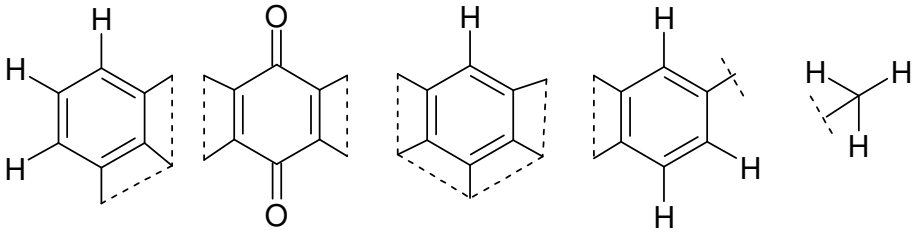
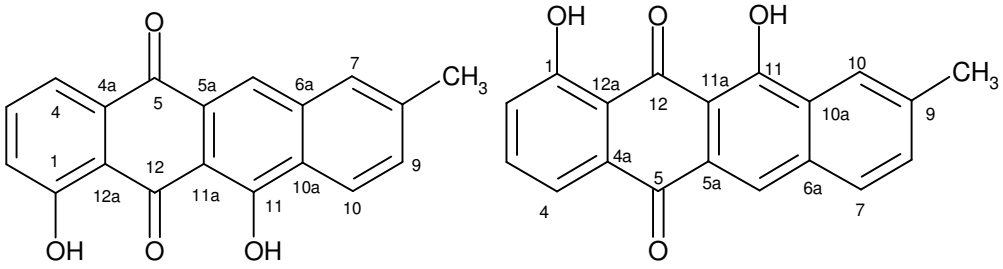


Figure 154: Suggested partial structures of Boshracin A (**278**).



278 **279**

To deduce the final structure, the compound was intensively studied using H,H COSY and HMBC experiments. Based on the HMBC experiment (Figure 155), the two protons at δ 8.22 (H-6) and 7.88 (H-4) exhibited ^3J cross signals with the carbonyl quinone C-5 (δ 181.9), confirming their location in *peri*-position to the non-chelated carbonyl. The methine H-6 showed an additional ^3J coupling with the *m*-coupling methine (C-7/10, δ_C: 129.7), which in turn displayed a cross signal with methyl singlet 8-CH_3 (δ_H: 2.57, δ_C: 22.0). The second quinone carbonyl C-12 (δ 191.8) showed no HMBC cross signals and must therefore be flanked by two chelated OH groups in *peri*-positions at C-1 (δ 162.6) and C-11 (δ 163.7), respectively, based on their ^2J correlations with the respective OH protons.

Finally, one of the *o*-coupled protons (H-10, δ 8.39) displayed long-range coupling (^3J) with the oxygenated carbon C-11 (δ 163.7), while its partner (H-9, δ 7.51) showed a coupling to the aromatic bound methyl (8-CH_3, δ_C: 22.0) and *vice versa*. So, the structure was assigned as 1,11-dihydroxy-8-methyl-naphthacene-5,12-dione (**278**) but not as 1,11-dihydroxy-9-methyl-naphthacene-5,12-dione (**279**). Angular quinones are excluded by the HMBC coupling of H-11 with 12-CO. According to the

literature, it is a new natural product, which we named boshracin A. The compound was, however, already obtained by synthesis[416].

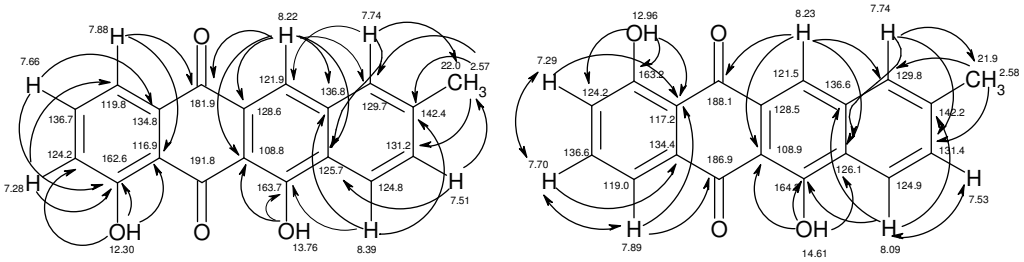


Figure 155: H,H COSY (↔) and HMBC (→) connectivities of Boshracins A (**278**) and B **280**.

Table 39: ^{13}C & ^{1}H NMR ($CDCl_3$) spectroscopic data for Bosrhacins A-B (**278**, **280**), *J* in [Hz].

Position	Boshracin A (**278**)		Boshracin B (**280**)	
	δ_H (300 MHz)	δ_C (150 MHz)	δ_H (300 MHz)	δ_C (150 MHz)
1	-	162.6	-	163.2
1-OH	12.30 (s)	-	12.96 (s)	-
2	7.28 (dd, 8.3, 1.1)	124.2	7.29 (dd, 8.4, 1.1)	124.2
3	7.66 (t, 7.5)	136.7	7.70 (t, 8.0)	136.6
4	7.88 (dd, 7.5, 1.1)	119.8	7.89 (dd, 7.6, 1.1)	119.0
4a	-	134.8	-	134.4
5	-	181.9	-	186.9
5a	-	128.6	-	108.9
6	8.22 (s)	121.9	-	164.2
6-OH	-	-	14.61 (s)	-
6a	-	136.8	-	126.1
7	7.74 (d,1.2)	129.7	8.39 (d, 8.5)	124.9
8	-	142.4	7.53 (dd, 8.6, 1.6)	131.4
8-CH_3	2.57 (s)	22.0	-	-
9	7.51(dd, 8.3, 1.1)	131.2	-	142.2
9-CH_3	-	-	2.58 (s)	21.9
10	8.39 (d, 8.5)	124.8	7.74 (d, 1.2)	129.8
10a	-	125.7	-	136.6
11	-	163.7	8.23 (s)	121.5
11-OH	13.76 (s)	-	-	-
11a	-	108.8	-	128.5
12	-	191.8	-	188.1
12a	-	116.9	-	117.2

5.14.2 Boshracin B

Compound **280**, a low polar yellow solid, was isolated from fraction I. It exhibited similar colour reactions as boshracin A (**278**), pointing to an additional *peri*-hydroxy quinone. Compound **280** showed the same molecular weight (304 Dalton)

and molecular formula ($C_{19}H_{12}O_4$) as boshracin A (**278**), as indicative for a second two positional isomer.

The ^{1}H NMR spectrum of **280** exhibited the same pattern as boshracin A (**278**): two chelated hydroxy groups (δ 14.61 and 12.96), three aromatic residues (each one 1,2,3-trisubstituted, 1,2,4-trisubstituted and pentasubstituted), and one aromatic bound methyl group (δ 2.58). Moreover, the compound showed ^{13}C/APT NMR spectra of high similarity in the chemical shifts with **278**.

The sole difference was the shift of the quinone-carbonyls (δ 188.1 and 186.6), exhibiting a smaller shift difference ($\Delta\delta$~2 ppm) than those for **278**. So, each of the two carbonyls must have a hydroxy group in *peri*-position, and two possible tetracenequinones were suggested therefore, 1,6-dihydroxy-9-methyl-naphthacen-5,12-dione (**280**) and 1,6-dihydroxy-8-methyl-naphthacen-5,12-dione (**281**).

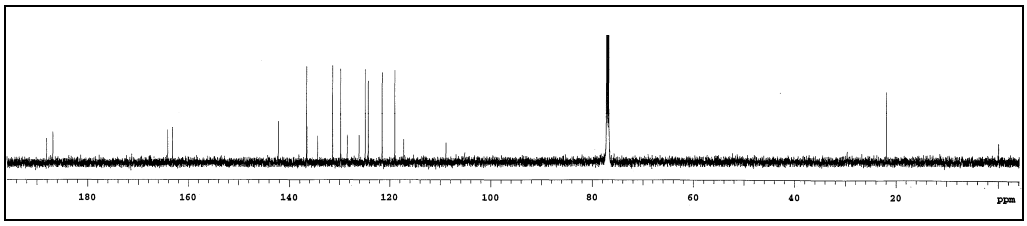
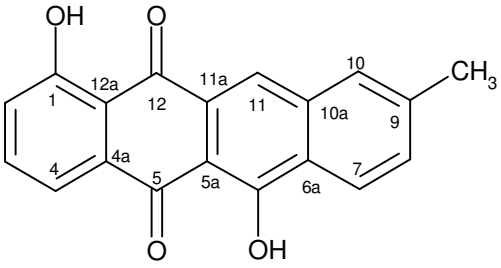
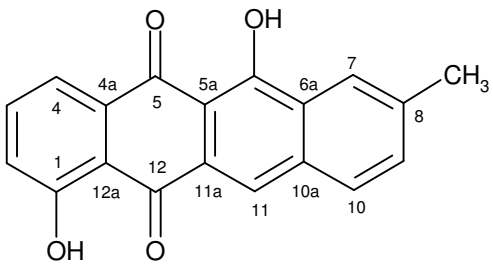


Figure 156: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of Boshracin B (**280**).



280



281

The singlet of H-11 (δ 8.23) showed a long-range coupling to the carbonyl at C-12 (δ 188.1) and to C-10 (δ 129.8), confirming the syn-periplanar orientation of H-10/11 with respect to 12-CO. According to its ^2J correlation, the *peri*-hydroxy proton at δ 14.61 was attached to C-6 (δ 164.2) in ring C. This was confirmed by the long range correlation of H-7 (δ 8.09, present in ring D) with C-6. The ^3J coupling between the *peri*-proton H-4 (δ 7.89) and C-5 (δ 186.9) confirmed the attachment of

the other hydroxy group (δ12.96) at C-1 in ring A. As in **278**, the methyl group (C-13) was connected with C-9 in ring D. Consequently, the compound was identified as 1,6-dihydroxy-9-methyl-naphthacen-5,12-dione (**280**), but was not 1,6-dihydroxy-8-methyl-naphthacen-5,12-dione (**281**). A search in the databases pointed to the novelty of **280** , which was named boshracin B.

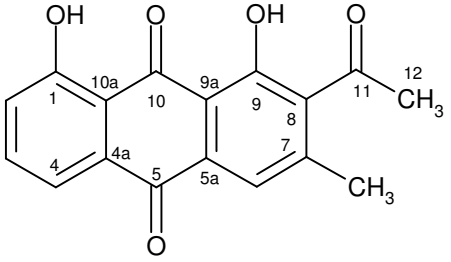
5.14.3 Boshracin C

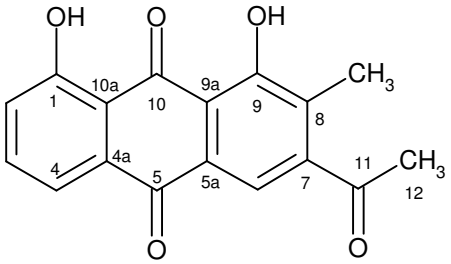
Compound **282** was isolated as middle polar yellow solid from fraction II. It showed an orange UV fluorescent band during TLC, which turned to red by treatment with diluted sodium hydroxide, as indication of a third *peri*-hydroxy-quinone. The EI-mass spectrum recognized its molecular weight as 296 Dalton, and the corresponding molecular formula was determined as $C_{17}H_{12}O_5$ by HREI-MS. The ^{1}H NMR spectrum of **282** established the presence of two *peri*-hydroxy signals (δ12.35 and 11.97) together with four aromatic signals corresponding to 1,2,3-trisubstituted and penta-substituted aromatic residues. In the aliphatic region, two 3H singlets were visible at δ2.60 and 2.39, which were most likely due to methyls linked to the aromatic system and/or carbonyl groups.

The ^{13}C/APT NMR spectra of compound **282** exhibited 17 carbon signals, three carbonyl signals corresponding to an acetyl (δ203.0) and the quinone system (δ192.6 and 181.4). The big shift difference ($\Delta\delta$ ~11) between both quinone carbonyls indicated the *peri*-location of the first carbonyl (δ192.6) between the two chelated hydroxy groups, as in boshracin A (**278**). Six of the remaining twelve sp^2 carbons were attributed to the four-methine and two hydroxy-carbons (δ162.6, 159.4). The previously discussed two singlets (δ31.9 and 20.3) were corresponding to an acetyl and an aromatic methyl, respectively.

Based on HMBC experiments, the two *peri*-protons of H-4 (δ7.82) and H-6 (δ7.67) showed long-range correlations with the carbonyl C-5 (δ181.9). This confirmed their *peri*-location. The chelated hydroxy protons (δ12.35 and 11.97) showed coupling with the oxygenated carbons, C-9 (δ159.4) and C-1 (δ162.6), respectively. Protons of the aromatic methyl group displayed strong cross-signals (^3J) with C-6 (δ122.2) and C-8 (δ136.2) as well as with the linked carbon (δ145.5), confirming its location at C-7 and not at C-8. So, the acetyl group was located at C-8 (δ136.2) as

shown by the ^{3}J cross signal between C-8 and the methyl protons at δ 2.60; the latter group was in turn connected to the corresponding carbonyl (203.0) *via* a ^{2}J coupling.





282 **283**

Consequently, the structure was identified as 8-acetyl-1,9-dihydroxy-7-methyl-anthraquinone (**282**), a new quinone, which we named boshracin C.

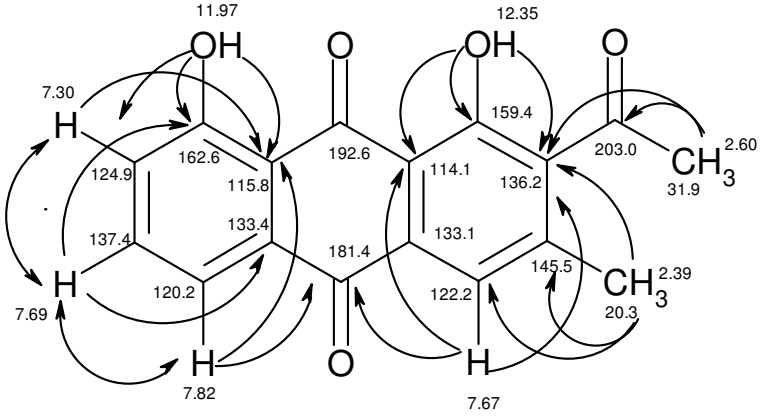


Figure 157: H,H COSY (↔) and HMBC (→) correlations of Boshracin C (**282**).

Table 40: ^{13}C and ^{1}H NMR ($CDCl_3$) spectroscopic data for Boshracins C-D (**282**, **284**), *J* in Hz.

Position	Boshracin C (**282**)		Boshracin D (**284**)	
	δ_H (300 MHz)	δ_C (150 MHz)	δ_H (300 MHz)	δ_C (150 MHz)
1	-	162.6	-	162.5
1-OH	11.97 (s)	-	12.03 (s)	-
2	7.30 (dd, 8.4, 1.2)	124.9	7.31 (dd, 8.4, 1.2)	124.7
3	7.69 (t, 8.3)	137.4	7.69 (t, 8.4)	137.3
4	7.82 (dd, 8.5, 1.2)	120.2	7.83 (dd, 7.5, 1.2)	120.1
4a	-	133.4	-	133.6
5	-	181.4	-	181.6
5a	-	133.1	-	132.3
6	7.67 (s)	122.2	-	121.5
6a	-	-	-	145.5
7	-	145.5	3.10 (dd, 17.8, 2.2), 2.87 (d, 17.9)	44.4
7-CH_3	2.39 (s)	20.0	-	-
8	-	136.2	-	68.5
8-CH_3	-	-	1.46 (s)	29.9
9	-	159.4	2.38 (td, 14.7, 2.0), 1.97 (dd, 14.7, 5.1)	40.6
9-OH	12.35 (s)	-	-	-
9a	-	114.1	-	-
10	-	192.6	5.30 (brm)	62.8
10a	-	115.8	-	132.2
11	-	203.0	-	161.2
11-OH	-	-	12.70 (s)	-
11a	-	-	-	113.8
12	-	-	-	192.7
12a	-	-	-	115.8
8 & 10-OH	-	-	3.45 (brs) and 3.58 (brs)	-

5.14.4 Boshracin D

Compound **284** was isolated as middle polar yellow solid, from fraction III. The compound showed an orange fluorescence under UV during TLC; the colour changed to red on treatment with diluted sodium hydroxide, referring to a fourth *peri*-hydroxy-quinone. The molecular mass of **284** was established as 340 Dalton by ESI-MS spectrum, and HRESI-MS deduced its corresponding molecular formula as $C_{19}H_{16}O_6$.

The ^{1}H NMR spectrum of **284** exhibited two chelated hydroxy protons (δ 12.70 and 12.03) along with 1,2,3-trisubstituted and pentasubstituted aromatic residues as in **282**. In the aliphatic region, a methyl singlet (δ 1.46), one oxymethine multiplet

(1H, δ 5.30) together with two exchangeable broad proton signals (δ 3.58 and 3.45) were seen. Finally, there were four 1H signals (δ 3.10, 2.87, 2.38 and 1.97), which were characteristic of two methylene groups included in a ring system as they displayed high coupling constants (J = 14.7 and 2.0 Hz).

The ^{13}C/APT NMR spectra of compound **284** exhibited 19 carbon signals, two of which were of the quinone system (δ 192.7 and 181.6). According to their shift, both hydroxy groups must again be chelated with the first carbonyl (δ 192.7), and the other carbonyl (δ 181.6) must be flanked in *peri*-position by two protons. Four of the remaining 12 sp^2 carbons were belonging to methines, while two were oxygenated (δ 162.5, 161.2). In the aliphatic region, the spectra displayed five carbon signals, belonging to a quaternary oxycarbon (δ 68.5), an oxymethine (δ 62.8), two methylenes (δ 44.4 and 40.6) as well as a methyl carbon (δ 29.9). Therefore, the compound possessed a tetracyclic quinone system, containing one aliphatic ring. This allowed ten positional isomers (Figure 158).

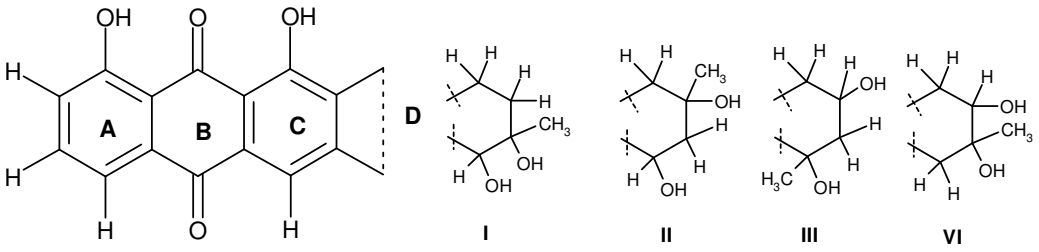


Figure 158: Eight of the ten possible alternatives of Boshracin D (**284**) according to **D** ring orientation *versus* the main quinone skeleton.

In the H,H COSY spectra, no correlations between protons of the two-methylene groups in ring D were detected, excluding their adjacent positions, and hence **D**-**I** was excluded. In contrast, the cross-signal between the oxymethine proton H-10 (δ 5.30) and one proton of the methylene group H_2-9 (δ 1.97) was indicative of their direct linkage, while the correlation with H_2-7 did not observed, excluding also the possibility **D**-**III**. Hence four possible structures **284**-**287** were left.

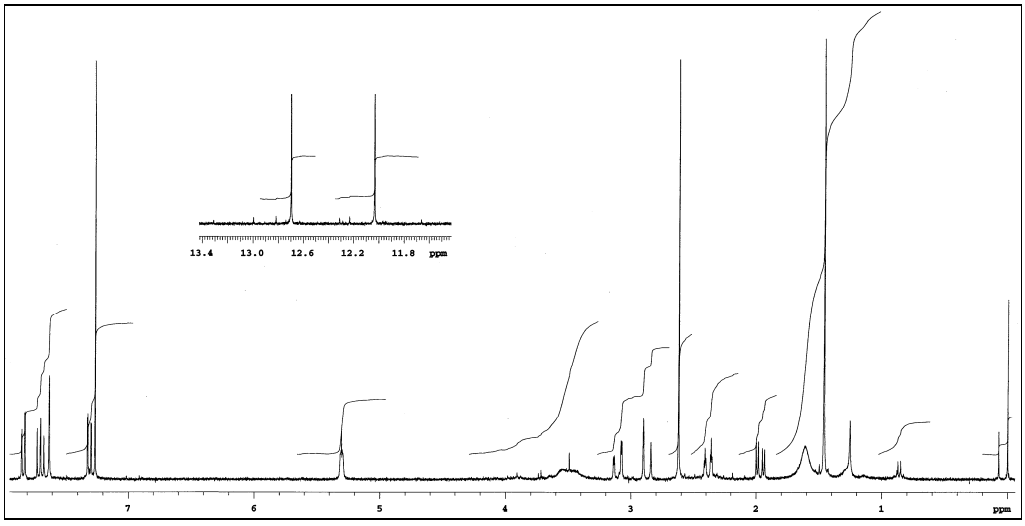
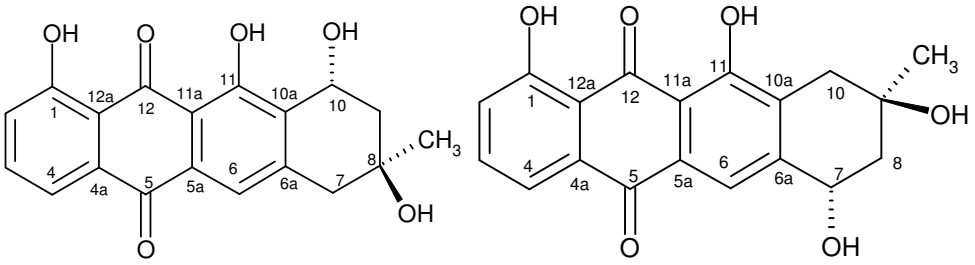
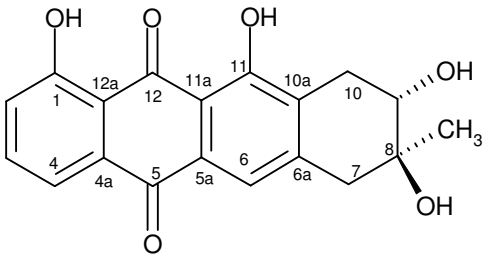


Figure 159: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Boshracin D (**284**).

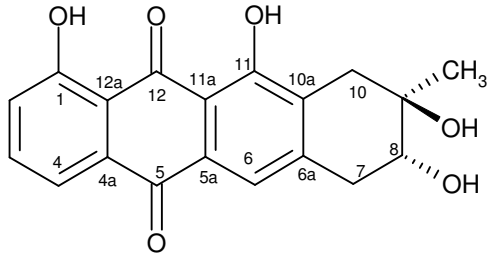


284

285



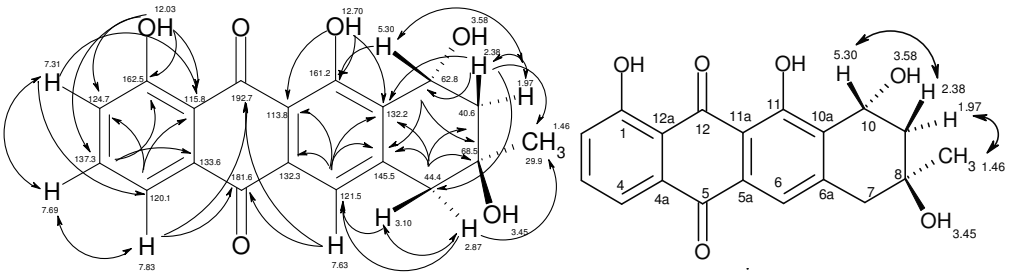
286



287

Therefore, the compound's structure was intensively studied using HMBC experiment (Figure 160a). Based on the HMBC ^{3}J correlations from oxymethine proton H-10 (δ5.30, δ_C: 62.8) with the oxygenated carbon C-11 (δ161.2) and from the methylene group H_2-7 with C-6 (δ121.5), and C-10a (δ132.2), the structures of **285** - **287** were excluded. Therefore, structure **284**, 1,8,10,11-tetrahydroxy-8-methyl-7,8,9,10-tetrahydro-naphthacen-5,12-dion, was confirmed as the sole consistent

structure. It had been mentioned before as synthetic product[417], however, without spectral data; we named it boshracin D.



a) b)

Figure 160: a) H,H COSY (↔) and HMBC (→), and b) NOESY correlations of Boshracin D (**284**).

Boshracin D (**284**) is optically active: it showed a positive specific rotation (+60°) and gave a CD effect. The relative configuration was assigned on the basis of NOESY data (Figure 160b), where strong correlations were found between the oxymethine (H-10, δ5.30), and one proton (δ2.38) of the neighbouring methylene group (C-9). However, a strong correlations were found between the methyl group (8-CH_3) and the other methylene proton at δ1.97 of the neighbouring methylene group (C-9). This assigned the chiral centers, C-8 and C-10 to be of (8*S**,10*R**)

All four boshracins were inactive in the agar diffusion test at 40 μg/disk against selected bacteria, fungi, and microalgae. However, boshracins A (278) and B (280) showed cytotoxic activity against human cell lines with a mean IC_{50} of > 10 μg/ml (Table 45). The biological importance of anthracyclines has already been discussed above (p. 235).

5.15 Marine *Streptomyces* sp. Isolate Merv8102

The marine *Streptomyces* sp. Merv8102 was isolated from sediments collected from the Mediterranean Sea at the Egyptian coast, and taxonomically identified in the National Research Centre, Egypt, and deposited there. The obtained crude extract was potently active against diverse bacterial isolates, and and moderately active against fungal isolates. TLC of the extract exhibited a major UV absorbing band of a middle polar component, which on spraying with anisaldehyde/sulphuric acid was stained pale yellow.

The strain was upscaled in a 10 L shaer fermentation using a medium[418] containing 75% of sea water. After working up and isolation using RP-18 column chromatography, a colourless solid of essramycin (**288**) was obtained (Figure 161). Details of the working up, isolation and identification of essramycin (**288**) were described in a recent article[419].

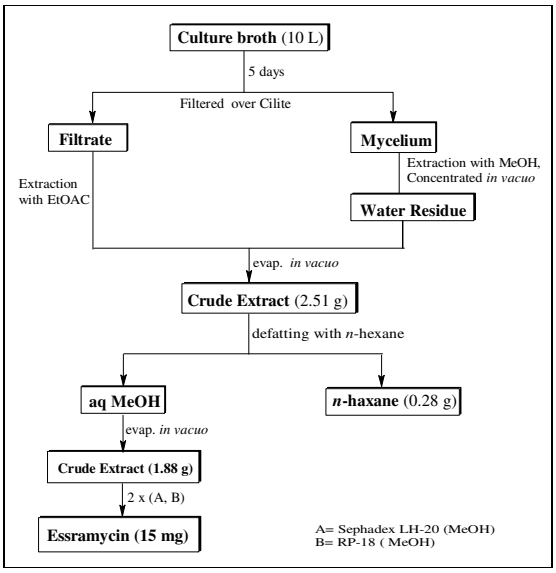


Figure 161: Working up scheme for extracts of *Streptomyces* sp. Merv8201.

5.15.1 Essramycin

The UV spectra (MeOH) of **288** displayed two strong bands at λ_{max} 244 and 277 nm in neutral solution. Under acidic conditions, the latter band showed a hypsochromic shift to λ_{max} 271 nm, while a bathochromic shift to λ_{max} 282 nm was observed in basic methanol ((Figure 162).

The IR spectra of **288** displayed a signal at ν 3350 cm^{-1} of NH or OH groups. Between ν 3080~2950, two absorption bands were indicative of aromatic and aliphatic C-H bonds, two strong bands at ν 1695 and 1645 cm^{-1} indicated conjugated carbonyl groups and an aromatic skeleton or double bonds. The molecular weight was determined by ESI MS. Two and three *quasi*-molecular ion peaks in positive and negative ESI MS mode, respectively, confirmed the molecular weight of **288** as 268 Dalton. (+)-HRESI MS of **288** delivered the molecular formula $C_{14}H_{12}N_4O_2$.

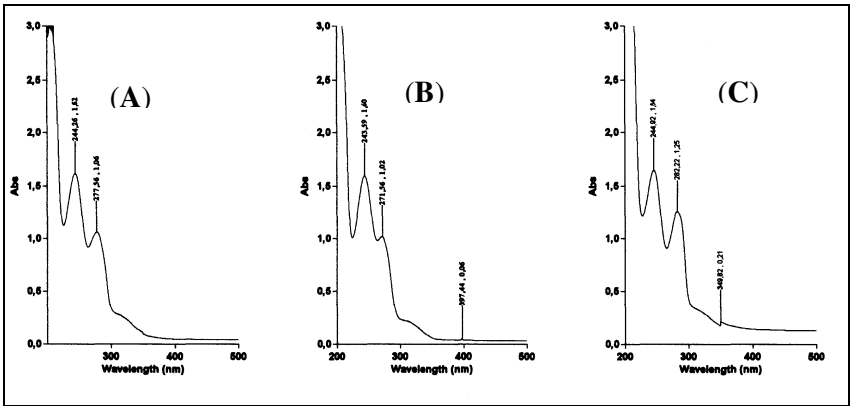


Figure 162: UV spectra of Essramycin (**288**) in: (A) neutral, (B) acidic, and (C) basic solutions.

The ^{1}H NMR spectrum of **288** showed the pattern of a monosubstituted aromatic system. The coupling constants (7.5-8, 1.0~1.3 Hz) were indicative of a benzene derivative, and the dd signal at δ8.04 (2H), and two td signals at δ7.66 (1H) and 7.54 (2H) pointed to an electron-withdrawing substituent. The spectra displayed only three additional singlets, an olefinic methine signal at δ5.76, a singlet of a methylene group at δ 4.49, and at δ2.28 the signal of a methyl group bound to an aromatic system, a double bond, or to a carbonyl group.

The ^{13}C/HMQC spectra of compound **288** exhibited 12 carbon signals, among them a carbonyl signal at δ195.0, and signals of four quaternary carbons at δ 155.6, 151.9, 151.4 and 136.0, the first three being due to O or N connected sp^2 carbon atoms. Furthermore, three sp^2 methine signals, two of them with double intensity, were due to the phenyl moiety. A fourth methine signal at δ 97.9 gave an HMQC correlation with the olefinic proton at δ5.76. In the aliphatic region, at δ 38.0 and 18.9, the expected carbon signals of a methylene and a methyl group, respectively, were visible. Both shifts excluded an attachment to hetero atoms.

In the HMBC spectrum, the *ortho*-coupled aromatic protons at δ 8.04 (H-2'/6') and 7.54 (H-3'/5') exhibited ^{2}J and ^{3}J couplings with the carbon signals at δ136.0 (C_q-1') and 133.4 (CH-4'), confirming the mono-substituted phenyl moiety. Additionally, the methine protons H-2'/6' (δ8.04) displayed a significant ^{3}J coupling with the carbonyl carbon at δ195.0, which in turn coupled (^{2}J) with the methylene protons (H_2-10, δ4.49). The latter protons displayed an additional cross signal with the

quaternary carbon at δ 159.0. As no further couplings of the methylene group were visible, and due to the downfield shift of the quaternary C atom, a guanidine fragment was assumed, resulting in partial structure **A** (Figure 163).

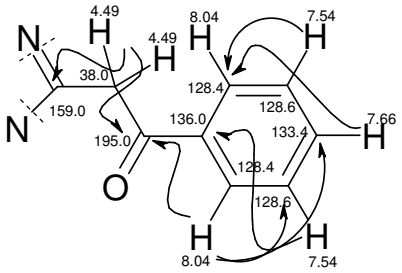
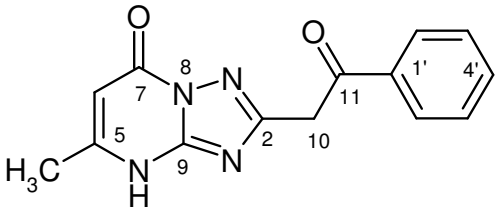


Figure 163: HMBC HMBC correlations in partial structure **A** of Essramycin (**288**)

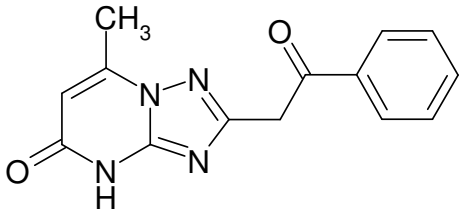
On subtracting the acetophenone substructure of **A**, 6 double bond equivalents were left for the remaining fragment $C_6H_5N_4O$. This pointed firstly to one of the four N-methylhypoxanthins; these were, however, easily excluded, as their N-methyl signals appear between δ = 3~4 and not at δ = 2.28 as in **288**.

According to the HSQC data, the olefinic proton (δ5.76) was attached to the upfield carbon at δ97.9. HMBC cross signals of this CH with the methyl group (Figure 164) and *vice versa* confirmed a propene fragment in which the methyl group was connected with the carbon at δ 151.9. The alkene shifts required a carbonyl group attached to the upfield carbon, whereby the former must be present as an amide to explain its shift. The methyl group coupled weakly with this carbonyl group and with an additional quaternary carbon at δ 151.4. However, due to overlapping with δ 151.9, it was not possible to distinguish between a weak ^3J or a strong ^4J coupling. This latter carbon showed no coupling with the methine proton at δ5.76, so that a ^3J coupling was excluded with respect to the CH and the CH_3 group.

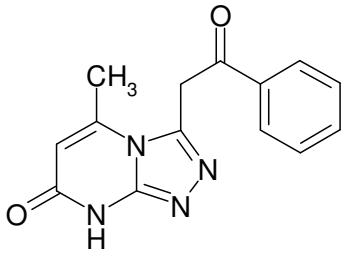
A calculation of all possible isomers containing substructure **A** and fulfilling the conditions listed above delivered 41 hits[420]. When the highly strained or chemically unstable structures were sorted out, only compounds **288-290** and further 5 prototrop-isomers with higher ground state energies[421] were left. Two of them were [1,2,4]triazolo[4,3-*a*]pyrimidines (e.g. **290**), with the methyl group and the acetophenone substituent in a *syn*-periplanar position. As essramycin did not show any nuclear Overhauser effect between the methyl group and the phenone moiety (solely an interaction between Me and 6-H is visible), these isomers were excluded.



288

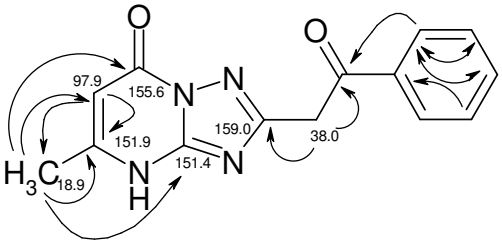


289



290

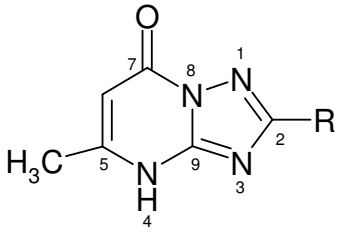
Figure 164: The three alternative skeletons **288-290** for Essramycin.



288

Figure 165: Selected (→) HMBC correlations of Essramycin (**288**).

It was not possible to distinguish between **288** and **289** on the basis of 2D CH correlations, and further measurements were hindered by the low yield. Comparison of the ^{13}C NMR data with values of related synthetic compounds of type **288** delivered, however, a much better agreement with the experimental values than with compounds of type **289**, confirming the skeleton of **288** [120,422]. A C-2 substituent affects the *ipso* C atom of 1,2,4-triazolo[1,5-a]pyrimidines, however, the influence on the ^{13}C shifts of the other ring atoms is negligible (Table 41). So, compound **288** was established as 5-methyl-2-(2-oxo-2-phenyl-ethyl)-4H-[1,2,4]triazolo[1,5-a]pyrimidine-7-one, a first triazolopyrimidine antibiotic isolated from nature, and named as essramycin.



291: R = H;
292: R = CH_2OH
293: R = $CH_2(CH_2)_5CH_3$
294: R = CH_2OCH_2COOH
295: R = CH_2SH

Table 41: ^{13}C Chemical shifts (δ values in [D_6]DMSO) of Essramycin (**288**) and further 2-substituted [1,2,4]triazolo[1,5-a]pyrimidine derivatives **291~295**[422].

No	C-2	C-5	C-6	C-7	C-9	5-CH_3
288	159.0	151.9	97.9	155.6	151.4	18.9
291	151.9	151.5	98.2	155.9	150.6	18.7
292	163.8	151.4	98.1	155.7	150.8	18.6
293	164.1	151.0	98.0	155.0	150.6	18.6
294	160.4	151.5	98.2	155.7	151.0	18.6
295	160.8	151.3	98.3	155.5	151.0	18.6

Table 42: ^{13}C and ^{1}H NMR spectral data for Essramycin (**288**) ([D_6]DMSO, shifts as δ values).

Position	δ_C (75 MHz)	δ_H (300 MHz, *J* in Hz)	HMBC
2	159.0	-	
5	151.9	-	
5-CH_3	18.9	2.28 (s)	3a, 5, 6, 7
6	97.9	5.76 (s, br)	5, CH_3-5
7	155.6	-	
9	151.4	-	
10	38.0	4.49 (s)	2, 11
11	195.0	-	
1’	136.0	-	
2’	128.4	8.04 (dd, 8.1, 1.0)	11, 3’, 4’
3’	128.6	7.54 (td, 7.5, 1.2)	1’, 2’
4’	133.4	7.66 (td, 8.4, 1.3)	
5’	128.6	7.54 (td, 7.5, 1.2)	
6’	128.4	8.04 (dd, 8.1, 1.0)	

Essramycin (**288**) is antibacterially active with minimum inhibitory concentrations (MIC) of 2 to 8 μg/ml against Gram-positive and Gram-negative bacteria, while it showed no antifungal activity[419].

Triazolopyrimidines are synthetic heterocycles with valuable bioactivity[61, 423-425]. They are useful in therapeutics, especially for the treatment and prevention of

cardiovascular diseases and in particular for the treatment of hypertension, cardiac insufficiency and diseases of the arterial wall, especially atherosclerosis [426]. They are known also as smooth muscle cell growth inhibitors [427] and are efficient analgesic and anti-inflammatory agents [428]. [1,2,4]Triazolo[1,5-a]pyrimidines have found a broad interest as fungicides [423a], herbicide safener [429,430], kinase inhibitors [431,432], antiparasitic [433] and plant protecting agents [434]. Thousands of compounds of this type have been described, however, to the best of our knowledge, not a single natural product is amongst them [61,105 ,435].

5.16 Marine-derived *Streptomyces* sp. Mei34

The marine *Streptomycete* sp. isolate Mei34 was selected, as its extract was highly active against Gram-positive (*B. subtilis*, S. *aureus*, *Streptomyces viridochromogenes* (TÜ57) and Gram-negative bacteria (*E. coli*), and microalgae (*Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*), based on the agar diffusion method. Moreover, the extract was moderately active against the yeast *Candida albicans* and the fungus *Mucor miehei* (Tü284).

On TLC, the extract displayed three polar bands, two of which turned to blue, while the third turned to greenish-yellow by spraying with anisaldehyde/sulphuric acid. HPLC-MS of the strain extract, showed a signal with a mass peak of 705/707, indicating a chlorine containing compound.

Large-scale fermentation on shaker at 28 °C for 10 days in 100 of 1-liter-Erlenmeyer flasks delivered a crude extract, which was subjected to flash chromatography on silica gel. Four fractions were separated according to TLC monitoring. Purification of the polar fraction III by a series of chromatographic steps lead nafisamycin (**297**). It formed to pale yellow crystals, was UV absorbing and turned to greenish-yellow by spraying with anisaldehyde/sulphuric acid. Purification of fractions I and II yielded, respectively, 2'-deoxy-thymidine and 2'-deoxy-uridine as colourless solids (Figure 166).

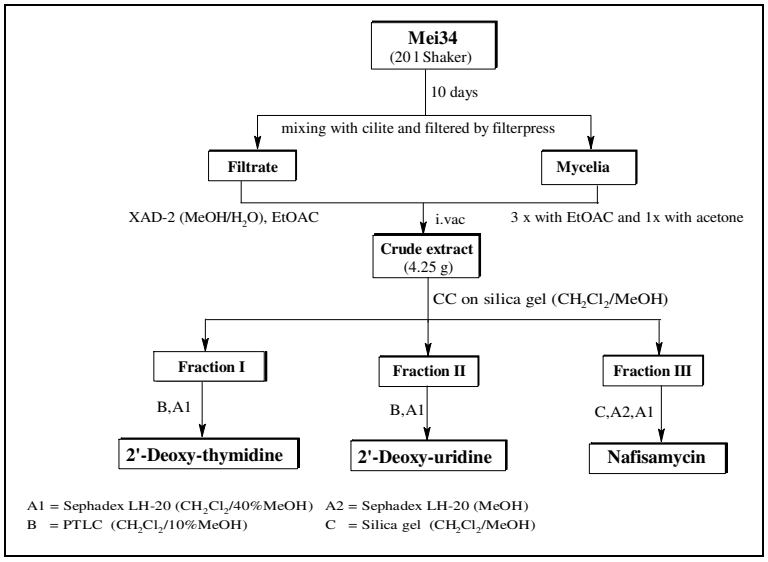


Figure 166: Working up scheme of the marine *Streptomyces* sp. isolate Mei34.

5.16.1 Nafisamycin

The UV spectra (Figure 167) of **297** displayed two strong bands at λ_{max} 289 and 327 nm, and the last band was bathochromically shifted (λ_{max} = 336 nm) in acidic solution. The molecular weight of compound was determined by ESI-MS to be 704 Dalton, and ESI-HRS mass spectrometry established its molecular formula as $C_{34}H_{38}Cl_2N_2O_{15}$.

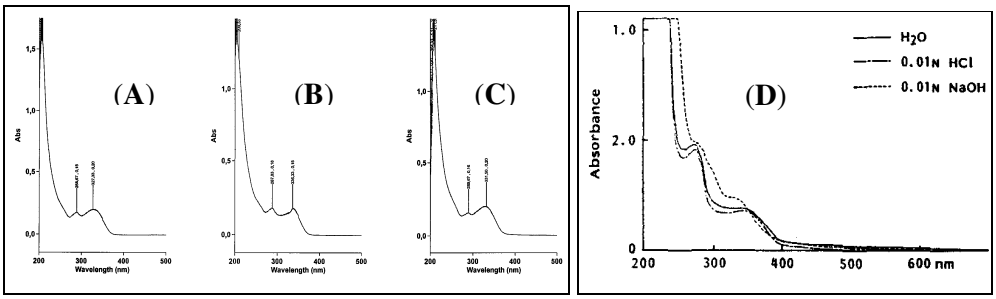


Figure 167: UV spectra of Nafisamycin (**297**) in: (**A**) neutral, (**B**) acidic, and (**C**) basic solutions, compared with those of antibiotic C-1027 (**D**; **300**)[436].

The proton NMR spectrum of **297** showed four 1H doublet signals located in the aromatic/olefinic regions. Two of these protons (δ 7.34, 6.77) were of a 1,2,3,4-tetrasubstituted aromatic ring (J ~8.0 Hz), while the remaining two (δ6.14 and 6.59) were of a 1,3,4,5-tetrasubstitituted aromatic system and showed a *m*-coupling (J ~2.1 Hz). The chemical shift of latter two protons was indicative of their neighbourhood

to hydroxy groups in a phenolic system. Three additional sp^2 methines were at δ6.95, 6.80, and 6.79 (J < 4 Hz), belonging most likely to a five-membered ring, e.g a cyclopentadiene or a heterocyclic system, e.g. a pyrrole.

In the aliphatic region, six oxymethine protons were detected, among them a 1H singlet at δ6.08, while the residual five signals were between δ4.78~2.97, attributed probably to a sugar system. A 1H signal was visible at δ 2.30, which was interpreted as a methine connected with nitrogen, together with two methylene groups. Shifts of two methylenes at δ4.51, 3.78 and δ2.69, 2.25 identified the first one of them as oxygenated, and the other one linked most likely to an sp^2 carbon. Their coupling pattern was indicative of their inclusion in a ring system. Four singlets of five methyl groups were shown, the first of which (δ2.48) was corresponding to an $N(CH_3)_2$ group, while that at δ1.93 could belong to an acetyl group. Finally, the residual two methyl singlets (δ 1.50 and 1.32) belonged to a $C(CH_3)_2$ according to the 2D experiments.

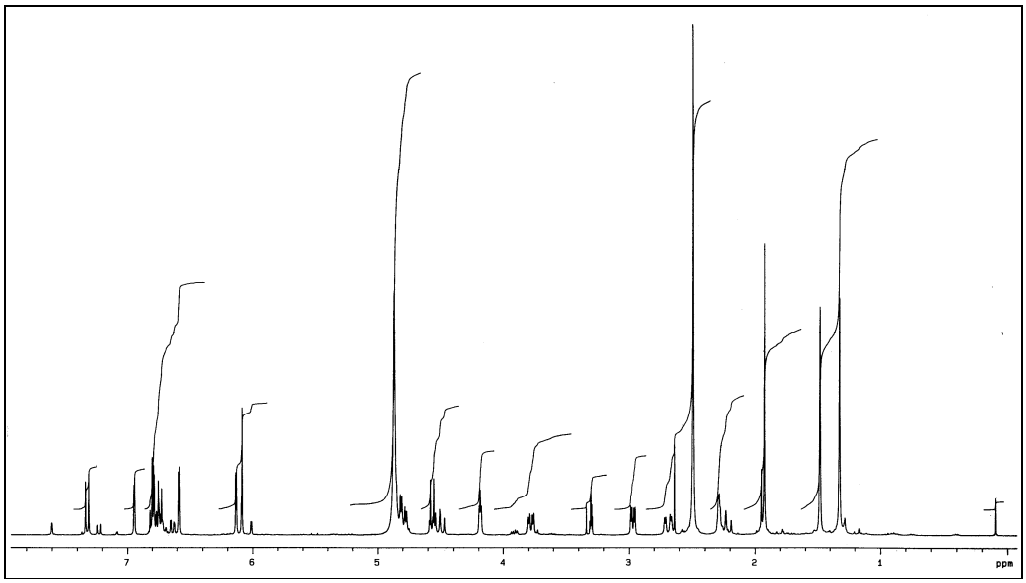


Figure 168: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of Nafisamycin (**297**).

The ^{13}C/APT NMR spectra indicated the presence of 32 carbon signals, two carbonyls of acid derivatives (δ172.3 and 170.5), 18 sp^2 carbons (7 of which were methines, and the remaining 11 sp^2 were quaternary); five atoms were possibly oxygenated (δ152.6~140.0). In the sugar region, one anomeric carbon (δ94.8) in addition to five oxy-methines (δ85.4~69.4) were observed. Furthermore, two sp^3 carbon sig-

nals (δ79.4, 66.7) of quaternary oxycarbons and/or methylene carbons, one methine signal (δ51.8) of an amino methine carbon were detected. Moreover, one methylene signal (δ43.8) was observed together with two methyl signals of N-$(CH_3)_2$ (δ45.3) and an acetyl group (δ22.6). Two remaining methyl (δ31.5 and 22.5) were recognized to be connected with the quaternary oxycarbon (δ79.4), resulting in the proposed $C(CH_3)_2$ moiety.

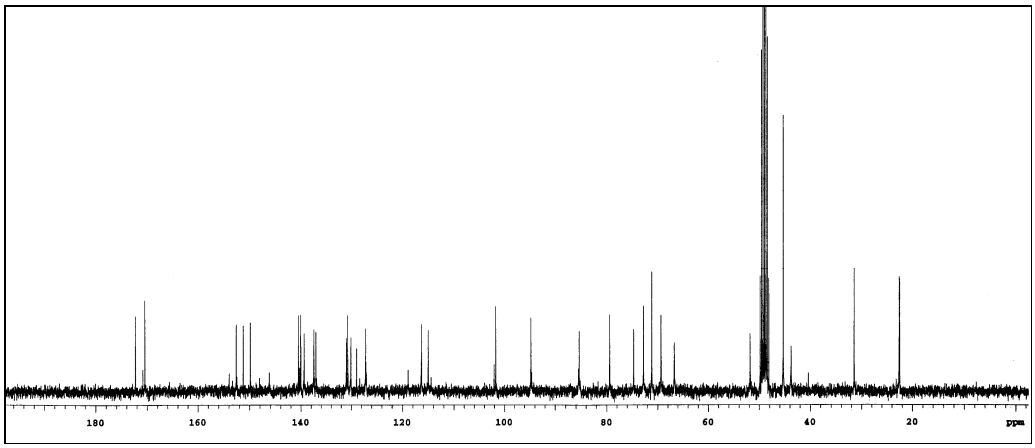


Figure 169: ^{13}C NMR spectrum (CD_3OD, 75 MHz) of Nafisamycin (**297**).

Based on the COSY and C,H correlation spectra (HMBC), the sugar part was confirmed as 5-dimethylamino-6,6-dimethyl-tetrahydro-pyran-2,3,4-triole, according to the following observations: The proton at δ 2.97 (2'-H) showed a ^2J correlation with the anomeric carbon C-1' (δ94.8). Both protons, H-1' and H-2' were assigned by H,H COSY connectivities and the coupling constant as vicinal in a bis-axial conformation (8.0 Hz). The N-$(CH_3)_2$ group was linked to C-4' (δ71.2), as the ^3J correlation from the methyl protons (δ2.48) with C-4' showed. The two methyl singlets (δ1.50 and 1.32) showed ^3J correlations with each other, as well as ^2J couplings with the oxycarbon C-5' (δ79.4), confirming that C-5' was connected with both methyls. One of the methyl singlets (δ1.32) showed a ^4J correlation towards C-1'. H-3' (δ4.19, δ_C 69.4) showed four significant correlations with C-1' (^3J), C-5' (^3J), C-2' (δ72.8, ^2J) and C-4' (δ71.2, ^2J) fixing its C-3' position. All correlations were further confirmed by the H,H COSY data (Figure 170).

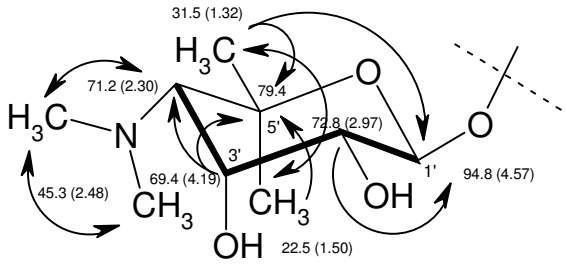


Figure 170: H,H COSY (▬) and HMBC (↔, →) correlations spectra of the sugar part.

A search with this amino sugar in AntiBase[61] delivered seven hits, all of them being endiynes or their cyclization products. The anomeric proton (δ 4.57) of the sugar moiety showed in the HMBC spectrum a ^3J correlation with C-9 (δ 101.8), confirming an O-glycosidic linkage between both amino-sugar and pentadiene moiety. According to H,H COSY and HMBC experiments, this pentadiene was forming a five membered ring, containing three methines H-10 (δ 6.80), H-11 (δ 6.79) and H-12 (δ 6.95), and two disubstitued carbons, C-9 (δ 101.8) and C-13 (δ 151.3). Accordingly, the proton of H-11 showed four correlations, two among them (^2J) with its neighbouring methine carbons C-10 (δ 137.5) and C-12 (δ 130.1), while the other two were directing to the quaternary C-1 (δ 151.3, ^3J) and to C-9 (δ 101.8) (Figure 171a).

The *o*-coupled proton at δ 7.34 (H-6) in the aromatic residue, exhibited three correlations with C-4 (δ 140.5, ^3J), C-2 (δ 137.1, ^3J) and C-7 (δ 149.9, ^2J), together with a ^3J coupling to the sp^3 oxycarbon C-8 (δ 85.4). The associated *o*-coupled proton, H-5 (δ 6.74) exhibited three correlations with C-4 (δ 140.5, ^2J), C-3 (δ 129.0, ^3J) and the aliphatic oxycarbon C-13 (δ 74.7, ^3J). This confirmed that C-4 was connected with C-13, and H-13 showed correspondingly a ^3J coupling with C-5 (δ 131.8) as well as with C-4 (δ 140.5). The chemical shift of C-3 pointed to its attachment with a chlorine atom[437]. So, in accordance, the partial structure b (Figure 171b) was proposed.

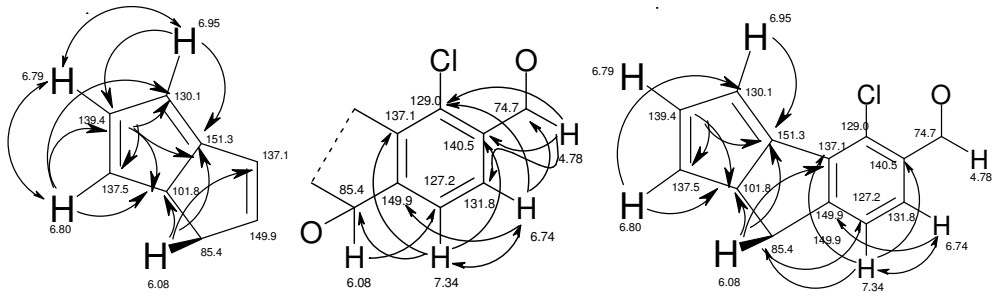


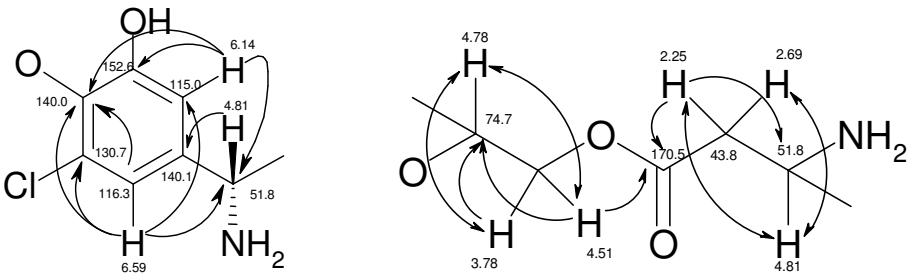
Figure 171: H,H COSY (↔) and HMBC (↔) correlations of structural constituents of Nafisamycin (**297**).

The sp^3 oxy-methine proton at δ6.08 (C-8), exhibited three important correlations, one of which was a ^2J coupling with C-9 at δ101.8. The two others were ^3J correlations with δ151.3 (C-1) and 137.1 (C-2). This confirmed the fusion of the cyclopentadiene (Figure 171a) and the aromatic residue (Figure 171b) through C_1–C_2 and C_8-C_9, to afford partial structure c (Figure 171c).

On other hand, the *m*-coupled aromatic proton H-23 (6.14) showed three correlations with the hydroxy sp^2 carbon C-22 (δ152.6, ^2J), the complimentary *m*-coupled C-19 (δ116.3, ^3J) and the nitrogenous bearing atom C-17 (δ51.8, ^3J). The *m*-coupled proton partner, H-19 (δ6.59) showed ^3J correlations with C-17 (δ51.8), C-23 (δ115.0) and C-21 (δ140.0), and a ^2J coupling with at C-20 (δ130.7). This confirmed the attachment of the amino carbon C-17 at C-18 between both *m*-coupled protons, while the other three aromatic quaternary carbons were due to two oxy-carbons (δ152.6, 140.0) and one chlorine-bearing carbon (δ130.7), respectively, establishing the partial structure d (Figure 172). The acidic protons of hydroxyl and amino groups appeared in DMSO as singlet (δ8.15) and doublet (8.41), respectively.

Additionally, the sp^3 oxymethine C-13 (δ74.7) showed a cross signal with the oxymethylene protons H_2-14 (δ3.78, 4.51) and *vice versa*, in addition to their H,H COSY correlations. One of the oxymethylene protons, H_a-14 (δ4.51) exhibited a ^3J coupling with the ester carbonyl C-15 (δ170.5). The latter carbonyl was further correlated by ^2J from H-16b (δ2.25), and this in turn coupled (^2J) with the amino-

methine carbon (C-17, δ51.8). So, the outlined fragment e was deduced (Figure 172).

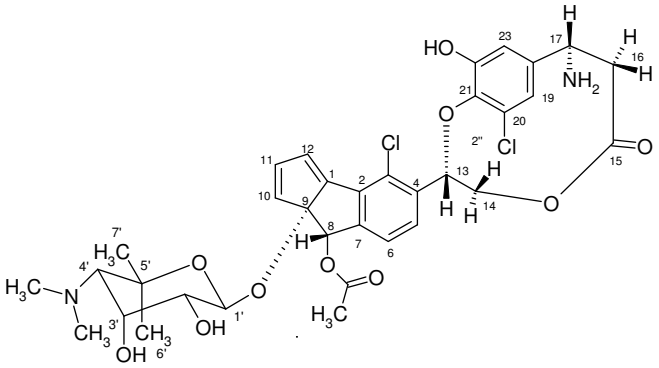


d **e**

Figure 172: H,H COSY and HMBC correlations of fragments **d** and **e** of Nafisamycin (**297**).

Finally, the residual two carbons at δ172.3 (C-24) and singlet methyl C-25 (δ_H 1.93, δ_C 22.6) were belonging to an acetyl moiety. As there were no more correlations observed from the acetyl group, two possible structures **296** and **297** were proposed. In **296**, the acetyl group was located at C-8. Consequently, an ether linkage results by ring closure *via* the phenolic oxygen at C-21 and C-13, forming an 11-membered macrolactone. In a second alternative, the acetyl group could be positioned at C-13, so that the oxycarbon C-8 (δ85.4) is now taking part in the ring closure *via* the oxygen at C-21 (δ140.0), giving compound **297**, a 16-membered macrolactone moiety.

Comparison of the experimental data with those of the related neo-C-1027 chromophores I or III (**300**; Table 43)[437] showed high similarity in their NMR data and UV spectra (Figure 167). The only difference between **297** and **300** was that the benzoxazinoneyloxy moiety (7-methoxy-2-methyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-5-carboxylic acid) in **300** was replaced by the acetyloxy in **297** at C-13, in addition to the chlorine at C-3 (Table 43). Full HMBC correlations of compound **297** were described in Figure 173.



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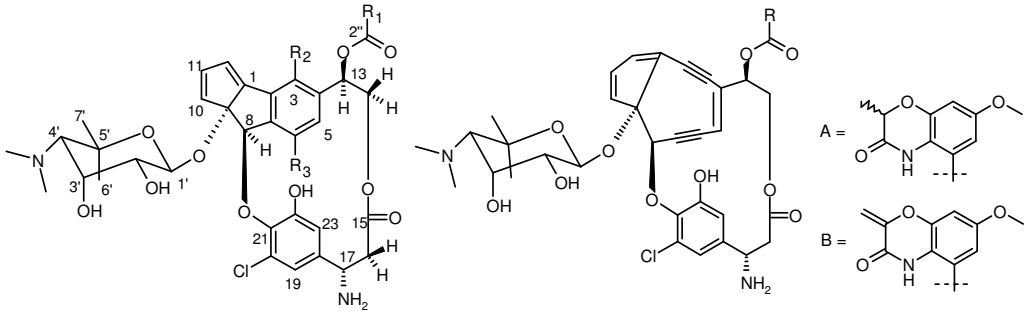


297: $R_1 = CH_3$, $R_2 = Cl$, $R_3 = H$
299: $R_1 = CH_3$, $R_2 = H$, $R_3 = Cl$
300: $R_1 = A$, $R_2 = H$, $R_3 = H$

298a: $R = CH_3$
298b: R = A (NeoC-1027 Chr-II)
298c: R = B (C-1027 Chr-IV)

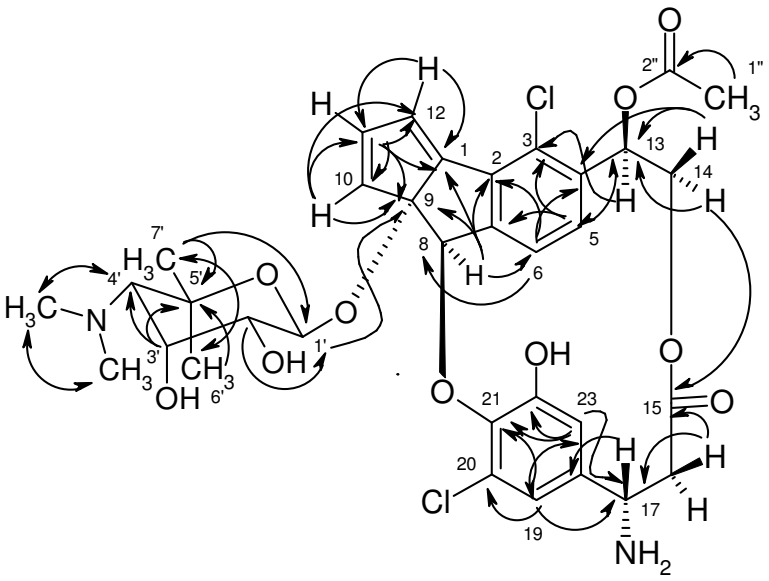


Figure 173: HMBC (→,↔) couplings in Nafisamycin (**297**).

During ESI-MS^2, compound **297** afforded three ion peaks at *m/z* 687, 669 and 499.9 which were attributed to the loss of one and two molecules water, respectively, and the sugar moiety **A** ($C_9H_{18}NO_4$) from the parent molecule. The sugar moiety **A** was the only part of the molecule **297** which could show water eliminations (Figure 174).

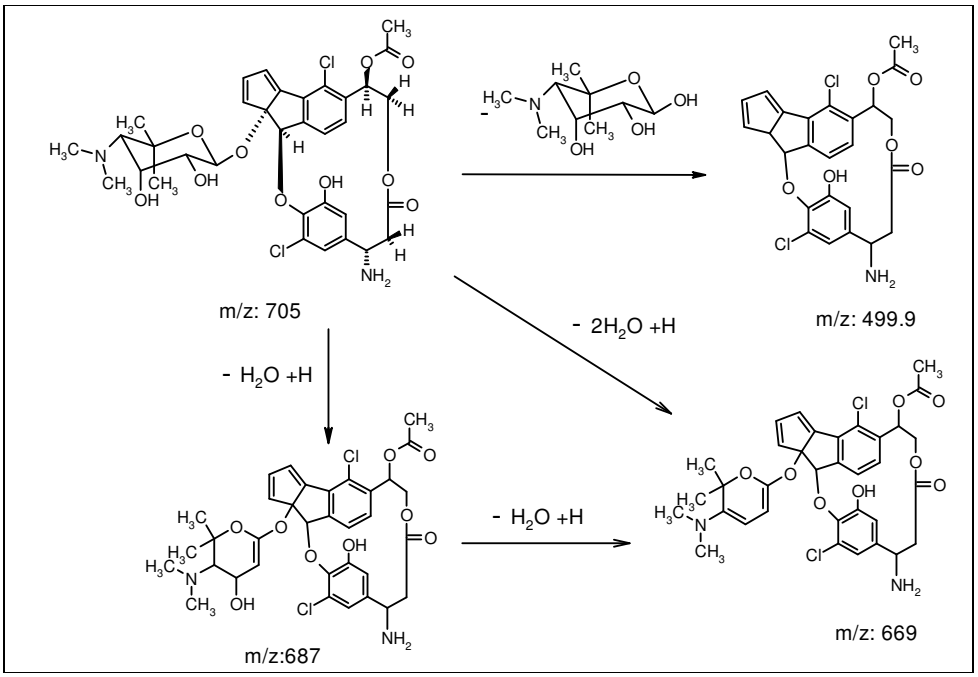


Figure 174: ESI-MS^2 Fragmentation pattern of Nafisamycin (**297**)

The stereochemistry of compound **297** was assigned on the basis of $[\alpha]_D$ (- 450°), CD spectra and NOESY experiments. The CD spectra exhibited three bands in the negative region (326, 240, and 223 nm), and one band in the positive region at 210 nm, (Figure 175). The CD spectra of nafisamycin (**297**) were very similar to those of NeoC-1027 Chr-II (**298b**) and indicated an identical absolute configuration at C-8, C-9, C-13 and C-17 to be of (8*R*,9*R*,13*R*,17*S*).

Table 43: ^{13}C and ^{1}H NMR assignments of Nafisamycin (**297**) in comparison with Neo-C-1027 Chromophor I (**300**) in CD_3OD (coupling constants *J* in [Hz]).

Position	Nafisamycin (**297**)		Neo-C 1027 Chromophor I (**300**)[449]	
	δ_C (75 MHz)	δ_H (300 MHz)	δ_C	δ_H
1	151.3	-	152.8	-
2	137.1	-	137.9	-
3	129.0	-	118.7	7.56 (br s)
4	140.5	-	141.4	-
5	131.8	6.74 (d, 8.0)	127.5	6.95 (dd, 8, 1.5)
6	127.2	7.34 (d, 7.8)	129.2	7.44 (d, 8.0)
7	149.9	-	148.4	-
8	85.4	6.08 (s)	86.0	6.11 (s)
9	101.8	-	102.0	-
10	137.5	6.80 (d, 0.4)	137.0	6.76 (d, 1.0)
11	139.4	6.79 (d, 1.9)	139.1	6.76 (d, 1.0)
12	130.1	6.95 (d, 1.8)	128.3	6.70 (br s)
13	74.7	4.78 (d, 4.0)	74.4	6.06 (t, 7.5)
14	66.7	4.51 (dd, 11.0, 16.1), 3.78 (dd, 3.8, 9.8)	67.0	4.26 (d, 7.5)
15	170.5	-	169.3	-
16	43.8	2.25 (dd, 8.4, 13.4), 2.69 (dd, 3.4, 13.5)	42.5	3.05 (dd, 12.0, 3.0), 2.59 (t, 12.0)
17	51.8	4.81 (d, 3.7)	52.9	4.40 (dd, 12.3)
18	140.1	-	134.5	-
19	116.3	6.59 (d, 2.1)	116.5	6.94 (d, 2.0)
20	130.7	-	131.5	-
21	140.0	-	141.9	-
22	152.6	-	153.4	-
23	115.0	6.14 (d, 2.0)	115.4	6.15 (d, 2.0)
1'	94.8	4.57 (d, 8.0)	93.8	4.52 (d, 8.0)
2'	72.8	2.97 (dd, 3.2, 8.0)	71.2	3.11 (dd, 8.0, 3.5)
3'	69.4	4.19 (t, 2.8, 5.6)	68.7	4.25 (overlapped)
4'	71.2	2.30 (d, 2.3)	72.0	1.8 (d, 3.0)
4'-$N(CH_3)_2$	45.3	2.48 (s)	44.9	2.98 (s)
5'	79.4	-	76.6	-
CH_3-6'	22.5	1.50 (s)	21.7	1.53 (s)
CH_3-7'	31.5	1.32 (s)	32.6	1.53 (s)
1"	22.6	1.93 (s)	-	-
2"	172.3	-	168.7	-
3"	-	-	74.1	4.68 (q, 7.0)
3"-CH_3	-	-	16.5	1.50 (d, 7.0)
4a"	-	-	146.2	-
5"	-	-	110.2	6.90 (d, 3.0)
6"	-	-	156.8	-
6"-OCH_3	-	-	56.5	3.84 (s)
7"	-	-	109.9	7.34 (d, 3.0)
8"	-	-	115.1	-
8"a	-	-	124.5	-
9"	-	-	166.5	-

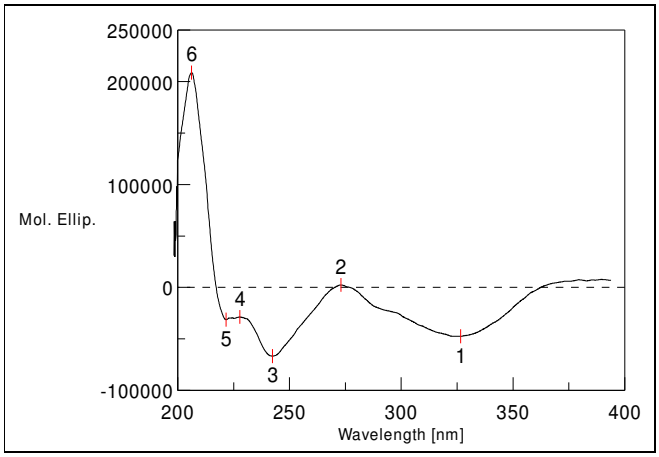


Figure 175: CD spectra (MeOH) of Nafisamycin (**297**).

The stereochemistry of the sugar moiety was further investigated on the basis of ^{1}H NMR coupling constants and NOESY experiment. Based on the coupling constants (~8.0 Hz), the oxy-methine protons H-1'/H-2' of the sugar residue were axially oriented.). This was further confirmed by an NOE effect of H-1' (δ4.57) with one of the cyclopentadiene protons, H-10 (δ6.80) and one of the geminal methyl groups (δ1.50, CH_3-6') located at C-5'. The protons H-2' and H-3' of sugar system were confirmed to be on the same side, where one of them was axial (H-2', δ2.97), while the other (H-3', δ4.19) was of equatorial conformation. On the other hand, the corresponding hydroxyl groups of C-2' and C-3' are equatorial and axial, respectively. The H-4' was deduced as axial (up direction), while its corresponding $N(CH_3)_2$ as equatorial (behind direction) (Figure 176). The stereochemistry of the sugar moiety was finally confirmed due to the high similarity with compounds containing the same sugar moiety[439] (e. g. **300**, Table 43).

The NeoC-1027 chromophore II (**298b**)[437], and others[440] were mostly obtained from streptomycetes and rare actinomycetes. Only seven compounds containing the amino sugar of **297** have been reported so far[61].

Accordingly, compound **297** was established to contain eight chiral centres, similar as the all reported enediyne antibiotics of the type C-1027 chromophores[446] These assigned stereocentres in nafisamycin (**297**) were deduced to be (8*R*,9*R*,13*R*,17*S*,1'*R*,2'*R*,3'*R*,4'*S*). A search for the structure of **297** in different data-

bases (AntiBase, DNP and CA) proved its novelty, and it was named nafisamycin[441].

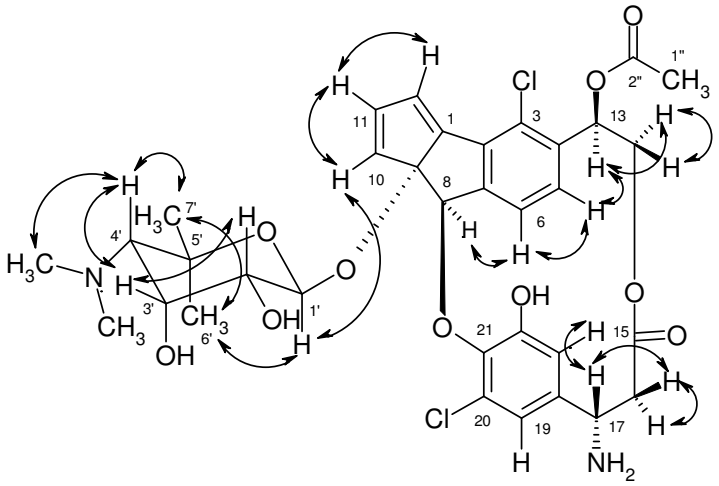


Figure 176: Key NOESY correlations of Nafisamycin (**297**).

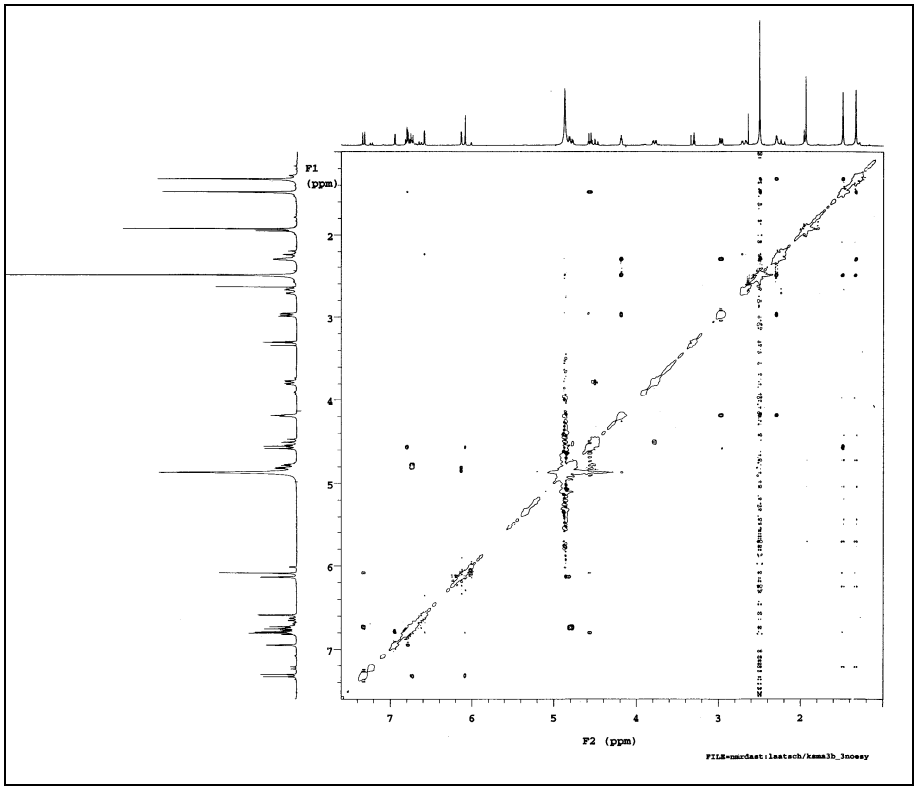


Figure 177: NOESY spectra (CD_3OD, 300 MHz) of Nafisamycin (**297**).

The close relation of nafisamycin (**297**) with the neo-C 1027 chromophor I (**300**) suggests that also their biosynthetic origin is similar: **300** may be formed by a

Masamune-Bergman cycloaromatization reaction (Figure 178)[442] from the highly reactive enediyn precursor **298b**.

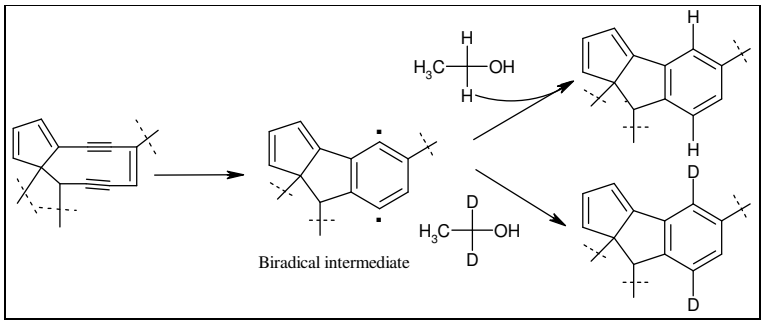
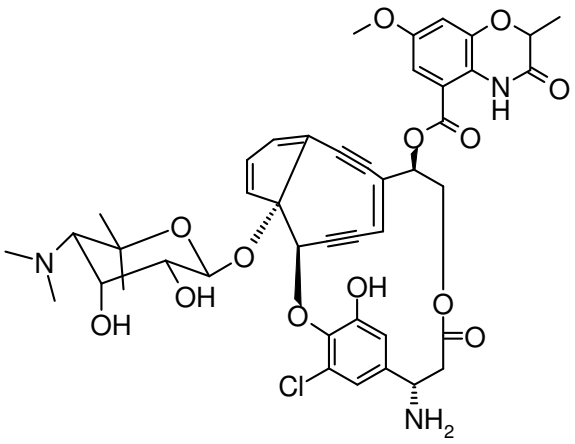


Figure 178: Masamune-Bergman cycloaromatization reaction.



298b

In a similar way, the formation of **297** could start with the cyclisation of **298** via a highly reactive diradical, which is trapped by chloride (Figure 179). As result, there are two possible orientations with respect to the chloride, which could be attached either at C-3 (**297**) or at C-6 (**299**). However, the isomer **299** was neither isolated nor detected by HPLC/MS, and might not be formed due to steric factors.

To find out if the formation of nafisamycin (**297**) occurred during the purification process (and the chlorine came from the solvent), a fresh shaker culture was extracted strictly avoiding all contacts with light and methanol or chlorinated solvents. On HPLC-MS, however, the crude extract showed only component **297** (*m/z*: 705 $[M+H]^+$), while its hypothetical acetylene precursor (**298**; *m/z* = 669) was not detectable due to its high instability or total absence. It is plausible therefore that **297** is in

fact a fermentation product which may, however, be formed from an endiyne precursor directly after its release.

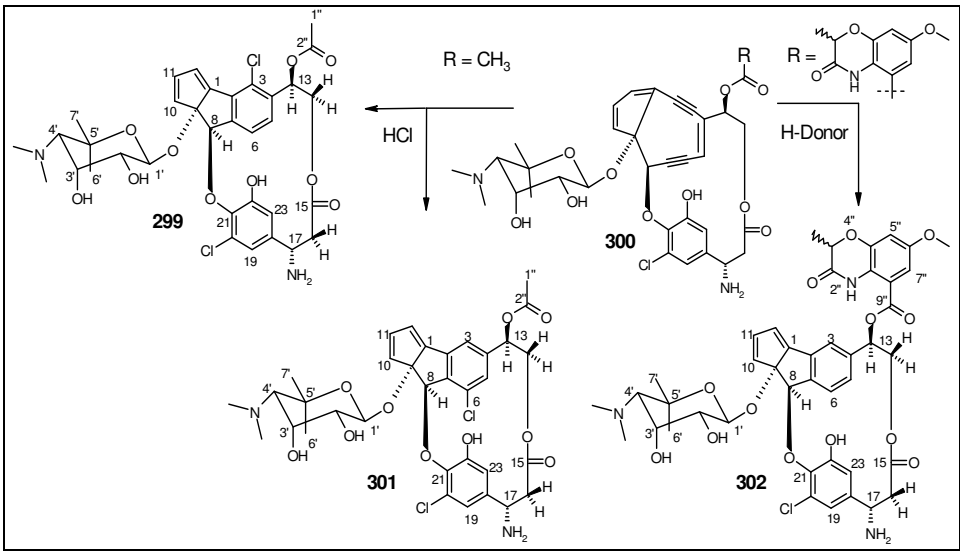


Figure 179: Formation of Nafisamycin (**297**) from a hypothetical precursor **298** by Masamune-Bergman cycloaromatization.

5.16.2 Biological Properties of Nafisamycin

Enediyne compounds are characterized by their high antitumor, as well as antibacterial activities, particularly against Gram-positive bacteria[443-449]. In the recent years, about 40 compounds containing this chromophor have been obtained[61]. One of them is C-1027 chromophore IV (**298c**)[450-451], a sequence-specific double-strand DNA cleavage agent, which is cytotoxic at very low concentration (1.5 x 10^{-17} M). The C-1027 chromophore is able to damage the DNA strand even in the absence of reducing agents[437,445], in contrast to other enediyne antibiotics that require a reducing agent.

Nafisamycin (**297**) was weakly cytotoxic, however, showed no antimicrobial activity against any of our test organisms, while the original crude extract was highly active (Table 85). As no other antibiotics were found, this may be a hint for unstable precursor enediyne **298**, in spite of the negative HPLC/MS experiment. The endiynes are very labile in alkali, light and solvents[452-453]. In contrast, they are slightly more stable in aqueous acids (for ~10 hrs) which may give a chance for another trial to isolate the postulated precursor **298**. Further experiments are needed, however, could not be performed here due to the lack of time.

6 Summary

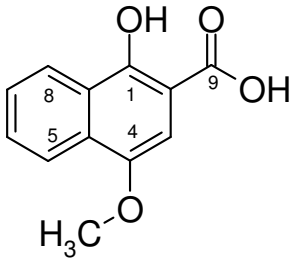
The search for new medically active compounds to overcome the countless diseases is one of the most relevant objectives worldwide. Medicinal plants were used previously in the traditional medicine and even now for the treatment of several infections. However, the appearance of new diseases and new drug resistances stimulated the search for new sources of bioactive agents. The marine environment comprises nearly three quarters of the earth's surface, and can be essentially considered as a soup of all imaginable types of microbes. Marine microorganisms, particularly bacteria, have provided new incentives for investigating marine natural products over the past 15 years, and also continue to be the subject of vigorous chemical investigation. This highlights their importance as a source of natural products. So, the search for new biologically active natural metabolites in bacteria may enable us to cure such unusual drug resistances and infectional diseases.

In the present study, 10 terrestrial Streptomycetes and 15 marine-derived bacterial strains were selected on the bases of a chemical and biological screening. The isolates were scaled up under standard conditions, and the culture broths was usually extracted with ethyl acetate or passed through Amberlite XAD-columns, while the mycelial cake was extracted with ethyl acetate, followed by acetone. The crude extracts were purified using various chromatographic methods, including silica gel columns, Sephadex LH-20, PTLC and HPLC. The isolated compounds were dereplicated by means of AntiBase, the Dictionary of Natural Products (DNP) and the Chemical Abstracts. Structure elucidation occurred with the aid of spectroscopic techniques (MS, MS/MS, 2D NMR, etc.). In some cases, simple derivatives were synthesized. In general, all isolated compounds were investigated for various biological activities (e.g. as antimicrobial, anticancer agents, etc.).

6.1 Secondary Metabolites from Terrestrial *Streptomyces* spp.

Three novel heterocyclic skeletons, karamomycins A-C (**73**, **75**, **78**), were isolated from *Streptomyces* sp. GW58/450, together with their precursor 1-hydroxy-4-methoxy-2-naphthoic acid (**71**) and actinomycin C_2. Karamomycins A and B (**73**, **75**) contain the rare 4-hydroxymethyl-4,5-dihydrothiazole and 4-hydroxymethyl-thiazole, respectively. Karamomycin C (**78**) has a complex tetracyclic skeleton, containing four heterocylic rings: a 4,5-dihydrothiazole (**A**), 1,3,5-oxathiazine (**B**), 1,4-

diazepine (**C**) and 3,4-dimethyl-thiazolidine (**D**). Details in the structure of **78** are still under consideration.



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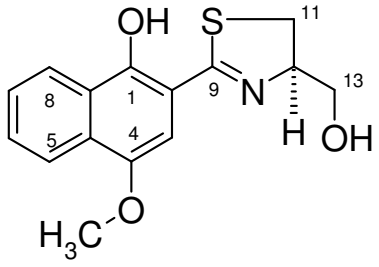

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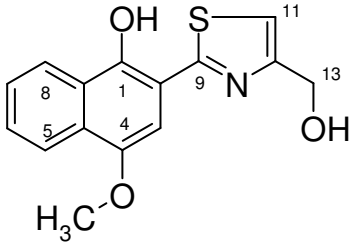

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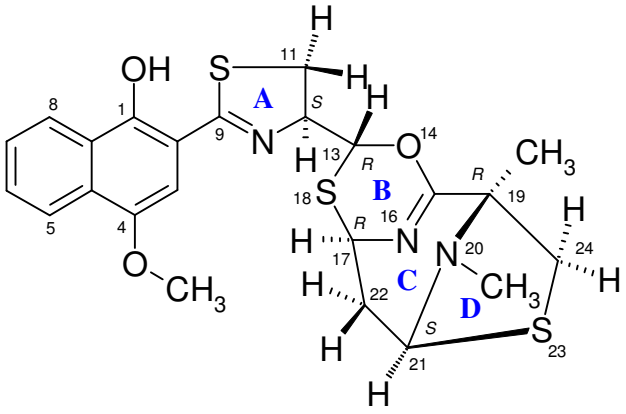
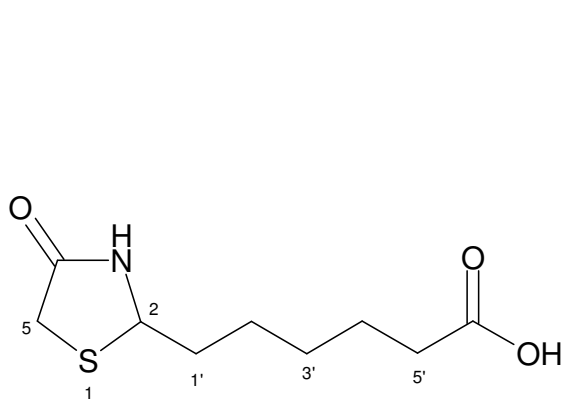


78

A fermentation of the terrestrial *Streptomyces* sp. TN58 delivered six compounds, some of them having interesting bioactivities: brevianamide F (**95**), N^{β}-acetyltryptamine (**96**), thiazolidomycin (**97**), 1-O-(2-aminobenzoyl)-α-L-rhamnopyranoside (**99**), 4-hydroxybenzoyl-α-L-rhamnopyranoside (**100**) and uracil. Rhamnopyranosides **99** and **100** are inhibitory agents of the enzyme 3α-hydroxysteroid-dehydrogenase (3α-HSD), in addition to their antibacterial activities against *S. aureus*.



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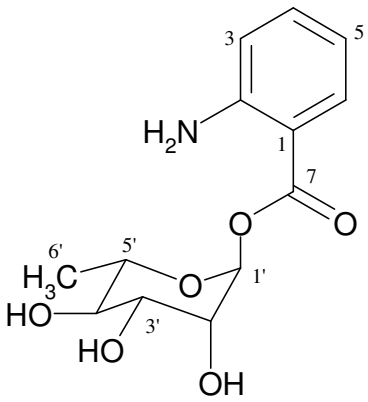

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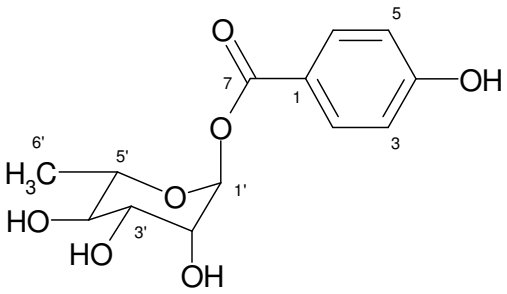

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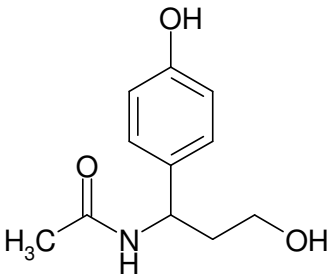
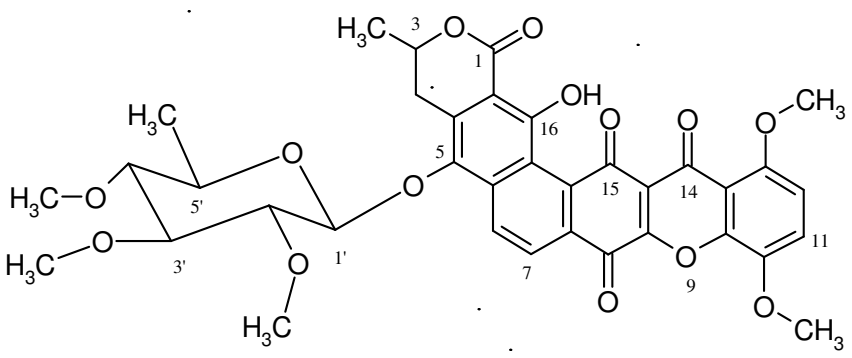


101

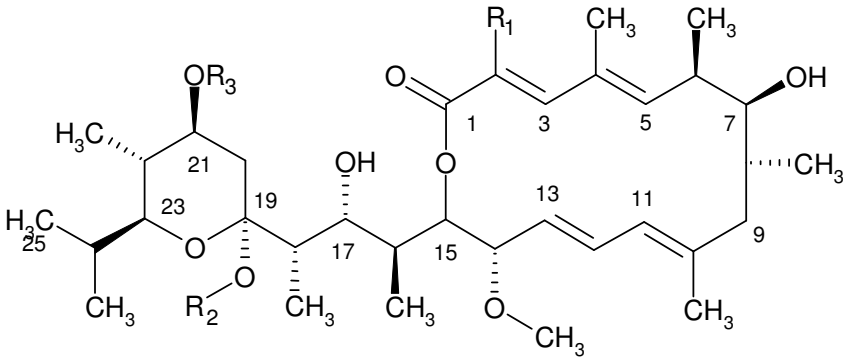
The primary screening of the terrestrial *Streptomyces* sp. GW19/5971 showed a component with a violet colouration on spraying with anisaldehyde/sulphuric acid. It was structurally assigned as isrocine (**101**), a new microbial natural product. Up-scaling on M_2 medium afforded phenazine-1-carboxylic acid (**104**) and phenazine-1-carboxylic acid methyl ester (**105**), and 2-acetamidophenol (**103**).

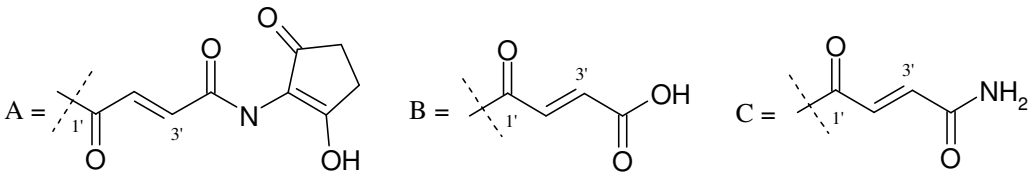
The terrestrial *Streptomyces* sp. isolate bl2/5831 delivered the derivative IB-00208 (**106**) in the high concentration of 130 mg/l. The xanthone possesses potent cytotoxic and antibiotic activities. Its biosynthesis is presently under investigation in the group of Prof. B. Piel (Bonn).



106

The terrestrial *Streptomyces* sp. bl47/4455 drew the attention because of its high antimicrobial activity and the interesting HPLC data. The strain afforded eight bafilomycin derivatives, bafilomycin A_1 (**108**), A_2 (**109**), B_1 (**110**), B_2 (**111**), C_1 (**112**), D (**114**), TS155 (**113**) together with the new bafilomycin K (**115**). TS155 (**113**) was reported here for the first time with its spectroscopic data, in addition to a full assignment of bafilomycin A_1 (**108**). Bafilomycins A_1 (**108**), K (**115**), C_1 (**112**) and TS155 (**113**) exhibited a potent activity against *Mucor miehei*, *Chlorella sorokiniana, Staphylococcus aureus* and *Scenedesmus subspicatus*. A further derivative, bafilomycin C_1-amide **116** was obtained from the marine-derived *Streptomyces* sp. Mei6-1,2.

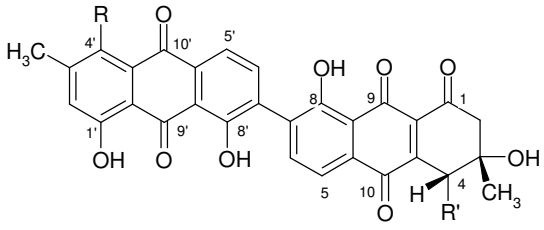




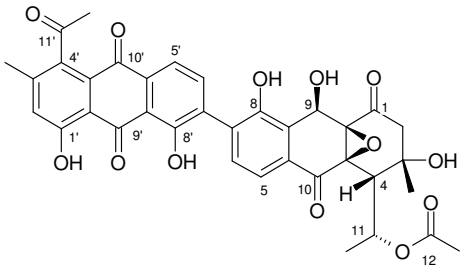
108: $R_1 = OCH_3$ $R_2 = R_3 = H$
109: $R_1 = OCH_3$ $R_2 = CH_3$ $R_3 = H$
110: $R_1 = OCH_3$ $R_2 = H$ $R_3 = A$
111: $R_1 = OCH_3$ $R_2 = CH_3$ $R_3 = A$
112: $R_1 = OCH_3$ $R_2 = H$ $R_3 = B$
113: $R_1 = CH_3$ $R_2 = H$ $R_3 = B$
115: $R_1 = CH_3$ $R_2 = R_3 = H$
116 $R_1 = OCH_3$ $R_2 = H$ $R_3 = C$

Fermentation of the terrestrial *Streptomyces* sp. isolate GW6225 afforded julichrome Q_6 glucuronide (**120**) as the first monomeric member of the julimycin-B complex in nature. It was isolated together with the julichromes $Q_{1\cdot2}$ (**117b**), $Q_{1\cdot5}$ (**117a**) and $Q_{3\cdot5}$ (**118**), which were fully assigned by 2D NMR spectra. The structures **117a** and **118** were fully characterized here for the first time. The co-occurrence of dimeric julichromes and potential monomeric precursors strongly supports the supposed biosynthetic origin of julichromes by phenol oxidation. Julichrome Q_6 glucuronide (**120**) displayed unselective moderate cytotoxic activity against a range of human tumor cell lines with IC_{50} of 12.3 μM and IC_{70} of 20.4 μM. A similar biological profile was observed for the julichromes $Q_{1\cdot5}$ and $Q_{3\cdot5}$ (**117a**, **118**), whose cytotoxic activity was approximately 2-fold more pronounced.

Furthermore, the new microbial products 4-acetylchrysophanol (**119a**) and N-phenyl-β-naphthylamine (**129**) together with the thiazolyl cyclopeptide, nosiheptide (**127**) were obtained. The high antibiotic activity of the extract was mainly due to **127**.



117a: R = $COCH_3$, R' = $CH(CH_3)OCOCH_3$
117b: R =R' = $CH(CH_3)OCOCH_3$



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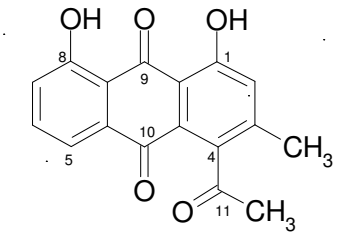
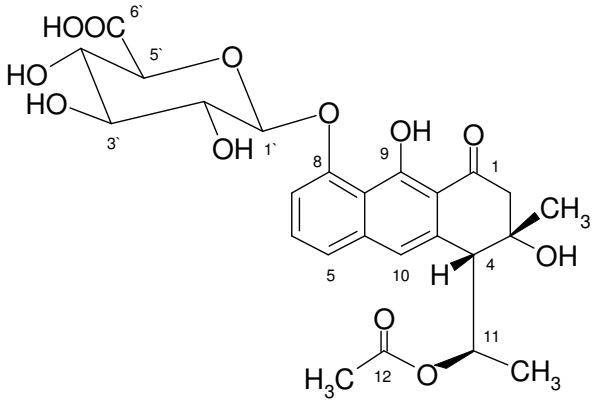


119a



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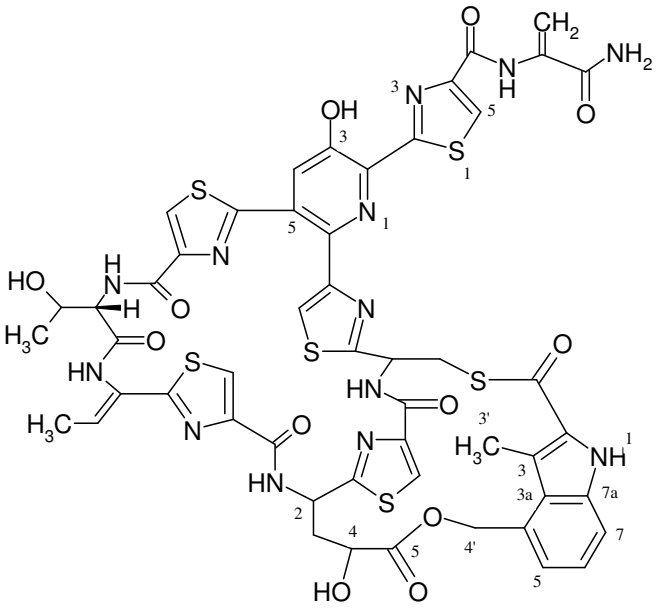
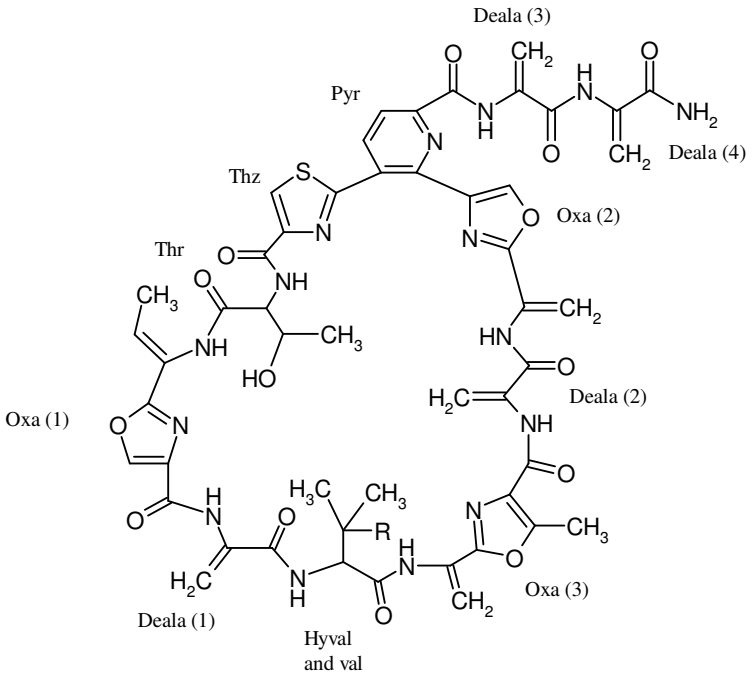


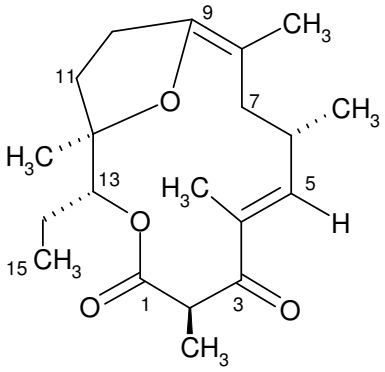
127

Val-Geninthiocin (**131**), a new member of the thiopeptide antibiotics, was isolated from the mycelial cake of the terrestrial *Streptomyces* sp. RSF18, together with the closely related geninthiocin (**130**). Chalcomycin A (**132**) was afforded from the water extract. Based on intensive NMR and MS studies, Val-geninthiocin (**131**) was identified as deoxy-geninthiocin, a thiopeptide containing several oxazole and thiazole units and a number of unusual amino acids. Val-geninthiocin (**131**) is potently active against Gram-positive bacteria and moderately active against fungal isolates.



130: R = OH; **131**: R = H

A 50 L shaker fermentation of the terrestrial *Streptomyces* sp. ANK26 lead to the isolation of three new macrolides, 10,11-dihydro-9,12-epoxy-8,9-anhydro-cromycin (**133**), 10,11-dihydro-9,12-epoxy-9-methoxy-picromycin (**134**), 10,11-dihydro-cromycin (**135**), and the two new seco derivatives, *seco*-decarboxy-cromycin (**136**) and (4*E*,8*E*)-4,8-dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**), together with cromycin (**138**), picromycin (**139**), picromycin C (**140**) and bafilomycins B_1~B_2 (**110**-**111**). The structures of the new compounds **133~137** were fully assigned using MS and NMR data. The full shift assignments of **138**, **139** and **140** according to 1D and 2D NMR experiments are reported here for the first time. Compounds **134** and **137** exhibited potent *in vitro* antibacterial activities against Gram-positive bacteria.



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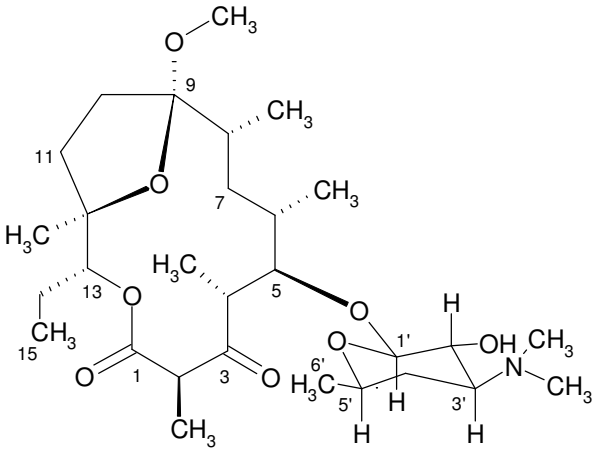

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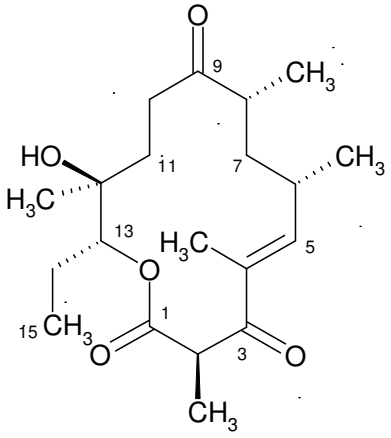

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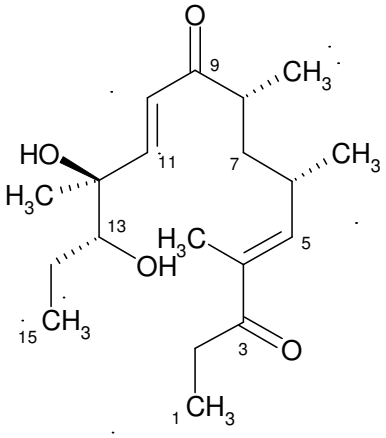

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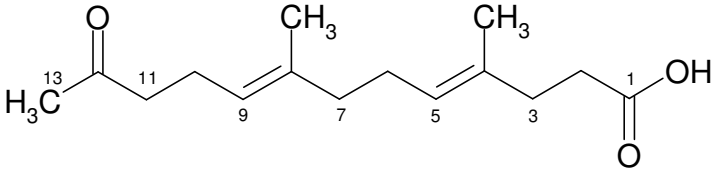

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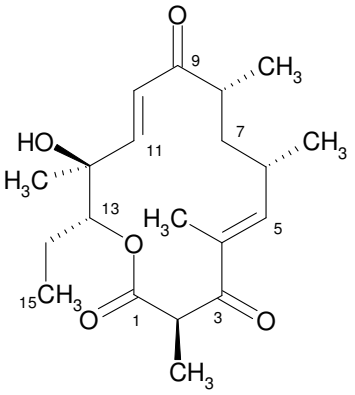

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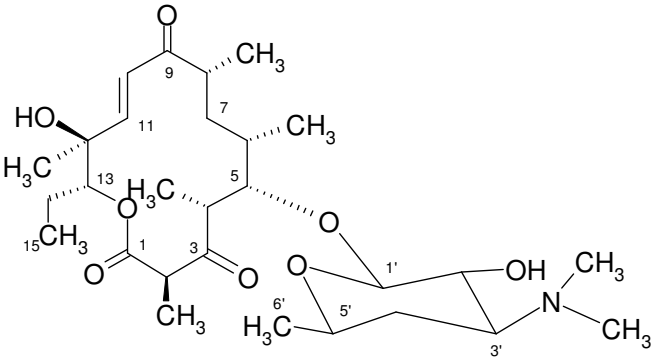

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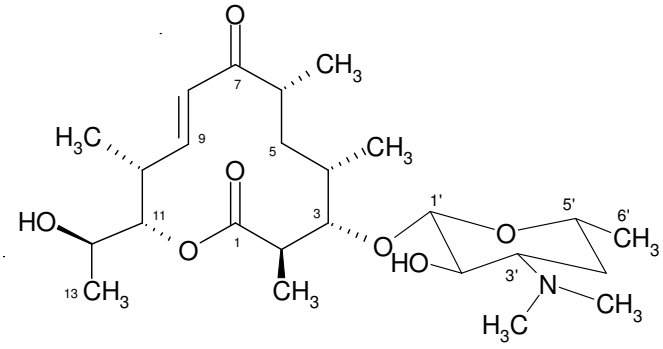
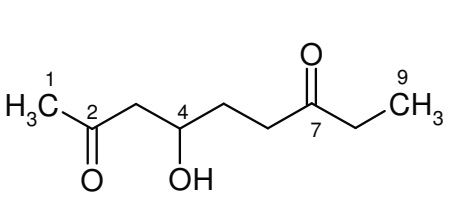


140

6.2 Secondary Metabolites from Marine-derived *Streptomyces* spp.

Three new bioactive compounds were obtained during the purification of the marine-derived *Streptomyces* sp. Act8970 extract. They are attiamycin A (**141**), attiamycin B (**143a**) and homonactic acid methyl ester (**145b**). The NMR data of the known bioactive metabolites homonactic acid (**145a**), dinactic acid, werramycin-B (**148**) and nonactic acid (**144a**) were fully assigned. Dinactin (**148**), attiamycin B (**143a**) and nonactic acid (**144a**) showed a high selective cytotoxicity against a range of human tumor cell lines with IC_{50} of 0.002~1.256 μg/ml and IC_{70} of 0.011~3.532 μg/ml, respectively.



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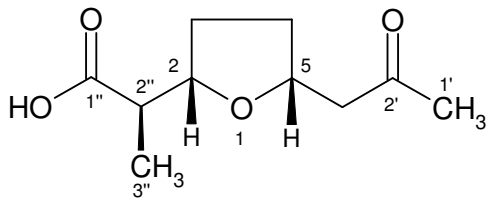
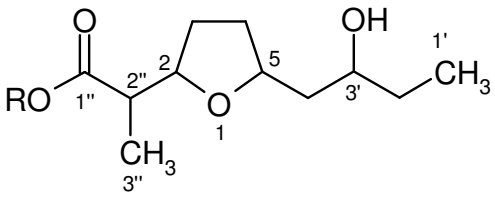
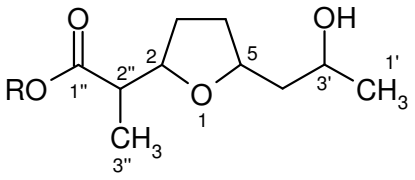


143a



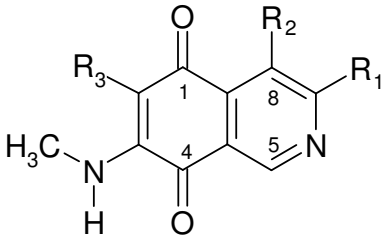
145 a: R = H; **145b**: R = CH_3



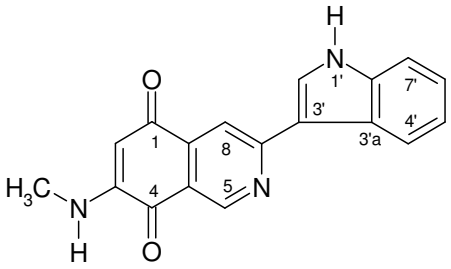
144a: R = H

The North Sea-derived *Streptomyces* sp. Mei37 was found to produce six novel isoquinoline-quinones, the mansouramycins A-C (**157**-**159**), D (**156**), E (**162**), and F (**163**), beside the new (4*S*)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (**233**). Additionally, 2,3-dihydroxy-1-(1H-indol-3-yl)-propan-1-one, albaflavenone (**167**), indole-3-acetic acid and indole-3-carboxylic acid were isolated.

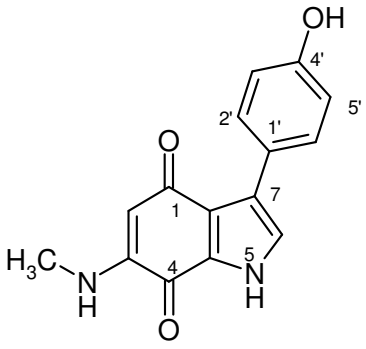
The isoquinoline-quinones proved to be highly cytotoxic in a panel of up to 36 tumor cell lines with a pronounced selectivity for non-small cell lung cancer, breast cancer, melanoma, and prostate cancer cells. Mansouramycin D (**156**) was the most active one with an overall potency of 0.089 μM. The antitumor potency of the mansouramycin molecules differed according to their substitution pattern, and variations at the C-8 or C-7 position of the isoquinoline quinone, respectively, strongly influenced cytotoxicity.



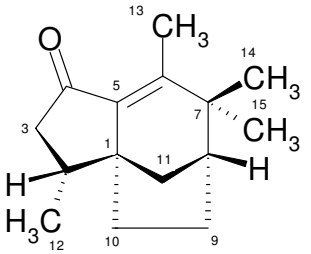
156: R_1 = $COOCH_3$ R_2 = H R_3 = H
157: R_1 = CH_3 R_2 = CH_3 R_3 = H
158: R_1 = CH_3 R_2 = H R_3 = H
159: R_1 = CH_3 R_2 = H R_3 = Cl



162

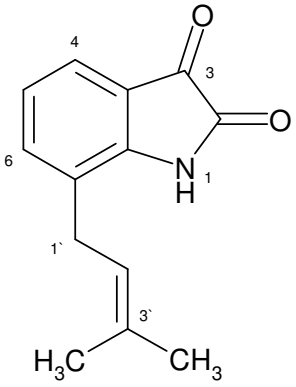


163

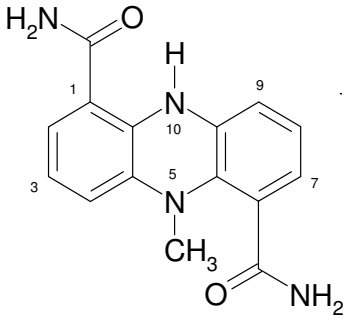


167

The crude extract of the marine *Streptomyces* sp. B7874 exhibited *in vitro* a potent antitumor activity against a number of cancer cell lines. Scale up fermentation of the strain using M_2^+ medium and working up resulted in the isolation of the new 7-(3-methyl-but-2-enyl)-isatin (**168**) and 5-methyl-5,10-dihydro-phenazine-1,6-dicarboxylic acid diamide (**178**).

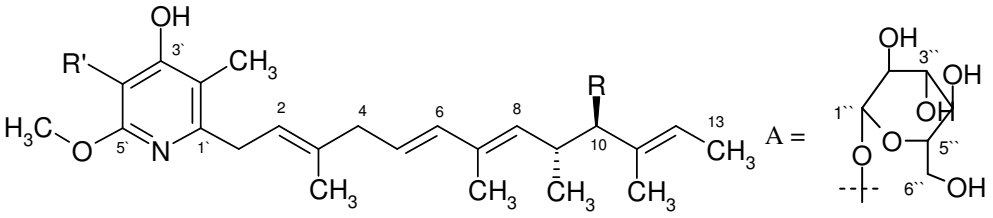


168

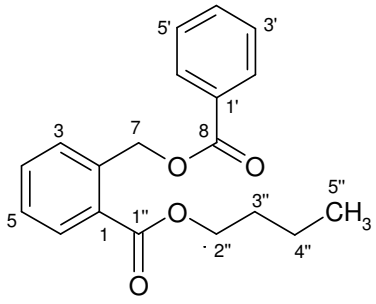


178

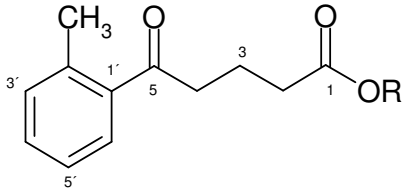
The marine *Streptomyces* sp. B8112 afforded the new colourless glucopiericidin C (**185**) together with two other new bacterial components; spatozoate (**186**) and 5-oxo-5-*o*-tolyl-pentanoic acid (**188a**). Additionally, piericidin-A (**182**), glucopiericidin A (**183**), 5-(*o*-tolyl)-4-pentenoic acid) (**187a**) and monensin-B (**191**) were isolated. The NMR data of monensin-B (**191**) were fully assigned here for the first time. Glucopiericidin C (**185**) is cytotoxic against a range of human tumor cell lines (IC_{50} of 2.0 μM, IC_{70} = 4.2 μM) with moderate selectivity. Compound **185** is potently activity against Gram-positive and Gram-negative bacteria, and microalgae. Spatozoate (**186**) was originally isolated from the brown algae *Spatoglossum variabile*, and might be formed there by associated bacteria.



182: R = OH, R' = OCH_3
183: R = A, R' = OCH_3
185: R = A, R' = H

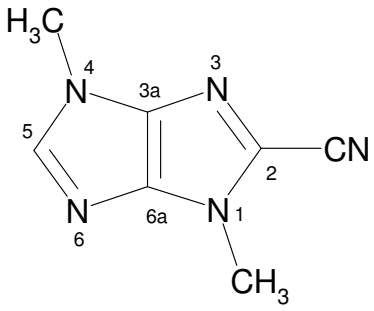


186



188a: R = H; **188b**: R = CH_3

Chemical investigation of the marine *Streptomyces* sp. isolate B8108 led to the isolation of two novel structures, 1,4-dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**) and 4,9-dihydroxy-9-methyl-decan-4-olide (**237**) together with 2-acetylamino-benzoic acid (**194a**), which is new as natural product. The imidazole **193** is a highly cytotoxic compound with IC_{50} 7.146 μg/ml. Compound **193** was also isolated from the marine *Streptomyces* sp. B8112.



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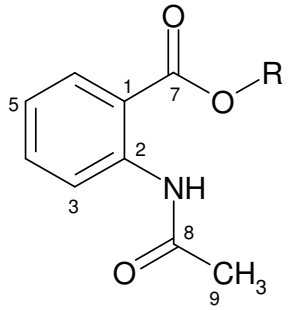
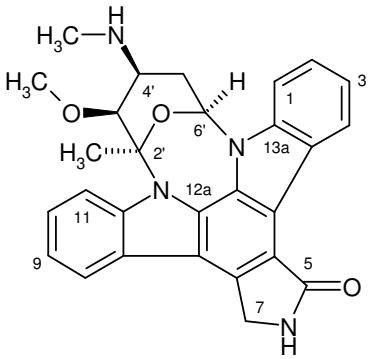
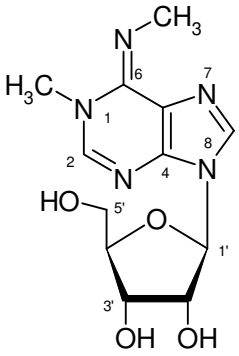


194a: R = H; **194b:** R = CH_3



198a

The marine derived *Streptomyces* sp. Mei02-8,1 showed antimicrobial activity against all our test microorganisms. On upscaling, three compounds were obtained, 4-hydroxy-10-methyl-11-oxododec-2-en-1,4-olide (**236**), staurosporine (**198**) and 1,N^6-dimethyladenosine (**199**). Compound **199** was firstly identified as a nucleoside in the urine of a lung cancer patient. Interestingly, the two methyl signals of **199** were very broad and overlapping in the ^{1}H NMR spectrum, due to coalescence phenomena; they were resolved at 600 MHz or at 300 MHz and 50 °C.



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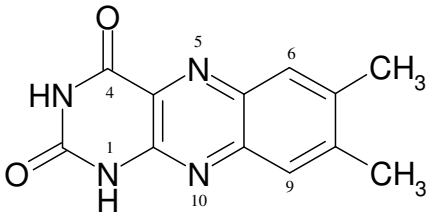

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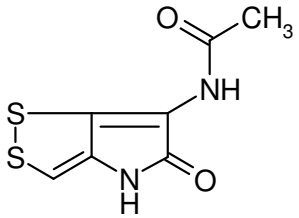
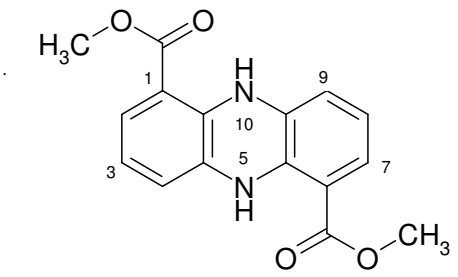


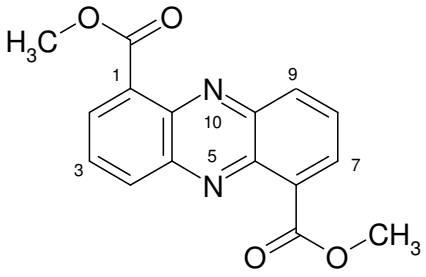
208

The pronounced antimicrobial activities of the North Sea *Streptomyces* sp. Mei4-1,23 led to the isolation of nine metabolites, among them lumichrome (**201**) and holomycin (**208**). Lumichrome (**201**) was recently isolated from a *Micromonospora* sp. Tü 6368 and was reported as the first known endogenous chemical with an affinity for the TCDD receptor. Holomycin (**208**) is a member of the pyrrothine group and was originally described as antibiotic from *Streptomyces griseus*, and *Streptomyces clavuliderus*.

Purification of the extract from the marine *Streptomyces* sp. B8300 afforded five metabolites, among them dihydrophencomycin methyl ester (**212**) and the naturally new phencomycin methyl ester (**215**) together with the known β-indomycinone (**219**) and saptomycin A (**220**).

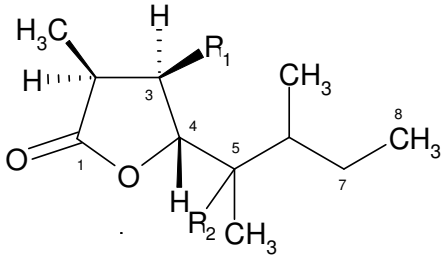


212



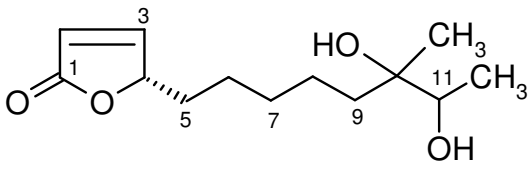
215

Based on bioassay guided fractionation, the water extract obtained from the marine *Streptomyces* sp. B7828 was selected for working up and purification using different chromatographic techniques. As a result, three new γ-butyrolactones were obtained as colourless oils: 4-hydroxy-2,5,6-trimethyl-octan-4-olide (**230**), 4,5-dihydroxy-2,5,6-trimethyl-octan-4-olide (**231**), and 3,4-dihydroxy-2,5,6-trimethyl-octan-4-olide (**232**). The three butanolides were inactive against a range of selected bacteria, fungi, and microalgae. They also had no influence on the spore formation of *Streptomyces viridochromogenes* (Tü 57) or of the producing strain. However, compound **230** displayed unselectively cytotoxic activity against a series of human tumor cell lines with IC_{50} of 22.09 μM and IC_{70} > 10 μM.



230: R_1=R_2=H;
231: R_1= H, R_2= OH
232: R_1= OH, R_2=

α,β-Unsaturated γ-butyrolactones are low polar UV absorbing colourless oils. They are mostly detected on spraying with anisaldehyde/sulphuric acid as violet spots and change later to blue. Saturated γ-butyrolactones exhibited the same colour reaction on TLC as their α,β-unsaturated analogues, except that they are not UV absorbing. As part of a project to discover new secondary metabolites from bacteria, we examined the crude extracts obtained from the fermentation of several marine derived *Streptomyces* sp. In accordance, from the marine-derived *Streptomyces* spp. Mei37 and Mei02-8,1 the new α,β-unsaturated γ-butyrolactone (4S)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4olide (**233**) and the known γ-butyrolactone 4-hydroxy-10-methyl-11-oxododec-2-en-1,4-olide (**236**) were obtained, respectively, while the marine *Streptomyces* sp. B8108 yielded 4,9-dihydroxy-9-methyl-decan-4-olide (**237**). On the other hand, the marine-derived *Actinomyces* sp. Act8015 exhibited high productivity of virginiae butanolide E (**242**), Graefe's Factors I (**249**) and III (**250**) in addition to the new butanolide 4,10-dihydroxy-10-methyl-dodecan-4-olide (**251**) along with its respected acid 4,10-dihydroxy-10-methyl-dodecanoic acid (**252**). A full signal assignment of virginiae butanolide E (**242**) is reported here for the first time.



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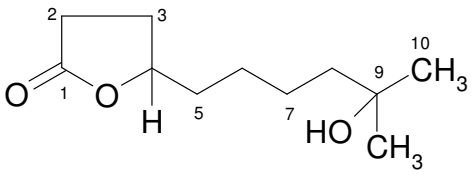

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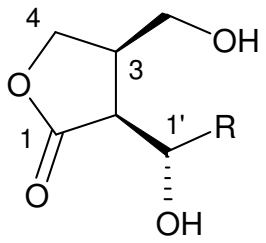
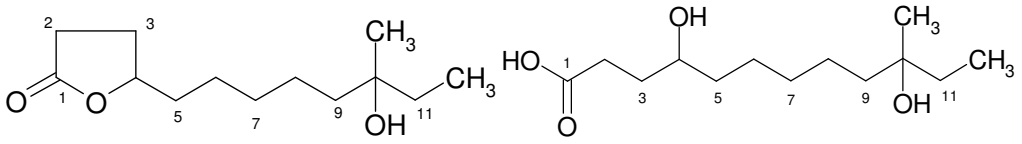
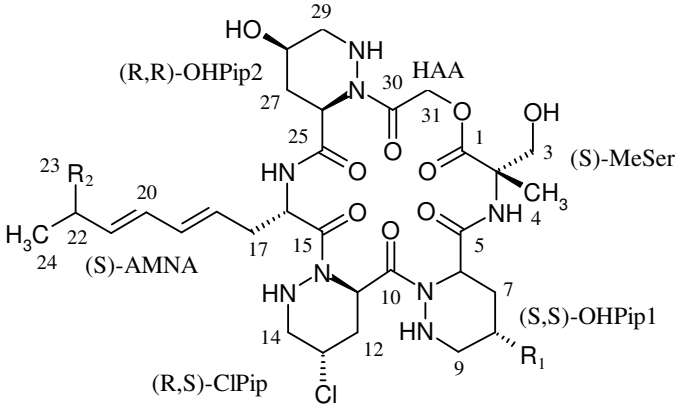


242: R = $-(CH_2)_2CH(CH_3)_2$
249: R = $-(CH_2)_4CH(CH_3)_2$
250: R = $-(CH_2)_5CH(CH_3)_2$



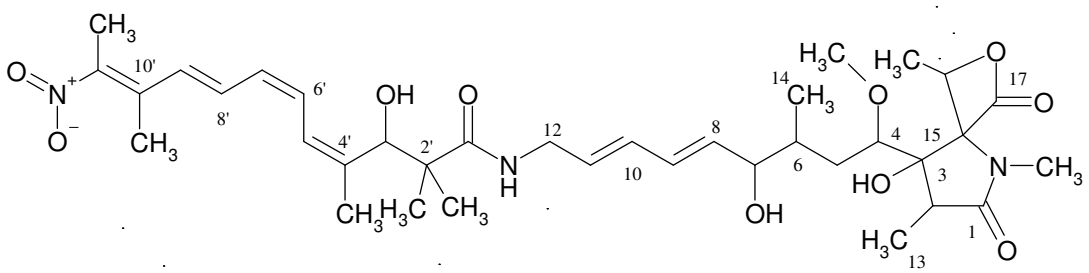
251 **252**

A large-scale cultivation of the marine-derived *Streptomyces* sp. Act8015 on M_2^+ medium produced the new peptidlactones, piperazimycins A and B (**254**, **255**). Furthermore, a number of γ-butrylocatones was isolated: 4,10-dihydroxy-10-methyl-dodecan-4-olide (**251**), together with the respective 4,10-dihydroxy-10-methyl-dodecanoic acid (**252**), virginiae butanolide E (**242**) and Graefe's Factors I (**249**) and III (**250**) were afforded. A full signal assignment of virginiae butanolide E (**242**) is reported here for the first time. Structures of piperazimycins A and B (**254**, **255**) were determined on the basis of intensive interpretation of spectroscopic data (1D and 2D) and HRESI-MS/MS analysis. They are constructed from the rare amino acids *R*-methylserine, γ-hydroxypiperazic acid, γ-chloropiperazic acid, 2-amino-8-methyl-4,6-nonadienoic acid and 2-amino-8-methyl-4,6-decadienoic acid together with hydroxy-acetic acid. Piperazimycin A (**254**) showed high but unselective cytotoxic activity against a range of human tumor cell lines with IC_{50} of 0.130 μg/ml and IC_{70} of 0.210 μg/ml. Additionally, **254** exhibited a mortality rate of 20% at 10 μg/ml according to the brine shrimp microwell assay. Compounds **254** and **255** exhibited substantial activities against Gram-positive and negative bacteria, fungi and microalgae.



254: R_1 = OH, R_2 = CH_3
255: R_1 = H, R_2 = CH_3

Up-scale fermentation (24 l) of the marine *Streptomyces* sp. B7729 afforded enterocin (**257**) and the new triene spiro-β-lactone-γ-lactam antibiotic 4',5'-dihydrolajollamycin (**263**) besids the closely related known lajollamycin (**262**). Moreover, polypropyleneglycol (**261**) was isolated and confirmed here as natural but not as artefact.



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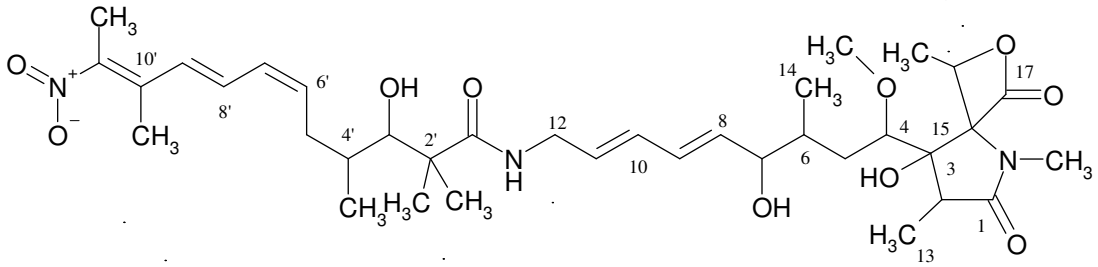

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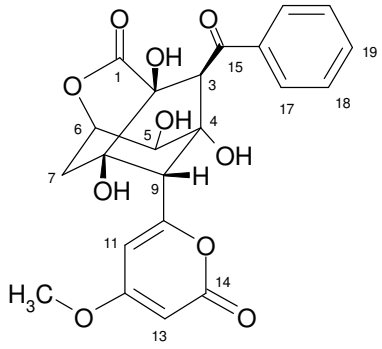
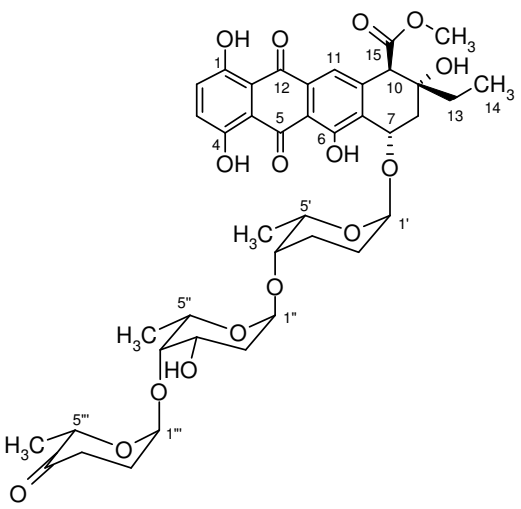
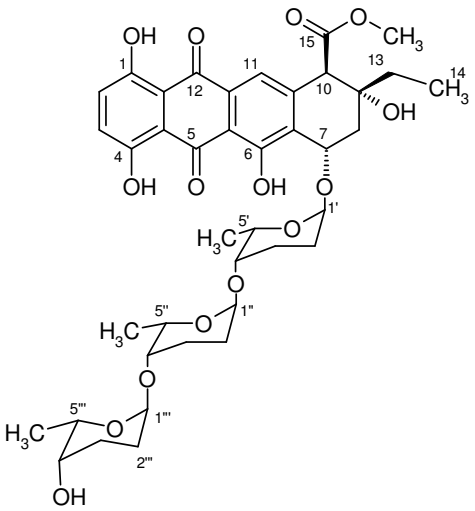


257

The crude extract produced by the marine *Streptomyces* sp. B9054 yielded η-pyrromycinone (**267**), ζ-pyrromycinone (**269**) together with the cinerubins A (**275**), B (**273**), X (**272**), Y (**271**) and the new anthracycline cinerubin K (**276**). Cinerubin A (**275**) exhibited microbial activity against all the tested microorganisms, while cinerubin B (**273**) showed only activity against *Staphylococcus aureus*.

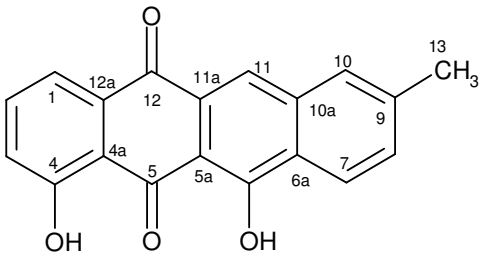


272



276

The marine-derived *Streptomyces* sp. Mei6-1,2, collected from the North Sea, produced four new tetracyclic quinones, which were assigned as boshracins A-D (**278**, **280**, **282**, **284**). Additionally, bafilomycin C_1-amide (**116**), B_1 (**110**) and B_2 (**111**) were isolated and structurally interpreted. The relative stereochemistry at C-8 and C-10 of boshracin D (**284**) was assigned as (8*S**, 10*R**) based on the NOESY experiment. The four quinones exhibited no antimicrobial activity against our set of pathogenic microorganisms.



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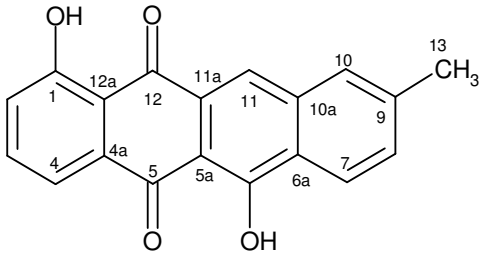

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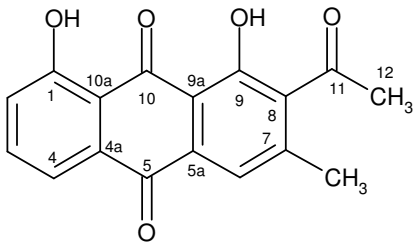

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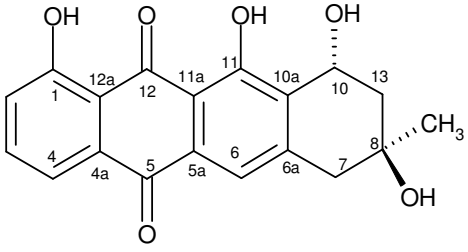
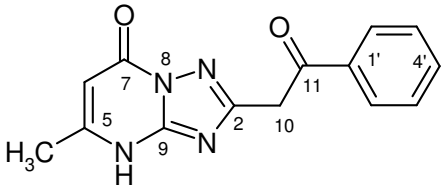


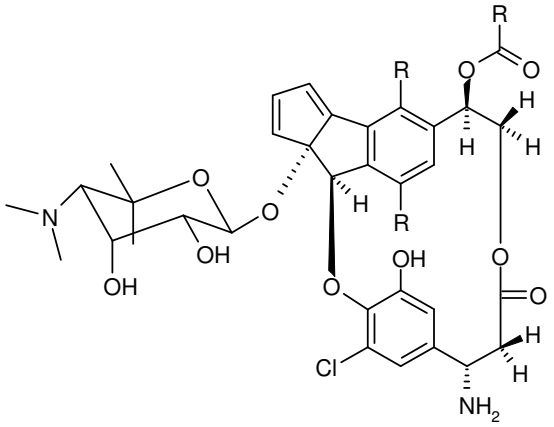
284

The marine *Streptomyces* sp. Merv8102 was isolated from sediments collected from the Mediterranean Sea on the Egyptian coast. The crude extract exhibited a potent antibacterial activity against both types of bacteria, in addition to its moderate antifungal activity. After a series of chromatographic purification steps, the extract afforded essramycin (**288**), the first triazolopyrimidine antibiotic isolated from nature, with an MIC of 2-8 μg/ml against Gram-positive and Gram-negative bacteria.

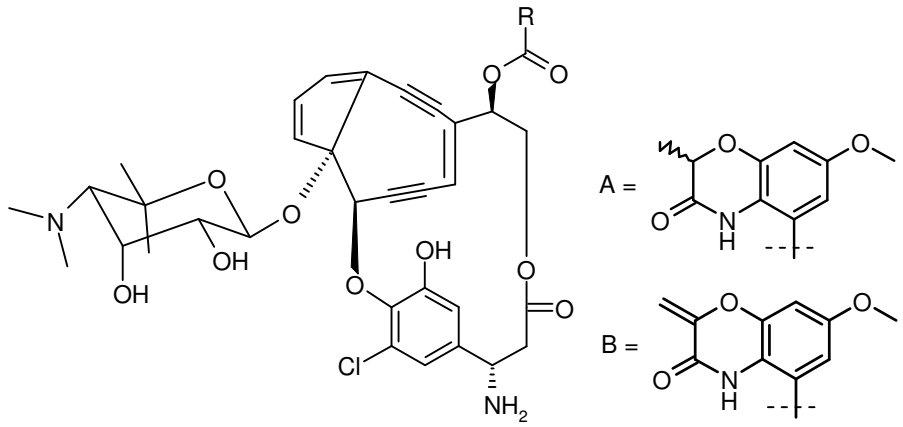
The marine streptomycete Mei34 was selected, due to the high activity of its extract against Gram-positive and Gram-negative bacteria and microalgae. Moreover, the extract was moderately active against yeast and fungi. An HPLC-MS analysis of the strain extract showed a signal of a mole peak at *m/z* 705/707, corresponding to a chlorinated structure. On upscaling, nafisamycin (**297**), a novel chlorinated enediyne derivative, was obtained, which is most likely a cyclisation product of the enediyne precursor **298a**. Enediyne antibiotics are characterized by a 1,5-diyn-3-ene core. Due to their high activities and low stabilities, enediynes often undergoing a Masamune-Bergman cycloaromatization during their isolation, resulting in stable benzene derivatives. Most enediynes are characterized by their high antitumor as well as antibacterial activity against Gram-positive bacteria, e.g. the antibiotics of the C-1027 series (**298b~c**).



288



297: $R_1 = CH_3$, $R_2 = Cl$, $R_3 = H$
299: $R_1 = CH_3$, $R_2 = H$, $R_3 = Cl$
300: $R_1 = A$, $R_2 = H$, $R_3 = H$



298a: R = CH_3

298b: R = A (NeoC-1027 Chr-II)

298c: R = B (C-1027 Chr-IV)

In the case of nafisamycin (**297**), the aromatization process could be attributed to a radicalic HCl addition to the highly active diradical intermediate. Of the two possible regioisomers **297** and **299**, only the first one has been isolated.

Despite of the high activity of the strain extract, nafisamycin (**297**) showed no activity against all our set of pathogenic microorganisms. This can be a hint for the presence of the endiyne precursor in the crude extract. Nafisamycin (**297**) has, however, been reproduced by strictly avoiding any contact with light, methanol or chlorinated solvents. An HPLC analysis of the extract proved the existence of **297** (*m/z*: 705 $[M+H]^+$) rather than its acetylene precursor (**298a**; *m/z* = 669), as an indicator of the high instability (or absence ?) of the latter.

6.3 Conclusion

Twenty five bacterial strains were selected and subjected to culture optimization, working up and isolation of their metabolic constituents. From the extracts of these strains, 140 compounds were isolated, among them 61 new compounds, of which twelve had new skeletons. The remaining compounds were classified into 24 quinones, 22 macrolides, 6 peptides, 8 sulphur compounds, 4 chlorine containing metabolites, 2 polyenes and one enediyne derivative (Table 44).

Table 44: Total number of isolated compounds from the bacterial strains in this thesis:

Strains	**Terrestrial** *Streptomyces* sp.	**Marine-derived** *Streptomyces* sp.		**Total**
	Strept.	***Strept.***	**North Sea *Strep.***	
Number of strains	10	10	5	25
Number of isolated compounds	44	72	24	140
No. of new compounds	21	26	14	61
Quinones	5	9	10	24
Macrolides	17	-	3	21
Peptides	4	2	1	7
N-containing compounds	18	31	14	63
S-containing compounds	7	-	1	8
Cl-containing compounds	-	1	3	4
Sugar-containing compounds	16	7	4	27
New skeletons	3	1	8	12
Polyenes	-	2	-	2
Enediynes	-	-	1	1

According to this study, it was found that there is a metabolic similarly between the terrestrial and marine *Streptomycetes*, so that their ability to produce new compounds is approximately the same. Finally, the results can be summarized as following:

- Terrestrial and marine *Streptomycetes* are an ideal target for discovery of novel potential bioactive compounds.
- The investigation of marine and terrestrial *Streptomycetes* should be continued to extend the probability of discovering new compounds with new skeletons.

7 Materials and Methods

7.1 General

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 (Perkin-Elmer, model 243), the concentration were given in [mg/ml]. – **^{1}H NMR spectra:** Varian Unity 300 (300.145 MHz), Bruker AMX 300 (300.135 MHz), Varian Inova 500 (499.8 MHz), Varian Inova 600 (600 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), Varian Inova 600 (150.7 MHz). Chemical shifts were measured relative 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 NOSY (Nuclear Overhauser Effect Spectroscopy). - **Mass spectra**: EI MS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosine as standard. DCI-MS: Finnigan MAT 95 A, 200 eV, Reactant gas NH_3. ESI MS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). ESI HRMS were measured on Micromass LCT mass spectrometer coupled with a HP1100 HPLC with a diode array detector. Reserpine (MW = 608) and leucine-enkephalin (MW = 555) were used as standards in positive and negative mode. High-resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). ESI MS/MS was performed with normalized collision energy of 35%. EI MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for EI HRMS, samples were infused with a flow rate of 2 µL/min. **CD-Spectra:** The Circulardichroismus (CD)-Spectra was messured by Jasco J 500 Spectrometer. The calculation of spectra were recorded by BMC IF 800 PC with Jasco IF 500 A/D-Wandler. The molar Elliptizitation θ were given in [10^{-1} grad cm^2 mol^{-1}]. – **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, 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 ten vertical neon tubes Philips TLD 15 W/25.

7.2 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):** 55 g Silica gel P/UV_{254} (Macherey-Nagel & Co.) 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 mesh (Macherey-Nagel & Co); silica gel (230-400 mesh) for flash chromatography: 30-60 µm (J. T. Baker); Sephadex LH-20 (Pharmacia) was used for size exclusion chromatography.

7.3 Spray Reagents

Anisaldehyde/sulphuric acid: 1 ml anisaldehyde was added to 100 ml of a stock solution containing 85 ml methanol, 14 ml acetic acid and 1 ml sulphuric acid. **Ehrlich's reagent:** 1 g 4-dimethylaminobenzaldehyde was dissolved in a mixture of 25 ml hydrochloric acid (37%) and 75 ml methanol, give red colouration with indol and yellow for other N-heterocycles. **Ninhydrin:** 0.3 g ninhydrin (2,2-dihydroxyindan-1,3-dione) 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. Ninhydrin in ethanol (0.1 %) was also directly used. **Vanillin/ sulphuric acid:** Vanillin (1.0 g) was dissolved in concentrated sulfuric acid (100 mL). **Orcin reagent:** Iron (III)-chloride (1.0 g) was dissolved in concentrated sulfuric acid (100 mL) and successively mixed with the same volume of orcin solution (6 % in ethanol). **Palladium(II)-chloride reagent:** Palladium(II) dichloride (0.5 g) was dissolved in water (100 mL) with some drops hydrochloride acid (25 %) as reagent for sulphur containing compounds. **Chlorine/*o*-dianisidin reaction**: The reagent was prepared from 100 ml (0.032%) *o*-dianisidin in 1 N acetic acid, 1.5 g $Na_2WO_4 \cdot 2\ H_2O$ in 10 ml water, 115 ml acetone and 450 mg KI. The moistened TLC plate was kept ca. 30 min in a chlorine atmosphere (from 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. **NaOH or KOH:** 2 N NaOH or KOH solutions are used to identify *peri*-hydroxyquinones by deepening of the colour from orange to violet or blue.

7.4 Microbiological Materials

Fermentor: 20 L fermentor (Fa. Meredos GmbH, Göttingen) consisting of culture container, magnet-coupled propeller stirrer, cooler with thermostat, control unit with pH and antifoam regulation. The 50 L fermentor type Biostat U consisted of a 70 L metallic container (50 L working volume), propeller stirrer, and culture container covered with thermostat for autoclaving, cooling and thermostating (Braun Melsungen, Germany). – **Storage of strains:** Deep-freeze storage in a Dewar vessel, 1'Air liquid type BT 37 A. - **Capillaries for deep-freeze storage:** diameter 1.75 mm, length 80 mm, Hirschmann Laborgeräte Eberstadt. – Soil for soil culture**:** Luvos

Heilerde LU-VOS JUST GmbH & Co. Friedrichshof (from the health shop). - **Ultra-turrax:** Janke & Munkel KG. – **Shaker :** Infors 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 discs:** 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 Merck, Darmstadt. - **Antifoam solution:** Niax PPG 2025; Union Carbide Belgium N. V. (Zwiijndrecht). - **Petri-dishes:** 94 mm diameter, 16 mm height, Fa. Greiner Labortechnik, Nürtingen. – **Celite:** Celite France S. A., Rueil-Malmaison Cedex. - **Sterile filters:** Midisart 2000, 0.2 µm, PTFE-Filter, Sartorius, 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).

7.5 Recipes

All cultures were autoclaved at 1.2 bar and 120 °C. Sterilisation time for 1 L shaker culture: 33 min, 2 L concentrated medium for fermentor: 50 min and fermentor containing 16 l water: 82 min.

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
tap water	ad 20 L

Trace element stock solution

H_3BO_3	0.611 g
$MnCl_2$	0.389 g
$CuSO_4$	0.056 g
$ZnSO_4 \cdot 7\ H_2O$	0.056 g
$Al_2(SO_4)_3 \cdot 18\ H_2O$	0.056 g
$NiSO_4 \cdot 6\ H_2O$	0.056 g
$CO(NO_3)_3 \cdot 6\ H_2O$	0.056 g
TiO_2	0.056 g
$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	0.056 g
LiCl	0.028 g
$SnCl_2$	0.028 g
KI	0.028 g
tap water	ad 1 L

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
tap water	ad 2 L

7.5.1 Nutrients

M_2 medium (without sea water)

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Tap water	ad 1 L

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of bacto agar

M_2^+ medium (M_2 medium 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.

M_2 100% sea water + $CaCO_3$

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
$CaCO_3$	0.5 g
Artificial sea water	1000 mL

The pH was adjusted to 7.3 using 2N NaOH. Solid medium was prepared by adding 18 g of bacto agar.

$CaCl_2$-Medium

Malt extract	40 g
Glucose	5 g
$CaCl_2$	45 g
Tap water	1000 mL

The pH was adjusted to 7.8 usin g 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* (Tü 284):

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

7.6 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
$MgSO_4 \cdot 7\ H_2O$	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.

7.7 Microbiological and Analytical Methods

7.7.1 Storage of Strains

All bacteria strains were stored in liquid nitrogen for long time. The strains were used to inoculate agar plates with the suitable media at room temperature.

7.7.2 Pre-Screening

The microbial isolates (obtained from culture collections) were cultured in a 1 L scale in 1 L-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.

7.7.3 Biological Screening

The crude extract was dissolved in $CHCl_3$/10% MeOH (at concentration of ~100 μg/platelet), in which the paper disks were dipped, dried under sterile conditions (flow box) and put on an agar plates inoculated with the Gram-positive bacteria; *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57), the Gram-negative *Escherichia coli*; the yeast, *Candida albicans*; and the fungi, *Mucor miehei* (Tü 284) along with the three microalgae; *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

The plates were incubated at 37 °C for bacteria (12 hours), 27 °C for fungi (24 hours), and 24-26 °C under day-light for micro-algae (96 hours). The diameter of the inhibition zones were measured by ruler.

7.7.4 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 at 254 nm and marked at 366 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.

7.7.5 Brine shrimp Microwell Cytotoxicity Assay

To a 500 ml separating funnel, filled with 400 ml of artificial sea water, 1 g 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 unhatched eggs dropped. In order to get a higher density of 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 and the dead larvae counted (number N). 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 counted in each well under the microscope (number A). The still living larvae were killed by addition of *ca*. 0.5 ml methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in%. Each test row was accompanied by a blind sample with pure DMSO (number B) and a control sample with 1 µg/test actinomycin D. The mortality rate M was calculated using the following formula:

$$M = \left[\frac{(A - B - N)}{(G - N)}\right] \cdot 100 \qquad \text{With}$$

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 brine shrimps

The mortality rate with actinomycin must be 100%.

7.7.6 Antitumor Test

A modified propidium iodide assay was used to examine the antiproliferative activity of the compounds against human tumor cell lines. The test procedure was described elsewhere[454]. Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice, or obtained from American Type Culture Collection, Rockville, MD, USA, National Cancer Institute, Bethesda, MD, USA, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

7.7.7 Fermentation in 20 L Fermentor

The 20 L glass fermentor was filled with 16 L of water and closed with the metal lid. The aeration, acid/base and antifoam systems were connected to the fermentor and 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 fermentor

was autoclaved for 90 minutes at 120 °C, after that it was taken out of the autoclave and the air supply, stirring motor and water circulation pumps were switched on. The acid (2N HCl), base (2N NaOH) and antifoam flasks (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 fermentor itself, two litres of medium containing suitable nutrients were prepared and autoclaved for 50 minutes at 120 °C. After cooling, the medium was added to the fermentor and the pre-culture was used to inoculate the fermentor.

7.8 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 on TLC. - **Toxicity test:** By counting survivors after 24 hrs, the mortality of the extracts was calculated (see above). The extracts, fractions or isolated compounds were considered inactive when the mortality rate was lower than 10% (-), from 10 to 59% as weakly active (+), from 60 to 95% as active (++) and over 95% as strongly active (+++). .

8 Origin of the Investigated Strains

All Streptomycetes with names starting with the signature "GW/BL" are of terrestrial origin and were obtained from the collection of the "Labor für Bodenkunde" (Dr. Grün-Wollny, Lohra-Kirchvers. The marine *Streptomyces* spp. (B-) and *Actinomyces* spp. (Act) are obtained from the collection of E. Helmke, Alfred-Wegener Institute for Polar and Marine Research, Bremerhaven. The North Sea bacteria strains (Mei) are from the Collection of Marine Streptomycetes (Prof. Meiners, Fachhochschule Emden). Origin of the few other strains is mentioned at the corresponding place.

Table 45: An *in vitro* antitumor activity of the selected bacteria strains crude extracts (2 concentrations - 6 cell line panel):

Code		Antitumor potency[a]		Tumor selectivity[b]				Action
		IC_{50}	IC_{70}	active/total*				
Strains	Oncotest	[μg/ml]	[μg/ml]	at 10 μg/ml		at 100 μg/ml		
B8108	MNEG127	<10	11,565	4/6	67%	6/6	100%	1. prio /10
B8112	MNEG128	<10	<10	6/6	100%	6/6	100%	1. prio /5
B7874	MNEG104	<10	<10	6/6	100%	6/6	100%	1. prio /2
B7729	MNEG103	<10	<10	6/6	100%	6/6	100%	1. prio /1
B8289	MNEG087	<10	10.0	5/6	83%	6/6	100%	1. prio /9
B7828	MNEG104	35.994	67.087	-	-	-	-	stop
ANK26	MNEG321	112.074	106.608	0/6	0%	1/6	17%	stop
Act8015	-	<10	<10	6/6	100%	6/6	100%	1. prio /5

[a] mean IC-values, determined as average of 6 human tumor cell lines tested.
[b] individual IC_{50} < ½ mean IC_{50}; e.g. if mean IC_{50} = 2.0 μM the threshold for above average sensitivity was IC_{50} < 1.0 μM

Table 46: *In vitro* antitumor activities of isolated compounds:

Compounds	Antitumor potency[a]		Tumor selectivity[b]		Compare Analysis
	Mean IC_{50} [µg/ml]	Mean IC_{70} [µg/ml]	n / total	%	
Mansouramycin D (**156**)	0.022	0.041	10/36	28%	
Butenolide (**233**)	9.759	10.265	1/36	3%	
Albaflavenone (**167**)	6.017	9.379	0/36	0%	
Imidazol (**193**)	7.146	> 10	0/36	0%	No positive correlation, low selectivity
Glucopiericidin C (**185**)	2.0	4.2	4/36	11%	
Piericidin A (**182**)	0.6	9.8	1/36	3%	
5-(2-Methylphenyl)-4-pentenoic acid) (**187a**)	7.8	9.3	2/36	6%	
5-Oxo-5-o-tolyl-pentanoic acid (**188a**)	>10	>10	0/36	0%	
Attiamycin A (**141**)	11.203	13.871	0/36	0%	
Attiamycin B (**143a**)	1.160	3.423	9/36	25%	
Nonactic acid (**144a**)	1.256	3.532	7/36	19%	
Homononactic acid (**145a**)	7.712	> 10	0/36	0%	
Dinactin (**148**)	0.002	0.011	9/36	25%	
Isatin **168:**	>10	>10	0/36	0%	
Phenazine **178:**	>10	>10	0/36	0%	
Butanolide (**230**)	22.093	>10	0/36	0%	-
IB-00208 (**106**)	0.002	0.007	5/36	14%	TOPO-II, Antimet
Karamomycin A (**73**)	6.783	11.198	0/36	0%	
Julichrome $Q_{1\text{-}5}$ (**117a**)[c]	5.5	10.0	2/36	6%	
Julichrome $Q_{3\text{-}5}$ (**118**)[c]	6.3	10.9	0/36	0%	
Julichrome Q_6 glucuronide (**120**)[c]	12.3	20.4	2/36	6%	

[a] mean IC-values, determined as average of 36 human tumor cell lines tested.
[b] individual IC_{50} < ½ mean IC_{50}; e.g. if mean IC_{50} = 2.0 μM the threshold for above average sensitivity was IC_{50} < 1.0 μM
[c] Conc. in [µM]

9 Metabolites from Selected Strains

9.1 Terrestrial *Streptomyces* sp. GW58/450

Culture of the terrestrial *Streptomyces* GW58/450 showed a white mycelium and the surrounding agar was white (using M_2 agar medium and incubation at 28 °C for four days).

9.1.1 Pre-screening

Fermentation of the strain as 1 L scale at 28 °C and 95 rpm using M_2 (for 10 and 3 days) and E-media (3 days) afforded dark yellowish-brown culture broth. After individual extraction of the filtrate and the mycelial cake with ethyl acetate (3 x) for each, three reddish-brown extracts were obtained.

The three extracts were tested antimicrobially and the results are presented in Table 47. The chemical screening exhibited on TLC confirmed the identity of the three extracts, in that they identically showed five middle polar UV fluorescent bands, at 366 nm. Three these bands were green-fluorescent, while the other were blue-fluorescent. On spraying with anisaldehyde/sulphuric acid and/or Ehrlich's reagent, these bands exhibited no colour staining.

Table 47: Antimicrobial activities of the crude extract of terrestrial *Streptomyces* sp. GW58/450 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]		
	M_2-Medium[a]	M_2-Medium[b]	E-Medium[b]
Bacillus subtilis	12	12	0
Streptomyces viridochromogenes (Tü 57)	20	0	16
Escherichia coli	12	0	0
Candida albicans	0	0	Nt
Mucor miehei (Tü 284)	0	0	0
Chlorella vulgaris	0	0	0
Chlorella sorokiniana	0	0	0
Scenedesmus subspicatus	0	0	0

[a] (10 days); [b](3 days)

9.1.2 Fermentation, Extraction and Isolation

The terrestrial *Streptomyces* sp. isolate GW58/450 was inoculated from its soil culture on three M_2 agar plates prepared with tap water. After incubation for 72 hours at 28 °C, the well-developed colonies were used to inoculate twelve of 1 liter-Erlenmeyer flasks, each containing 200 ml of M_2 medium. The cultures were shaken at 110 rpm for 6 days at 28 °C. The seed culture obtained was served to inoculate a 25-liter jar fermentor. The cultivation procedure was carried out at the same conditions for additional 6 days to give a dark brown culture broth. The broth was mixed with *ca.* 1 kg diatom earth and pressed through a pressure. Both filtrate and residue

were extracted with ethyl acetate, and one time with acetone for the mycelial cake. The acetone extract was concentrated to a small volume *in vacuo* and then extracted with EtOAc. As the TLC of both extracts showed similar compositions, they were combined and evaporated at 38 °C under vacuum, to yield 3.56 g of a reddish-brown crude extract.

The extract was subjected to column chromatography (3 x 60 cm, 220 g silica gel), the column being eluted with CH_2Cl_2-MeOH gradient (0 to 10% MeOH) and separated under TLC control into fractions **I** (1.6 g), **II** (382 mg), **III** (70 mg). By applying the low polar fraction **I** to fractionation and purification using Sephadex LH-20 (MeOH), actinomycin C_2 (3 mg), 1-acetyl-β-carboline (**228**, 2.5 mg), and the three karamomycins A (**73**, 12.8 mg), B (**75**, 3.5 mg) and C (**78**, 21.3 mg) were obtained. Purification of fraction II by Sephadex LH-20 (MeOH) led to 1-hydroxy-4-methoxy-2-naphthoic acid (**71**, 150.7 mg). Finally, purification of the polar fraction III by Sephadex LH-20 (MeOH) led to uracil (20.3 mg).

Table 48: Cytotoxic activities of Karamomycin A (**73**).

Compound	Potency		Tumor selectivity		
	Mean IC50 [μg/ml]	Mean IC70 [μg/ml]	Selectivity* Total	% selectivity	Rating**
Karamomycin A (**73**)	6.783	11.198	0/36	0%	-

* individual IC70 <1/3 mean IC70; e.g. if mean IC70 = 2.1 µM the threshold for above average sensitivity was IC < 0.7 µM; ** - (%selective = <4%); + (4%> %selective >= 10%); ++ (10%> %selective >= 20%); +++ (%selective >20%)

1-Hydroxy-4-methoxy-2-naphthoic acid (71): $C_{12}H_{10}O_4$ (218); colourless powder, an UV blue fluorescent (254, 366 nm). **– R_f** = 0.66 (DCM/5% MeOH). **– UV/VIS** (MeOH): λ_{max} (log ε) = 221 (4.06), 259 (4.07), 349 (3.51) nm. **– ^{1}H** and **^{13}C/APT NMR**, see Table 1 and Table 2. **– (-)-ESI MS**: *m/z* (%) = 457 ([2M-2H+Na]$^-$, 85), 217 ([M-H]$^-$, 100). – **EI MS:** *m/z* (%) = 218 [M]$^+$, 28), 200 [M-H_2O]$^+$, 100), 185 (9), 157 (19), 144 (30), 129 (46), 101 (20). – **HREI MS**: *m/z* 218.05700 (calcd. 218.05736 for $C_{12}H_{10}O_4$). **– (-)-HRESI MS**: *m/z* 217.050661 [M-H]$^-$ (calcd. 217.05062 for $C_{12}H_9O_4$).

Karamomycin A (73): $C_{15}H_{15}NO_3S$ (289); Yellow solid, an UV -green fluorescent (254, 366 nm), with no coloure change with anisaldehyde/sulfuric acid, brown with $PdCl_2$. **– R_f** = 0.55 (CH_2Cl_2). **– $[\alpha]_D^{20}$** = - 100 (*c* 0.1, MeOH). **– UV/VIS**: λ_{max} (log ε) = (MeOH): 217 (4.44), 246 (4.18), 271 (4.36), 292 (3.70) sh, 331 (3.52),

369 (3.73), 437 (3.05) sh, 460 (2.95) sh; (MeOH/HCl): 219 (4.37), 275 (4.31), 282 (4.32), 313 (3.90) sh, 412 (3.78); (MeOH/NaOH): 218 (4.42), 246 (4.16), 272 (4.31), 332 (3.55), 3.83 (3.77) nm. – **IR** (KBr): ν = 3421, 2957, 2853, 1720, 1650, 1633, 1590, 1562, 1456, 1397, 1381, 1344, 1265, 1217, 1134, 1103, 1075, 1042, 821, 802, 768 cm^{-1}. – **^{1}H** and **^{13}C/APT NMR**, see Table 3. – **(+)-ESI MS**: *m/z* (%) = 290 $[M+H]^+$. – **(-)-ESI MS**: *m/z* (%) = 288 $[M-H]^-$. – **(+)-ESI MS^2**: *m/z* (%) = 290 ($[M+H]^+$, 35), 274 (25), 258 (15), 200 (100). – **EI MS**: *m/z* (%) = 289 $[M]^{+\cdot}$, 100), 274 $[M-CH_3]^{+\cdot}$, 80), 258 $[M-OCH_3]^{+\cdot}$ 28), 199 (32), 184 (48), 91 (24), 73 (40), 45 (20). – **HREI MS**: *m/z* 289.07520 (calcd. 289.07672 for $C_{15}H_{15}NO_3S$). – **(+)-HRESI MS**: *m/z* 290.084803 $[M+H]^+$ (calcd. 290.08454 for $C_{15}H_{16}NO_3S$), 312.066667 $[M+Na]^+$ (calcd. 312.06649 for $C_{15}H_{15}NO_3SNa$). **– H,H COSY**, **HMBC** and **NO-ESY:** see Figure 14 and Figure 15.

Karamomycin B (**75**)**:** $C_{15}H_{13}NO_3S$ (287); Yellow solid, green fluorescence (366 nm), no coloure change with anisaldehyde/sulfuric acid, brown with $PdCl_2$. – R_f = 0.60 (CH_2Cl_2). – **UV/VIS**: λ_{max} (log ε) = (MeOH): 208 (3.92), 230 (3.90) sh, 280 (3.81) sh, 294 (3.84), 373 (3.52), 395 (3.50) sh; (MeOH/HCl): 205 (3.96), 230 (3.92) sh, 280 (3.78) sh, 295 (3.83), 375 (3.48), 395 (3.46) sh; (MeOH/NaOH): 211 (3.96), 230 (3.90) sh, 299 (3.76), 403 (3.55) nm. – **^{1}H** and **^{13}C NMR**, see Table 3. – **EI MS:** *m/z* (%) = 287 ($[M]^{+\cdot}$, 100), 272 ($[M-CH_3]^{+\cdot}$, 34), 254 ($[M-OCH_3]^{+\cdot}$, 96), 184 (10), 134 (10), 71 (36), 45 (8). **– (+)-HRESI MS** *m/z* 288.0689920 $[M+H]^+$ (calcd. for $C_{15}H_{14}NO_3S$, 288.068895 $[M+H]^+$). **–H,H COSY**, **HMBC:** see Figure 18.

Karamomycin C (78): $C_{23}H_{25}N_3O_3S_3$ (487); Yellow solid, an UV-green fluorescent (366 nm), no coloure change with anisaldehyde/sulfuric acid, brown with $PdCl_2$. – R_f = 0.91 (CH_2Cl_2). – $[\alpha]_D^{20}$ = - 336 (*c* 0.025, MeOH). – **UV/VIS**: λ_{max} (log ε) = (MeOH): 216 (4.55), 248 (4.27), 274 (4.45), 373 (3.81), 460 (3.25) sh; (MeOH/HCl): 217 (4.50), 248 (4.16), 275 (4.41), 390 (3.73), 450 (3.67) sh; (MeOH/NaOH): 218 (4.54), 248 (4.23), 274 (4.39), 377 (3.78), 390 (3.78), 450 (3.33) sh, nm. – **IR** (KBr): ν = 3430, 2925, 1635, 1585, 1561, 1506, 1453, 1378, 1344, 1242, 1210, 1137, 1102, 1048, 822, 769, 701 cm^{-1}. – **^{1}H** and **^{13}C/APT NMR**, see Table 4. **–(+)-ESI MS**: *m/z* (%) = 997 ($[2M+Na]^+$, 100), 510 ($[M+Na]^+$, 22); **–(-)-ESI MS**: *m/z* (%) = 995 ($[2M-2H+Na]^-$, 100), 486 ($[M-H]^-$, 28): **– EI MS:** *m/z* (%) = 487 ($[M]^+$, 48), 444 (20), 287 (8), 258 (20), 229 (6), 201 (98), 183 (16), 138 (56),

114 (100), 71 (9), 56 (13). – **(+)-HRESI MS**: *m/z* 488.11307 (calcd. 488.11308 for $C_{23}H_{25}N_3O_3S_3$). **– H,H COSY**, **HMBC** and **NOESY:** see Figure 22 and Figure 23.

9.2 Terrestrial *Streptomyces* sp. TN58

The terrestrial *Streptomyces* sp. TN58 was isolated from Tunisian soil and taxonomically identified and described by Prof. Mellouli[125].

9.2.1 Pre-screening, Isolation and Working up

The biological and chemical screenings of the strain TN58 strain were performed and reported in details in our recent article[127]. TN58 strain was showed antibacterial activities against *M. luteus* LB 14110 (A) and *E. coli* ATCC 8739 (B)[126]. A largescale fermentation of the strain was carried out as 25 l shaker culture using M_2-medium (pH 7.8). After harvesting, the obtained broth (greenish-yellow) was filtered and passed through a column of XAD-2. after final extraction and evaporation (in-vacuo), affording 1.30 g as brown crude extract. After a sereise of chromatographic purification of the extract, the follwing compounds were delivered: brevianamide F (**95**; 2.1 mg, colourless solid), N^{β}-acetyltryptamin (**96**; 8.1 mg, colourless solid), thiazolidomycin (**97**; 35.7 mg, white crystals) and 1-O-(2-aminobenzoyl)-α-L-rhamnoside (**99**; 7.3 mg, white solid), uracil (20.7 mg, white solid) and 4-hydroxybenzoyl α-L-rhamnopyranoside (**100**; 2.3 mg, colorless solid), (Figure 26).

The complete spectroscopic data of the isolated compounds were described in details in our recent published article[127].

9.3 Terrestrial *Streptomyces* sp. GW19/5671

The terrestrial *Streptomyces* sp. GW19/5671 was isolated and taxonomically identified by bioLeads (Heidelberg). The strain showed a white mycelium characteristic for the *Streptomyces* sp. by incubation on M_2-, casein- or M_2^+ media for 10 days at 28 °C.

9.3.1 Pre-screening

The primary screening of the strain GW19/5671 was first carried out on casein-medium and showed middle polar violet band by spraying with anisaldehyde/sulphuric acid. A cultivation of the strain on M_2-medium, showed two rather

different main non-polar greenish-yellow bands, turned orange by anisaldehyde/sulphuric acid. A comparative antimicrobial screening of both extracts (Table 49) displayed their similarity in the activity.

Table 49: Antimicrobial activity of the crude extract from *Streptomyces* sp. GW19/5671 (1 mg/ml), using Casein- and M_2-media.

Tested microorganisms	Inhibition zone ∅ [mm]	
	Casein-medium	M_2-medium
Bacillus subtilis	19	22
Escherichia coli	15	19
Staphylococcus aureus	18	18
Streptomyces viridochromogenes	11	11

9.3.2 Casein-medium

9.3.2.1 Fermentation and Working up

A subculture of the terrestrial strain *Streptomyces* GW19/5671 was seeded to 20 l shaker culture using casein-medium (10 g/l casein, 5 g/l yeast extract, 10 g/l NaCl, and 5 g/l glucose in tap water, set to pH 7.8 before sterilisation). The fermentor broth was harvested after 10 days and mixed with Celite and filtered. The filtrate and mycelia were subjected to extraction separately using ethyl acetate for the water phase and acetone for the biomass. The combined organic phases were evaporated to dryness to afford a brown crude extract (1.04 g).

9.3.2.2 Isolation

The strain extract was analysed with the aid of TLC which showed a similarity with the result mentioned above in the primary screening giving a violet band of a polar component after spraying with anisaldehyde/sulphuric acid, in addition to faint non-polar yellow bands. The strain extract was subjected to column chromatography on silica gel using a chloroform-methanol gradient (1.5 L $CHCl_3$, 0.5 L $CHCl_3$/5% MeOH, 0.5 L $CHCl_3$/10% MeOH, 0.3 L $CHCl_3$/20% MeOH, 0.5 L $CHCl_3$/50% MeOH, 0.4 L MeOH) giving four fractions. Fractions II (150 mg), III (110 mg), IV (100 mg), showed a violet band after spraying with anisaldehyde/sulphuric acid and gave a faintly UV absorbing zone at 254 nm. The compound was isolated from the mentioned fractions by subjecting them to Sephadex LH-20 (MeOH) followed by

PTLC (CH_2Cl_2/7% MeOH) and further purification by Sephadex LH-20 (CH_2Cl_2/MeOH 6:4, 2×) to afford an oily colourless substance (**101**; 16 mg).

Isrocine (N-[3-Hydroxy-1-(4-hydroxy-phenyl)-propyl]-acetamide) (101): $C_{11}H_{15}NO_3$ (209). **– R_f =** 0.37(CH_2Cl_2/10% CH_3OH). **– UV** (MeOH): λ_{max} (log ε) = 276 nm (2.91). **– IR** (KBr): ν = 3279, 2959, 1666, 1542, 1516, 1456, 1263, 1061 and 834 cm^{-1}. **– ^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 9.15 (s br, H/D exchangeable, 1H, OH), 8.12 (d, ^3J = 7.2 Hz, 1H, NH-4), 7.06 (d, ^3J = 8.3 Hz, 2 H, 2'/6'-H), 6.68 (d, ^3J = 8.3 Hz, 2H, 3'/5'-H), 4.78 (q, ^3J = 7.2 Hz, 1 H, 3-H), 4.42 (s br, 1H, OH), 3.33 (m, 2 H, 1-H_2), 1.78 (s, 3H, 6-CH_3), 1.78 (m, 2 H, 2-H_2). **– ^{13}C NMR** ([D_6]DMSO, 75 MHz): δ = 168.3 (C_q, C-5), 155.9 (C_q, C-4'), 134.0 (C_q, C-1'), 127.5 (2 CH, C-2'/6'), 114.9 (2 CH, C-3'/5'), 57.9 (CH_2, C-1), 48.9 (CH, C-3), 39.8 (CH_2, C-2), 22.7 (CH_3, C-6). **- (+)-ESI MS**: *m/z* (%) = 441 ($[2 M + Na]^+$, 100), 232 ($[M + Na]^+$, 42). **- (-)-ESI MS**: *m/z* (%) = 417 ($[2 M - H]^-$, 100), 208 $[M - H]^-$, 35). **– EI MS** (70 eV): *m/z* (%) = 209 ($[M]^+$, 28), 164 ($[M-CH_2CH_2OH]^+$, 54), 122 ($[M-COCH_3]^+$, 100), 43 ($[NHCOCH_3]^+$, 22). **- HREI MS:** *m/z* = 209.1052 (Calcd. for $C_{11}H_{15}NO_3$, 209.1052).

9.3.3 M_2-medium

The terrestrial *Streptomyces* sp. GW19/5671 subculture was used to incubate a 20 l shaker culture using M_2-medium (pH 7.8 before sterilisation). The fermentor broth was harvested after 10 days and the resulting greenish yellow culture broth was subjected to filtration on Celite using pressure on filter press. The filtrate and mycelia were subjected to extraction separately by ethyl acetate (water phase) and acetone for the biomass. The combined organic phases were evaporated to dryness under vacuum, to give a dark yellowish-green crude extract (2.39 g).

9.3.3.1 Isolation

The crude extract was subjected to column chromatography on silica gel under monitoring by TLC (UV and spraying reagents), using a chloroform-methanol gradient (2.0 l $CHCl_3$, 0.5 l $CHCl_3$/5% MeOH, 0.5 l $CHCl_3$/10% MeOH, 0.5 l $CHCl_3$/20% MeOH, 0.2 $CHCl_3$/50% MeOH, 0.2 MeOH) to give four fractions: I (350 mg), II (120 mg), III (270 mg) and IV (400 mg). The yellow bands were isolated and further purified to afford three compounds, two of which were yellow solids and the third one was a colourless solid (Figure 32).

***o*-Hydroxy-acetanilid**; **2-Acetamidophenol (103):** $C_8H_9NO_2$ (151), colourless solid (13 mg), UV absorbing (254 nm) yellow with anisaldehyde/sulphuric acid. **– R_f =** 0.52 (CH_2Cl_2/5% CH_3OH). **– ^{1}H NMR** ([D_6]acetone, 300 MHz): δ = 9.44 (s br, 1H), 9.28 (s br, 1H), 7.42 (dd, ^3J = 8 Hz, ^4J = 1.2 Hz, 1 H, 3-H), 7.01 (td, ^3J = 8 Hz, ^4J = 1 Hz, 1 H, 5-H), 6.92 (dd, ^3J = 8 Hz, ^4J = 1.1 Hz, 1 H, 6-H), 6.78 (td, ^3J = 8 Hz, ^{4}J = 1.1 Hz, 1 H, 4-H), 2.22 (s, 3 H, CH_3). **– (+)-ESI MS**: *m/z* (%) = 325 ([2 M + Na]$^+$, 100), 174 ([M + Na]$^+$, 60). **– EI MS** (70 eV): *m/z* (%) = 151 ([M], 32), 109 [M - $COCH_3$ + H]$^+$, 100), 80 (20), 43 (20).

Phenazine-1-carboxylic acid (Tubermycine B) (104): $C_{13}H_8N_2O_2$ (224), yellowish-green solid (8 mg). **– R_f** = 0.66 ($CHCl_3$ /10% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 15.62 (s br, H/D exchangeable, 1 H, OH), 8.98 (dd, ^3J = 7.1 Hz, ^4J = 1.4 Hz, 1 H, 6-H), 8.53 (dd, ^3J = 8.8 Hz, ^4J = 1.4 Hz, 1 H, 9-H), 8.37 (dd, ^3J = 8.8, ^4J =1.4 Hz, 2-H), 8.26 (dd, ^3J = 8.8, ^4J = 1.4 Hz, 1 H, 4-H), 8.07-7.95 (m, 3 H, 3-, 7-, 8-H). **– ^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 8.48 (s br, H/D exchangeable, 1 H, OH), 8.23 (m, 2H, Ar-H), 8.07 (d, ^3J = 8.8 Hz, 1 H, Ar-H), 7.92 (m, 2H, Ar-H), 7.82 (t, ^3J = 8.6, Ar-H), 7.72 (d, ^3J = 8.8 Hz, 1 H, Ar-H). **– (+)-ESI MS:** *m/z* (%) = 471 ([2 M + Na]$^+$], 100), 247 ([M + Na]$^+$, 20), 225 ([M + H]$^+$, 10). **– (-)-ESI MS:** *m/z* (%) = 223 ([M - H]$^-$, 100). **– EI MS** (70 eV): *m/z* = 224 ([M]$^{+\cdot}$, 12), 207 ([M-OH]$^{+\cdot}$, 46), 180 ([M-CO_2]$^{+\cdot}$, 100), 90 (10), 73 (24), 44 (20).

Phenazine-1-carboxylic acid methyl ester (105): $C_{14}H_{10}N_2O_2$ (238), yellowish-green solid (6 mg). **– R_f** = 0.85 ($CHCl_3$/7% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.145 MHz): δ = 8.40 (dd, ^3J = 8 Hz, ^4J = 1 Hz, 1 H, Ar-H), 8.35 (m, 1 H, Ar-H), 8.25 (dd, ^3J = 7 Hz, ^4J = 1 Hz, 2 H, Ar-H), δ 7.94-7.83 (m, 3 H, 3 Ar-H), 4.15 (s, 3 H, 1-OCH_3). **– (+)-ESI MS:** *m/z* (%) = 499 ([2 M + Na]$^+$, 100), 261 ([M + Na]$^+$, 58), 239 ([M + H]$^+$, 4).

9.4 Terrestrial *Streptomyces* spp. bl 2/5831, bl 4/5844 and bl 10/5742

Terrestrial *Streptomyces* spp. isolates bl 2/5831, bl 4/5844 and and bl 10/5742 exhibited white aerial mycelia by cultivation on agar plates using C- medium at 28 °C for 3 days. The strains afforded slightly brown culture broths after inoculation into 1 L shaker, maintenance on C-medium (28 °C for 6 days).

9.4.1 Pre-screening

Chemical screening of the afforded three brown extracts using TLC displayed one unique major UV absorbing band, which turned brown and intensive reddish-brown by spraying with anisaldehyde/H_2SO_4 and Ehrlich's spraying reagents, respectively. Antimicrobial screening of the extracts are litsted in Table 50.

Table 50: A representative antimicrobial activities of the terrestrial *Streptomyces* spp. bl 2/5831and bl 10/5742 extracts (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]	
	bl 2/5831	bl 10/5742
Bacillus subtilis	10	11
Staphylococcus aureus	14	0
Chlorella vulgaris	19	10
Chlorella sorokiniana	20	10
Scenedesmus subspicatus	15	12

9.4.2 Fermentation, Extraction and Isolation of Extracts from Strains bl 2/5831 and bl 4/5844

Well grown plates of terrestrial *Streptomyces* spp. isolates bl 2/5831 and bl 4/5844 were inoculated into 2 L and 4 L respectively, using C- medium at 28 °C on shaker (95 rpm) for 6 days. After extraction with ethyl acetate gave and concentration *in vacuo*, 0.40 g and 0.85 g as yellowish-brown crude extracts, were obtained respectively. Purification of both extracts using the ame chromatographic techniques (column chromatography on silica gel and Sephadex LH-20) delivered pyrrol-2-carboxylic acid (150 mg, colourless solid).

9.4.3 Optimization of the Terrestrial *Streptomyces* sp. bl 2/5831

An optimization of the terrestrial-derived *Streptomyces* sp. bl 2/5831 was carried out using 5 different nutritional media (1 liter), pH (6.5, 7.8), temperature (28, 35 °C), and shaking speed (95, 110 rpm), at fixed cultivation period (6 days) (Table 51). TLC and microbial examinations indicated that C-medium with 50% sea was the optimum one for production of promising metabolites with high antimicrobial activities, (Figure 34).

Table 51: Optimization of the terrestrial *Streptomyces* sp. bl 2/5831

Medium	pH	Ingredients (per litre)	Shaking rate (rpm)	Temp.	Time (d)
C-medium (50% SW)	7.8, 6.5	10 g glucose, 2 g peptone, 1 yeast, 1 g meat extract.	95, 110	28, 35 °C	6
C- medium (TW)	7.8, 6.5	10 g glucose, 2 g peptone, 1 yeast, 1 g meat extract	95, 110	28, 35 °C	6
SM-Medium (TW)	7.8, 6.5	20 g soja fatt frei, 20 g mannite	95, 110	28, 35 °C	6
F-Medium (TW)	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	95, 110	28, 35 °C	6
A-Medium (M_2) (TW)	7.8, 6.5	10 g malt extract, 5 g yeast extract 5 g glucose	95, 110	28, 35 °C	6

Table 52: Antimicrobial activities of the crude extracts produced by the optimization of the terrestrial *Streptomyces* sp. bl 2/5831 (1 mg/ml, diameter of inhibition zones in mm).

Tested microorganismis	Inhibition zone ∅ [mm]				
	A	B	C	D	E
Bacillus subtilis	12	0	0	0	0
Staphylococcus aureus	15	11	10	0	10
Streptomyces viridochromogenes (Tü 57)	10	14	0	0	0
Escherichia coli	14	0	0	0	0
Candida albicans	0	n*	0	0	0
Mucor miehei (Tü 284)	0	0	0	0	0
Chlorella vulgaris	20	15	10	0	10
Chlorella sorokiniana	20	20	0	0	11
Scenedesmus subspicatus	18	15	0	0	11

A = C-medium with 50% sea water; **B** = C-medium; **C** = SM-medium; **D** = F-medium; **E** = A-Medium; n*= did not tested.

9.4.3.1 Fermentation, Extraction and Isolation of Extracts from *Streptomyces* sp. bl 2/5831

The strain bl 2/5831 was cultivated as 16 1 L Erlenmeyer flasks each containing 250 ml of C-medium containing 50% sea water (at 28 °C with 95 rpm, and pH 7.8) for six days using a linear shaker. After fermentation, the dark brown broth obtained was harvested and filtered. The mycelial cake and filtrate were exhaustively extracted by ethyl acetate (3 and 4 times, respectively). The organic phases were combined and concentrated *in vacuo* to dryness to give 4.20 g a brownish oily crude extract. The extract was purified using silica gel column chromatography (column 2 × 50 cm, 70 g), with CH_2Cl_2/MeOH gradient to give a pure orange powder of IB-00208 (**106**; 520 mg).

Table 53: Cytotoxic activities of IB-00208 (**106**)

Compound	Antitumor potency [a]		Tumor selectivity [b]		Compare analysis
	Mean IC_{50} [µM]	Mean IC_{70} [µM]	n/total	%	
IB-00208 (**106**)	0.002	0.007	5/36	14%	TOPO-II, Antimet

[a] mean IC-values, determined as average of 36 human tumor cell lines tested.
[b] individual IC_{50} < ½ mean IC_{50}; e.g. if mean IC_{50} = 2.0 μM the threshold for above average sensitivity was IC_{50} < 1.0 μM.

IB-00208 (106): $C_{36}H_{34}O_{14}$ (690), UV absorbing (254 nm), orange powder, no colour reaction with anisaldehyde/sulphuric acid. – $[\alpha]_D^{20}$ = -230 (*c* 0.1, $CHCl_3$).– R_f = 0.70 (CH_2Cl_2/10% MeOH). –**IR** (KBr): ν = 3444, 2922, 1697, 1650, 1616, 1478, 1420, 1288, and 1056 cm^{-1}. – **UV/VIS** (MeOH): λ_{max} (log ε) = 390 (3.08), 321(3.41), 252 (3.65), 225 (3.68) nm. – **^{1}H** and **^{13}C NMR**, see Table 7. – **(+)-ESI MS:** *m/z* (%) = 1403 ($[2M+Na]^+$, 100), 713 ($[M+Na]^+$, 20). – **(+)-ESI MS/MS:** *m/z* (%) = 713 ($[M+Na]^+$, 2), 525 ([M-glucopyranosyl moiety+Na]$^+$, 100). **–H,H COSY** and **HMBC:** see Figure 38.

9.5 Terrestrial *Streptomyces* sp. bl 47/4455

The terrestrial *Streptomyces* sp. bl47/4455 was obtained, taxonomically identified and deposited in bioLeads (Heidelberg). The strain showed white aerial mycelia during incubation either on M_2 or C-medium for 10 days at 28 °C.

Well-developed colonies of the strain in agar plates were used to inoculate 12 of 1L-Erlenmeyer flasks each containing 250 ml of C- medium. The fermentation was carried out on a shaker (95 rpm) for 10-days at 28 °C. After harvesting, the yellowish-green broth was filtered, and then both of supernatant and the mycelial cake were exhaustively extracted by ethyl acetate (3 times).

9.5.1 Pre-screening

Using agar diffusion disk method, the strain extract was antimicrobially screened (Table 54). Chemical screening of the extract during TLC exhibited various UV absorbing bands (254 nm), most them stained to reddish-brown beside to one intensive yellow appeared after spraying with anisaldehyde/H_2SO_4 and heating. The HPLC-MS analysis of the crude extract showed several compounds' peaks of reten-

tion time ranged between 21~24 minutes (LC MS). This peaks were of corresponding mole masses of 604, 588, 622, 606, 915, 720, 734 and 718 Daltons.

Table 54: Comparative antimicrobial assays for the crude extracts of the terrestrial *Streptomyces* isolate bl 47/4455 using M_2 - and C- media.

Tested microorganisms	Inhibition zone ∅ [mm]	
	(M_2- medium)	(C- medium)
Bacillus subtilis	14	0
Staphylococcus aureus	23	12
Streptomyces viridochromogenes (Tü 57)	35	0
Escherichia coli	14	10
Candida albicans	11	0
Mucor miehei (Tü 284)	30	43
Chlorella vulgaris	0	0
Chlorella sorokiniana	0	0
Scenedesmus subspicatus	0	0

9.5.2 Fermentation, Extraction and Isolation

Well grown agar sub-cultures of the terrestrial *Streptomyces* sp. bl 47/4455 were inoculated into 100 sterilized 1 L Erlenmeyer flasks, each containing 200 ml of C-medium (meat extract medium). The fermentation was continued for 10 days at 28 °C on a linear shaker (110 rpm). The obtained culture broth (20-liter) was mixed with ca. 1 kg diatom earth and filtered through a press filter. The bacterial cells were extracted (4 times) with ethyl acetate to give extract A. The filtrate was passed through XAD-16, and the adsorbant was then eluted by methanol, followed by concentration *in vacuo*. The remaining water residue was re-extracted with ethyl acetate to give extract B. Both extracts A and B were identical from TLC, therefore they combined. After evaporation, the obtained yellowish-oil crude extract (5.20 g) was chromatographed on Sephadex LH-20 column using CH_2Cl_2/MeOH (60:40) eluent system. After TLC monitoring, two fractions I and II were delivered. Only fraction II was antimicrobially active against *Mucor miehei* (Tü 284) and *Scenedesmus subspicatus*. So fraction II was subjected to further purifications to afford bafilomycin A_1 (**108**; 46 mg, white amorphous solid), A_2 (**109**; 12 mg, white amorphous solid), B_1 (**110**; 25 mg, yellow amorphous solid), B_2 (**111**; 20 mg, yellow amorphous solid), C_1 (**112**; 38 mg, white amorphous powder), TS155 (**113**; 6 mg, white amorphous solid),

D (**114**; 54 mg, white amorphous solid), and K (8; 5 mg, colourless amorphous solid), (Figure 41).

Bafilomycins: A_1 (**108**), C_1 (**112**) and TS155 (113) and K (**115**) were examined as antibacterial, antifungal and antialgal agents using agar diffusion method with 9 mm paper discs at concentration of 40 μg per disk (Table 55).

Table 55: Antimicrobial activities of Bafilomycins: A_1 (**108**), C_1 (**112**), TS155 (**113**) and K (**115**) (∅ of inhibition zones, mm).

Compounds	Conc. (µg/disk)	BS[a]	CA[b]	SA[c]	EC[d]	MM[e]	CS[f]	SS[g]
Bafilomycin A_1(**108**)	40	0	0	0	0	11	0	15
Bafilomycin K (**115**)	40	0	0	0	0	13	0	16
Bafilomycin C_1 (**112**)	40	10	13	11	0	20	22	13
TS155 (**113**)	40	0	0	11	0	20	30	27

[a]*Bacillus subtilis*, [b]*Candida albican*, [c]*Staphylococcus aureus*, [d]*Escherichia coli,*[e]*Mucor miehi,* [f]*Chlorella sorokiniana,* [g]*Scenedesmus subspicatus*

Bafilomycin A_1 (108): $C_{35}H_{58}O_9$ (622), white amorphous of UV absorbance, turned reddish-brown by spraying with anisaldehyde/sulphuric acid and heating. **- R_f** = 0.28 (CH_2Cl_2/5% MeOH). **- ^{1}H** and **^{13}C NMR**, see Table 9. **- (+)-ESI MS:** *m/z* (%) = 1267 ($[2M + Na]^+$, 100), 645 ($[M + Na]^+$, 62) **- (-)-ESI MS:** *m/z* = 621 ($[M - H]^-$).

Bafilomycin C_1 (112): $C_{39}H_{60}O_{12}$ (720), white amorphous of UV absorbance, turned reddish-brown by spraying with anisaldehyde/sulphuric acid and heating. **-R_f** = 0.10 ($CHCl_3$/10% MeOH). **- ^{1}H NMR**, see Table 8. **- (+)-ESI MS:** *m/z* (%) = 1463 ($[2M+Na]^+$, 100), 743 ($[M+Na]^+$, 96). **- (-)-ESI MS:** *m/z* (%) = 1439.5 ($[2M-H]^-$, 100), 719 ($[M-H]^-$, 78).

TS155 (113): $C_{39}H_{60}O_{11}$ (704), white amorphous, having UV absorbance which stained to reddish-brown by spraying with anisaldehyde/sulphuric acid followed by heating. **- R_f** = 0.11 (CH_2Cl_2/10% MeOH). **- ^{1}H** and **^{13}C NMR**, see Table 8. **- (+)-ESI MS:** *m/z* (%) = 1431 ($[2M+Na]^+$, 20), 727 ($[M+Na]^+$, 100). **- (-)-ESI MS:** *m/z* = 703 ($[M-H]^-$). – **(+)-HRESI MS**: *m/z* = 727.4025100 $[M+Na]^+$ (calcd. 727.4027653for $C_{39}H_{60}O_{11}Na$). **– H,H COSY** and **HMBC:** see Figure 44.

Bafilomycin D (114): $C_{35}H_{56}O_8$ (604), white amorphous solid (54 mg), UV absorbing (254 nm), intense-yellow with anisaldehyde/sulphuric acid spraying reagent. **- R_f** = 0.56 (CH_2Cl_2/10% MeOH). **- ^{1}H** and **^{13}C NMR**, see Table 8.**- (+)-ESI MS:** *m/z* (%) = 1231 ($[2M+Na]^+$, 100), 627 ($[M+Na]^+$, 60).

Bafilomycin K (115): $C_{35}H_{58}O_8$ (622), colourless amorphous, an UV absorbing, stained to reddish-brown by spraying with anisaldehyde/sulphuric acid and heating. **-** R_f = 0.40 (CH_2Cl_2/5% MeOH). **– UV/VIS** (MeOH): λ_{max} = 234, 268 (sh) nm. **- IR (KBr):** ν = 3438, 2928, 2856, 2361, 1735, 1660, 1458, 1385, 1278, 1247, 1102, 917, 764, 704, 640 cm^{-1}. **- ^{1}H** and **^{13}C NMR**, see Table 9. **- (+)-ESI MS:** *m/z* (%) = 1235 ($[2M+Na]^+$, 100), 629 ($[M+Na]^+$, 80). –**HRESI MS**: *m/z* (%) = 629.402290 $[M+Na]^+$ (calcd. 629.402938 for $C_{35}H_{58}O_8Na$). **–H,H COSY** and **HMBC:** see Figure 46.

9.6 Terrestrial *Streptomyces* sp. GW6225

The terrestrial *Streptomyces* sp. GW6225 displayed a yellow aerial mycelium during its cultivation using M_2 medium.

9.6.1 Pre-screening, Fermentation, Working up and Isolation

Precereening of the strain cornfirmed its high antimicrobial activities against noumerous pathogenic microbes[174]. Chemical and HPLC-MS screening of the strain extract revealed the presence of several promising bands. Fermenation of the strain was performed using 20 l shaker on M_2 medium, at 28 °C (5 days). After harvesting and extraction of the broth, 6.30 g of a greenish-brown crude extract were obtained. During a a consequent purification series of the extract with the aid of diveres chrmatographic techniques lead to isolation the shown below compounds. They were N-phenyl-β-naphthylamine (**129**, 16.3 mg, colorless solid), 4-acetylchrysophanol (**119a**, 3.6 mg, yellow solid), julichrome $Q_{1\cdot2}$ (**117b**, 1.3 mg, red solid), Julichromes $Q_{1\cdot5}$ (**117a**, 3.6 mg, red solid), $Q_{3\cdot5}$ (**118**, 6.3 mg, orange solid), nosiheptide (**127**, 6.6 mg, yellow solid) and julichrome Q_6 glucuronide (**120**, 6.3 mg, yellowish-green solid) (Figure 47).

Recently, we published the detailed biological activities (antimicrobial, cyto-toxictiy) and spectroscopic data of the compounds; **129**, **119a**, **117a**, **118** and **120**.[174]

Julichrome $Q_{1\cdot2}$ (**117b**): Red solid, R_f = 0.42 (CH_2Cl_2/4% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 12.55 (s, 2H, 8-OH, 8'-OH), 12.14 (s, 1H, 1'-OH), 7.88 (d, 7.8 Hz, 1H, H-5'), 7.88 (d, 7.8 Hz, 1H, H-6'), 7.83 (d, 8.1, 1H, H-5), 7.81 (d, 8.1, 1H, H-6), 7.20 (s, 1H, H-2'), 5.82 (m, 2H, H-11), 4.15 (m, 1H, H-11'), 3.58 (d, 1.5, 2H,

H-4), 2.78 (s, 2H, CH_2-2), 2.36 (s, 3H, 3'-CH_3), 1.91 (s, 3H, CH_3-12), 1.44 (s, 3H, 3-CH_3), 1.22 (d, 6.6, 3H, 11-CH_3), 1.24 (d, 6.6, 3H, 11'-CH_3), 1.91 (s, 3H, CH_3-12'),. – **(+)-ESI MS:** *m/z* = 719 $[M+Na]^+$. – **(+)-HRESI MS:** *m/z* = 697.1916680 (calcd. for $[M+H]^+$, $C_{38}H_{33}O_{13}$, 697.191565).

Nosiheptide (**127**): Yellow solid, weak gree-yellow UV fluorescence on TLC, strong in solution. – **^{1}H NMR** (DMSO, 300 MHz): see Table 13. – **(+)-ESI MS**: *m/z* = 1242 $[M+Na]^+$. – **(-)-ESI MS:** *m/z* = 1220 $[M-H]^-$. – **(+)-HRESI MS:** *m/z* = 1222.15746 $[M+H]^+$, 1244.13737 $[M+Na]^+$ and 1283.06961 $[M+Cu-H]^+$ (calcd. for $[M+H]^+$, $C_{51}H_{44}N_{13}O_{12}S_6$, 1222.15511, calcd. for $[M+Na]^+$, $C_{51}H_{43}N_{13}O_{12}S_6Na$, 1244.13706 and calcd for $[M+Cu-H]^+$, $C_{51}H_{42}N_{13}O_{12}S_6Cu$, 1283.06907).

9.7 Terrestrial *Streptomyces* sp. RSF18

The terrestrial *Streptomyces* sp. isolate RSF18 was collected, isolated and identified by the research group of Prof. Shahida Hasnain; Department of Microbiology and Molecular Genetics, University of the Punjab, Pakistan. During the primary screening of the strain cultivated on M_2 medium (7-days at 28 °C), three middle polar UV-absorbing bands were detected during TLC. Two of these bands turned yellow and the third as dark blue by anisaldehyde/sulphuric acid. HPLC MS/MS of the extract showed two peak signals with masses of 1131 and 1115, corresponding to sulphur containing compounds. The extract exhibited a strong activity against Gram-positive and Gram-negative bacteria (Table 56). The extract displayed cytotoxic activity with mortality of 13.49% against the brine schrimp *Artemia salina*, as test organism.

Table 56: Antimicrobial activity of the crude extract from RSF18 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	17
Staphylococcus aureus	16
Streptomyces viridochromogenes (Tü 57)	12
Escherichia coli	15

9.7.1 Fermentation and working up

The upscale fermentation (15 l, as shaker culture, M_2 medium at 28 °C, for 7 days), isolation and working up of the obtained extract (6.85 g) were published re-

cently by us in details[194]. Consequently, the follwing compounds were deliverd in pure forms and tested against diveres pathogenic bacterial and fungal strains: Geninthiocin (**130**; 78.6 mg), val-geninthiocin (**131**; 27.5 mg).and chalcomycin A (**132**; 19.8 mg); Figure 56. For MS/MS studies and detailed spectroscopic data of Geninthiocin (130) and Val-Geninthiocin **(131)**: see our recent publication[194].

Chalcomycin A (132): $C_{35}H_{56}O_{14}$ (700), UV absorbing colourless solid, dark-blue coloration with anisaldehyde/H_2SO_4 spraying reagent. **– R_f** = 0.55 ($CHCl_3$/10% MeOH). **– ^{13}C NMR**, see Table 15 . – **(+)-ESI MS:** *m/z* (%) = 1423 ($[2M+Na]^+$, 100), 723 ($[M+Na]^+$, 94). **– (-)-ESI MS:** *m/z* (%) = 745 ($[M+HCOO]^-$, 100), 699 ($[M-H]^-$, 20). – **(-)-HRESI MS:** *m/z* = 701.3742780 $[M+H]^+$ and 723.3561690 $[M+Na]^+$ (calcd. 701.3742653 $[M+H]^+$, for $C_{35}H_{57}O_{14}$ and 723.3562153 $[M+Na]^+$, for $C_{35}H_{56}O_{14}Na$).

9.8 Terrestrial *Streptomyces* sp. ANK26

The terrestrial *Streptomyces* sp. isolate ANK26 obtained from the collection of Prof. Anke (Institute for Biotechnology and Drug Research (IBWF), Kaiserslautern, Germany). The strain was inoculated from its soil culture on three agar plates using M_2 medium and incubated for 96 hours at 28 °C, showing a white mycelium characteristic for the *Streptomyces* sp.

9.8.1 Pre-screening

Well-developed colonies were used to inoculate 12 of 1L-Erlenmeyer flasks, each containing 250 ml of M_2 medium followed by cultivation on a shaker (95 rpm for 7-days at 28 °C). The afforded yellowish-brown broth was harvested, filtered and extracted (3×) with ethyl acetate. Antimicrobilally, the obtained extract exhibited strong activities against Gram-positive and Gram-negative bacteria., but no activity against fungi, yeast or microalgae; Table 57.

In the chemical screening during TLC, semi-polar zones were detected, which stained as unusual yellow, dark green, violet and black on spraying with anisaldehyde/sulphuric acid. HPLC MS/MS assay of strain extract revealed the existence of diveres interesting peaks in the positive mode with MS/MS of an sugar molecule with mass of 158 Dalton (**A**), which resulted directly in the identification of picro-

mycin derivatives, as they are the only type of molecules containing this sugar moiety based on a search in AntiBase[61].

Table 57: Antimicrobial activity of the crude extract from ANK26 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	28
Staphylococcus aureus	25
Streptomyces viridochromogenes (Tü 57)	28
Escherichia coli	30
Candida albicans	0
Mucor miehei (Tü 284)	0
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.8.2 Fermentation of Isolate ANK26

Well-developed colonies of the strain were used to inoculate 200 of 1 litre-Erlenmeyer flasks each containing 250 ml of M_2 medium, and incubated for 7 days as shaker culture (95 rpm) at 28 °C. After harvesting, the broth was mixed with *ca.* 1.5 kg diatomaceous earth and pressed through a pressure filter to give filtrate and biomass. The fractions, water and mycelial phases were extracted separately. For the water phase, it was subjected to adsorption on Amberlite XAD-16, and subsequently extracted with methanol. The methanol extract was concentrated, and the resulting aqueous residue was re-extracted (4 times) by ethyl acetate and followed by evaporation *in vacuo* until dryness. The biomass was extracted (4 times) with ethyl acetate followed by acetone (1 time). The acetone was evaporated, and the water residue obtained was re-extracted by ethyl acetate. Both organic extracts were combined, and evaporated to dryness, yielding 17.5 g of an oily yellow crude extract.

9.8.3 Isolation

The crude extract was subjected to flash chromatography on silica gel, and eluted gradually with CH_2Cl_2-MeOH gradient (2L CH_2Cl_2, 1 L 3% MeOH, 1 L 5% MeOH, 1 L 10% MeOH, 0.5 L 20%, 0.5 L 50% MeOH, 0.2 L 100% MeOH) and monitored by TLC to deliver six fractions. Fractions I-IV were detected as fats of altogether 11.2 g. Re-fractionation and purification of fraction V (2.1 g) using silica gel column chromatography was followed by preparative TLC and finally purified on

columns of Sephadex LH-20 to deliver: 10,11-dihydro-9,12-epoxy-8,9-anhydro-cromycin (**133**, 41.3 mg, colourless oil), (4*E*,8*E*)-4,8-dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**, 2.7 mg, colourless solid), cromycin (**138**, 56.4 mg, white amorphous), 10,11-dihydro-cromycin (**135**, 10.3 mg, colourless oil), *seco*-decarboxy-cromycin (**136**, 18.7 mg, colourless oil), bafilomycin B_1 (**110**, 10.8 mg, yellow amorphous) and bafilomycin B_2 (**111**, 3.1 mg, yellow amorphous). Likewise, on purification of fraction VI (1.8 g), picromycin (**139**, 350 mg, white amorphous), picromycin-C (**140**, 10.7 mg, white amorphous) and 10,11-Dihydro-9,12-epoxy picromycin-9-methylether (**134**, 52 mg, colourless oil) were afforded (Figure 61).

9.8.4 Biological Properties

Antibacterial and antifungal activities were evaluated using the agar diffusion method for the isolated compounds (**133~140**) in comparison with erythromycin A (**4**). Although a pronounced antibacterial activity of most picromycins and cromycins against Gram-positive bacteria have been observed, only the two new compounds **134** and **137** along with picromycin (**139**) and picromycin-C (**140**) were active against Gram-positive *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57) in addition to the Gram-negative bacteria *Bacillus subtilis*. The minimum inhibitory concentration (MIC) of the new compounds **134** and **137** was carried out against the Gram-positive *Bacillus subtilis*, in comparison with erythromycin A (**4**). Starting with concentration of 100 µg/ml, followed serial dilutions to give 11 concentrations (50~0.0975 µg/ml), the MIC for compound **134** ranged between 50 and 25 μg/ml, while those for compound **137** were higher than 100 μg/ml, and for erythromycin A (**4**) it was between 0.195 and 0.0975 μg/ml (Table 59). The crude extract showed, however, a moderate and rather unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 112.074 μM (mean IC_{70} = 106.608 μM); Table 45.

Table 58: Antibacterial activity of the isolated compounds (**133~140**) compared with Erythromycin A (**4**) (40 μg/disk).

Compounds	Tested microorganisms (Inhibition zone ∅, mm)			
	BS[a]	SA[b]	SV[c]	EC[d]
133	0	0	0	0
134	20	12	18	0
135	0	0	0	0
136	0	0	0	0
137	10.5	12	17	0
138	0	0	0	0
139	18	14	15	0
140	21	17	24	0
Erythromycin A (**4**)	25	25	17	0

[a]*Bacillus subtilis*; [b] *Staphylococcus aureus*; [c] *Streptomyces viridochromogenes* (Tü 57); [d] *Escherichia coli*

Table 59: MIC of compounds **134** and **137** compared with Erythromycin A (**4**) as reference using *Bacillus subtilis*.

Compounds	MIC against *Bacillus subtilis*
134	< 50 µg/ml
137	>100 µg/ml
Erythromycin A (**4**)	< 0.195 µg/ml

10,11-Dihydro-9,12-epoxy-8,9-anhydro-cromycin (133): Colourless oil, as UV absorbing turned to yellow by anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.88 (CH_2Cl_2/2% MeOH). – $\boldsymbol{[\alpha]_D^{20}}$ = + 144 (*c* 0.1, MeOH). – **UV/VIS:** λ_{max} (log ε) = (MeOH): 225 (4.06); (MeOH/HCl): 231 (4.05) 204 (3.96); (MeOH/NaOH): 236 (4.00), 209 (4.11) nm. **– ^{1}H** and **^{13}C NMR**, see Table 16. – **(+)-ESI MS:** *m/z* (%) = 691 $[2M+Na]^+$, 48), 357 $[M+Na]^+$, 100). – **(+)-HRESI MS:** *m/z* 335.2217240 $[M+H]^+$, and 357.20366 $[M+Na]^+$]$^+$, (calcd. 335.22167 $[M+H]^+$, for $C_{20}H_{31}O_4$ and 357.20363 $[M+Na]^+$, for $C_{20}H_{30}O_4$ Na). **–H,H COSY**, **HMBC** and **NOE:** see Figure 64 and Figure 66.

10,11-Dihydro-9,12-epoxy picromycin-9-methylether (**134**): Colourless oil, initially yellow then turned to dark-green and 1 hour latter to violet with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.52 (CH_2Cl_2/10% MeOH). – $\boldsymbol{[\alpha]_D^{20}}$ = + 17 (*c* 0.1, MeOH). – **UV/VIS:** λ_{max} (log ε) = (MeOH): 285 (2.86), 203 (3.60); (MeOH/HCl): 283 (2.86), 206 (3.40); (MeOH/NaOH): 283 (2.86), 206 (3.81) nm. – **^{1}H** and **^{13}C NMR**, see Table 16. – **(+)-ESI MS**: *m/z* (%) = 1105 $[2M+Na]^+$, 100), 1083

$[2M+H]^+$, 92), 564 $[M+Na]^+$, 28), 542 $[M+H]^+$, 44). – **(+)-HRESI MS:** *m/z* 542.368587 $[M+H]^+$, (calcd. 542.36873 $[M+H]^+$ for $C_{29}H_{52}NO_8$). **–H,H COSY**, **HMBC** and **NOE:** see Figure 64 and Figure 66.

10,11-Dihydro-cromycin (135): Colourless oil, as UV absorbing, yellow with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.34 (CH_2Cl_2/2% MeOH). – $\boldsymbol{[\alpha]_D^{20}}$ = + 33 (*c* 0.1, MeOH). – **UV/VIS:** λ_{max} (log ε) = (MeOH): 231 (4.08); (MeOH/HCl): 230 (4.06); (MeOH/NaOH): 279 (3.27), 231 (4.07) nm. – **^{1}H** and **^{13}C NMR**, see Table 17. – **(+)-ESI MS:** *m/z* (%) = 727 $[2M+Na]^+$, 100), 375 $[M+Na]^+$, 58). – **(+)-HRESI MS:** *m/z* 375.214171 $[M+Na]^+$, (calcd. 375.21419 $[M+Na]^+$, for $C_{20}H_{32}O_5$ Na). **–H,H CO-SY**and **HMBC:** see Figure 69.

***seco*-Decarboxy-cromycin (136):** colourless oil, as UV absorbing, turned to violet by anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.12 (Cyclohexane/70% EtOAc), R_f = 0.22 (CH_2Cl_2/2% MeOH). – $\boldsymbol{[\alpha]_D^{20}}$ = + 4 (c 0.23, MeOH). – **UV/VIS:** λ_{max} (log ε) = (MeOH): 230 (4.45); (MeOH/HCl): 231 (4.45); (MeOH/NaOH): 230 (4.44) nm. – **^{1}H** and **^{13}C NMR**, see Table 17 . – **(+)-ESI MS:** *m/z* (%) = 671 $[2M+Na]^+$, 28), 347 $[M+Na]^+$, 100). – **(+)-HRESI MS:** *m/z* 347.2192080 $[M+Na]^+$, 325.2372120 $[M+H]^+$ (calcd. 347.21928 $[M+Na]^+$ for $C_{19}H_{32}O_4$ Na, and calcd 325.23733 $[M+H]^+$, for $C_{19}H_{33}O_4$). **–H,H COSY**and **HMBC:** see Figure 69.

(4*E*,8*E*)-4,8-Dimethyl-12-oxo-trideca-4,8-dienoic acid (**137**): colourless solid, initially yellow then turned to green with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.25 (CH_2Cl_2/2% MeOH). – **UV/VIS:** λ_{max} (log ε) = (MeOH): 204 (4.07); (MeOH/HCl): 203 (4.04); (MeOH/NaOH): 207 (4.06) nm. – **^{1}H** and **^{13}C NMR**, see Table 18. – **(+)-ESI MS:** *m/z* (%) = 549 ($[2M+2H+2Na]^+$, 100), 297 ($[M+H+2Na]^+$, 15), 275 ($[M+Na]^+$, 92). – **(+)-HRESI MS:** *m/z* 275.1619260 $[M+Na]^+$, (calcd. 275.16177 $[M+Na]^+$, for $C_{15}H_{24}O_3$ Na). **–H,H COSY**and **HMBC:** see Figure 72.

Cromycin (**138**): white amorphous, as UV absorbing, initially yellow turned to greenish-blue with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.32 (CH_2Cl_2/2% MeOH). – **^{1}H** and **^{13}C NMR**, see Table 19. – **(+)-ESI MS:** *m/z* (%) = 723 $[2M+Na]^+$, 100), 373 $[M+Na]^+$, 40). – **(+)-HRESI MS:** *m/z* 373.198546 $[M+Na]^+$, (calcd. 373.19854 $[M+Na]^+$, for $C_{20}H_{30}O_5$ Na). **–H,H COSY**and **HMBC:** see Figure 73.

Picromycin (139): White amorphous, as UV absorbing, initially yellow then turned to dark-blue with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.39 (CH_2Cl_2/10%

MeOH). – **^{1}H** and **^{13}C NMR**, see Table 19. – **(+)-ESI MS:** *m/z* (%) = 1051 ([2M+H]$^{+}$, 98), 526 ([2M+H]$^{+}$, 100). – **(-)-ESI MS:** *m/z* = 524 [M+H]$^{-}$. – **(+)-HRESI MS:** *m/z* 526.3373990 [M+H]$^{+}$ (calcd. 526.33743 [M+H]$^{+}$, for $C_{28}H_{48}NO_8$). **– H,H COSY**and **HMBC:** see Figure 73.

Picromycin-C (140): White amorphous, as UV absorbing, dark-green with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.21 (CH_2Cl_2/10% MeOH). – **^{1}H** and **^{13}C NMR**, see Table 19. – **(+)-ESI MS:** *m/z* (%) = 939 [2M+H]$^{+}$, 40), 470 [M+H]$^{+}$, 100). – **(+)-HRESI MS:** *m/z* 470.3112430 [M+H]$^{+}$, (calcd. 470.31122 [M+H]$^{+}$, for $C_{25}H_{44}N_1O_7$). **– H,H COSY**and **HMBC:** see Figure 73.

9.9 Marine-derived *Streptomyces* sp. Act8970

The marine-derived *Streptomyces* sp. Act8970 formed a white aerial mycelium and the surrounding agar was stained white.

9.9.1 Pre-screening

The strain was cultivated at 28 °C for 72 hrs on three agar plates with M_2^+ using 50% artificial sea water. The seed agar plates were cut into small pieces and used to inoculate 12 of 1L-Erlenmeyer flasks each containing 250 ml of M_2^+ using 50% artificial sea water. Fermentation was carried out on a shaker at 95 rpm for 3-days at 28 °C. The broth was harvested after 3 days and both the filtrate and the mycelial cake were extracted three times with ethyl acetate. A chemical screening of the extract was visualized during TLC. The extract displayed a number of UV non-absorbing bands, stained either blue or brown by anisaldehyde/sulphuric acid. Based on agar disc diffusion method, the extract showed activity against a set of pathogenic microorganisms, as summarized in Table 60.

Table 60: Antimicrobial activities of the crude extract (1 mg/ml).

Test microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	12
Escherichia coli	14
Staphylococcus aureus	12
Streptomyces viridochromogenes (Tü57)	0
Candida albicans	11
Mucor miehei (Tü 284)	11
Chlorella vulgaris	15
Chlorella sorokiniana	18
Scenedesmus subspicatus	15

9.9.2 Fermentation, Extraction and Isolation

The marine-derived *Streptomyces* sp. Act8970 was pre-cultivated on M_2^+ medium (+50% artificial sea water) agar plates at 28 °C for 3 days. With pieces of well grown agar subcultures of the strain, 20 l shaker culture was carried out using 100 of 1l-Erlenmyer flasks, each containing 200 ml of M_2^+ (50% sea water) for 11 days at 28 °C on a linear shaker (110 rpm). After the usual work-up, the brown crude extract obtained was subjected to flash chromatography on silica gel and eluted with MeOH/CH_2Cl_2 gradient to afford three fractions (I, II, III). Purification of the fast fraction **I** (2.1 g) using Sephadex followed by silica gel led to colourless oils of attiamycin A (**141**; 16 mg) and attiamycin B (**143a**; 160.8 mg). Similarly, Purification of the middle polar fraction **II** (1.6 g) gave three colourless oils of homononactic acid (**145a**; 77.3 mg), homononactic acid methyl ester (**145b**; 3.1 mg) and dinactin (**148;** 330 mg). Also, working up of fraction **III** (1.3 g) using the same conditions afforded two colourless oils of nonactic acid (**144a**; 66.4 mg) and dinactin (**148**; 1.2 g) (Figure 74).

Attiamycin A; 4-Hydroxy-nonane-2,7-dione (141): $C_9H_{16}O_3$ (172), colourless oil turned as yellow by anisaldehyde/sulphuric acid and changed later to brown. – $\boldsymbol{R_f}$ = 0.37 (CH_2Cl_2/5% MeOH). – $\mathbf{[\alpha]_D^{20}}$ = + 44 (*c* 0.1, MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 4.01 (m, 1H, 4-CH), 2.61 (m, 4H, 3/6-H_2), 2.46 (q, 7.3 Hz, 2H, 8-H_2), 2.18 (s, 3H, 1-H_3), 1.73 (m, 2H, 5-H_2), 1.06 (t, 7.3 Hz, 3H, 9-H_3). – **^{13}C NMR** ($CDCl_3$, 75 MHz): δ = 211.7 (C_q-7), 209.7 (C_q-2), 66.8 (CH-4), 50.0 (CH_2-3), 38.1 (CH_2-6), 36.0 (CH_2-8), 30.6 (CH_3-1), 29.9 (CH_2-5), 7.8 (CH_3-9). – **ESI MS:** *m/z* (%) = 195 ([M+Na]$^+$). – **(+)-HRESI MS:** *m/z* = 195.09914 [M+Na]$^+$ (calcd. 195.099165 for $C_9H_{16}O_3Na$). **–H,H COSY** and **HMBC:** see Figure 76.

Attiamycin B; 2-[5-(2-Oxo-propyl)-tetrahydro-furan-2-yl]-propionic acid (143a): $C_{10}H_{16}O_4$ (200), colourless oil, turned brown by anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.46 (CH_2Cl_2/5% MeOH). **– $\mathbf{[\alpha]_D^{20}}$ = -** 16 (*c* 0.1, MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 6.30 (br s, 1H, COOH), 4.32 (m, 1H, 5-H), 4.08 (m, 1H, 2-H), 2.82 (dd, 15.8, 6.5 Hz, 1H, H_A-3'), 2.56 (dd, 15.1, 6.4 Hz, 1H, H_B-3'), 2.51 (q, 7.1 Hz, 1H, H-2"), 2.18 (s, 3H, H_3-1'), 2.10 (m, 1H, 4-HA), 1.90 (m, 1H, 3-H_A), 1.60 (m, 1H, 3-H_B), 1.50 (m, 1H, 4-H_B), 1.17 (d, 7.1 Hz, 3H, 3"-H_3). – **APT/ ^{13}C NMR** ($CDCl_3$, 75 MHz): δ = 207.5 (C_q-2'), 179.7 (C_q-1"), 80.3 (CH-2), 75.5 (CH-5), 49.6

(CH_2-3'), 45.1 (CH-2"), 30.9 (CH_2-4), 30.8 (CH_3-1'), 28.4 CH_2-3), 13.2 (CH_3-3"). – (+)-**ESI MS**: *m/z* (%) = 223 ($[M+Na]^+$, 65), 423 ($[2M + Na]^+$, 100). – (-)-**ESI MS**: *m/z* (%) = 199 ($[M-H]^-$, 100), 399 ($[2M-H]^-$, 55). – (+)-**HRESI MS**: *m/z* = 223.094111 $[M+Na]^+$ (calcd. 223. 094075 for $C_{10}H_{16}O_4Na$), and 218.138710 $[M+NH_4]^+$ (calcd. 218. 138685 for $C_{10}H_{20}NO_4$). **–H,H COSY**, **HMBC** and **NOESY:** see Figure 79 and Figure 80.

Attiamycin B methyl ester; 2-[5-(2-Oxo-propyl)-tetrahydro-furan-2-yl]-propionic acid methyl ester (143b): $C_{11}H_{18}O_4$ (214), middle polar colourless oil, UV non-absorbing or fluorescence, turned as violet by spraying with anisaldehyde/sulphuric acid. It was obtained during the poring of 1 ml diazomethane to 7 mg of 2-[5-(2-Oxo-propyl)-tetrahydro-furan-2-yl]-propionic acid (**143a**) in DCM at 0 °C for 1 min. The mixture was evaporated *in vacuo* to yield 5.3 mg of **143b**. – R_f = 0.51 (CH_2Cl_2/4% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 4.23 (m, 1H, 5-H), 4.03 (m, 1H, 2-H), 3.69 (s, 3H, -$COOCH_3$), 2.77 (dd, 15.7, 6.7 Hz, 1H, H_A-3'), 2.65-2.51 (m, 2H, H_B-3', H-2"), 2.17 (s, 3H, H_3-1'), 2.10 (m, 1H, 4-HA), 1.98 (m, 1H, 3-H_A), 1.67 (m, 1H, 3-H_B), 1.56 (m, 1H, 4-H_B), 1.12 (d, ^3J = 7.1 Hz, 3H, 3"-H_3). – **ESI MS**: *m/z* (%) = 237 ($[M+Na]^+$, 6), 451 ($[2M+Na]^+$, 10).

Homononactic acid methyl ester (**145b**): $C_{12}H_{22}O_4$ (230), middle polar colourless oil, showing the same chromatographic properties of **143a** and **143b.** – R_f = 0.34 (CH_2Cl_2/5% MeOH). **– $[\alpha]_D^{20}$** = 0 (*c* 0.1, MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 4.15 (m, 1H, 6-H), 3.99 (m, 1H, 3-H), 3.75 (m, 1H, H-8), 3.69 (s, 3H, 1-$COOCH_3$), 2.54 (dq, *J* = 8.3, 7.0 Hz, H-2), 2.00 (m, 2H), 1.70 (m, 4H, $2H_2$), 1.50 (m, 2H, H_2), 1.13 (d, 3H, *J* = 7.0 Hz, H_3-2), 0.93 (t, 3H, *J* = 7.4 Hz, H_3-10). – **ESI MS**: *m/z* (%) = 253 $[M+Na]^+$, 66), 483 $[2M+Na]^+$, 100).

Nonactic acid (144a): $C_{10}H_{18}O_4$ (202), colourless oil, exhibiting the same chromatographic properties as in **145b**. **–** R_f = 0.12 (Cyclohexane/70% EtOAc), 0.15 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** (CD_3OD, 600 MHz): δ = 4.00 (m, 2 H, 2-H, 5-H), 3.91 (m, 1 H, 2'-H), 2.41 (m, 1 H, 2''-H), 1.98 (m, 2 H, 3-H_A, 4-H_A), 1.63 (m, 1 H, 3-H_B), 1.58 (m, 2 H, CH_2-3'), 1.53 (m, 1 H, 4-H_B), 1.15 (d, 6.3 Hz, 3 H, CH_3-1'), 1.08 (d, 7.0 Hz, 3 H, 2''-CH_3). **– ^{13}C NMR** (CD_3OD, 125 MHz): δ = 180.0 (C-1''), 82.2 (C-2), 77.8 (C-5), 66.1 (C-2'), 47.5 (C-2''), 46.2 (C-3'), 32.3 (C-4), 29.4 (C-3), 24.1 (CH_3-1'), 14.1 (2''-CH_3). **– (+)-ESI MS**: *m/z* = 225 $[M+Na]^+$. **– (-)-ESI MS**:

m/z = 201 $[M-H]^-$. **– (+)-HRESI MS**: *m/z* = 225.109714 $[M+Na]^+$ ([calcd 225.11027 for $C_{10}H_{18}O_4Na$). **–H,H COSY** and **HMBC:** see Figure 81.

Homononactic acid (145): $C_{11}H_{20}O_4$ (216), colourless oil, appearing the same chromatographic characterization of **6a**. **–** R_f = 0.18 (Cyclohexane/70% EtOAc). **– ^{1}H NMR** ($CDCl_3$, 600 MHz): δ = 4.20 (m, 1 H, 2-H), 3.96 (m, 1 H, 5-H), 3.75 (m, 1 H, 3'-H), 2.50 (m, 1 H, 2''-H), 2.02 (m, 2 H), 1.76 (m, 4 H), 1.49 (m, 2 H), 1.15 (d, 7.0 Hz, 3 H, 2''-CH_3), 0.91 (d, 7.4 Hz, 3 H, CH_3-1'). **–(+)-ESI MS**: *m/z* (%) = 477 ($[2M-H+2Na]^+$, 100), 455 ($[2M+Na]^+$, 20), 239 ($[M+Na]^+$, 45). **– (-)-ESI MS**: *m/z* = 215 $[M-H]^-$. **– (+)-HRESI MS**: *m/z* = 239.12538 $[M+Na]^+$ (calcd 239.125375 for $C_{11}H_{20}O_4Na$). **– H,H COSY**: see Figure 82.

9.9.3 Cytotoxic Activity

The cytotoxic activities of attiamycin A (**141**), attiamycin B (**143a**), nonactic acid (**144a**), homononactic acid (**145a**) and dinactin (**148c**) are listed in Table 61. Dinactin (**148**), attiamycin B (**143a**) and nonactic acid (**144a**) showing a highly selective cytotoxic activity against a range of human tumor cell lines.

Table 61: Cytotoxic activities of compounds **141**, **143a**, **144a**, **145a** and **148**.

Compound	Antitumor potency[a]		Tumor selectivity[b]	
	Mean IC_{50} [μg/ml]	Mean IC_{70} [μg/ml]	n / total	%
Attiamycin A (**141**)	11.203	13.871	0/36	0%
Attiamycin B (**143a**)	1.160	3.423	9/36	25%
Nonactic acid (**144a**)	1.256	3.532	7/36	19%
Homononactic acid (**145a**)	7.712	> 10	0/36	0%
Dinactin (**148**)	0.002	0.011	9/36	25%

[a] mean IC-values, determined as average of 36 human tumor cell lines tested.
[b] individual IC_{50}<½ mean IC_{50}; e.g. if mean IC_{50} = 2.0 μM the threshold for above average sensitivity was IC_{50} < 1.0 μM.

9.10 Marine-derived *Streptomyces* sp. Mei37

The marine derived *Streptomyces* sp. isolate Mei37 was pre-cultivated on calcium carbonate medium agar plates at 28 °C for 7 days. With small pieces of well grown agar subcultures of the strain, 120 1-liter Erlenmeyer flasks, each containing 250 ml of sterilized calcium carbonate medium with pH of 7.4 were inoculated. The cultivation was carried out on shaker (110 rpm) at 28 °C for 6 days. The culture broth obtained was mixed with ca. 1.5 kg Celite and filtered through a pressure filter. The filtrate was extracted by passing through a XAD-2 column (10 × 120 cm), which was finally eluted with MeOH. The MeOH extract was concentrated *in vacuo* and the

afforded aqueous residue was re-extracted by ethyl acetate. The mycelium was subjected to extraction by ethyl acetate for several times, and one time by acetone. Both ethyl acetate extracts were combined and evaporated *in vacuo* to dryness, from which 3.7 g of dark-red crude extract were yielded.

The extract was subjected to fractionation on Sephadex LH-20 (column 3 × 70 cm, CH_2Cl_2/40% MeOH) giving two fractions, I and II. The main fraction II was re-fractionated to afford four sub-fractions (IIA, IIB, IIC and IID) during a silica gel column chromatography and elution with CH_2Cl_2-MeOH gradual system. Purification of the first two low polar sub-fractions IIA and IIB by using different chromatographic methods led to the isolation of albaflavenone (**167**; 35.7 mg), (4S)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (**233**; 58.0 mg), indole-3-acetic acid (10.7 mg) and indole-3-carboxylic acid (3.0 mg). Further purification of the middle-polar sub-fraction IIC on Sephadex LH-20 (column 3 x 70 cm, CH_2Cl_2/40% MeOH) led to uracil (20.7 mg) and 2,3-dihydroxy-1-(1*H*-indol-3yl)-propan-1-one (3.0 mg). In the same way, the polar sub-fraction IID afforded 2'-deoxy-thymidine (5.1 mg) and 2'-deoxy-uridin (4.3 mg), along with mansouramycin D (**156**; 2.3 mg); Figure 86.

9.10.1 Biological Activity

Mansouramycin D (**156**), showed a high and selective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 0.022 µM (mean IC_{70} = 0.041 µM). However, (4S)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (**233**) and albaflavenone (**167**), showed a moderate and rather unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 9.759 µM (mean IC_{70} = 10.265 µM) and 5.017 µM (mean IC_{70} = 9.379 µM) respectively, Table 62 and Table 63.

Table 62: Cytotoxic activities of **156**, **233** and **167**.

Compound	Antitumor potency[a]		Tumor selectivity[b]	
	Mean IC_{50} [µg/ml]	Mean IC_{70} [µg/ml]	n / total	%
Mansouramycin D (**156**):	0.022	0.041	10/36	28%
Butenolide (**233**):	9.759	10.265	1/36	3%
Albaflavenone (**167**):	6.017	9.379	0/36	0%

[a] mean IC-values, determined as average of 36 human tumor cell lines tested.
[b] individual IC_{50} < ½ mean IC_{50}; e.g. if mean IC_{50} = 2.0 μM the threshold for above average sensitivity was IC_{50} < 1.0 μM.

Mansouramycin D (156): Dark red solid. – R_f = 0.55 (CH_2Cl_2/5% MeOH), blue-violet with anisaldehyde/sulphuric acid or conc. sulphuric acid. – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 9.36 (s, 1H, 5-H), 8.71 (s, 1H, 8-H), 6.13 (m br, 1H, 3-NH), 5.90 (s, 1H, 2-H), 4.07 (s, 3H, 9-OCH_3), 2.99 (d, ^3J = 5.5 Hz, 3H, 3-NCH_3). – **^{13}C NMR** (150.8 MHz, $CDCl_3$): δ = 180.6 (C_q-4), 177.4 (C_q-1), 164.3 (C_q-9), 153.3 (C_q-7), 148.3 (C_q-3), 148.0 (CH-5), 140.5 (C_q-8a), 126.0 (C_q-4a), 120.7 (CH-8), 101.5 (CH-2), 54.4 (9-OCH_3), 29.3 (3-NCH_3). – **EI MS** (70 eV): *m/z* (%) = 246 ($[M]^{+\cdot}$, 6), 188 ($[M\text{-}COOCH_3]^{+\cdot}$, 16), 82 (100), 76 (37), 63 (12), 57 (16), 50 (25), 43 (18), 41 (11). – **(+)-ESI MS:** *m/z* (%) = 247 ($[M+H]^+$, 4), 269 ($[M+Na]^+$, 23), 515 ($[2M+Na]^+$, 18). – **(+)-HRESI MS:** *m/z* = 247.07136 $[M+H]^+$ (calcd. 247.07188 for $C_{12}H_{11}N_2O4$). – **HMQC** and **HMBC:** see Figure 88.

(4S)-4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide (233): $C_{13}H_{22}O_4$, colourless solid, showing a blue coloration by spraying with anisaldehyde/sulphuric acid. — R_f = 0.29 (CH_2Cl_2/5% MeOH). — **CD** (6.24E-005 mol/L MeOH)**:** +35363 $[\theta]_{202}$, +32375 $[\theta]_{207}$. — **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.44 (dd, ^3J = 5.6 Hz, ^4J = 1.5 Hz, 1H, 3-H), 6.10 (dd, ^3J = 5.6 Hz, ^3J = 1.9 Hz, 1H, 2-H), 4.98 (m, 1H, 4-H), 3.58 (q, ^3J = 6.4 Hz, 1H, 11-H), 2.83 (brs, 2H, 10-OH, 11-OH), 1.78-1.20 (m, 10H, 5-H_2, 6- H_2,7-H_2, 8-H_2, 9-H_2), 1.10 (d, ^3J = 6.5 Hz, 3H, 12-H_3), 1.02 (s, 3H, 10-CH_3). — **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 173.3 (C_q, C-1), 156.6 (CH, C-3), 121.2 (CH, C-2), 83.4 (CH, C-4), 74.7 (C_q, C-10), 72.6 (CH, C-11), 38.7 (CH_2, C-9), 32.8 (CH_2, C-5), 29.7 (CH_2, C-7), 24.8 (CH_2, C-6), 22.8 (CH_2, C-8), 20.2 (10-CH_3), 17.3 (CH_3, C-12). — **(+) ESI MS:** *m/z* (%) = 507 ($[2M+Na]^+$, 100), 265 ($[M+Na]^+$, 18). — **HRESI MS:** *m/z* = 265.14096 $[M+H]^+$ (calcd. 265.14104 for $C_{13}H_{22}O_4Na$). – **HMQC** and **HMBC:** see Figure 125.

Albaflavenone (167): $C_{15}H_{22}O$ (218), colourless oil, stained to intense orange (one hour later to violet) by anisaldehyde/sulphuric acid. – R_f = 0.57 (CH_2Cl_2), 0.14 (CH_2Cl_2/50% Cyclohexane). – **^{1}H** and **^{13}C NMR**, see Table 21. – **EI MS** (70 eV): *m/z* (%) = 218 $[M]^+$ (88), 203 (18), 175 (100), 147 (45), 133 (65). – **(+) ESI MS:** *m/z* = 219.3 $[M+H]^+$. – **HRESI MS:** *m/z* = 219.1743120 $[M+H]^+$ (calcd. 219.174335 for $C_{15}H_{23}O$). – **HMQC** and **HMBC:** see Figure 90.

Table 63: Cytotoxic activity of Mansouramycins A (**157**) ~ F (**163**) against tumor cell lines in a monolayer proliferation assay.

Tumor type	Cell line	IC_{50} [μM]				
		157	**158**	**159**	**156**	**163**
Bladder	BXF 1218L	28.19	10.26	1.38	0,134	>37.31
	BXF T24	12.58	0.61	n.d.[a]	0,008	>37.31
Glioblastoma	CNXF 498NL	14.39	5.21	1.34	0,13	>37.31
	CNXF SF268	14.41	1.8	n.d.	0,008	>37.31
Colon	CXF HCT116	9.11	3.62	n.d.	0,114	>37.31
	CXF HT29	4.63	1.98	n.d.	0,146	>37.31
Stomach	GXF 251L	31.9	3.6	1.75	0,167	>37.31
Head & neck	HNXF 536L	0.71	0.38	n.d.	0,146	21.56
Lung	LXF 1121L	27.14	6.56	n.d.	0,15	>37.31
	LXF 289L	19.05	3.04	5.96	0,13	>37.31
	LXF 526L	18.99	10.62	n.d.	0,11	>37.31
	LXF 529L	12.42	4.88	1.54	0,089	>37.31
	LXF 629L	4.1	1.18	1.23	0,016	>37.31
	LXF H460	7.11	3.92	n.d.	0,134	>37.31
Breast	MAXF 401NL	47.76	17.31	3.55	0,195	>37.31
	MAXF MCF7	2.34	1.11	n.d.	0,012	37.31
Melanoma	MEXF 276L	2.44	0.35	0.36	0,008	>37.31
	MEXF 394NL	15.57	5.72	n.d.	0,106	>37.31
	MEXF 462NL	49.93	18.97	5.64	0,179	>37.31
	MEXF 514L	2.6	2.02	n.d.	0,012	>37.31
	MEXF 520L	5.45	0.24	n.d.	0,012	>37.31
Ovary	OVXF 1619L	13.15	4.01	n.d.	0,045	>37.31
	OVXF 899L	34.89	6.6	13.91	0,134	>37.31
	OVXF OVCAR3	32.31	7.64	2.0	0,012	>37.31
Pancreas	PAXF 1657L	26.03	4.93	1.81	0,061	>37.31
	PAXF PANC1	26.75	3.83	n.d.	0,549	>37.31
Prostate	PRXF 22RV1	21.74	4.69	5.67	0,671	23.9
	PRXF DU145	1.25	0.34	n.d.	0,992	>37.31
	PRXF LNCAP	21.67	6.36	n.d.	1,431	>37.31
	PRXF PC3M	32.56	6.04	3.23	0,215	35.69
Mesothelioma	PXF 1752L	46.3	6.02	5.19	0,13	>37.31
Kidney	RXF 1781L	16.36	9.89	n.d.	0,114	>37.31
	RXF 393NL	18.65	4.16	1.57	0,122	>37.31
	RXF 486L	59.14	50.62	17.89	1,646	>37.31
	RXF 944L	18.1	5.43	n.d.	0,02	>37.31
Uterus	UXF 1138L	18.02	2.23	1.68	0,012	>37.31
Mean		13.44	3.49	2.7	0.089	36.25
Selectivity[b]		6/36	6/36	1/18	10/36	0/36

[a] n.d.: not determined, [b] IC_{50} < 1/2 mean IC_{50} value / total

Table 64: Cytotoxic activity of Mansouramycin B (**158**) against cell suspensions derived from tumor xenografts in a clonogenic assay.

Tumor type	Tumor model	IC_{50} [µM]
Blood stem	AHS NSB005	4.58
cells	AHS NSB008	4.53
Bladder	BXF 1218	4.34
Colon	CXF 243	10.32
Liver	LIXF 575	8.35
Lung	LXFA 526	4.31
	LXFA 629	1.63
Breast	MAXF 1322	8.35
	MAXF 401	3.89
Melanoma	MEXF 276	0.33
	MEXF 514	0.05
Ovary	OVXF 899	5.35
Kidney	RXF 631	7.81
Mean		2.94

9.11 Marine *Streptomyces* sp. B7874

The marine *Streptomyces* sp. B7874 showed white colonies during incubation on agar using M_2^+ medium for three days at 28 °C

9.11.1 Pre-screening

The well-developed colonies were used to inoculate 12 of 1L-Erlenmeyer flasks each containing 250 ml of M_2^+ medium. Fermentation was carried out on a shaker at 95 rpm for 7-days at 28 °C. After harvesting, the obtained yellowish-brown broth was lyophilized, and the residue was exhaustively extracted by ethyl acetate. In the biological screening using the agar diffusion test method, the extract was inactive at 100 µg/disc against bacteria of Gram-positive (*Bacillus subtilis, Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57), and Gram-negative (*Escherichia coli*), yeast (*Candida albicans*), fungi (*Mucor miehei*) and microalgae (*Chlorella vulgaris, Chlorella sorokiniana* and *Scenedesmus subspicatus*), Table 65. On other hand, the extract exhibited a potent *in vitro* antitumor activity against cancer cells, Table 45. On the bases of TLC analysis, and evaluation by UV lamp and spraying reagents, the extract exhibited several coloured bands (yellow-red). These bands showed no colour changing by spraying with anisaldehyde/sulphuric acid.

Table 65: Antimicrobial activities of the marine *Streptomyces* sp. B7874 extract (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	0
Staphylococcus aureus	0
Streptomyces viridochromogenes (Tü 57)	0
Escherichia coli	0
Candida albicans	0
Mucor miehei (Tü 284)	0
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.11.2 Fermentation, Extraction and Isolation

Well grown subcultures of the marine *Streptomyces* sp. B7874 on agar plates (cultivated on M_2^+ medium) were inoculated into 96 of 1-L Erlenmeyer flasks each containing 250 ml and allowed to fermentation on shaker for 7 days at 28 °C. The obtained faint yellow culture broth was filtered on celite. The mycelial cake was extracted with ethyl acetate, while the filtrate was passed through a XAD-16 column followed by extraction with MeOH. The methanol extract was concentrated *in vacuo*, and the remaining water residue was re-extracted by ethyl acetate. Both ethyl acetate extracts were combined, and concentrated *in vacuo* to dryness. The afforded brown crude extract (8.21 g) was then chromatographed on silica gel and eluted with CH_2Cl_2-MeOH gradient (CH_2Cl_2, 2 L; 3% MeOH, 1 L; 5% MeOH, 1 L; 7% MeOH, 1 L; 15% MeOH, 1 L; 30% MeOH, 0.5 L; 50% MeOH, 0.5 L; 100% MeOH, 0.2 L). After TLC monitoring, five fractions were furnished: **I** (0.8 g), **II** (1.6), **III** (3.4 g), **IV** (0.65 g) and **V** (0.6). Further fractionation of fraction IV on Sephadex LH-20 (CH_2Cl_2/40% MeOH), followed by preparative TLC (CH_2Cl_2/3% MeOH) afforded two crude components. On purification of the two crude components using Sephadex LH-20 (MeOH), ferulic acid **(181**; 13.9 mg, colourless solid) and 7-(3-methyl-but-2-enyl) isatin (**168;** 6.9 mg, yellow-orange solid) were obtained. Purification of the polar fraction **V** (0.6) on Sephadex LH-20 (MeOH) led to 5-methyl-5,10-dihydrophencomycin amide (**178**; 4.3 mg, yellow-orange solid) (Figure 91).

9.11.3 Biological Activity

The isatin **168** and phenazine **178** showing unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} > 10 µg/ml (mean IC_{70} > 10 μg/ml), Table 66.

Table 66: Cytotoxic activities of the Isatin **168** and Phenazine **178**.

Compound	Antitumor potency[a]		Tumor selectivity	
	Mean IC_{50} [µg/ml]	Mean IC_{70} [µg/ml]	n / total	%
Isatin **168:**	>10	>10	0/36	0%
Phenazine **178:**	>10	>10	0/36	0%

[a]mean IC-values, determined as average of 36 human tumor cell lines tested.

7-(3-Methyl-but-2-enyl)-isatin (168): $C_{13}H_{13}NO_2$ (215), a yellow-orange solid stained to light-red by treatment with NaOH. **– R_f** = 0.14 (CH_2Cl_2). **– UV/VIS** (MeOH): λ_{max} (log ε) = 213 (4.21), 243 (4.20), 306 (3.51), 422 (2.84) nm; (MeOH/HCl) = 211 (4.21), 243 (4.18), 306 (3.47), 422 (2.69) nm; (MeOH/NaOH) = 213 (4.11), 251 (4.27), 320 (3.31), 468 (2.59) nm. **– ^{1}H** and **^{13}C NMR**, see Table 22. **– EI MS** (70 eV): *m/z* (%) = 215 ($[M]^{+\cdot}$, 100), 187 (20), 172 (60), 159 (46), 144 (86), 131 (20), 117 (20), 115 (18), 103 (12), 91 (8), 77 (10). **– HRESI MS**: *m/z* = 216.1019140 $[M+H]^+$, (calcd. 216.10191 for $C_{13}H_{14}NO_2$), 238.08389 $[M+Na]^+$ (calcd. 238.08385 for $C_{13}H_{13}NO_2Na$) and 254.05788 $[M+K]^+$, ([calcd. 254.05779 for $C_{13}H_{13}NO_2K$). **– H,H COSY**, **HMQC** and **HMBC:** see Figure 92 and Figure 94.

5-Methyl-5,10-dihydro-phencomycin amide (178): $C_{15}H_{14}N_4O_2$ (282), a yellow-orange solid (4.3 mg), stained to light-red by treatment with NaOH. **– R_f** = 0.29 (CH_2Cl_2/4% MeOH). **– ^{1}H** and **^{13}C NMR**, see Table 22. **– EI MS** (70 eV): *m/z* (%) = 282 ($[M]^{+\cdot}$, 80), 265 (40), 250 (20), 237 (100), 192 (18). **– (+)-ESI-MS**: *m/z* = 587.0 ($[2M+Na]^+$), 305 ($[M + Na]^+$). **– (-)-ESI-MS**: *m/z* = 281 ($[M - H]^-$). **– HR-ESIMS**: *m/z* 305.10090 $[M+Na]^+$ ([calcd. 305.10144 $C_{15}H_{14}N_4O_2Na$). **–H,H COSY**, **HMQC** and **HMBC:** see Figure 95.

9.12 Marine *Streptomyces* sp. B8112

The marine *Streptomyces* sp. B8112 showed characteristic white colonies for the *Streptomyces* species during the incubation on agar plates using M_2^+ medium for three days at 28 °C

9.12.1 Pre-screening

Based on the agar diffusion method, the extract showed a moderate activity against both Gram-positive and negative bacteria, as well as yeast (*Candida albicans*), while it was strongly active against fungi (*Mucor miehei*; Tü284), (Table 67). The extract showed an *in vitro* antitumor activity against cancer cells at very low concentrations, (Table 45). According to TLC, extract of the marine *Streptomyces* sp. B8112 exhibited numerous UV absorbing bands, two of which were stained as blue with anisaldehyde/sulphuric acid, one of which turned later to dark green. An additional band turned to purple by the same reagent. HPLC-MS of the extract combined with AntiBase[61] revealed the presence of new piericidin components e.g. piericidin-A (**182**). The new piericidin glucoside; glucopiericidin C (**185**) (*m/z* 548.2 $[M+H]^+$, R_t = 15.8 min, MeOH-H_2O) was not found in AntiBase, pointing to its novelty.

Table 67: Antimicrobial activity of *Streptomyces* sp. B8112 extract (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	14
Staphylococcus aureus	14
Streptomyces viridochromogenes (Tü 57)	0
Escherichia coli	14
Candida albicans	11
Mucor miehei (Tü 284)	25
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.12.2 Fermentation, Extraction and Isolation

With pieces of a well grown agar subcultures, 35 L M_2^+ medium were inoculated and cultivated on shaker. After 7 days of cultivation at 28 °C, a brown-yellow culture broth was obtained, which was filtered over celite with the aid of a filter press. The filtrate was subjected to adsorption on Amberlite XAD-16, and subsequently extracted with methanol. The methanol extract was concentrated, and the resulting aqueous residue was re-extracted by ethyl acetate. The mycelial phase was dissolved in acetone, which was in turn filtered and concentrated *in vacuo* delivering an aqueous residue. The aqueous solution was further extracted by ethyl acetate. Both ethyl acetate extracts showed similarities from TLC, and subsequently, they were combined brought to dryness to give 5.80 g of brown extract.

On application of the crude extract to column chromatography on Sephadex LH-20 (CH_2Cl_2/50% MeOH), two fractions, I (0.35 g) and II (3.6 g) were delivered. Fraction I was subjected to column chromatography on flash silica gel and eluted with CH_2Cl_2-MeOH gradient to afford five sub-fractions: FIA (0.37 g), FIB (0.30 g), FIC (0.25 g), FID (0.95 g), FIE (0.41 g). Purification of fast mobile sub-fraction FIA using silica gel, PTLC, followed by Sephadex LH-20 led to spatozoate (**186**; 16.3 mg). On purification of sub-fraction, FIC by 3 x Sephadex LH-20 (MeOH), 5-(2-methylphenyl)-4-pentenoic acid (**187a**; 153.3 mg) and monensin-B (**191**, 53.7 mg) were obtained. Similarly, purification of the middle polar sub-fraction FID afforded 5-oxo-5-o-tolyl-pentanoic acid (**188a**; 16.3 mg), piericidin-A (**182**; 120.7 mg), glucopiericidin A (**183**; 35.1 mg), phenyl acetic acid (**189**; 10.3 mg) and 1,4-dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**; 3.7 mg). The polar sub-fraction FIE afforded Glucopiericidin C (**185**; 22.4 mg), 2`-deoxy-uridin (6.3 mg) and 2`-deoxy-thymidin (11.3 mg) during PTLC purification, followed by Sephadex LH-20. Indole-3-carboxylic acid (10.3 mg) and uracil (15.3 mg) were isolated from fraction II using silica gel, (Figure 97).

9.12.3 Biological Properties

Glucopiericidin C (**185**) was similar to glucopiericidin A (**183**) in their activity against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative *Escherichia coli.* It showed, additionally, an activity against the fungal *Candida albicans*, and the micro algae *Chlorella vulgaris*; (Table 68). On other hand, **185** displayed selective and moderate cytotoxicity against a range of human tumor cell lines with a mean IC_{50} of 2.0 µM (mean IC_{70} = 4.2 µM). A similar biological profile was observed for 5-(2-methylphenyl)-4-pentenoic acid (**187a**) (Table 69).

Table 68: Antimicrobial activity of Glucopiericidin C (**185**) compared with Glucopiericidin A (**183**) (40 µg/disc, diameter of inhibition zones in mm).

Compounds	Amount (µg/disk)	BS[a]	SA[b]	SV[c]	EC[d]	CA[e]	MM[f]	CV[g]	CS[h]	SS[g]
Glucopiericidin C (**185**):	40	10	11	0	10	13	0	15	0	0
Glucopiericidin A (**183**):	40	10	11	0	10	13	0	15	0	0

[a]*Bacillus subtilis*, [b]*Staphylococcus aureus*, [c]*Streptomyces viridochromogenes* (Tü 57), [d]*Escherichia coli,* [e]*Candida albican*, [f]*Mucor miehi,* [g]*Chlorella vulgaris,*[h]*Chlorella sorokiniana,* [i]*Scenedesmus subspicatus*

Table 69: Cytotoxic activities of Glucopiericidin C (**185**), Piericidin-A (**182**), 5-(*o*-Tolyl)-4-pentenoic acid (**187a**) and 5-Oxo-5-o-tolyl-pentanoic acid (**188a**).

Compound	Antitumor potency		Tumor selectivity	
	Mean IC_{50} [µM]	Mean IC_{70} [µM]	n / total	%
Glucopiericidin C (**185**)	2.0	4.2	4/36	11%
Piericidin-A (**182**)	0.6	9.8	1/36	3%
5-(2-Methylphenyl)-4-pentenoic acid (**187a**)	7.8	9.3	2/36	6%
5-oxo-5-o-tolyl-pentanoic acid (**188a**)	>10	>10	0/36	0%

[a] mean IC-values, determined as average of 36 human tumor cell lines tested.
[b] individual IC_{50} < ½ mean IC_{50}; e.g. if mean IC_{50} = 2.0 μM the threshold for above average sensitivity was IC_{50} < 1.0 μM

Piericidin-A (182): $C_{25}H_{37}NO_4$ (415), Yellow oil, UV absorbing, stained to blue with anisaldehyde/sulphuric acid spraying. **–** R_f = 0.34 (CH_2Cl_2). **–** **^{1}H** and **^{13}C NMR**, see Table 24. **– (+)-ESI MS**: *m/z* (%) = 853 ($[2M+Na]^+$, 30), 438 ($[M+Na]^+$, 100), 416 ($[M+H]^+$, 12). **–(-)-ESI MS**: *m/z* = 414 ($[M-H]^-$.

Glucopiericidin A (183): $C_{31}H_{47}O_9N$ (577), an amorphous powder, turned as blue by anisaldehyde/sulphuric acid and heating, which changed later to dark green. **–** R_f = 0.2 (CH_2Cl_2/5% MeOH). **–** **^{1}H** and **^{13}C NMR**, see Table 23. **–(+)-ESI MS**: *m/z* (%) = 1177 ($[2M+Na]^+$, 58), 600 ($[M+Na]^+$, 100), 578 ($[M+H]^+$, 74). **–(-)-ESI MS**: *m/z* = 576 ($[M - H]^-$).

Glucopiericidin C (185): $C_{30}H_{45}NO_8$ (547), Yellow oil, UV absorbing, Soluble in DMSO, MeOH, EtOH and EtOAc; insoluble in hexane, benzene and H_2O, turned as blue by anisaldehyde/sulphuric acid and heating, which changed later to dark green.. **–** R_f = 0.19 (CH_2Cl_2/10% MeOH). **– UV/VIS:** λ_{max} (log ε): (MeOH): 223 (4.26), 239 (4.30); (MeOH/HCl): 204 (4.36), 235 (4.27), 271 (3.74); (MeOH/NaOH): 209 (4.37), 238 (4.36), 270 (3.38) nm.**–** **^{1}H** and **^{13}C NMR**, see Table 24.**– (+)-ESI MS**: *m/z* (%) = 1117 ($[2M+Na]^+$, 100), 570 ($[M+Na]^+$, 78), 548 ($[M+H]^+$, 12). **–(-)-ESI MS**: *m/z* (%) = 1093 ($[2M-H]^-$, 100), 546 ($[M-H]^-$, 82). **–HRESI MS**: *m/z* = 548.321770 $[M+H]^+$ ([calcd 548.32233 for $C_{30}H_{46}NO_8$). **–** **$[\alpha]_D^{20}$** = -13 (*c* 0.2, MeOH). **– CD** (1.83 E-005 mol/L MeOH): $[\theta]_{235}$ -9383, $[\theta]_{216}$ +10572.

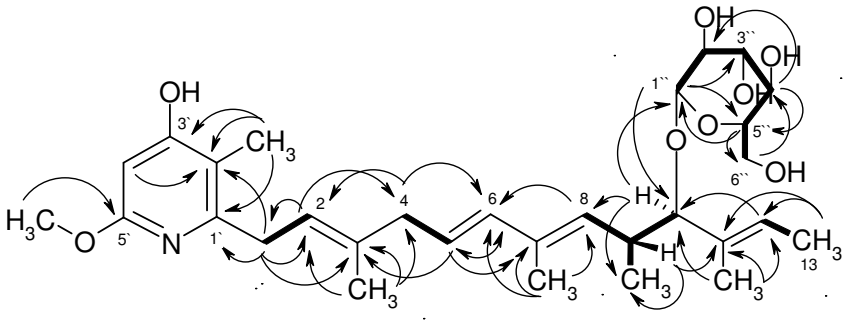


Figure 180: H, H COSY (▬, ↔) and selected HMBC (→) connectivities of Glucopiericidin C (**185**).

Spatozoate (186): Colourless oil, UV-absorbing, which turned to blue by anisaldehyde/sulphuric acid. **–*R*$_f$** = 0.41 (Cyclohexane/10% EtOAc). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.76 (dd, 7.5, 1.5 Hz, 1H, H-6), 7.72 (m, 1H, H-3), 7.54 (td, 7.5, 1.5 Hz, 1 H, H-5). 7.52 (td, 7.5, 1.5 Hz, 1H, H-4), 7.46 (dd, 8.3, 1.8 Hz, 2H, H-2'), 7.38 (td, 8.3, 1.8 Hz, 2H, H-3'), 7.30 (dd, 8.5,1.8 Hz, 1H, H-4), 5.36 (s, 2H, H-7), 4.20 (t, 7.0 Hz, 2H, H-2"), 1.39 and 1.65 (4H, m, H-4"/3"), 0.92 (t, 7.4 Hz, 3H, H-5").- **^{13}C NMR** (75.5 MHz, $CDCl_3$): δ = 167.5 (C-8), 167.2 (C-l"), 135.3 (C-2), 132.3 (C-1), 131.6 (C-1'), 131.0 (C-6), 130.8 (C-3), 128.8 (C-5), 128.7 (C-4), 128.4 (C-2'), 128.2 (C-3'), 128.2 (C-4'), 67.2 (C-7), 65.4 (C-2"), 30.3 (C-3"), 19.0 (C-4"), 13.6 (C-5"). – **EI MS** (70 eV): *m/z* (%) = 312 ([M]$^{+\cdot}$, 10), 206 ([M-(PhCO)]$^{+\cdot}$, 34), 149 (100), 91 (50).

5-(2-Methylphenyl)-4-pentenoic acid (187a): $C_{12}H_{14}O_2$ (190), Colourless semi-solid, which stained as purple by spraying with anisaldehyde/sulphuric acid. – ***R*$_f$** = 0.75 ($CHCl_3$/10% MeOH). – **^{1}H** and **^{13}C NMR**, see Table 25. – **EI MS** (70 eV): *m*/z (%) = 190 ([M] $^{+\cdot}$, 73), 164 (48), 131 ([M-CH_2COOH] $^{+\cdot}$, 92), 105 (100), 91 (70). – **HRESI MS:** *m/z* = 191.1067770 $[M+H]^+$ and 213.0888090 $[M+Na]^+$(calcd. 191.106655 $[M+H]^+$ for $C_{12}H_{15}O_2$ and 213.088595 $[M+Na]^+$ for $C_{12}H_{14}O_2Na$).

5-(2-Methylphenyl)-4-pentenoic acid methyl ester (187b): $C_{13}H_{16}O_2$ (190), Colourless semi-solid, was obtained after treatment of 5.3 mg from **187a** with diazomethane in dry methylene chloride. **– *R*$_f$** = 0.78 (CH_2Cl_2). **- ^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.38 (t, *J* = 4.5 Hz, 1 H), 7.15 (m, 3 H), 6.63 (d, *J* = 15.8 Hz, 1 H), 6.06 (dd, *J* = 15.8, *J* = 6.4 Hz, 1 H), 3.69 (s, 3 H, OMe), 2.53 (m, 4 H, 2 CH2), 2.32 (s, 3 H, CH_3).

5-Oxo-5-*o*-tolyl-pentanoic acid (188a): Colourless wax-like solid, UV-absorbing and turned to purple by anisaldehyde/sulphuric acid. **- R_f** = 0.18 (CH_2Cl_2/3% MeOH). **- ^{1}H** and **^{13}C NMR**, see Table 25. **- DCI MS:** *m*/z (%) = 430 ($[2M+NH_4]^+$, 8), 224.2 ($[M+NH_4]^+$, 100), 207.1 ($[M+H]^+$, 16).

5-Oxo-5-o-tolyl-pentanoic acid methyl ester (188b): Colourless oil, obtained by methylation of 5-oxo-5-o-tolyl-pentanoic acid (**188a**) with diazomethane. **– R_f** = 0.78 (CH_2Cl_2/4% MeOH). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.65 (dd, 8.3, 1.2 Hz, 1 H, H-6`), 7.40-7.25 (m, 3 H, H-3`, H-4`, H-5`), 3.70 (s, 3H, 1-OCH_3), 2.96 (t, 7.2 Hz, 2H, CH_2-4), 2.50 (s, 3 H, 2`-CH_3), 2.44 (t, 7.1 Hz, 2H, CH_2-2), 2.05 (m, 2H, CH_2-2).– **EI MS** (70 eV): *m/z* (%) = 220 ($[M]^{+\cdot}$, 8), 189 (14), 161 (16), 119 (100), 91 (96), 65 (32).

Monensin-B (191): C_{35} H_{60} O_{11} (657), white amorphous solid, UV non-absorbing, which turned to orange-red by anisaldehyde/sulphuric acid spraying reagent. **–R_f** = 0.34 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR**, see Table 26. **– (+)-ESI MS**: *m/z* = 680 ($[M+Na]^+$. **–(-)-ESI MS**: *m/z* = 656 $[M-H]^-$.

9.13 Marine *Streptomyces* sp. B8108

The marine *Streptomyces* sp. B8108 showed a white mycelium, as characteristic for *Streptomyces* sp., by incubation on M_2^+ medium (using 50% sea water) for 10 days at 28 °C.

9.13.1 Pre-screening

In the agar diffusion test, crude extracts of *Streptomyces* isolate B8108 exhibited high bioactivity against *Mucor miehei* (Tü 284) and moderate activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Table 70). Additionally, the strain crude extract showed *in vitro* antitumor activity against cancer cells at very low concentrations (Table 45). The chemical screening of the strain extract using TLC, established the existence of one UV non-absorbing band, which stained initially as blue and later to pink with anisaldehyde/sulphuric acid. In addition, several dark UV absorbing bands were observed. Some were detected under blue fluorescence, which turned yellow with anisaldehyde/sulphuric acid.

Table 70: Biological activity of *Streptomyces* sp. B8108 crude extract (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	10
Staphylococcus aureus	11
Streptomyces viridochromogenes (Tü 57)	0
Escherichia coli	10
Candida albicans	0
Mucor miehei (Tü 284)	25
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.13.2 Fermentation, Extraction and Isolation

With pieces of well-grown agar sub-cultures, the marine isolate B8108 served for the inoculation into 24 L shaker culture using M_2^+ medium. After 6 days of cultivation at 28 °C, the yellowish-brown culture broth obtained was filtered over Celite. The afforded filtrate was extracted using Amberlite XAD-2, while the mycelial cake was extracted by ethyl acetate and then by acetone. Both ethyl acetate extracts were combined and concentrated *in vacuo* to afford 3.52 g as brown crude extract.

The afforded extract was separated on silica gel with a CH_2Cl_2/MeOH gradient and monitored by TLC, UV, and staining reagents to afford six fractions: I (0.3 g fat), II (0.25 g fat), III (0.5 g), IV (1.35 g), V (0.2 g) and VI (0.15 g). PTLC, silica gel column and Sephadex LH-20 of fraction III afforded 1,4-dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (**193**; 7.3 mg), piericidin-A (**182**; 25.6 mg) and 5-(2-Methylphenyl)-4-pentenoic acid (**187a**; 7.3 mg). Purification of fraction IV yielded 4,9-dihydroxy-9-methyl-decan-4-olide (**237**; 14.7 mg), 2-acetylamino-benzoic acid (**194**a; 36.7 mg), (±)-homononactic acid (**145a**; 1.7 mg), tyrosol (**229**; 3.1 mg), anthranilic acid (5.3 mg), indole-3-acetic acid (86.1 mg), 3-(hydroxyacetyl)-indole (2.3 mg) and indole-3-carboxylic acid (10.7 mg). Further fractionation and purification of fractions V and VI afforded the known compounds, 2'-deoxy-uridine (14.2 mg), 2`-deoxy-denosine (4.2 mg), 5'-methyl-thioadenosine (3.6 mg), uracil (25.0 mg), (±)-nonactic acid (**144a**; 1.2 mg), glucopiericidin A (**183**; 3.2 mg) and the highly biologically active staurosporine (**198**; 4.2 mg).

1,4-Dimethyl-1,4-dihydro-imidazo[4,5-d]imidazole-2-carbonitrile (193): $C_7H_7N_5$ (161), colourless solid, UV absorbing. **–** $\boldsymbol{R}_f$ = 0.45 (CH_2Cl_2/5% MeOH). **–**

UV/VIS: λ_{max} (log ε) = (MeOH): 205 (3.92), 254 (3.79); (MeOH/HCl): 203 (3.92), 254 (3.77); (MeOH/NaOH): 208 (3.75), 254 (3.77) nm. **– IR** (KBr): ν_{max} = 3428, 3104, 2924, 2235, 1653, 1560, 1437, 1353, 1268, 1192, 1144, 1057 cm^{-1}. **– ^{1}H** and **^{13}C NMR,** see Table 27. **– (+)-ESI MS**: *m/z* (%) = 345 ([2M+Na]$^+$, 10), 184 ([M+Na]$^+$, 100). **– EI MS** (70 eV): *m/z* (%) = 161 ([M]$^{+\cdot}$, 100), 135 ([M-CN]$^{+\cdot}$, 30), 120 (36), 107 (50), 67 (68), 42 (26). **– HREI MS**: *m/z* = 161.06970 [M]$^{+\cdot}$, (calcd. 161.07014 for $C_7H_7N_5$). **– HMQC** and **HMBC**, see Figure 109.

4,9-Dihydroxy-9-methyl-decan-4-olide (**237**)**:** $C_{11}H_{20}O_3$ (200), colourless oil, UV non absorbing or fluorescence, stained as blue by anisaldehyde/sulfuric acid and latter turned to pink. **– R_f** = 0.19 (Cyclohexane/50% Ethyl acetate). **– ^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.27 (m, 1H, H-4), 2.65 (m, 2H, 2-CH_2), 2.00-1.40 (m, 11H, 3-CH_2, 5-CH_2, 6-CH_2, 7-CH_2, 8-CH_2, 9-OH), 1.23 (s, 3H, H_3-10), 1.22 (s, 3H, 9-CH_3). **– ^{13}C NMR** (75 MHz, $CDCl_3$): δ = 175.8 (C-1), 80.7 (C-4), 70.5 (C-9), 39.2 (C-8), 34.9 (C-2), 34.7 (C-5), 31.1 (C-7), 29.7 (CH_3-10), 28.9 (9-CH_3), 28.2 (C-5), 23.0 (C-3). **– (+)-ESI MS**: *m/z* (%) = 423 ([2M+Na]$^+$, 100), 223 ([M+Na]$^+$, 78). **– (+)-HRESI MS**: *m/z* 201.14865[M+H]$^+$, 223.13062 [M+Na]$^+$ ([calcd. 201.14906 for $C_{11}H_{21}O_3$, calcd. 223.13101 for $C_{11}H_{20}O_3Na$). **–H,H COSY** and **HMBC**, see Figure 127.

2-Acetylamino-benzoic acid (194a): $C_9H_9NO_3$ (179), white solid, UV absorbing/blue fluorescence, turned pale yellow with anisaldehyde/sulphuric acid. **– R_f** = 0.38 (CH_2Cl_2/10% MeOH). **– ^{1}H** and **^{13}C/APT NMR,** see Table 42. **– EI MS** (70 eV): *m/z* (%) = 179 ([M]$^{+\cdot}$, 38), 137 (62), 119 (100), 92 (20), 43 (28). **– H,H COSY** and **HMBC**, see Table 28.

2-Acetylamino-benzoic acid methyl ester (194b): $C_{10}H_{11}NO_3$ (193), was obtained after treatment of 2-acetylamino-benzoic acid (**194a**) with diazomethane in dry dichloromethane. As Colourless solid, showing the same chromatographic properties of **3a**. **– R_f** = 0.38 (CH_2Cl_2). **– ^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 11.06 (brs, 1 H, NH), 8.70 (d, 8.4, 1 H, 3-H), 8.03 (dd, 8.0, 1.6 Hz, 1 H, 6-H), 7.55 (dt, 7.5 Hz, 1.6 Hz, 1 H, 5-H), 7.08 (dt, 8.4 Hz, 1.1 Hz, 1 H, 4-H), 3.93 (s, 3 H, 7-OCH_3) 2.25 (s, 3 H, 9-CH_3). **– EI MS** (70 eV): *m/z* (%) = 193 ([M]$^{+\cdot}$, 28), 151 (58), 119 (100), 92 (17), 43 (24).

Staurosporine (198a): $C_{28}H_{26}N_4O_3$, white powder. – R_f = 0.27 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 9.43 (d, ^3J = 7.9 Hz, 1H, 4-H), 7.93 (d, ^3J = 8.6 Hz, 1H, 11-H), 7.88 (dd, ^3J = 7.9 Hz, ^4J = 0.8 Hz, 1H, 8-H), 7.50-7.20 (m, 5H), 6.57 (s, 1H, NH), 6.55 (dd, ^3J = 5.2, 1.1 Hz, 1H, 6'-H), 5.01 (s, 2H, 7-H_2), 3.87 (d, ^3J = 3.8 Hz, 1H, 3'-H), 3.41 (s, 3H, OMe), 3.34 (q, ^3J = 4.2 Hz, 1H, 4'-H), 2.74 (ddd, ^3J = 14.7, 5.3, 3.6 Hz, 1H, 5'-H_A), 2.40 (m, 1H, 5'-H_B), 2.35 (s, 3H, 2'-Me), 1.54 (s, 3H, NCH_3). – **^{13}C/APT NMR** (DMSO, 75.7 MHz): δ = 172.1 (C_q, C-5), 139.2 (C_q, C-11a), 136.3 (C_q, C-13a), 132.0 (C_q, C-4c), 129.9 (C_q, C-12a), 129.9 (C_q, C-12b), 126.5 (CH, C-4), 125.5 (CH, C-2), 124.8 (C_q, C-7c), 124.3 (CH, C-10), 123.8 (C_q, C-4a), 120.8 (CH, C-8), 119.7 (CH, C-9), 119.0 (CH, C-3), 118.8 (C_q, C-7a), 114.8 (C_q, C-4b), 114.2 (CH, C-11), 113.5 (C_q, C-7b), 108.3 (CH, C-1), 91.3 (CH, C-2'), 82.3 (CH, C-3'), 80.0 (CH, C-6'), 57.5 (CH_3, OMe), 50.5 (CH, C-4'), 45.3 (CH_2, C-7), 32.8 (CH_3, NMe), 29.4 (CH, H-5'), 29.0 (CH_3, 2'-Me). – **(+)-ESI MS:** *m/z* (%) = 933 ($[2M+H]^+$, 44), 955 ($[2M+Na]^+$, 100), 467 ($[M+H]^+$, 88). – **(+)-HRESI MS:** *m/z* = 467.2078950 $[M+H]^+$ (calcd. 467.20777 for $C_{28}H_{27}N_4O_3$).

9.14 Marine-derived *Streptomyces* sp. Mei02-8,1

The marine-derived *Streptomyces* sp. Mei02-8,1 was pre-cultivated on agar plates using M_2, (containing 100% sea water + $CaCO_3$). The incubation was performed at 28 °C for 3 days, exhibiting white mycelium.

9.14.1 Pre-screening

The above subcultures were served to inoculate 2 L on a rotary shaker (95 rpm) at 28 °C for 7 days giving a yellow culture broth. After extraction with ethyl acetate, a yellow oil crude extract was afforded. The extract was applied to antimicrobial assay, displaying an activity against all tested microorganisms (Table 71). The chemical screening showed an UV blue fluorescence during TLC in addition to several UV absorbing spots, which were mostly stained as greenish-blue after spraying with anisaldehyde/sulphuric acid reagent.

Table 71: Antimicrobial activity of the crude extract from the *Streptomyces* sp. Mei02-8,1 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ (mm)
Bacillus subtilis	10
Staphylococcus aureus	15
Streptomyces viridochromogenes (Tü 57)	20
Escherichia coli	10
Candida albicans	13
Mucor miehei (Tü 284)	13
Chlorella vulgaris	13
Chlorella sorokiniana	14
Scenedesmus subspicatus	15

9.14.2 Fermentation, Extraction and Isolation

With pieces of well grown agar subculture of the strain, 80 of 1 L Erlenmeyer flasks, each containing 250 ml of M_2 100% sea water + $CaCO_3$, were inoculated and cultivated on shaker (95 rpm) at 28 °C for 7 days. The broth obtained was mixed with ca. 1.5 kg celite and filtered using filter press. The mycelium and filtrate were separately extracted with ethyl acetate. The organic phases showed similar compositions and were combined, followed by concentration in vacuo to dryness. The oily residue (3.90 g) was chromatographed on silica gel (column 3 x 60 cm, 150 g) using CH_2Cl_2/MeOH gradient (CH_2Cl_2, 2 L; 3% MeOH, 1.5 L; 7% MeOH, 1 L; 10% MeOH, 0.5 L; 15% MeOH, 0.7 L; 20% MeOH, 0.5 L; 50% MeOH, 0.7 L; 100% MeOH, 0.5 L). According TLC visualization, three fractions were collected; I (1.5 g), II (0.59 g) and III (1.1 g). Purification of fraction I on Sephadex LH-20 (column 3 x 70 cm, CH_2Cl_2/40% MeOH) followed by silica gel (CH_2Cl_2) yielded 4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**236**; 40 mg, colorless oil). On a twice application of fraction II to a column of Sephadex LH-20 (column 3 x 70 cm, CH_2Cl_2/40% MeOH) afforded staurosporine (**198**; 7 mg, white powder), 2'-deoxyuridine (25 mg, colourless solid) and 1,N^6-dimethyladenosine (**199**; 3.6 mg, colourless solid). Purification of fraction III using PTLC (CH_2Cl_2/10% MeOH) followed by Sephadex LH-20 (column 3 x 70 cm, CH_2Cl_2/40% MeOH) led to 2'-deoxy-adenosine (4 mg, colourless solid) and 2'-deoxy-thymidine (12 mg, colourless solid), Figure 111.

4-Hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (236): $C_{13}H_{20}O_3$, colourless oil, violet, changed (after ~20 minutes) later to blue with anisaldehyde/sulphuric acid. – R_f = 0.35 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 7.48 (dd, ^3J = 5.6 Hz, ^4J = 1.5 Hz, 1H, 3-H), 6.12 (dd, ^3J = 5.6 Hz, ^4J = 2.0 Hz, 1H, 4-H), 5.06 (m, 1H, 4-H), 2.52 (m, 1H, 10-H), 2.13 (s, 3H, 12-CH_3), 1.78 (m, 1H), 1.63 (m, 2H), 1.50-1.20 (m, 10H), 1.10 (d, ^3J = 7.0 Hz, 3H, 13-CH_3). – **^{13}C NMR** ($CDCl_3$, 50.3 MHz): δ = 212.8 (C_q-11), 173.1 (C_q-1), 156.2 (CH-3), 121.5 (CH-2), 83.3 (CH-4), 47.0 (CH-10), 33.0 (CH_2-5), 32.6 (CH_2-9), 29.2 (CH_2-7), 28.0 (12-CH_3) 26.9 (CH_2-8), 24.7 (CH_2-6), 16.2 (CH_3-13). – **(+)-ESI MS:** *m/z* (%) = 471 ([2M+Na]$^+$, 100), 247 ([M+Na]$^+$, 25).

1, N^6-Dimethyladenosine (199): $C_{12}H_{17}N_5O_4$ (295), white solid, UV absorbing, turned greenish-blue with anisaldehyde/sulphuric acid. - R_f = 0.36 (CH_2Cl_2/4% MeOH). - **^{1}H NMR** ($CDCl_3$, 600 MHz): δ = 8.42 (s, 1H, 2-H), 8.29 (s, 1H, 6-H), 6.03 (d, 5.9 Hz, 1 H, 1'-H), 4.62 (t, ^3J = 5.4 Hz, 1H, 2'-H), 4.31 (dd, 5.0, 3.3 Hz, 1H, 3'-H), 4.16 (q, 5.8 Hz, 1H, 4'-H), 3.88, 3.77 (*ABX*, ^3J = 12.3, 2.8 Hz, 1H, 5'$_a$-H, 5'$_b$-H), 3.67 (s, 3H, CH_3), 3.61 (brs, 3H, CH_3). - **^{13}C NMR** (CD_3OD, 150 MHz): δ = 149.5 (C_q-6), 141.2 (CH-2, CH-8, C_q-4), 121.6 (C_q-5), 91.0 (CH-1'), (CH-1'), 87.8 (CH-4'), 76.0 (CH-2'), 72.3 (CH-3'), 63.0 (CH_2-5'), 40.4 (N_6-CH_3), 38.3 (1-CH_3). - **(+)-ESI MS:** *m/z* (%) = 613 ([2M+Na]$^+$, 40), 318 ([M+Na]$^+$, 100). – **EI MS** (70 eV): *m/z* (%) = 295 ([M]$^{+\cdot}$, 20), 206 (30), 192 (60), 164 ([M-Sugar residue]$^{+\cdot}$, 56), 148 (26), 134 ([Furanose residue]$^{+\cdot}$, 100). – (+)-**HRESI MS**: *m/z* = 296.1353480 [M+H]$^+$ (calcd. 296.135325 for $C_{12}H_{18}N_5O_4$).

9.15 Marine-derived *Streptomyces* sp. Mei4 1-2,3

The marine derived *Streptomyces* sp. isolate Mei4 1-2,3 was isolated and deposited in Emden. The strain showed a white aerial mycelium after incubation on M_2^+ agar medium (28 °C & 4 days). For primary screening, 1 L of sterilized M_2^+ medium containing seed cells of the strain were incubated on shaker (95 rpm) for 10 days at 28 °C. The faintly brown culture broth produced was extracted with ethyl acetate to give an oily yellowish-brown oily crude extract.

9.15.1 Pre-screening

In biological screening, the extract was examined against a set of pathogenic microorganisms using agar diffusion test (Table 72). The chemical screening of the extract during TLC revealed the presence numerous violet-blue grey spots after spraying with anisaldehyde/sulphuric acid. the TLC showed also several UV absorbing spots, and one blue fluorescence band. These bands were turned as greenish-blue and/or reddish brown with anisaldehyde/sulphuric acid and heating. The TLC exhibited one yellow band in day light which changed as brown by spraying with sulphur reagent ($PdCl_2$ reagent).

Table 72: Antimicrobial activity of the crude extract from the marine-derived *Streptomyces* sp. Mei4 1-2,3 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	10
Staphylococcus aureus	18
Streptomyces viridochromogenes (Tü 57)	23
Escherichia coli	10
Candida albicans	11
Mucor miehei (Tü 284)	0
Chlorella vulgaris	18
Chlorella sorokiniana	17
Scenedesmus subspicatus	18

9.15.2 Fermentation, Extraction and Isolation

Small pieces of well grown agar plates of *Streptomyces* sp. Mei4 1-2,3 were inoculated into 160 of 1 L sterilized Erlenmeyer flasks. The flasks (each was fill with 250 ml of M_2^+ medium containing 100% sea water + $CaCO_3$ were applied to cultivation on shaker culture at the same previous conditions. The dark brown broth obtained was harvested and filtered on Celite. On other hand, the filtrate was passed through a column of XAD-16, followed by washing with demineralised water (20 L) and extraction by MeOH (10 L). The methanol extract was evaporated *in vacuo*, and the remaining water residue was re-extracted by ethyl acetate. The ethyl acetate extracts were combined based on their TLC similarities and evaporated to dryness yielding 9.35 g, as yellowish-brown crude extract. The extract was subjected to a silica gel column chromatography (column 3 × 70 cm, 200 g) using CH_2Cl_2/MeOH gradient (CH_2Cl_2, 1 L; 3% MeOH, 0.5 L; 6% MeOH, 10% MeOH, 0.5 L; 15%

MeOH, 0.7 L; 20% MeOH, 0.6 L; 50% MeOH, 0.8 L; 100% MeOH, 0.3 L). After TLC monitoring, eight fractions I-VIII were afforded: I (1.3 g), II (0.7 g), III (30 mg), IV (30 mg), V (0.28 g), VI (40 mg), VII (0.35) and VIII (0.850 g). Further purification of fraction II using silica gel column (and elution with CH_2Cl_2-MeOH gradient) followed by Sephadex LH-20 (CH_2Cl_2/40% MeOH) and preparative HPLC afforded holomycin (**208**; 3.3 mg, yellow solid) and lumichrome (**201**; 35 mg, pale yellow solid). Purification of fractions III-VIII using diverse chromatographic methods (silica gel, Sephadex LH-20 and PTLC) afforded homononactic acid (**145a**; 28.7 mg, colourless oil), nonactic acid (**144a**; 60.8 mg, colourless oil), dinactin (**148**; 181.2 mg, colourless oil), uracil (38 mg, white solid), 2'-deoxy-uridine (16.8 mg, colourless solid) and 2'-deoxy-thymidine (21.9 mg, colourless solid). Further purification fraction VII using PTLC (CH_2Cl_2/11% MeOH) and Sephadex LH-20 (column 3 x 70 cm, CH_2Cl_2/40% MeOH) lead to *p*-hydroxyphenyl acetic acid (**190**) as colourless solid (4 mg), Figure 113.

Lumichrome (201): $C_{12}H_{10}N_4O_2$ (242), as UV absorbing (254 nm), blue fluorescence (366 nm), light yellow solid, yellow with anisaldehyde/sulphuric acid spraying reagent. – $\boldsymbol{R_f}$ = 0.36 (CH_2Cl_2/10% MeOH). – $\boldsymbol{R_t}$ = 13.5 min (RP-HPLC). – **^{1}H NMR** ([D_6]DMSO, 300 MHz): δ = 11.65 (br s, 2H, 2NH, 1-H, 3-H), 7.90 (s, 1H, 6-H), 7.70 (s, 1H, 9-H), 2.49 (s, 3H, 8-CH_3), 2.46 (s, 3H, 7-CH_3). – **^{13}C NMR** ([D_6]DMSO, 75 MHz): δ = 160.3 (C_q-4), 149.8 (C_q-2), 146.2 (C_q-10a), 144.4 (C_q-8), 141.5 (C_q-9a), 138.6 (C_q-7), 138.2 (C_q-5a), 129.9 (C_q-4a), 128.5 (CH-6), 125.7 (CH-9), 20.0 (8-CH_3), 19.3 (7-CH_3). – **(+)-ESI MS:** *m/z* (%) = 507 ($[2M+Na]^+$, 36), 265 ($[M+Na]^+$, 100). – **(-)-ESI MS:** *m/z* (%) = 505 ($[2M-2H+Na]^-$, 90), 241 ($[M-H]^-$, 100). – **EI MS** (70 eV): *m/z* (%) = 242 ($[M]^{+\cdot}$, 100), 199 ($[M-CHNO]^{+\cdot}$, 7), 171 ($[M-C_2HNO_2]^{+\cdot}$, 44), 156 ($[M-C_2H_2N_2O_2]^{+\cdot}$, 48). – HMBC, see Figure 116.

9.16 Marine *Streptomyces* sp. B8300

The marine *Streptomyces* sp. B8300 formed a yellow aerial mycelium and the surrounding agar was stained yellow. The strain was cultivated at 28 °C for 72 h on three agar plates with M_2^+ using 50% artificial sea water. The agar from one plate was cut into small pieces and used to inoculate 12 of 1L-Erlenmeyer flasks each containing 250 ml of M_2^+ using 50% artificial sea water. Fermentation was carried out

on a shaker at 95 rpm for 3-days at 28 °C. The broth was harvested after 3 days and both the filtrate and the mycelial cake were extracted three times with ethyl acetate.

9.16.1 Pre-screening

The chemical screening of this marine strain extract using TLC showed a number of yellow spots, together with numerous UV absorbing spots. Two of the UV absorbing bands were stained blue and a third one as reddish-brown on spraying with anisaldehyde/sulphuric acid. The extract showed a strong activity against a set of microorganisms (Table 73).

Table 73: Antimicrobial activities of the crude extract (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	20
Staphylococcus aureus	0
Streptomyces viridochromogenes (Tü 57)	11
Escherichia coli	27
Candida albicans	27
Mucor miehei (Tü 284)	0
Chlorella vulgaris	-
Chlorella sorokiniana	10
Scenedesmus subspicatus	13

9.16.2 Fermentation, Extraction and Isolation

The marine isolate B8300 was pre-cultivated on M_2^+ agar plates (with 50% artificial sea water) at 28 °C for 3 days. The well grown agar subcultures were served to inoculate 20 L shaker culture using the same medium and cultivated for 5 days at 28 °C. After harvesting, the yellowish-brown broth obtained was filtered over Celite with the aide of a filter press. Both phases filtrate and mycelia were extracted separately: the filtrate was subjected to adsorption on Amberlite XAD-16, and the latter subsequently extracted with methanol. The methanol extract was concentrated, and the resulting aqueous residue was extracted with ethyl acetate. The mycelial phase was extracted with ethyl acetate (3 times) and acetone (one time), the solution was concentrated *in vacuo*, and the aqueous residue obtained was extracted with ethyl acetate. The two ethyl acetate extracts showed the same TLC characterization and hence were combined and brought to dryness and yielded 4.66 g of yellowish-brown crude extract.

Chromatography of the obtained crude extract (4.66 g) using silica gel column chromatography with a CH_2Cl_2-MeOH gradient (CH_2Cl_2 (1L), 2% MeOH (1.5L), 1 L 5% MeOH, 10% MeOH (0.5L), 20% MeOH (0.5L), 50% MeOH (0.5L), 50% MeOH (0.5L), 100% MeOH (0.5L) afforded three fractions by monitoring of TLC. Purification of fraction I (0.65 g) on PTLC (CH_2Cl_2/5% MeOH) followed by Sephadex-LH20 (CH_2Cl_2/40% MeOH) resulted in phencomycin methyl ester (**215**; 10.6 mg) as yellow crystals. Further fractionation and purification of fraction II (1.4 g) using silica gel column chromatography (CH_2Cl_2/MeOH-gradient) followed by PTLC and then Sephadex-LH20 resulted in the isolation of 2,5-furandimethanol (35.7 mg, as colourless oil), dihydrophencomycin methyl ester (**212**; 3.7 mg, redish-brown solid), β-indomycinone (**219**; 1.1 mg, yellowish-orange solid) and saptomycin A (**220**; 1.3 mg, yellow amorphous powder). The polar fraction III (1.15 g) was further purified using PTLC followed by Sephadex LH-20 to deliver adenine (colourless solid), adenosine (colourless solid) and 2`-deoxy-adenosine (colourless solid), (Figure 117).

Dihydrophencomycin methyl ester; 5,10-Dihydrophenazine-1,6-dicarboxylic acid dimethylester (212): $C_{16}H_{14}N_2O_4$ (298), reddish-brown solid, stained pale-yellow by spraying with anisaldehyde/sulphuric acid reagent. **–** R_f = 0.89 (CH_2Cl_2). **–** ^{1}**H** and ^{13}**C NMR**, see Table 30. **– EI MS** (70 eV): *m/z* (%) = 298 ($[M]^{+\cdot}$, 46), 266 (26), 238 (72), 206 (100), 178 (40), 152 (20), 117 (56), 103 (46), 90 (30), 76 (20). **– H,H COSY** and **HMBC**, see Figure 118.

Phencomycin methyl ester; Phenazine-1,6-dicarboxylic acid dimethyl ester (215): $C_{16}H_{12}N_2O_4$ (296), yellow crystals, stained yellowish-green on spraying with anisaldehyde/sulphuric acid. **–** R_f = 0.65 (CH_2Cl_2/5% MeOH).**–** ^{1}**H** and ^{13}**C NMR,** see Table 30. **– EI MS** (70 eV): *m/z* (%) = 296 ($[M]^{+\cdot}$, 42), 265 (30), 238 (100), 222 (18), 169 (16). **– (+)-HRESI MS**: *m/z* = 297.08698 $[M+H]^+$, ([calcd 297.08699 for $C_{16}H_{13}N_2O_4$), 319.06893 $[M+Na]^+$, ([calcd. 319.06893 for $C_{16}H_{12}N_2O_4Na$). **– H,H COSY** and **HMBC**, see Figure 119.

9.17 Marine *Streptomyces* sp. B7828

The marine *Streptomyces* sp. B7828 formed a white aerial mycelium and the surrounding agar was stained white. The strain was cultivated at 28 °C for 72 hours

on three agar plates with M_2^+ using 50% artificial sea water. The agar from one plate was cut into small pieces and used to inoculate 12 1L-Erlenmeyer flasks each containing 250 ml of M_2^+ using 50% artificial sea water. Fermentation was carried out on a shaker at 95 rpm for 3-days at 28 °C. The broth was harvested after 3 days and both the filtrate and the mycelial cake were extracted three times with ethyl acetate.

9.17.1 Pre-screening

The antimicrobial assays of the extract are listed in Table 74. The chemical screening of this marine strain extract on TLC showed an UV absorbing spot at 254 nm and stained to blue by spraying with anisaldehyde/sulphuric acid after heating, a middle polar blue fluorescence band, some middle polar spots showed no UV activity and stained to blue by anisaldehyde/sulphuric acid, in addition, a middle polar blue fluorescence band staining to yellow by anisaldehyde/sulphuric acid was observed.

Table 74: Antimicrobial activities of the crude extract (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	0
Staphylococcus aureus	13
Streptomyces viridochromogenes (Tü 57)	12
Escherichia coli	0
Candida albicans	0
Mucor miehei (Tü 284)	0
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.17.2 Fermentation, Extraction and Isolation

The marine isolate B7828 was pre-cultivated on M_2^+ agar plates (with 50% artificial sea water) at 28 °C for 3 days. With pieces of a well grown agar subculture, a 25 L shaker culture was inoculated on M_2^+ medium. After 13 days of cultivation at 28 °C, a brown-yellow culture broth was obtained, which was filtered over Celite with the aide of a filter press. Both phases filtrate and mycelia were extracted separately, at which the filtrate was subjected to adsorption on Amberlite XAD-2, and the latter subsequently extracted with methanol. The methanol extract was concentrated, and the resulting aqueous residue was extracted with ethyl acetate. The mycelial

phase was extracted with acetone, the solution concentrated *in vacuo* and the obtained aqueous residue was extracted with ethyl acetate. The two ethyl acetate extracts were separately brought to dryness and yielded 0.7 g and 0.6 g of brown extracts from filtrate and mycelia, respectively.

Chromatography of the filtrate extract (0.70 g) was performed using silica gel with a MeOH/CH_2Cl_2 gradient followed by another silica gel column chromatography with an ethyl acetate/cyclohexane gradient. The butanolide fractions (blue with anis aldehyde/sulfuric acid) were further purified on Sephadex LH-20 to deliver 4-hydroxy-2,5,6-trimethyl-octan-4-olide (**230**; 23.7 mg), 4,5-dihydroxy-2,5,6-trimethyl-octan-4-olide (**231**; 3.7 mg), and 3,4-dihydroxy-2,5,6-trimethyl-octan-4-olide (**232**; 2.3 mg).

Fractionation and purification of the mycelial extract (0.6 g) by silica gel column chromatography with a MeOH/CH_2Cl_2 gradient followed by PTLC and Sephadex LH-20 resulted in 1-acetyl-β-carboline (**228**; 2.1 mg), 2'-deoxy-uridine (7.2 mg), tyrosol (**229**; 3.1 mg), and 1-hydroxy-4-methoxy-2-naphthoic acid (**71**; 1.8 mg); Figure 86.

Table 75: Cytotoxic activities of 4-Hydroxy-2,5,6-trimethyl-octan-4-olide (**230**)

Compound	Antitumor potency[a]		Tumor selectivity[a]		Compare analysis
	Mean IC_{50} [µM]	Mean IC_{70} [µM]	n/total	%	
γ-Butyrolactone (**230**):	22.093	>10	0/36	0%	-

[a] mean IC-values, determined as average of 36 human tumor cell lines tested.
[b] individual IC_{50} < ½ mean IC_{50}; e.g. if mean IC_{50} = 2.0 μM the threshold for above average sensitivity was IC_{50} < 1.0 μM.

4-Hydroxy-2,5,6-trimethyl-octan-4-olide (**230**)**:** Brownish oil, blue coloration with anisaldehyde/sulfuric acid. **–R_f** = 0.18 (cyclohexane/50% CH_2Cl_2). **– $[\alpha]_D^{20}$** = + 66 (*c* 0.1, MeOH). **– ^{1}H** and **^{13}C NMR**, see Table 31. **– (+)-ESI MS:** *m/z* (%) = 391 ($[2M+Na]^+$, 35), 239 ($[M+CH_3OH+Na]^+$, 100), 207 ($[M+Na]^+$, 15). **– (+)-ESI MS:** *m/z* (%) = 391 ($[2M+Na]^+$, 35). **–EI MS** (70 eV): *m/z* (%) = 184 ($[M]^{+\cdot}$, 2), 142 (16), 127 (36), 98 (20), 70 (24), 69 (28), 56 (100), 43 (92), 41 (52). **– HREI MS:** *m/z* = 184.1458 (calcd. for $[M]^+$, $C_{11}H_{20}O_2$, 184.1458). **– H,H COSY**, **HMBC** and **NOESY**, see Figure 123 and Figure 124.

4,5-Dihydroxy-2,5,6-trimethyl-octan-4-olide (**231**)**:** Colorless oil, blue coloration with anisaldehyde/sulfuric acid. **–R_f** = 0.52 (CH_2Cl_2/3% MeOH). **– ^{1}H** and **^{13}C**

NMR, see Table 31. **–DCI MS** (NH_3)**:** *m/z* (%) = 418 ($[2M+NH_4]^+$, 2), 235 ($[M+NH_3+NH_4]^+$, 8), 218 ($[M+NH_4]^+$, 100). **–HRESI MS** *m/z* 201.14865 [M+H], 223.13061 [M+Na] (calcd. for $[M+H]^+$, $C_{11}H_{21}O_3$ 201.14851 and for $[M+Na]^+$, $C_{11}H_{20}O_3Na$ 223.13047). **–H,H COSY** and **HMBC**, see Figure 123.

3,4-Dihydroxy-2,5,6-trimethyl-octan-4-olide (**232**)**:** Colorless oil, blue coloration with anisaldehyde/sulfuric acid. **–R_f** = 0.40 (CH_2Cl_2/3% MeOH). **– ^{1}H NMR**, see Table 31. **–DCI MS:** (NH_3) *m/z* = 418 $[2M+NH_4]^+$, 235 $[M+NH_3+NH_4]^+$, 218 $[M+NH_4]^+$. **– (-)-ESI MS:** *m/z* (%) = 399 ($[2M-H]^-$, 100), 199 ($[M-H]^-$, 34). **–(+)-ESI MS:** *m/z* (%) = 423 ($[2M+Na]^+$, 30), 254 ($[M+CH_3OH+Na]^+$, 100), 223 ($[M+Na]^+$, 40). **– (+)-HRESI MS:** *m/z* = 201.14863 [M+H], 223.13065 [M+Na] (calcd. for $[M+H]^+$, $C_{11}H_{21}O_3$ 201.14851 and for $[M+Na]^+$, $C_{11}H_{20}O_3Na$, 223.13048).

9.18 Marine-derived *Streptomyces* sp. Act8015

The marine-derived *Streptomyces* sp. Act8015 showed a white aerial mycelium. Eight 1 L Erlenmeyer flasks, each containing 250 ml of M_2^+ medium, were incubated at 28 °C on a linear shaker (95 rpm), to afford a light brown broth. The broth was lyophilized and the residue was extracted by ethyl acetate followed by concentration *in vacuo*. The yielded yellowish-brown oily extract was applied to biological and chemical screening.

9.18.1 Pre-screening

The chemical screening of the extract during TLC showed appeared several UV absorbing spots, which stained with anisaldehyde/sulphuric to yellowish-brown and/or reddish brown. Further non-UV absorbing spots were detected by the same reagent as violet-blue and which later changed to greenish-blue. Biologically, the extract exhibited only antimicrobial activities against *Staphylococcus aureus* and *Candida albicans* using agar disc diffusion method (Table 76). The extract was examined against a set of cancer cells, and it showed a potent *in vitro* antitumor activity against all selected cells (Table 45).

Table 76: Antimicrobial activity of the crude extract from the marine-derived *Streptomyces* sp. Act8015 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	0
Staphylococcus aureus	18
Streptomyces viridochromogenes (Tü 57)	-
Escherichia coli	0
Candida albicans	10
Mucor miehei (Tü 284)	0
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.18.2 Fermentation, Extraction and Isolation

The strain was cultured as 20 L, using 80 of 1 L Erlenmeyer flasks each containing 250 ml of M_2^+ medium at 28 °C for four days using a linear shaker (95 rpm). The brown culture broth was harvested and filtered to separate the mycelium. The mycelial cake was extracted with ethyl acetate, while the filtrate was passed through a column of XAD-2. Afterward, the column was eluted with 25 L demineralised to exclude the unwanted water soluble materials, followed by elution with 15 L methanol. The methanolic extract which was concentrated under reduced pressure and the water residue were re-extracted by ethyl acetate. Both ethyl acetate extracts were combined based on TLC. The yielded crude extract (2.3 g) was chromatographed on silica gel column (3×60 cm, 150 g) and eluted with CH_2Cl_2/MeOH-gradient (CH_2Cl_2, 0.5 L; 3% MeOH, 1.5 L; 6% MeOH, 0.5 L; 10% MeOH, 0.3 L; 20 % MeOH, 0.5 L; 50% MeOH, 0.4 L; 100% MeOH, 0.2 L). After TLC, six fractions (I-VI) were collected. From them fraction I (0.2 g) was determined as fat. Purification of fraction II (0.1 g) using silica gel column (CH_2Cl_2) followed by Sephadex LH-20 (CH_2Cl_2/40% MeOH) delivered virginiae butanolide E (**242**; 20.9 mg, colourless oil). Application of fraction III (0.85 g) to Sephadex LH-20 (MeOH) lead to the sub-fractions IIA, IIB, which on further purification using different chromatographic methods, they delivered eight compounds: tryptophol (5.3 mg, white solid), ferulic acid (**181**; 40 mg, colourless solid), mixture of Graefe's Factors I (**249**) and III (**250**) (8.2 mg, colourless oil), piperazimycin A (**254**; 29.8 mg, white crystals), piperazimycin B (**255**; 3.6 mg, white solid), 4,10-dihydroxy-10-methyl-dodecan-4-olide (**251**; 18.7 mg, colour-

less oil), 4,10-dihydroxy-10-methyl-dodecanoic acid (**252**; 5.7 mg, colourless oil). Individual purifications of the remained three fractions IV (0.3 g), V (0.21 g) and VI (0.4) on Sephadex LH-20 resulted in indole-3-carboxylic acid (2.3 mg, white solid), staurosporine (**198**; 3.1 mg, white powder) and adenine (25 mg, white powder), respectively, (Figure 132).

9.18.3 Biological Activity

Antibacterial, antifungal and antialgal activities were *semi* quantitatively determined using the agar diffusion method with 9 mm paper disks and 40 μg of the pure compounds. Virginiae butanolide E; VB-E (**242**), 4,10-dihydroxy-10-methyl-dodecan-4-olide (**251**), 4,10-dihydroxy-10-methyl-dodecanoic acid (**252**), piperazimycins A (**254**) and B (**255**) were tested. Only compounds **254** and **255** were antimicrobially active against all test microbes except three algae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus* for **254** and *Scenedesmus subspicatus* for **255** (Table 77). Additionally, Piperazimycin A (**254**) showed unselective high cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 0.130 μg/ml (mean IC_{70} = 0.210 μg/ml). In addition, a cytotoxicity examination of **254** using brine shrimp microwell assay and showed a 20% cytotoxic activity.

Table 77: Antibacterial, antifungal and anti micro-algal activities of compounds **242**, **251**, **252**, **254** and **255** in agar diffusion test (∅ of inhibition zones, mm).

Compounds	(μg/disk)	BS[a]	SA[b]	SV[c]	EC[d]	CA[e]	MM[f]	CV[g]	CS[h]	SS[i]	RS[j]	PU[k]
242	40	0	0	0	0	0	0	0	0	0	0	0
251	40	0	0	0	0	0	0	0	0	0	0	0
252	40	0	0	0	0	0	0	-	-	-	-	-
254	40	14	22	26	17	0	15	0	0	0	0	0
255	40	14	21	25	16	14	24	16	14	0	++	+++

[a]*Bacillus subtilis*, [b]*Staphylococcus aureus*, [c]*Streptomyces viridochromogenes* (Tü 57), [d]*Escherichia coli*, [e]*Candida albicans*, [f]*Mucor miehei*, [g]*Chlorella vulgaris*, [h]*Chlorella sorokiniana*, [i]*Scenedesmus subspicatus*, [j]*Rhizoctonia solani*, [k]*Pythium ultimum*; ++ = active, +++ = highly active; - = not tested

Table 78: Cytotoxic activities of the Piperazimycin A (**254**) against tumor cell lines in a monolayer proliferation assay.

Tumor type	Cell line	IC_{50} [μg/ml]	IC_{70} [μg/ml]
Bladder	BXF 1218L	0.113	0.176
	BXF T24	0.098	0.159
Glioblastoma	CNXF 498NL	0.088	0.149
	CNXF SF268	0.097	0.156
Colon	CXF HCT116	0.105	0.163
	CXF HT29	0.098	0.154
Stomach	GXF 251L	0.123	0.201
Head & neck	HNXF 536L	0.871	1.405
Lung	LXF 1121L	0.105	0.167
	LXF 289L	0.117	0.187
	LXF 526L	0.123	0.190
	LXF 529L	0.103	0.167
	LXF 629L	0.102	0.166
	LXF H460	0.098	0.154
Breast	MAXF 401NL	0.110	0.177
	MAXF MCF7	0.103	0.165
Melanoma	MEXF 276L	0.127	0.207
	MEXF 394NL	0.098	0.158
	MEXF 462NL	0.107	0.175
	MEXF 514L	0.125	0.186
	MEXF 520L	0.111	0.182
Ovary	OVXF 1619L	0.127	0.202
	OVXF 899L	1.102	1.818
	OVXF OVCAR3	0.113	0.184
Pancreas	PAXF 1657L	0.125	0.212
	PAXF PANC1	0.100	0.163
Prostate	PRXF 22RV1	0.092	0.157
	PRXF DU145	0.105	0.163
	PRXF LNCAP	0.118	0.176
	PRXF PC3M	0.099	0.157
Mesothelioma	PXF 1752L	0.110	0.173
Kidney	RXF 1781L	0.143	0.252
	RXF 393NL	0.103	0.163
	RXF 486L	1.129	1.798
	RXF 944L	0.096	0.161
Uterus	UXF 1138L	0.101	0.162
Mean		0.130	0.210

Virginiae butanolide E; VB-E (242): $C_{11}H_{20}O_4$ (216), colourless oil, UV non-absorbing, turned initially violet and later to greenish-blue by anisaldehyde/sulphuric acid, $\boldsymbol{R_f}$ = 0.33 (CH_2Cl_2/5% MeOH). $[\alpha]_D^{20}$ = - 74 (*c* 0.2 MeOH). **– ^{1}H NMR** (300 MHz, $CDCl_3$): see Table 32. **– ^{13}C NMR** (75 MHz, $CDCl_3$): see Table 32. **– (+)-ESI-MS**: *m/z* (%) = 455 ($[2M+Na]^+$, 22), 239 ($[M+Na]^+$, 100), 217 ($[M+H]^+$, 8). **– (-)-ESI-MS**: *m/z* = 261 ($[M+HCOO]^-$. **– (+)-HRESI-MS**: *m/z* = 217.1435550 $[M+H]^+$, 239.1255210 $[M+Na]^+$ ([calcd. 217.14343 for $C_{11}H_{21}O_4$, and calcd. 239.12537 for $C_{11}H_{20}O_4Na$). **– H,H COSY** and **HMBC**, see Figure 128.

Graefe's Factor I (249) and Graefe's Factor III (250): colourless oil, UV non-absorbing, turned blue-violet by anisaldehyde/sulphuric acid. **– R_f =** 0.54 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.39 (m, 2H, 2(CH-1'), 4.08 (m, 4H, 2 (3-CH_2), 3.70 (m, 4H, 2(CH_2-4)), 2.81 (m, 2H, 2(CH-2)), 2.54 (m, 2H, 2 (CH-3)), 2.40 (brs, 2H, 2OH), 1.57-1.17 (m, 20H, 9CH_2, 2CH), 0.86 (d, 6.6 Hz, 6H, 2CH_3), 0.85 (d, 6.6 Hz, 6H, 2CH_3). **–(+)-ESI-MS**: *m/z* (%) = 539 ($[2M_2+Na]^+$, 40), 525 ($[M_1M_2+Na]^+$, 75), 281 ($[M_2+Na]^+$, 70), 267 ($[M_1+Na]^+$, 100). **–(-)-ESI-MS**: *m/z* = 289 $[M_1+HCOO]^-$, 303 $[M_2+HCOO]^-$.

4,10-Dihydroxy-10-methyl- dodecan-4-olide (251): $C_{13}H_{24}O_3$ (228), colourless oil, UV non absorbing, detected as violet by anisaldehyde/sulphuric acid and changed later to greenish-blue. **– R_f** = 0.47 (CH_2Cl_2/5% MeOH). **– $[\alpha]_D^{20}$** = - 40 (*c* 0.1, MeOH). **– ^{1}H** and **^{13}C NMR**, see Table 33. **– (+)-ESI MS**: *m/z* (%) = 479 ($[2M+Na]^+$, 100), 251 ($[M+Na]^+$, 95), 229 ($[M+H]^+$, 12). **– (+)-HRESI-MS**: *m/z* = 229.17991 $[M+H]^+$, 251.1617780 $[M+Na]^+$ (calcd. 229.179815 for $C_{13}H_{25}O_3$, calcd. 251.1617653 for $C_{13}H_{24}O_3Na$). **– H,H COSY** and **HMBC**, see Figure 129 and Figure 130.

4,10-Dihydroxy-10-methyl-dodecanoic acid (252): $C_{13}H_{26}O_4$ (246.1), colourless oil, UV non absorbing, stained initially as violet by anisaldehyde/sulphuric acid and afterward changed to greenish-blue. **– R_f** = 0.10 (CH_2Cl_2/5% MeOH). **– $[\alpha]_D^{20}$** = 0 (*c* 0.1, MeOH). - **^{1}H** and **^{13}C NMR**, see Table 33. **–(+)-ESI-MS**: *m/z* = 269 $[M+Na]^+$. **– (-)-ESI-MS**: *m/z* (%) = 513 ($[2M-2H+Na]^-$, 50), 491 ($[2M-H]^-$, 30), 245 ($[M-H]^-$, 100). **– (+)-HRESI-MS**: *m/z* = 269.1724060 $[M+Na]^+$, ([calcd. 269.172325 for $C_{13}H_{26}O_4Na$). **– H,H COSY** and **HMBC**, see Figure 131.

4,10-Dihydroxy-10-methyl-dodecanoic acid methyl ester (253): $C_{14}H_{28}O_4$ (260), was obtained during the treatment of **252** with using diazomethane in dry methylene chloride, colourless oil (2.1 mg), UV inactive, stained with anisaldehyde/ sulphuric acid as pink. **– R_f** = 0.25 (CH_2Cl_2/5% MeOH). – **^{1}H NMR (300 MHz, $CDCl_3$):** δ = 3.65 (s, 3H, 1-OCH_3), 3.55 (brs, 1H, H-4), 2.35 (t, 7.0 Hz, 2H, CH_2-2), 1.60 (m, 2H, CH_2-3), 1.65-1.30 (m, 12H, CH_2-5, CH_2-6, CH_2-7, CH_2-8, CH_2-9, CH_2-11), 1.11 (s, 3H, 10-CH_3), 0.85 (t, 7.4 Hz, 3H, CH_3-12).

Piperazimycins A (254): $C_{31}H_{47}N_8O_{10}Cl$ (726.3), white crystals, UV absorbing, stained to blue by exposing to chlorine/tolidine reagent. **–R_f** = 0.26 (CH_2Cl_2/5%

MeOH).– **^{1}H NMR** (600 MHz, $CDCl_3$): see Table 34. – **^{13}C NMR** (125 MHz, $CDCl_3$): see Table 34. – **(+)-ESI-MS**: *m/z* (%) = 1475 ($[2M+Na]^+$, 30), 749 ($[M+Na]^+$, 100). – **(-)-ESI-MS**: *m/z* = 725 $[M-H]^-$. – **(+)-HRESI-MS**: *m/z* 727.3176330 $[M+H]^+$, (calcd 727.3176353 for $C_{31}H_{48}N_8O_{10}Cl$), 749.2995570 $[M+Na]^+$ (calcd 749.299575 for $C_{31}H_{47}N_8O_{10}Cl$ Na). – **CID- MS/MS**, see Figure 138.

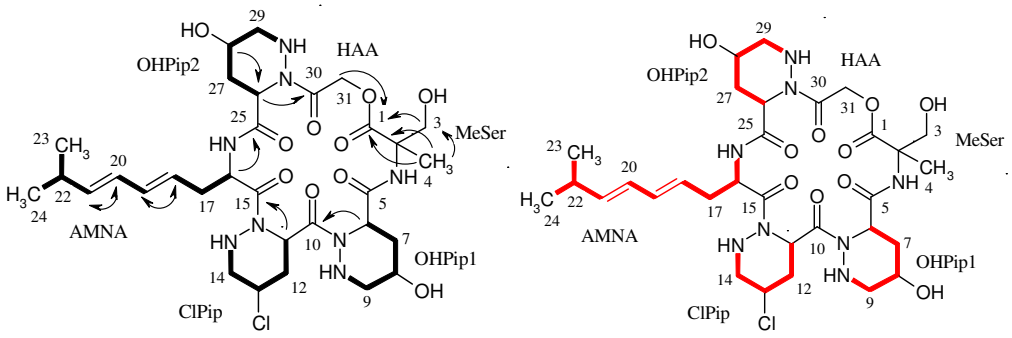


Figure 181: (▬, ↔) HH COSY, (▬, =) TCOSY and selected (→) HMBC coupling in Piperazimycin A (**254**)

Piperazimycins B (255): $C_{31}H_{47}N_8O_9Cl$ (710), white solid, as UV absorbing, showed the same colour reaction as **254** with chlorine/tolidine reagent. **–R_f** = 0.33 (CH_2Cl_2/5% MeOH). – **(+)-ESI MS**: *m/z* (%) = 1443 ($[2M+Na]^+$, 28), 733 ($[M+Na]^+$, 100). – **(-)-ESI MS**: *m/z* (%) = 755 ($[M+HCOO]^-$, 100), 709 ($[M-H]^-$, 60). – **(+)-HRESI MS**: *m/z* = 711.3227100 $[M+H]^+$, (calcd. 711.3227153 for $C_{31}H_{48}N_8O_9Cl$), 709.3069980 $[M-H]^-$ (calcd. 709.308154 for $C_{31}H_{46}N_8O_9Cl$). – **CID-MS/MS**, see Figure 138.

9.19 Marine *Streptomyces* sp. B7729

The marine *Streptomyces* sp. B7729 showed white colonies characteristic for the *Streptomyces* sp. by incubation on agar plates using M_2^+ medium at 28 °C (3 days). The well-developed colonies were used to inoculate 12 of 1L-Erlenmeyer flasks each containing 250 ml of M_2^+ medium. Fermentation was carried out on a shaker at 95 rpm for 7-days at 28 °C. The obtained yellowish-brown broth was harvested, lyophilized and the residue was exhaustively extracted (3 times) by ethyl acetate.

9.19.1 Pre-screening

For biological screening, the extract was tested against a set of microorganisms using agar diffusion test method. The extract showed high activity against two Gram-positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus*, one Gram-negative bacteria; *Escherichia coli* and one yeast; *Candida albicans* (Table 79). Also, it was subjected to antitumor assays, in which it exhibited *in vitro* antitumor activity against set of cancer cells at very low concentrations (Table 45). During TLC, the chemical screening of the extract exhibited two UV absorbing bands (254 nm), and one yellow band in day light, which turned as brown by treatment with H_2SO_4.

Table 79: Antimicrobial activity of the crude extract from the marine *Streptomyces* sp. B7729 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	28
Staphylococcus aureus	20
Streptomyces viridochromogenes (Tü 57)	0
Escherichia coli	15
Candida albicans	11
Mucor miehei (Tü 284)	0
Chlorella vulgaris	0
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.19.2 Fermentation, Extraction and Isolation

Well grown agar plates of the marine *Streptomyces* sp. strain B7729 were divided into small pieces and inoculated into 96 of 1-L Erlenmeyer flasks, each containing 250 ml, and allowed to ferment on shaker using M_2^+ medium (for 7 days, at 28 °C). The obtained yellow culture broth was filtered on Celite. The filtrate was chromatographed on XAD-16 followed by extraction with MeOH. The methanol extract was evaporated *in vacuo*, and the resulted aqueous solution was re-extracted with ethyl acetate. The mycelial cake was exhaustively extracted (4 times) by ethyl acetate, and the last time by acetone. The ethyl acetate extracts were combined and concentrated *in vacuo* to dryness. The yielded yellowish brown extract (6.97 g) was then chromatographed on silica gel column (100 x 3 cm) and eluted by CH_2Cl_2-MeOH gradient (CH_2Cl_2, 1.5 L; 2% MeOH, 1 L; 5% MeOH, 1.5 L; 10% MeOH, 0.5 L; 20% MeOH, 0.5 L; 40% MeOH, 0.5 L; 100% MeOH, 0.5 L). After monitoring by

TLC, five fractions were obtained: **I** (2.1 g), **II** (0.3), **III** (0.3 g), **IV** (1.21 g) and **V** (0.21). Purification of fraction III on PTLC (CH_2Cl_2/5% MeOH) and Sephadex LH-20 (CH_2Cl_2/40% MeOH) delivered cinnamic acid (**260**; 40.6 mg, colourless solid). On fractionation of fraction IV using Sephadex LH-20 (CH_2Cl_2/50% MeOH), followed by preparative TLC (CH_2Cl_2/3% MeOH) lead to isolation of polyisopropylenglycol (**261**; 37.7 mg, colourless oil), and a mixture of bright-yellow solid (11.3 mg) of lajollamycin (**262**) and 4',5'-dihydro-Lajollamycin (**263**). Purification of the polar fraction V by PTLC (Cyclohexane/90% EtOAc), followed by Sephadex LH-20 (CH_2Cl_2/40% MeOH) afforded enterocin (**257**; 43.8 mg, colourless solid), (Figure 139).

Enterocin (257): $C_{22}H_{20}O_{10}$ (444), colourless solid, UV absorbing (254 nm), turned as yellowish-green by anisaldehyde/H_2SO_4, and as pale red by Ehrlich's reagent. – R_f = 0.21 CH_2Cl_2/10% MeOH), 0.29 (Cyclohexene/85% EtOAc). – R_t = 8.4 min (LC-MS). – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 7.92 (d, ^3J = 7.7 Hz, 2H, 17-H, 17'-H), 7.57 (t, ^3J = 7.5 Hz, 1H, 19-H), 7.46 (t, ^3J = 7.4 Hz, 2H, 18-H, 18'-H), 6.39 (dd, J = 2.2, 0.5 Hz, 1H, 11-H), 5.62 (d, ^4J = 2.2 Hz, 1H, 13-H), 4.79 (m, 1H, 6-H), 4. 72 (d, ^3J = 2.7 Hz, 1H, 9-H), 4.69 (s, 1H, 3-H), 4.65 (d, ^3J = 4.5 Hz, 1H, 5-H), 3.86 (s, 3H, 12-OCH_3), 2.63 (dd, ^2J = 14.6 Hz, ^3J = 3.0 Hz, 1H, 7-H_{ax}), 1.86 (dd, ^2J = 14.6 Hz, ^3J = 2.9 Hz, 1H, 7-H_{eq}). – **^{13}C NMR** (CD_3OD, 125 MHz): δ = 197.7 (C_q-15), 175.6 (C_q-1), 173.4 (C_q-12), 167.1 (C_q-14), 162.2 (C_q-10), 140.6 (C_q-16), 134.1 (CH-19), 129.5 (CH-18, CH-18'), 129.4 (CH-17, CH-17'), 107.2 (CH-11), 89.0 (CH-13), 80.7 (C_q-2), 79.9 (CH-6), 77.5 (C_q-8), 77.3 (C_q-4), 71.0 (CH-5), 57.0 (CH-3), 56.5 (12-OCH_3), 54.5 (CH-9), 36.5 (CH_2-7). – **(+)-ESI-MS**: *m/z* (%) = 911 ($[2M+Na]^+$, 100), 467 $[M+Na]^+$, 10). – **(-)-ESI-MS**: *m/z* (%) = 887 ($[2M-H]^-$, 100), 489 ($[M+HCOO]^-$, 48). 443 ($[M-H]^-$, 40). –**HRESI MS**: *m/z* (%) = 443.0983350 $[M-H]^-$ (calcd. 443.0983647 for $C_{22}H_{19}O_{10}$).

Lajollamycin (262): $C_{36}H_{53}N_3O_{10}$ (687), bright-yellow solid, brown with sulphuric acid. **– R_f** = 0.12 (CH_2Cl_2/5% MeOH). **– ^{1}H** and **^{13}C NMR**, see Table 36. **– (+)-ESI MS**: *m/z* = 710 $[M + Na]^+$. **– (-)-ESI MS**: *m/z* = 686 $[M-H]^-$. **–HRESI MS**: *m/z* 688.3805680 $[M+H]^+$, ([calcd. 688.3803553 for $C_{36}H_{54}N_3O_{10}$). **– (+)-ESI MS/MS:** *m/z* = 710.3 $[M+Na]^+$, 690.3, 666.3, 663.3, 619.4, 575.5, 549.2, 445.1,

429.0, 375.2. **– (-)-ESI MS/MS:** *m/z* = 686.4 $[M-H]^-$, 667.4, 639.5, 465.3, 392.9, 361.3. **– HMBC**, see Figure 143.

4', 5'-Dihydro-lajollamycin (263): $C_{36}H_{55}N_3O_{10}$ (689), bright-yellow solid, brown with sulphuric acid. **–** R_f = 0.12 (CH_2Cl_2/5% MeOH). – R_t = 18.5 min (LC-MS). **– ^{1}H** and **^{13}C NMR**, see Table 36. **– (+)-ESI MS**: *m/z* = 712 $[M+Na]^+$. **– (-)-ESI MS**: *m/z* = 688 $[M-H]^-$. **–HRESI MS**: *m/z* 690.3961770 $[M+H]^+$, ([calcd. 690.396005 for $C_{36}H_{56}N_3O_{10}$). **– (+)-ESI MS/MS:** *m/z* = 712.3 $[M+Na]^+$, 668, 621, 589, 575, 552, 445, 429, 401, 375, 358. **– (-)-ESI MS/MS:** *m/z* = 688 $[M-H]^-$, 641, 534, 465, 395, 361 (see Figure 142). **– HMBC**, see Figure 143.

9.20 Marine *Streptomyces* sp. B9054

The marine *Streptomyces* sp. B9054 exhibited a pink aerial mycelium and the surrounding agar was stained as red. This appeared after cultivation of the strain on agar plates at 28 °C for 72 hours using M^+_2 medium.

9.20.1 Pre-screening

Well grown agar plates of the strain were served to inoculate 12 of 1L-Erlenmeyer flasks each containing 200 ml of M^+_2 medium. Fermentation was carried out on a shaker at 95 rpm for 3-days at 28 °C. After harvesting, red broth was collected and lyophilized followed by an exhaustive extraction using ethyl acetate. Chemical screening of the extract visualized by TLC analysis, showed several unpolar reddish-orange bands, appeared as UV orange-fluorescence, and turned as blue by treatment with 2N NaOH. Based on Agar diffusion test, the extract showed moderate antibacterial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Chlorella sorokiniana* as shown in Table 80.

Table 80: Activity of the crude extract from strain B9054 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Escherichia coli	15
Bacillus subtilis	16
Staphylococcus aureus	15
Streptomyces viridochromogenes	18

9.20.2 Fermentation and Isolation

The marine isolate *Streptomyces* sp. B9054 was inoculated from its soil culture on three M_2^+ agar plates. After incubation for 72 hours at 28 °C, the well-developed colonies were used to inoculate 96 1 liter-Erlenmeyer flasks, each containing 250 ml of M_2^+ medium. The cultures were shaken at 110 rpm for 5 days at 28 °C. The red culture broth obtained was mixed with *ca.* 1 kg diatom earth, passed through a pressure filter and the both filtrate and residue were extracted with ethyl acetate, and one time with acetone for the mycelial cake. The acetone extract was concentrated to a small volume *in vacuo* and then extracted with EtOAc. As the TLC of both extracts showed similar compositions, they were combined, and evaporated at 38 °C under vacuum to yield 1.32 g of a reddish-brown crude extract.

9.20.3 Isolation and Purification

The crude extract was subjected to column chromatography (3 x 60 cm, 220 g silica gel), the column being eluted with CH_2Cl_2-MeOH gradient (0 to 10% MeOH) and divided into six fractions, after TLC monitoring: fraction I (20 mg), II (30 mg), III (70 mg), IV (40 mg), V (130 mg), VI (200 mg). Purification of fraction I using silica gel (CH_2Cl_2) and Sephadex LH-20 (CH_2Cl_2/40% MeOH) yielded η-Pyrromycinone (**267**, 3.5 mg). As the same, fraction II afforded ζ-pyrromycinone (**269**, 6.5 mg) and cinerubin Y (**271**, 2.1 mg). Fraction III gave cinerubin X (**272**, 15 mg) after purification on silica gel column chromatography (CH_2Cl_2/2.5% MeOH) followed by Sephadex LH-20 (CH_2Cl_2/40% MeOH). From fraction IV, 3 mg of cinerubin K (**276**, 3.0 mg) was obtained as red solid after purification during PTLC followed by Sephadex LH-20 (2 x 100 cm, CH_2Cl_2/40% MeOH). Finally, cinerubin B (**273**, 57 mg) cinerubin A (**275**, 25 mg) were obtained from fractions V and VI, alternatively (Figure 144).

9.20.4 Biological Activity

Antibacterial and antifungal activities of the isolated compounds (**267**, **269**, **271~273**, **275** and **276**) were *semi* quantitatively determined using the agar diffusion method with 9 mm paper discs and 20 µg /disk. Only cinerubin A (**275**) exhibited activity against all the tested microorganisms, while cinerubin B (**273**) showed only activity against *Staphylococcus aureus* (Table 81).

Table 81: Antibacterial and antifungal activities of tetracyclins**273** and **275** in agar diffusion test at 20 μg/disk (∅ of inhibition zones, mm).

Compounds	BS[a]	SA[b]	SV[c]	EC[d]	CA[e]	MM[f]
Cinerubin B (**273**):	0	10	0	0	0	0
Cinerubin A (**275**):	12	12	15	12	10	14

[a]*Bacillus subtilis*, [b]*Staphylococcus aureus*, [c]*Streptomyces viridochromogenes* (Tü 57), [d]*Escherichia coli,* [e]*Candida albican*, [f]*Mucor miehi*.

η-Pyrromycinone (267): $C_{22}H_{16}O_7$ (392), red powder, on TLC under UV orange fluorescent, which turned to violet with 2N NaOH. **– R_f** = 0.64 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 13.84 (s, 1H, OH), 13.10 (s, 1H, OH), 12.31 (s, 1H, OH), 8.52 (d, ^3J = 9 Hz, 1H, 8-H), 8.25 (s, 1H, 11-H), 7.60 (d, ^3J = 9 Hz, 1H, 7-H), 7.28 (s, 2H, 2-H, 3-H), 4.10 (s, 3H, $COOCH_3$), 2.81 (m, 2H, 13-H_2), 1.32 (t, ^3J = 9.4 Hz, 3H, 14-H_3). **– (+)-ESI MS:** *m/z* = 807 ($[2M+Na]^+$). **– (-)-ESI MS:** *m/z* (%) = 805 ($[2M-2H+Na]^-$, 100), 391 ($[M-H]^-$, 58).

ζ-Pyrromycinone (269): $C_{22}H_{20}O_8$ (412), orange-red solid, turned to violet with NaOH. **– R_f** = 0.55 (CH_2Cl_2/5% 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* = 847 $[2M+Na]^+$. **– (-)-ESI-MS:** *m/z* (%) = 845 ($[2M-2H+Na]^-$, 100), 411 ($[M-H]^-$, 65).

Cinerubin Y (271): $C_{40}H_{46}O_{16}$ (782), orange-red solid, UV (366 nm) orange fluorescence, turned to blue-violet with 2N NaOH. **– R_f** = 0.80 (CH_2Cl_2/4% MeOH). - **^{1}H NMR** ($CDCl_3$, 600 MHz): δ = 12.97 (s, 1-OH), 12.80 (s, 6-OH), 12.25 (s, 4-OH), 7.73 (s, 1 H, 11-H), 7.34 (d, ^3J = 9.3 Hz, 1 H, 2-H), 7.32 (d, ^3J = 9.4 Hz, 3-H), 5.42 (d, ^3J = 3.3 Hz, 1 H, 1'-H), 5.32 (d, ^3J = 2.4 Hz, 7-H), 5.05 (d, ^3J = 2.7 Hz, 1H, 1'''-H), 5.20 (d, ^3J = 3.5 Hz, 1H, 1''-H), 4.58 (s, 1 H, 9-OH), 4.13 (s, 1 H, 10-H), 4.10 (m, 1H, 5'-H), 4.80 (m, 1H, 5'' -H), 4.05 (s, 1H, 4''-H), 4.00 (m, 1H, 2'''-H), 3.99 (m, 1H, 5'''-H), 3.72 (s, 3 H, 15-OCH_3), 3.57 (s, 1H, 4'-H), 2.55 (dd, ^3J = 4.29 Hz, J = 15.0 Hz, 1H, 8-Ha), 2.55 (m, 2 H, 3'''-H_2), 2.37 (d, J = 15.1 Hz, 1 H, 8-Hb), 2.04 (m, 1H, 2'-Ha), 1.75 (m, 1H, 2''-Ha), 1.74 (m, 2H, 3'-H_2), 1.73 (m, 1 H, 13-Ha), 1.67 (m,

3 H, 2'-Hb, 3"-H_2), 1.54 (m, 1H, 2"-Hb), 1.49 (m, 1 H, 13-Hb), 1.25 (d, ^3J = 6.5 Hz, 3H, 6"-H_3), 1.20 (d, ^3J = 6.6 Hz, 3H, 6"'-H_3), 1.16 (d, ^3J = 6.7 Hz, 3H, 6'-H_3), 1.08 (t, ^3J = 7.3 Hz, 3H, 14-H_3). **– ^{13}C/APT NMR** ($CDCl_3$, 150 MHz): see Table 37. **– (+)-ESI MS:** *m/z* (%) = 1587 ([2M+Na]$^+$, 100), 805 ([M+Na]$^+$, 98). **– (-)-ESI MS:** *m/z* (%) = 1585 ([2M-2H+Na]$^-$, 85), 781 ([M-H]$^-$, 100). **– (+)-ESI MS/MS:** *m/z* = see Figure 147.

Cinerubin K (276): $C_{40}H_{50}O_{15}$ (770), red powder, turned to violet by treatment with 2N NaOH, and as an UV orange fluorescent (366 nm). **–R_f** = 0.73 (CH_2Cl_2/4% MeOH), 0.34 (CH_2Cl_2/2% MeOH). **– $[\alpha]_D^{20}$** = + 105 (0.566 mg/ ml MeOH). **– UV/VIS** (MeOH): λ_{max} (log ε) = 258 (3.92), 288 (3.64), 300 (3.58), 483 (3.69), 495 (3.62), 514 (3.52), 529 (3.45) nm. **– IR** (KBr): ν_{max} = 3430, 2925, 1734, 1654, 1458, 1384, 1293, 1262, 1165, 1116, 1023, 803 cm^{-1}. – **^{1}H** and **^{13}C/APT NMR**, see Table 38. – **(+)-ESI-MS:** *m/z* (%) = 1563 [2M+Na]$^+$, 80) 793 [M+Na]$^+$, 100). – **(-)-ESI-MS:** *m/z* (%) = 1562 [2M+Na-H]$^-$, 74), 769 [M-H]$^-$, 100). – **(+)-HRESI MS:** *m/z* 793.30408 [calcd 793.30473 for $C_{40}H_{50}O_{15}$+Na]$^+$. **– (+)-ESI MS/MS:** *m/z* = see Figure 150. – **H,H COSY** and **HMBC**, see .Figure 151.

9.21 Marine-derived *Streptomyces* sp. Mei6-1,2

The Marine-derived *Streptomyces* sp. Mei6-1,2 showed a white mycelium characteristic for the *Streptomyces* sp. by incubation on M_2^+ medium with 100% artificial sea water with addition of calcium carbonate media for 10 days at 28 °C.

9.21.1 Pre-screening

The chemical screening of the strain extract showed low polar yellow bands with an UV yellow-fluorescence (366 nm), turned to red by treatment with dilute sodium hydroxide, as indication of *peri*-hydroxy quinones along with some colourless solids by its cultivation on M_2^+ using 100% artificial sea water and 0.5 g/l Calcium carbonate.

Table 82: Antimicrobial activity of the crude extract from marine-derived *Streptomyces* sp. Mei6-1,2 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	10
Escherichia coli	10
Staphylococcus aureus	12
Streptomyces viridochromogenes (Tü57)	11
Candida albicans	-
Chlorella vulgaris	10
Chlorella sorokiniana	11
Scenedesmus subspicatus	11

Table 83: Cytotoxic activities of Boshracins A (**278**) and B (**280**).

Compound	Potency		Tumor selectivity		
	Mean IC_{50} [μg/ml]	Mean IC_{70} [μg/ml]	Selectivity*/Total	% selectivity	Rating**
Boshracin A (**278**):	>10	>10	0/36	0%	-
Boshracin B (**280**):	>10	>10	0/36	0%	-

* individual IC70 <1/3 mean IC70; e.g. if mean IC70 = 2.1 µM the threshold for above average sensitivity was IC < 0.7 µM, ** - (%selective = <4%); + (4%> %selective >= 10%); ++ (10%> %selective >= 20%); +++ (%selective >20%)

9.21.2 Fermentation and Isolation

The marine *Streptomyces* sp. Mei6-1,2 was fermented as 20 L (using M_2^+ containing 100% artificial sea water) on shaker culture (95 rpm) at 28 °C for 10 days. After harvesting, the obtained dark brown broth was mixed with Celite and applied to filtration with the aide of pressure filter press. The two phases, filtrate and mycelial cake were exhaustively extracted by ethyl acetate, and acetone, respectively. The acetone extract was concentrated under vacuum, and the resulting water residue was re-extracted by ethyl acetate. TLC monitoring allowed the combining of both organic extracts, which on evaporation *in vacuo*, gave 5.180 g of yellow oil extract. This extract was subjected to fractionation using column chromatography on Sephadex LH-20 (DCM/MeOH, 1:1) giving four fractions; FI (1.6 g), FII (0.35 g) and FIII (0.6 g). Re-fractionation of the yellow fraction I on Sephadex LH-20 (DCM/MeOH, 6:4) followed by silica gel column chromatography (eluted with cyclohexane-DCM, 1:1) gave two yellow crude components. These two components were purified by PTLC (cyclohexane-DCM, 2:1) giving two yellow solids of boshracins A (**278**, 12.1 mg) and B (**280**, 7.1 mg). Purification of fraction II using 2 x Sephadex LH-20

(DCM/MeOH, 6:4) followed by PTLC afforded boshracin C (**282**, 1.3 mg, a yellow solid). Purification of fraction III by PTLC (DCM-MeOH, 98:2) and Sephadex LH-20 (DCM/MeOH, 6:4) led to boshracin D (**284,** 2.7 mg, a yellow solid). Purification of the middle polar fraction IV using silica gel column (DCM/2% MeOH), PTLC (DCM/2% MeOH) and Sephadex LH-20 (DCM/MeOH, 6:4), respectively, yielded the bafilomycins B_1 (**110**; pale yellow solid, 6 mg), B_2 (**111**; pale yellow solid, 13 mg) and C_1-amide (**116**; white solid, 43 mg), (Figure 152)

9.21.3 Biological Activity

In the agar diffusion tests, boshracins A-D (**278**, **280, 282 and 284**) were inactive at 40 μg/disk against *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces viridochromogenes*, *Bacillus subtilis*, *Candida albicans*, and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

Boshracin A (278): $C_{19}H_{12}N_2O_4$ (304), yellow solid turned red by 2N NaOH. – R_f = 0.73 (cyclohexane/DCM, 1:1), 0.21 (cyclohexane/DCM, 2:1). – **UV** (MeOH): λ_{max} = 241, 259, 290, 452 nm. – **IR** (KBr): ν = 3447, 2922, 1671, 1616, 1595, 1461, 1385, 1244, 1210, 1025, 749 cm^{-1}. – **^{1}H** and **^{13}C/APT NMR**, see Table 39. – **EI MS**: *m/z* = 304 $[M]^{+\cdot}$. – **HREI MS**: *m/z* = 304.0775 (calcd. 304.07356 for $C_{19}H_{12}O_4$). – **H,H COSY** and **HMBC**, see Figure 155.

Boshracin B (280): $C_{19}H_{12}N_2O_4$ (304), yellow solid turned red by 2 N NaOH. – R_f = 0.77 (cyclohexane/DCM, 1:1). –**UV** (MeOH): λ_{max} = 243, 258, 285 (sh), 295 (sh), 451 nm. –**IR** (KBr): ν = 3448, 2924, 1617, 1471, 1385, 1275, 1060, 782 cm^{-1}. – **^{1}H** and **^{13}C/APT NMR**, see Table 39. – **(+)-EI MS**: *m/z* = 304 $[M]^{+\cdot}$. – **HREI MS**: *m/z* = 304.0775 (calcd. 304.07356 for $C_{19}H_{12}O_4$). – **H,H COSY** and **HMBC**, see Figure 155.

Boshracin C (282): $C_{17}H_{12}N_2O_5$ (296), yellow solid turned red by 2N NaOH. – R_f = 0.43 (DCM). –**UV** (MeOH): λ_{max} = 225, 254, 286, 428 nm. –**IR** (KBr): ν = 3440, 2926, 1703, 1674, 1605, 1596, 1451, 1374, 1374, 1273, 1090, 1054, 752 cm^{-1}. – **^{1}H** and **^{13}C/APT NMR**, see Table 40. –**EI-MS**: *m/z* (%) = 296 ($[M]^{+\cdot}$, 62), 281 ($[M\text{-}CH_3]^{+\cdot}$, 100), 254 ($[M\text{-}COCH_3]^{+\cdot}$, 16), 43 ($[COCH_3]^{+\cdot}$, 8). – **HREI MS**: *m/z* = 296.0685 (calcd. 296.06847 for $C_{17}H_{12}O_5$). – **H,H COSY** and **HMBC**, see Figure 157.

Boshracin D (284): $C_{19}H_{16}O_6$ (340), yellow solid turned red by 2 N NaOH. **– R_f** = 0.73 (DCM/2% MeOH). –**UV** (MeOH): λ_{max} = 227, 255, 288, 430 nm. –**IR** (KBr): ν = 3428, 2924, 1619, 1470, 1387, 1258, 1214, 1105, 1016, 755 cm^{-1}. – **$[\alpha]_D^{20}$** = + 60 (*c* 0.02, MeOH). **– ^{1}H** and **^{13}C/APT NMR**, see Table 40. (-)-**ESI MS**: *m/z* (%) = 339 [[M-H]$^-$, 100), 701 ([M-2H+Na]$^-$, 42). **– (+)-HRESI MS**: *m/z* = 363.0837870 (calcd. 363.08445 for $C_{19}H_{16}O_6Na$). **– H,H COSY, HMBC** and **NOESY**, see Figure 160.

Bafilomycin C_1-amide (116): $C_{39}H_{61}NO_{11}$ (719), white solid, UV absorbing, turned reddish-brown by spraying with anisaldehyde/sulphuric acid and heating.- **HPLC MS:** R_t (min) = 20 (H_2O/70% MeOH). - **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 7.00 (d, ^3J = 15.5 Hz, 3'-H), 6.82 (d, ^3J = 15.5 Hz, 2'-H), 6.70 (s, 1H, 3-H), 6.57 (dd, ^3J = 14.7 Hz, 10.8, 1H, 12-H), 6.02, 5.90 (s br, 1H, 4'-NH_2), 5.79-5.82 (td, ^3J = 10.0 Hz, ^4J =1.2 Hz, 2H, 5-H, 11-H), 5.59 (s br, 1H, OH), 5.15 (dd, ^3J = 14.7 Hz, ^4J = 9.3 Hz, 1H, 13-H), 5.02 (m, 1H, 21-H), 4.96 (dd, ^3J = 8.3 Hz, 1.5 Hz, 1H, 15-H), 4.68 (s br, 1H, OH), 4.13 (m, 1H, 17-H), 3.89 (dd, ^3J = 9.3 Hz, 8.3 Hz, 1H, 14-H), 3.62 (s, 3 H, OCH_3), 3.48 (dd, ^3J = 10.7 Hz, 2.0 Hz, 1H, 23-H), 3.30 (m, 1H, 7-H), 3.22 (s, 3 H, OCH_3), 2.55 (m, 1H, 6-H), 2.35 (dd, ^3J = 11.9, 4.7 Hz, 1H, 20-H_A), 2.10 (m, 2H, CH_2-9), 2.16 (m, 1H, 16-H), 1.97 (s, 3H, 4-CH_3), 1.92 (s, 3H, 10-CH_3), 1.88 (m, 1H, 8-H), 1.93 (m, 1H, 24-H), 1.78 (m, 1H, 18-H), 1.61 (m, 1H, 22-H), 1.29 (m, 1H, 20-H_B), 1.04 (d, ^3J = 6.8 Hz, 3H, 6-CH_3), 0.99 (d, ^3J = 6.8 Hz, 3H, 31-CH_3), 0.95 (d, ^3J = 6.8 Hz, 3H, 24-CH_3), 0.91 (d, ^3J = 6.8 Hz, 3H, 8-CH_3), 0.87 (d, ^3J = 6.8 Hz, 3H, 16-CH_3), 0.83 (d, ^3J = 6.8 Hz, 3H, 22-CH_3), 0.81 (d, ^3J = 6.8 Hz, 3H, CH_3-25). - **^{13}C NMR**, see Table 10. - **(+)-ESI MS:** *m/z* (%) = 1462 ([2M+Na]$^+$, 44), 742 ([M+Na]$^+$, 100). - **(-)-ESI MS:** *m/z* = 764 ([M+HCOO]$^-$).

9.22 Marine *Streptomyces* sp. Isolate Merv8102

9.22.1 Taxonomy, Fermentation and Isolation

The isolate Merv8102 was isolated from the sediments collected from the Mediterranean Sea on the Egyptian coast, and identified by Dr. M. El-Gendy and deposited in the Department of Chemistry of Natural Compounds, National Research Centre, Dokki, Egypt. Working up, isolation and identification, the entire bioactive constituent; essramycin (**288**) was fully described in our recent article[419].

Essramycin (288): $C_{14}H_{12}N_4O_2$ (268), colourless solid, UV absorbing, which turned pale yellow by spraying with anisaldehyde/sulphuric acid; Soluble in DMSO, MeOH, EtOH and EtOAc; Insoluble in hexane, benzene and H_2O. – $\boldsymbol{R_f}$ = 0.34 (CH_2Cl_2/5% MeOH), 0.52 (CH_2Cl_2/10% MeOH). **– Mp** = 219-221 °C. **– IR** (KBr): ν = 3350, 3080, 2950, 1695, 1645, 1610, 1520, 1456, 1382, 1270 cm^{-1} **– UV/VIS:** λ_{max} (log ε) = (MeOH): 244 (4.16), 277 (3.96); (MeOH/HCl): 243 (4.16), 271 (3.95); (MeOH/NaOH): 244 (4.17), 282 (4.05) nm. –**^{1}H** and **^{13}C NMR**, see Table 42. – **(+)-ESI MS:** *m/z* (%) = 291 ($[M+Na]^+$, 24), 539 ($[2M+Na]^+$, 100). – **(-)-ESI MS:** *m/z* (%) = 267 ($[M-H]^-$, 100), 535 ($[2M-H]^-$, 72), 557 ($[2M-2H+Na]^-$, 54). **– (+)-HRESI MS:** *m/z* = 269.103367 $[M+H]^+$, 291.085319 $[M+Na]^+$ (calcd. 269.103306 $[M+H]^+$, for $C_{14}H_{13}N_4O_2$ and 291.085246 $[M+Na]^+$, for $C_{14}H_{12}N_4O_2Na$).

9.23 Marine *Streptomyces* sp. Mei34

The marine *Streptomyces* sp. Mei34 formed a pale yellow aerial mycelium and the surrounding agar was stained pale yellow.

9.23.1 Pre-screening

The strain was cultivated (12 hrs, at 28 °C) as agar plates using M_2^+ medium (containing 0.5 g/l Calcium carbonate and 100% artificial sea water). These agar plates were served to inoculate 12 of 1L-Erlenmeyer flasks each containing 250 ml of the same M_2^+ medium, and fermented on a shaker (95 rpm) for 3-days at 28 °C. The pale-yellow broth obtained was harvested, followed by lyophilization, and the residue was extracted with ethyl acetate.

In the chemical screening, TLC of the EtOAc extract exhibited three polar bands, two of which turned to blue, while the third turned greenish-yellow on spraying with anisaldehyde/sulphuric acid. The HPLC-MS of the crude extract, showed a signal with mole peak of 705/707, for a chloride- containing compound. Based on the agar diffusion method, the extract showed a high antimicrobial activity against all tested microorganisms which are listed in Table 84.

Table 84: Antimicrobial activity of the crude extract from Mei34 (1 mg/ml).

Tested microorganisms	Inhibition zone ∅ [mm]
Escherichia coli	30
Bacillus subtilis	26
Staphylococcus aureus	33
Streptomyces viridochromogenes	24
Candida albicans	14
Mucor miehei	14
Chlorella vulgaris	21
Chlorella sorokiniana	22
Scenedesmus subspicatus	18

9.23.2 Fermentation of the Marine *Streptomyces* sp. Isolate Mei34

Well-developed colonies of Mei34 on agar plates were used to inoculate 100 1 liter-Erlenmeyer flasks each containing 250 ml of M_2^+ medium; same as the pre-screening. The latter was incubated for 10 days on shaker (95 rpm) at 28 °C. The resulting grey culture broth was mixed with *ca.* 1 kg diatomaceous earth, and passed through a pressure filter. Both the water and mycelia phases were extracted separately. The water phase was applied to XAD-2 extraction followed by MeOH/H_2O, and the resulting extract was evaporated, and the water residue was finally extracted by ethyl acetate. The biomass was extracted (3 times) with ethyl acetate followed by acetone (1 time). The acetone extract was concentrated, and the afforded water residue was also extracted with ethyl acetate. Both organic phases were combined, and evaporated to dryness, yielding 4.25 g of a greenish-brown crude extract.

9.23.3 Isolation and Purification

The extract was subjected to column chromatography (3 × 80 cm, 220 g silica gel). The column being eluted with CH_2Cl_2-MeOH gradient (0~50% MeOH) to deliver three fractions; I (50 mg), II (73 mg), III (230 mg). Further purification of the polar fraction III using Sephadex LH-20 (DCM/MeOH) and silica gel column chromatography led to pale yellow crystals of nafisamycin (**297**; 34 mg), an UV-absorbing substance (254 nm), which turned to greenish-yellow by spraying with anisaldehyde/sulphuric acid. Purification of fractions I and II applying the same method for **2**, afforded two colourless solids of 2'-deoxy-thymidine (15.3 mg) and 2'-deoxy-uridine (10.7 mg), respectively (Figure 166).

Table 85: Antimicrobial activities of Nafisamycin (**297**) in comparison with the strain extract (diameter of inhibition zones in mm).

	EC	BS	SV	SA	CA	MM	CV	CS	SS
Strain extract	30	26	24	33	14	14	21	22	18
Nafisamycin (**297**)	0	0	0	0	0	0	0	0	0

EC = *Escherichia coli*, SA = *Staphylococcus aureus*, SV = *Streptomyces viridochromogenes*, BS = *Bacillus subtilis*, CA = *Candida albicans*, MM = *Mucor miehei* (Tü284), CV = *Chlorella vulgaris*, CS = *Chlorella sorokiniana*, SS = *Scenedesmus subspicatus*.

Nafisamycin (297): $C_{34}H_{38}Cl_2N_2O_{10}$ (704). **– R_f** = 0.31 (CH_2Cl_2/10% MeOH). – ***RP*-HPLC** (R_t = 8.45 min; CH_3CN/H_2O). – **$[\alpha]^{25}_D$** = - 452 (*c* 0.1, MeOH). **– UV/VIS**: λ_{max} (log ε) = (MeOH): 289 (3.72), 327 (3.77); (MeOH/HCl): 288 (3.72), 336 (3.72); (MeOH/NaOH): 290 (3.67), 331 (3.77) nm. **– IR** (KBr): ν = 3489, 3421, 3078, 2928, 1732, 1645, 1578, 1483, 1449, 1373, 1299, 1233, 1133, 1090,1051, 936, 868, 583, 550, 479 cm^{-1}. **– CD** (λ^{MeOH} nm (θ): 331 (-54699), 276 (-3521), 245 (-74237), 230 (-35321), 224 (-38050), 208 (207159), 201 (74196) nm. **– ^{1}H** and **^{13}C/APT NMR,** see Table 43. **– (+)-ESI MS**: *m/z* = 705 $[M+H]^+$. **– (-)-ESI MS:** *m/z* (%) = 749 ($[M+HCOO]^-$, 100), 703 ($[M-H]^-$, 45). – **(+)-HRESI MS**: *m/z* 705.19737 (calcd. 705.197615 for $C_{34}H_{39}Cl_2N_2O_{10}$). – **(+)-ESI MS^2:** *m/z* = 705 $[M+H]^+$, 687 $[M-H_2O+H]^+$, 669 $[M-2H_2O+H]^+$, 500 $[M-C_9H_{18}NO_4$ (amino sugar)$+H]^+$. – **HMBC** and **NOESY**, see Figure 173 and Figure 176.

9.24 References

10 References

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Khaled Attia Shaaban Mahmoud

Lebenslauf

Am 01.01.1978 wurde ich in Karam, Tanah, El-Mansoura, Ägypten als Sohn des Khaled Attia Shaaban Mahmoud geboren.

Von 1984 bis 1990 besuchte ich die Al-Mogamma Grundschule in Tanah, El-Mansoura, Ägypten, anschließend von 1990 bis 1993 die Orientierungsstufe an der Al-Shinnawy Perschool in Tanah. 1993 wechselte ich auf das Al-Shinnawy-Gymnasium in Tanah, das ich mit dem Abitur 1996 verließ.

Ab 1996 studierte ich an der El-Mansoura-Universität in Ägypten an der Fakultät der Wissenschaft Chemie und schloss im Jahr 2000 mit dem B. Sc. Chemistry ab.

Im Institut für Organische & Biomolekulare Chemie der Georg-August-Universität Göttingen beschäftige ich mich seit Oktober 2002 unter der Anleitung von Herrn Prof. Dr. H. Laatsch mit dem Thema: "New Secondary Metabolites from Marine Bacteria: Barycin and 3,8-Dihydroxy-1-hydroxymethylanthraquinone" und fertigte meine Diplomarbeit im Januar 2005 an.

Im Rahmen dieser Arbeit war ich in der Zeit von Juli 2004 bis Januar 2005 in einem Kooperationsprojekt von Prof. Dr. H. Laatsch und Prof. Dr. M. Meiners, FH Oldenburg, zum Thema: "Isolation and Structure Elucidation of New Bioactive Secondary Metabolites from North Sea Streyptomycetes" angestellt.

Nach der Diplomprüfung im Januar 2005 begann ich mit meiner Doktorarbeit: "Nafisamycin, Cyclisation Product of a New Enediyne Precursor, Highly Cytotoxic Mansouramycins, Karamomycins Possessing a Novel Heterocyclic Skeleton and Further Unusual Secondary Metabolites from Terrestrial and Marine Bacteria". Als wissenschaftlicher Assistent betreute ich den theoretischen und praktischen Chemieunterricht für Biologiestudenten des 3. Semesters.

In diesen Zeitraum fällt auch der Auslandsaufenthalt in Abteilung von Prof. Dr. S. Qin, Institute of Oceanology, Chinese Academy of Sciences in Qingdao/China vom 27.03. bis 25.04.2007. Arbeitsschwerpunkt dort: "Isolation of Bioactive Secondary Metabolites from Marine Fungi" und ein von mir gehaltener Vortrag zum The-

ma: "Dereplication Methods and Unusual Secondary Metabolites from *Streptomyces* sp.".

Der sich anschließende Aufenthalt in Lexington, USA vom 02.11. bis 13.12.2007 im Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Abteilung Prof. Dr. J. Rohr hatte das Forschungsthema "I. Studies on Mithramycin Oxygenase MtmOIII, II. Screening and Working up of *Streptomyces* sp. EG6" zum Inhalt.

Göttingen, den 10.12.2008

Khaled Attia Shaaban Mahmoud

Publications List

[**1**] Hong-Xia Cui, Song Qin, **Khaled Shaaban** and Hartmut Laatsch, "Screening of marine *Streptomyces* with antibacterial activity from JiaoZhou Bay and studies on metabolites from strain M097", *Zhongguo Tianran Yaowu*, **2006**, *4* (4), 308-311.

[**2**] Hong-Xia Cui, **Khaled A. Shaaban**, Hartmut Laatsch, and Song Qin, "Two Anthraquinone Compounds from a Marine Actinomycete Isolate M097 Isolated from Jiaozhou Bay", *World J. Microbiol. Biotechnol*. **2006**, *22* (12), 1377-1379.

[**3**] Hongxia Cui, Fuchao Li, Binlun Yan, **Khaled Shaaban**, Song Qin, Hartmut Laatsch, "*Streptomyces* sp. M095 from Jiaozhou bay producing inhibitory-fungal antibiotic, holomycin", *Zhongguo Haiyang Yaowu*, **2006**, *25* (1), 11-15.

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[**5**] M. M. A. El-Gendy, M. Shaaban, A. M. EL-Bondkly and **K. A. Shaaban**, "Bioactive Benzopyrone Derivatives from New Recombinant Fusant of Marine *Streptomyces*", *Egypt. Pharm. J.* (*NRC*), **2007**, *6* (2), 177-191.

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[**8**] Mervat M. A. El-Gendy, Mohamed Shaaban, **Khaled A. Shaaban**, Ahmed M. El-Bondkly, Hartmut Laatsch, "Essramycin: A First Triazolopyrimidine Antibiotic Isolated from Nature", *J. Antibiot*. **2008**, *61* (3), 149-157.

[**9**] Mervat M. A. El-Gendy, M. Shaaban, A. M. El-Bondkly, **K. A. Shaaban**, "Bioactive benzopyrone derivatives from new recombinant fusant of marine *Streptomyces*", *Appl. Biochem. Biotechnol*. **2008**, *150* (1), 85-96

[**10**] Imran Sajid, **Khaled A. Shaaban**, Holm Frauendorf, Shahida Hasnain and Hartmut Laatsch, "Val-Geninthiocin: Structure Elucidation and MS^n Fragmentation of Thiopeptide Antibiotics Produced by *Streptomyces* sp. RSF18", *Z. Naturforsch*. **2008**, *63b*, 1223-1230.

[**11**] A. M. El-Bondkly, H. I. Abd-Alla, M. Shaaban and **Khaled A. Shaaban**, "The Electrospray Ionization - Mass spectra of Erythromycin A obtained from a marine *Streptomyces* sp. Mutant", *Ind. J. Pharm. Sci*. **2008**, *70* (3), 310-319.

[**12**] **Khaled A. Shaaban**, Mohamed Shaaban, Petrea Facey, Serge Fotso, Elisabeth Helmke and Hartmut Laatsch, "Electrospray Ionization-Mass Spectra of Piperazimycins A and B and γ-Butyrolactones from Marine-derived *Actinomycete* sp.", *J. Antibiot*. **2008**, *61* (12), 736-746.

[**13**] Howaida I. Abd-Alla, Mohamed Shaaban, **Khaled A. Shaaban**, Nagat S. Abu-Gabal, Nagwa M. M. Shalaby and Hartmut Laatsch, "New Bioactive Compounds from *Aloe Hijazensis*", *Nat. Prod. Res.* (Accepted: May **2008**).

[**14**] Raoudha Ben Ameur Mehdi, **Khaled A. Shaaban**, Ines Karray-Rebai, Slim Smaoui, Samir Bejar and Lotfi Mellouli, "Five naturally bioactive molecules including two rhamnopyranoside derivatives isolated from the *Streptomyces* sp. strain TN58", *Nat. Prod. Res.* (Accepted: Juli **2008**).

[**15**] I. Sajid, C. B. Fotso Fondja Yao, **K. A. Shaaban**, S. Hasnain and H. Laatsch, "Antifungal and antibacterial activities of indigenous *Streptomyces* isolates from saline farmlands: prescreening, ribotyping and metabolic diversity", *World J. Microbiol. Biotechnol*. (Accepted: November **2008**).

[**16**] H. Rahman, M. Shaaban, **K. A. Shaaban**, M. Saleem, E. Helmke, I. Grün-Wollny, and H. Laatsch, "Secondary Metabolites from Streptomycetes B7967 and GW 5127", *Nat. Prod. Comm.* (Submitted: November **2008**).

[**17**] **Khaled A. Shaaban**, Mohamed Shaaban, Hnin Yu Win, Lei Lei, Elisabeth Helmke, Heidrun Anke and Hartmut Laatsch, "Structure Elucidation and Synthesis of Isatins from Streptomycetes", *Eur. J. Org. Chem.* (Submitted: November **2008**).

[**18**] U. W. Hawas, M. Shaaban, **Khaled A. Shaaban**, M. Speitling, M. Meiners, H. Laatsch, "Mansouramycins A~E, novel Isoquinoline Quinones from Marine Streptomycetes", *J. Nat. Prod.*, submitted **2008**.

[**19**] M. Shaaban, **K. A. Shaaban**, M. Speitling, E. Helmke, H. Laatsch, "Mansouramycins F-I, further Isoquinolinequinones from Marine Streptomycetes", *J. Antibiot*, to be submitted **2008**.

[**20**] **K. A. Shaaban**, R. Al-Salman, M. Al Zoubi, D. Schröder, E. Helmke, I. Grün-Wollny, H. Laatsch. New γ-Butyrolactones from marine and terrestrial *Streptomyces* isolates, *Nat. Prod. Comm.*, submitted **2008**.

Poster Prize

- **Khaled A. Shaaban**, M. Shaaban, M. Meiners und H. Laatsch. Aktuelle Entwicklungen in der Naturstoff-Forschung, 18-Irseer Naturstofftage der DECHEMA e.V., 22-24 Feb. **2006**: Nafisamycin, ein neues En-diin-Derivat aus einem Nordsee-Streptomyceten. This poster was awarded "Best Poster of the 18-Irseer Naturstofftage der DECHEMA e.V. with best compound structure". See Newsletter No. 4, der Fakultät für Chemie, Mai **2006**, page 5 (http://www.uni-goettingen.de/de/sh/28469).

- **K. A. Shaaban**, M. Shaaban, H. Rahman, I. Grün-Wollny and H. Laatsch. Aktuelle Entwicklungen in der Naturstoff-Forschung, 19-Irseer Naturstofftage der DECHEMA e.V., 21-23 Feb. **2007**: Karamomycine, komplexe Heterocyclen aus dem Schüttelkolben. This poster was awarded "Best Poster of the 19-Irseer Naturstofftage der DECHEMA e.V. with best compound structure". See 7th Newsletter der Fakultät für Chemie, Dezember **2007**, Page 5 (http://www.uni-goettingen.de/de/sh/28481).

- Imran Sajid, **Khaled A. Shaaban**, Shahida Hasnain and H. Laatsch. Val-Geninthiocin: A New Thiopeptide Antibiotic Produced by *Streptomyces* sp. RSF18. Institute of Organic and Biomolecular Chemistry, University of Goettingen, Tammannstrasse 2, D-37077 Göttingen, Germany; 1st Göttinger Chemie-Forum, June 29th, **2007**. This poster was awarded "Best Poster of the 1st Göttinger Chemie-Forum".

- **Khaled A. Shaaban**, DAAD Prize of the best researcher in Göttingen University, December **2008**; Nr. 278/2008-05.12.2008 (See http://www.uni-goettingen.de/de/3240.html?cid=3149)