Serge Fotso

Highly Cytotoxic Kettapeptin, Bhimamycins Possessing Unusual Chromophores and Further New Secondary Metabolites from Terrestrial and Marine Bacteria

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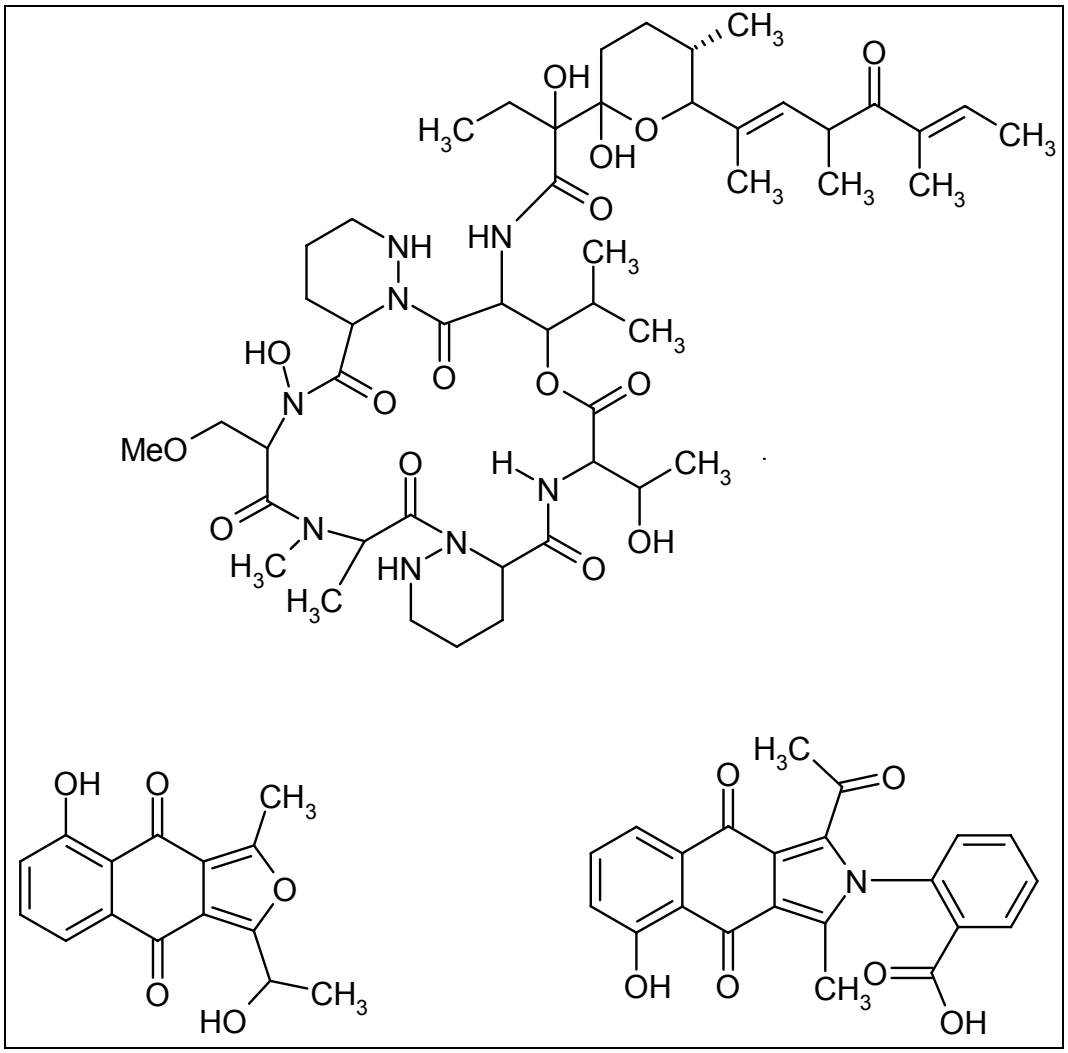

Dissertation

Highly Cytotoxic Kettapeptin, Bhimamycins Possessing Unusual Chromophores and Further New Secondary Metabolites from Terrestrial and Marine Bacteria

Dissertation

zur Erlangung des Doktorgrades

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der Georg-August-Universität zu Göttingen

vorgelegt von

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aus

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D7

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

Für meine Eltern und
meine Verlobte

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1 Introduction

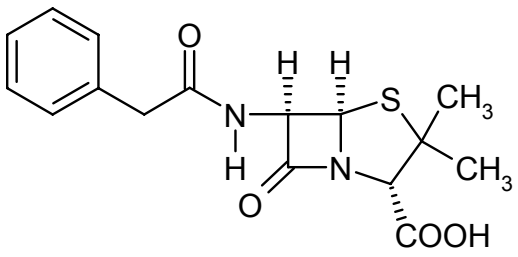
Nature is a source of medicinal agents since thousands of years, and an impressive number of modern drugs has been isolated from natural habitats, many based on their use firstly in traditional medicine and later in the developed countries as single agent drugs on a scientific basis. In the past six decades, however, microorganisms have played an increasing role in the production of antibiotics and other drugs for the treatment of some serious diseases.[1] The increase of new diseases such as AIDS, Ebola and SARS, the development of resistance in infectious microorganisms to existing drugs, and the menacing presence of resistant organisms requires the discovery and development of new drugs.[2] Microorganisms and especially marine bacteria are being examined as a source of novel antibiotics that are active against antibiotic resistant human pathogens.

1.1 New drugs from the Sea

Natural products are naturally derived metabolites and/or by-products from microorganisms, plants or animals.[3] Based on traditional medicine, these products have been exploited for human use and represent a rich source of biologically active compounds, a good source of novel and clinically important drugs and are an example of molecular diversity with recognized potential in drug discovery and development.[4-6]

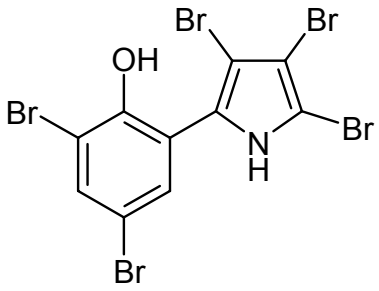
The marine environment covers a wide thermal range from below freezing temperatures in Antarctic waters to about 350 °C in deep hydrothermal vents, and a pressure range up to 1000 atm.[7] Its variability has facilitated extensive evolutionary process at all phylogenetic levels, from microorganisms to mammals. Despite this biodiversity in marine environment compared to plants, research on marine natural products is still in its infancy and to a large extent unexplored compared to the vast domain of explored terrestrial plant habitats, and this may be due to the lack of ethno-medical history and the difficulties involved in the collection of marine organisms, and the non-culturability of their majority.[8] Near the problem of collection, there are some advantages of having microorganisms as natural product sources: In principle one bacterial cell is enough for cultivation, fermentations are independent of climate and seasons, and in addition, by feeding experiments new metabolites may be generated.

According to Joffe about 25% of all pharmaceuticals sales are drugs derived from plants and an additional 12% are microbial produced natural products.[9] After Pasteur discovered that fermentation is caused by living cells, scientists seriously began to investigate microbes as sources for bioactive natural products. Then, scientific serendipity and the power of observation provided the impetus to Fleming with the discovery of the first antibiotic penicillin (**1**): A contamination by *Penicillium notatum* exhibited inhibitory effects against two Gram positive bacteria (*Staphylococcus* and *Streptococcus* sp.) on agar plates. Since then, scientists have been engaged with the discovery and application of microbial metabolites from different microorganisms like fungi, mushrooms, actinomycetes, etc. with activity against both plant and human pathogens.[10]



1

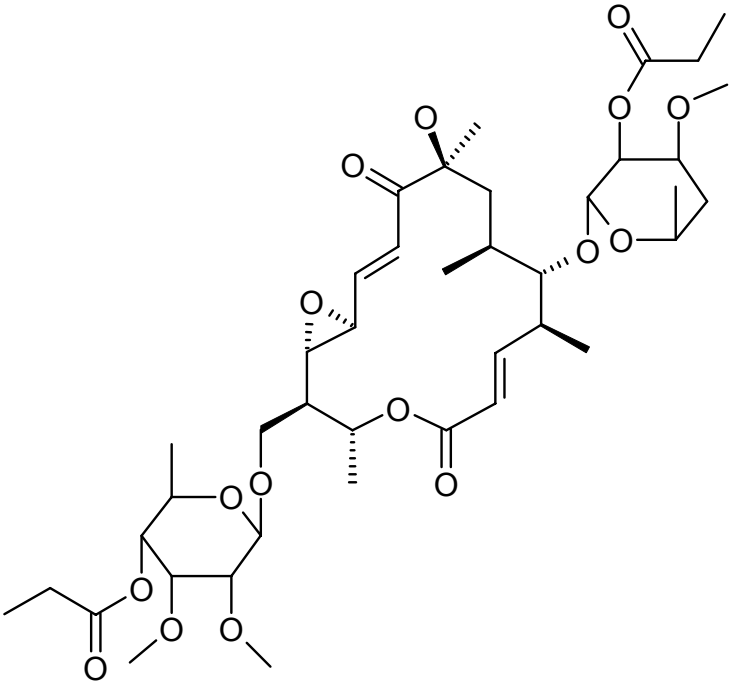
There is nothing new in using marine products for medicinal purposes, with seahorses being used for centuries in traditional treatments for sexual disorders, respiratory and circulatory problems, kidney and liver diseases, amongst other aliments, in China, Japan and Taiwan.[11] Marine natural products are small to medium molecular weight compounds produced by marine plants, invertebrates and microbes that have stimulated interdisciplinary studies by chemists and biologists.[12] They are collected from tropical and cold temperate ocean habitats and continue to be the subject of vigorous chemical investigation. Their extracts are being examined as a source of novel cytotoxic secondary metabolites that are potential leads for the development of new drugs. The first marine bacterial natural product to be reported was the highly brominated pyrrole antibiotic pentabromopseudiline (**2**) by Burkholder and coworkers in 1966 from a culture of *Pseudomonas bromoutilis*.[13] It shows impressive antibiotic activity against Gram-positive bacteria.



2

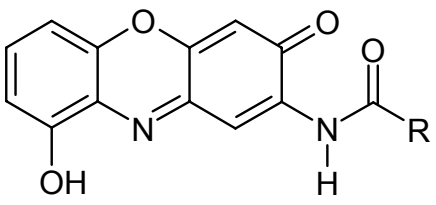
Although the research for marine natural products has been going on for only about 40 years, over 7000 compounds have been published and various reviews dealing with different aspects of the chemistry of marine natural products have appeared in the literature.[14-16] From 1969-1999 approximately 300 patents on bioactive marine compounds were issued, with hundreds of new compounds been discovered every year.

The rich variety of chemically novel and biologically active metabolites serves to indicate that marine bacteria are a source and big reservoir natural compounds of interesting and biologically active metabolites. Chalcomycin B (**3**) for example isolated from the marine *Streptomyces* sp. B7064 exhibited very strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Streptomyces viridochromogenes* (Tü 57).[17]



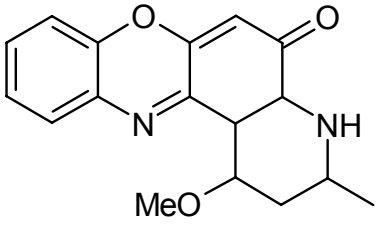
3

The phenoxazin-3-one antibiotics chandrananimycins A (**4a**), B (**5**) and C (**4b**) were isolated in our research group from the culture of *Actinomadura* sp. derived from the sediment from Jiaozhou Bay in China. Chandrananimycins A-C were active against human tumour cell lines CCL HT29 (colon carcinoma), MEXF 514 L (melanoma), LXFA 526L (lung carcinoma), MACL MCF-7(breast carcinoma) and PRCL PC3M, RXF 631L (kidney tumor) with IC_{70} values of ~1.4 μg/ml, while **C (4b)** exhibited potent activity against the fungus *Mucor miehei* and the bacteria *B. subtilis* and *E. coli*, and antialgal activity against the microalgae, *Chlorella vulgaris*, *C. sorokiniana* and *Scenedesmus subspicatus*.[18]



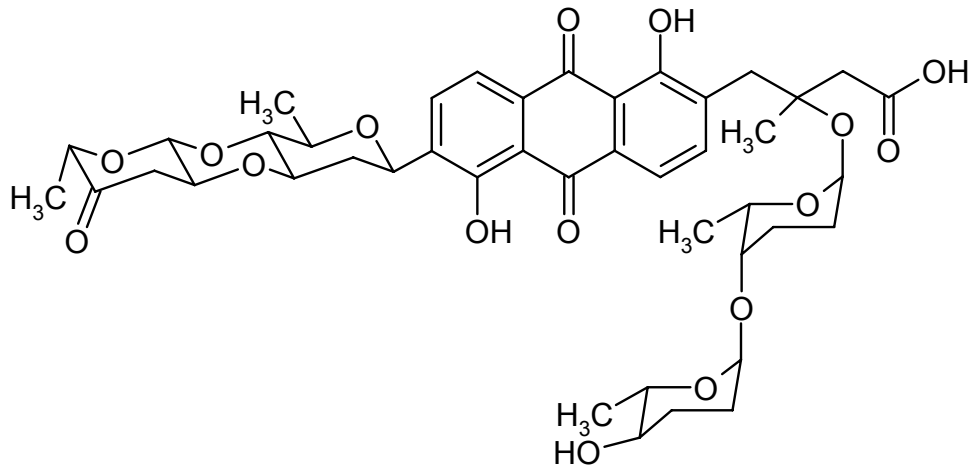
4a R= Me

4b R=CH_2OH



5

The himalomycins A (**6**) and B[19] (**7**) are C-glycosidic anthraquinones, which exhibited antibacterial and antitumor activities and were isolated from a marine *Streptomyces* sp. in our group. Compounds possessing C-glycosidic moieties are rather rare in nature compared with O-glycosides.



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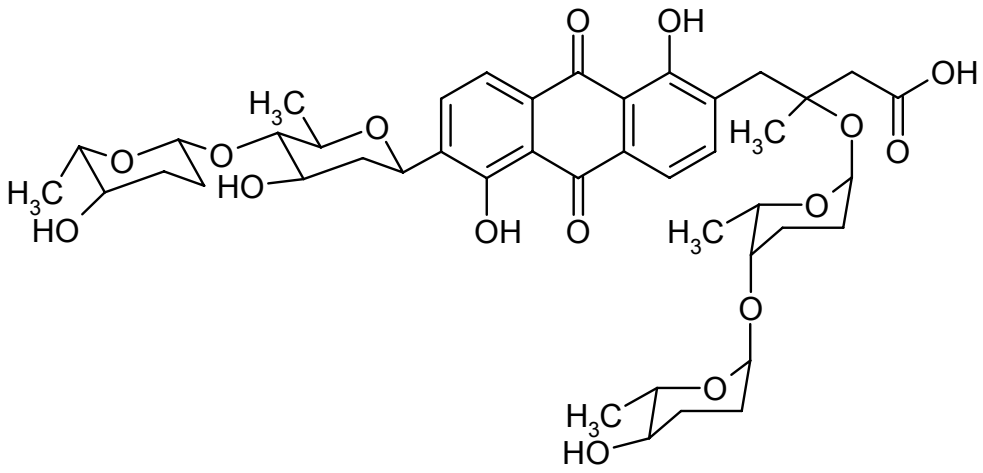
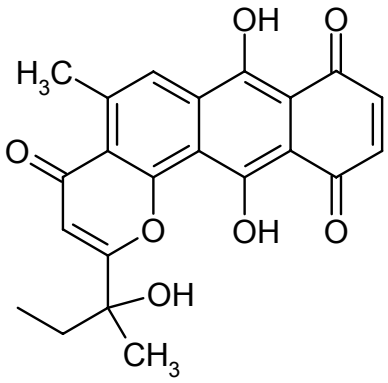


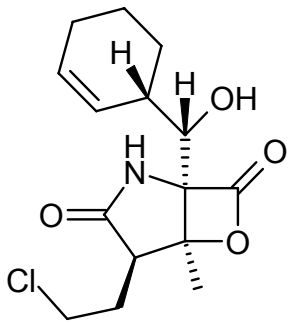
7

Parimycin (**8**), a new anthraquinone, was formed by a *Streptomyces* sp. isolated from sediment of the Laguna de Terminos, Gulf of Mexiko. Parimycin (**8**) had moderate activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Streptomyces viridochromogenes* (Tü 57) in addition to activity against a number of human tumor cell lines.[20]



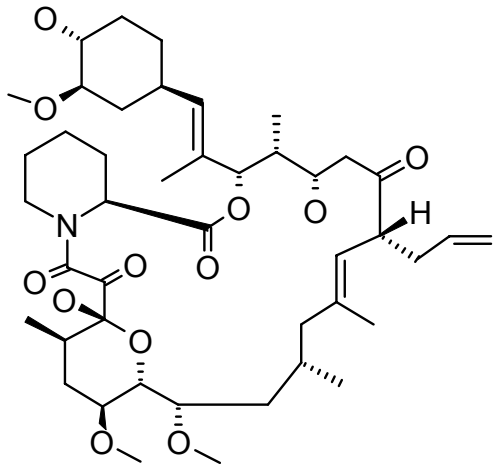
8

From a culture of the marine actinomycete *Salinospora* sp., the potent protease inhibitor salinosporamide A **(9)** was isolated and its structure including the absolute stereochemistry deduced by X-ray crystallography and spectral analyses. Salinosporamide A **(9)** showed a potent and selective *in vitro* cytotoxicity against various cell lines. It s also exhibited highly potent inhibition of the proteasomal chymotrypsin-like proteolytic activity of purified 20S proteasome. The unique functionalisation of the core-fused γ-lactam-β-lactone bicyclic ring structure of salinosporamide A **(9)** appears to contribute to its potency.[21]



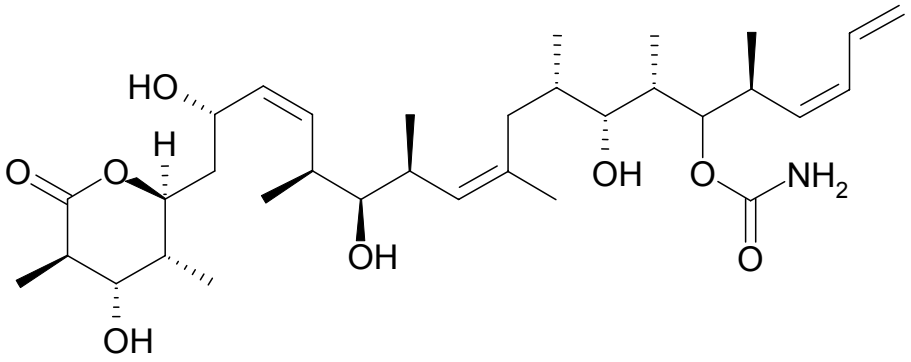
9

Various studies have shown that the metabolites obtained from microorganisms are structurally more diverse, and exhibit more interesting bioactivities compared to those of plant origin. Instead of monotonous alkaloids from plants, microorganisms produce many different classes of chemical structures, such as polyketides [e.g. FK506 (**10**)], terpenes, polysaccharides (e.g. acarbose), polyether, and nitrogen-containing compounds, such as indoles, peptides, pyrroles or glycopeptides.



10

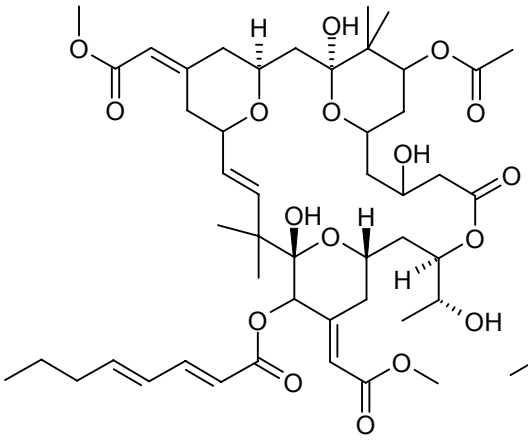
Marine bacteria have recently emerged as an entirely new source of structurally novel natural products for the development of new drug candidates.[22] It is noteworthy that presently published new antimicrobial compounds are to a great extend found in marine organisms, some of them got already into preclinical phase and may lead to pharmaceutical products.[10] Investigations are done with promising compounds, such as the polyhydroxylated lactone antitumor and immunosuppressive compound discodermolide (**11**) isolated from the deep-water sponge *Discodermia* sp.



11

Another example is the antineoplastic bryostatin-1 (**12**) isolated from the common fouling bryozoan *Bugula neritina*[23]. It is the first potent anticancer compound of marine origin, exhibited an *in vivo* and *in vitro* activity against solid tumors and showed also activity against leukemia. Ecteinascidin 743 (ET-743, **13**), a marine tetrahydro-isoquinoline isolated from *Ecteinascidia turbinata,* an inhabitant of Caribbean mangroves, contains a new skeleton of 9 cycles. It is active against B16

melanoma and shows a unique mode of action, which is different from that of other DNA-interacting drugs, constituting a new subclass of antitumor agents that could be active against resistant cell lines.[24] Recent research shows that ET-743 (**13**) may even be able to prevent tumors from becoming drug-resistant. Bryostatin (**12**) and ET-743 (**13**) are actually in phase II of clinical trials. Supply of material for clinical trials of bryostatin was a problem until it was demonstrated that *Bugula neritina* was suitable for aquaculture. Recently evidence favouring a symbiotic origin for bryostatin (**12**) have been presented,[25] opening the way to biotechnological manipulation of the biosynthetic genes.[26] It is important to note that the majority of marine natural products actually in trials or under clinical evaluation is produced by invertebrates such as sponges, tunicates, molluscs or bryozoans, but not algae.[27]



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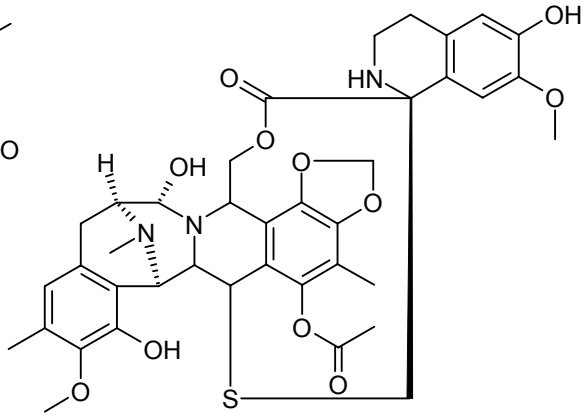


13

At the beginning, it was expected that marine bacteria may produce metabolites different from those from terrestrial habitats; however, it seems now that many metabolites from marine microorganisms and especially those from *Streptomyces* are derived from or are identical with those of terrestrial origin. It is therefore not easy to decide if a microorganism is truly marine. In some cases it is postulated that the stable relationship of a bacterial community associated with sponges or tunicates may corresponds to similarities in the natural product profiles of both host and guest.[27] Symbiotic and endophytic microbes associated with terrestrial and marine macroorganisms are supposed to be responsible for at least some metabolites in their hosts.

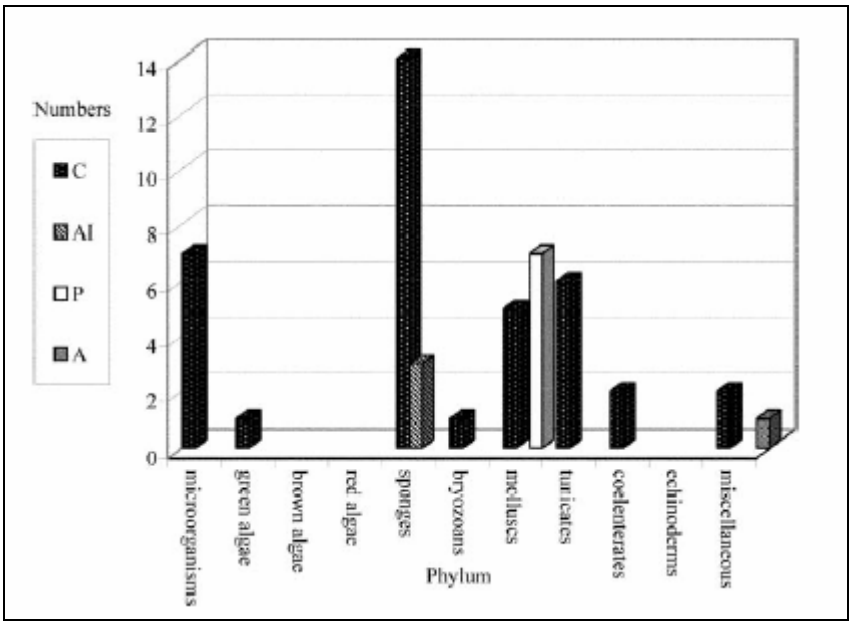
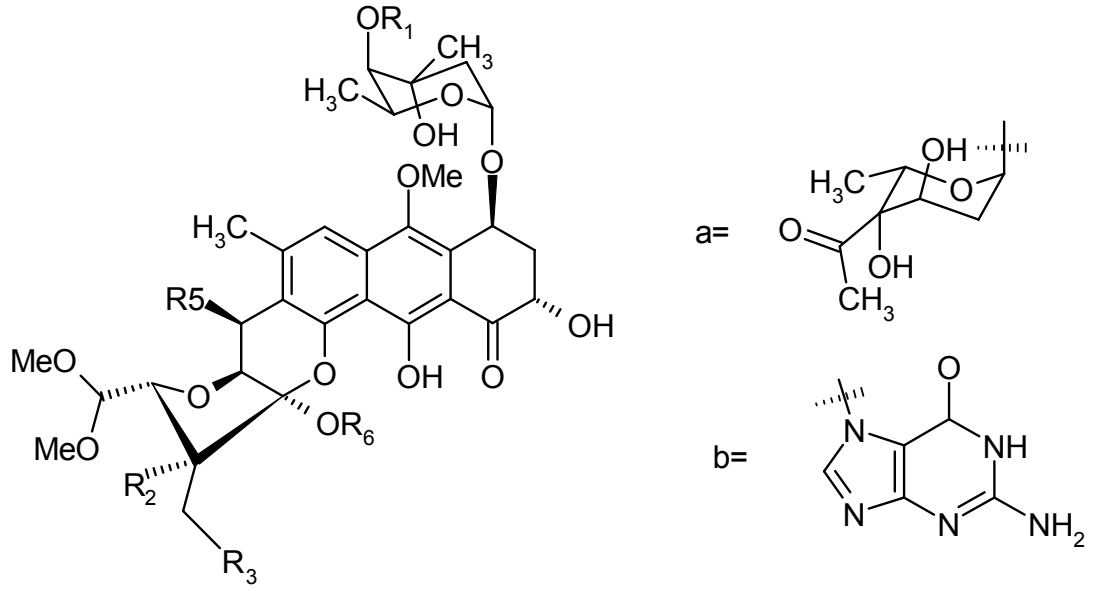


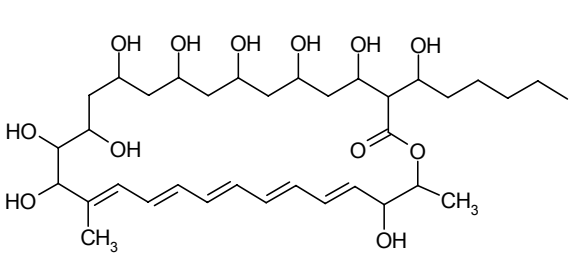
Figure 1: Number and distribution of marine and marine-derived compounds in clinical and Pre-clinical trials (C: anticancer drugs, AI: anti-inflammatory drugs, P: drugs for intractable pain, A: Alzheimer)[28]

Marine Streptomyces produced also complexe compounds like gutingimycin[29] (**14a**) and trioxacarcines D-F (**14b-c**) isolated from the strain *Streptomyces* B8652. The latest exhibited antitumor, antibacterial and high anti-malaria activity.[30] Among the trioxacarcines, trioxacarcine D (**14b**) was found to be the most active of all, and possessed extremely high antiplasmodial activity.



14a	R_1= $COCH_3$	R_2=b	R_3= OH	R_4= O	R_5= O	R_6=a
14b	R_1= H	R_2=O	R_3= O	R_4= O	R_5= O	R_6=a
14c	R_1= $COCH_3$	R_2=OH	R_3= OH	R_4= OH	R_5= OH	R_6=H
14d	R_1= $COCH_3$	R_2=OH	R_3= OH	R_4= OH	R_5= OH	R_6=a

Microorganisms provide also a large number of polyene antibiotics such as fungichromin (**15)** isolated first from a *Streptomyces* sp. and possessing antifungal and antiprotozoal activity.[31] Ansatrienine A[32] (**16**), asukamycin[33] (**17**) and manumycin A[34] (**18**), which show activity against fungi, L1210 leukemia cells and bacteria and constituted a task and challenge for synthetic chemistry.



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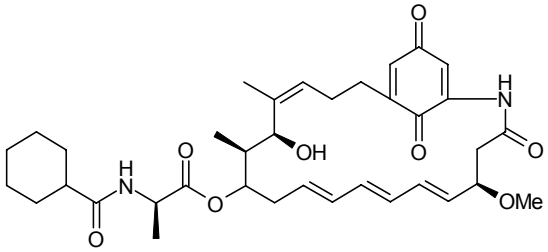

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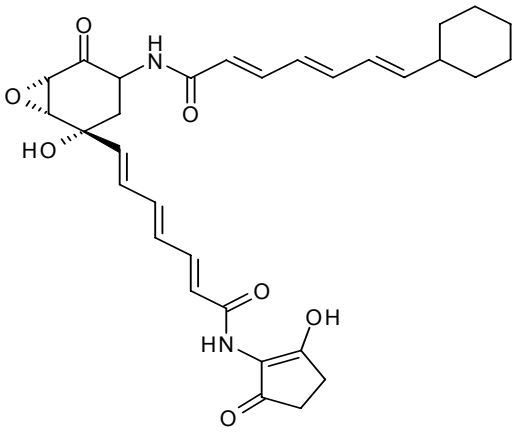

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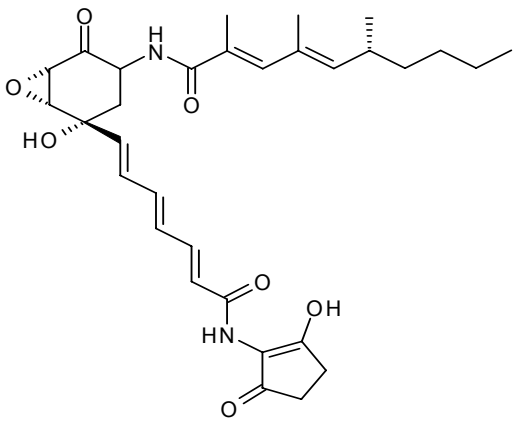
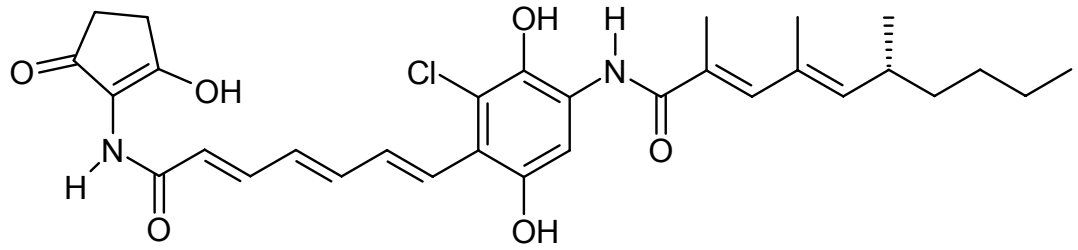


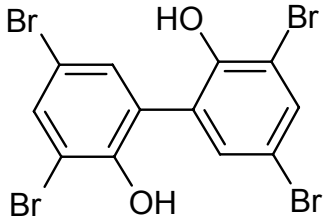
18

Chinikomycin A (**19a**) and B (**19b**) isolated in our group belongs also to this polyene class. It exhibited an unusual *para*-orientation of the side chain, showed antitumor activity against different human cancer cell lines, however, was inactive in antiviral and phytotoxicity tests.[35]



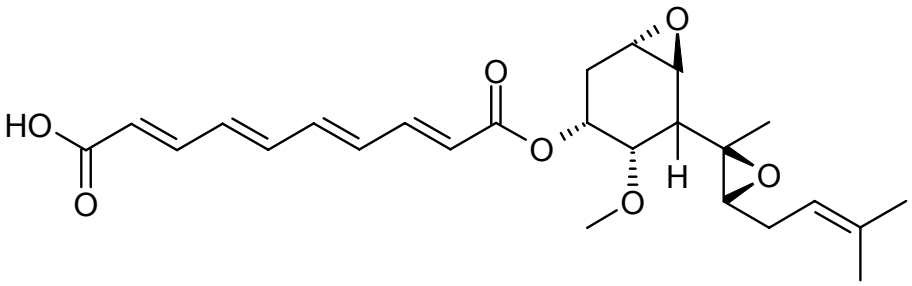
19a: chinicomycin A
19b: quinone form of **19a**

The bactericidal compound **20**, obtained from a culture of a new marine species *Pseudoalteromonas phenolica* sp. nov., isolated from seawater collected of Ogasawara Island (Japan),[36] had potent activity against methicillin-resistant *S. aureus* (MRSA) and was also strongly active against *Enterococcus serolicida*, *E. faecium* and *E. faecalis*.[37] This compound is available commercially, but this is the first reported isolation as a natural product.



20

Eble *et al*.[38] have isolated from the marine derived fungus *Aspergillus fumigatus* the compound fumagillin (**21**), which possesses a wealth of biological activities including amoebicidal, anticancer, antiparasitic and antibacterial properties.



21

Not all the isolated natural products serve as antibiotics. Some of them function as signalling substances between microorganisms. One of the most intensively studied bacterial intercellular signal substance is the A-factor (γ-butylrolactone), which stimulates the production of streptomycin by *Streptomyces griseus* and it is also responsible for the formation of mycelium and pigments.[39]

Natural products have not only an impact on the modern medicine: About 40% of prescribed drugs are made of them,[2] but they also and frequently stimulated the development of new approaches to structure elucidation. This is especially true in NMR spectroscopy, where natural products were among the first examples of successful application of multidimensional methods.

Despite changing strategies in natural product research, such as sample selection and collection, isolation techniques, structure elucidation, biological evaluation, *semi*synthesis, dereplication, biosynthesis, as well as optimisation of downstream processing,[40] the discovery rate of truly novel natural product drugs has actually de-

creased.[41] Reasons for this fact are related to high costs and time consuming of conventional techniques,[42] which led to the exploitation of modern high-throughput screening and combinatorial strategies by the pharmaceutical industry, to generate new lead structures.[5, 41, 43] However, far from being competitive, combinatorial and natural product chemistry should complement on a synergistic perspective, since nature continues to be the most diverse and active compound library known.[44] In the beginning of the area of antibiotics it seemed that all diseases could be defeated, but with the extended use of some antibiotics resistances developed. This drug resistances cost ca. 17 million lives every year due to untreatable infections.[45] For this reason, there is worldwide a need of new medicinal sources, new antibiotics or old antibiotics with new activity. It has been estimated by the World Health Organization that 80% of the people on earth mainly depend on the traditional medicines for their health care.[46] In developing countries, the search for biologically active compounds obtainable from locally available plants and from microorganisms, may particularly help to reduce public health costs, which have significantly been raised due to acquisition of synthetic drugs from industrialised countries. Exploring marine organisms will be one of the main focuses in the next years and should be highly successful.

2 Aim of the present work

The main objectives of this work are the isolation and structure elucidation of new and preferably biologically active secondary metabolites from bacteria. To achieve this purpose, chemical (TLC/ HPLC-MS) and biological screening for the desired strains needs to be done in a sequence as outlined in the following steps:

- After an adaptation screening of the culture conditions (p_H, duration of the fermentation, medium type) of the selected strains, the fermentation mode on big scale should be carried out to isolate the different constituents.
- The crude extract obtained from the fermentation must be separated using various chromatographic methods (silica gel, Sephadex LH-20, PTLC, HPLC, etc.) into its pure components. The latter will be identified using spectroscopic methods (NMR, MS, IR, UV, X-ray crystal analysis if possible) and with the help of databases (AntiBase, Dictionary of Natural Products and Chemicals Abstracts).
- The isolated pure metabolites are submitted for different bioassays (i.e. antimicrobial test, brine shrimp test). Also known compounds might reveal new bioactivities when tested against new targets.

3 General techniques

3.1 Collection of strains

The strains for this investigation were obtained *via* cooperations with various microbiological groups. All the *Streptomycetes* investigated here were obtained from the strain collection of bioLeads in Heidelberg and from the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven. The North Sea bacteria were collected near the isle of Helgoland by the group of M. Meiners (Emden). The collected organisms were described at the beginning temporarily by colour, morphology, the presence of mucus etc. In some cases, the taxonomy was determined later in detail.

3.2 Pre-screening

Among the received strains, around 30% were usually able to produce metabolites with bioactivity or further interesting properties. To select these strains, a so-called pre-screening is performed. In this method, strains are selected by a number of suitable qualitative or quantitative criteria, like biological, chemical or physical interactions of metabolites with test systems.

The strains are sub-cultured on agar plates for 3-7 days and microscopically examined for contaminations. Small pieces of the agar culture were then used to inoculate 1 L Erlenmeyer flasks with inflections containing 250 ml of medium, followed by incubation on a rotary shaker at 28 °C. The culture broth was then lyophilised and the dried residue extracted with ethyl acetate. The obtained crude extract was used for biological, chemical and pharmacological screenings and also for HPLC-MS.

3.3 Chemical screening

The search and isolation of pure bioactive compounds from bacteria is a multiple step procedure and an expensive task. For this reason it is important to eliminate unnecessary work like the re-isolation of known metabolites from the crude extract or from a partially purified fraction. Chemical screening is a method, which allows

reaching this aim at the earliest stages of separation and is therefore economically very important.

The TLC (thin layer chromatography) is one of the cheapest and simplest methods used for the detection of bacterial constituents in the crude extract. Compared with other methods like HPLC it is easy to perform, quick, requires simple equipment and is sufficiently reproducible. A spot of the crude extract is developped on a TLC with a CH_2Cl_2/MeOH sovent system. The developed TLC plate is visualized under UV light, and interesting zones are further localized by exposure to spray reagents. Many sprays reagents are available for the detection, some specific, other universal. In our group, only the following spray reagents are used routineously:

- Anisaldehyde/sulphuric acid gives different colour reactions with many structural elements.
- Ehrlich's reagent is a specific reagent used to determine indoles and some other nitrogen containing compounds; indoles turn pink, blue or violet, pyrroles and furans become brown, anthranilic acid derivatives change to yellow.
- Concentrated sulphuric acid is especially used for polyenes. Short conjugated chains are showing a brown or black colour, carotenoids develop a blue or green colour.
- NaOH is used for the detection of *peri*-hydroxy-quinones, which turn red, blue or violet. The deep red prodigiosins are showing the colour of the yellow base.
- Chlorine/*o*-dianisidin is used as universal reagent for the detection of peptides.

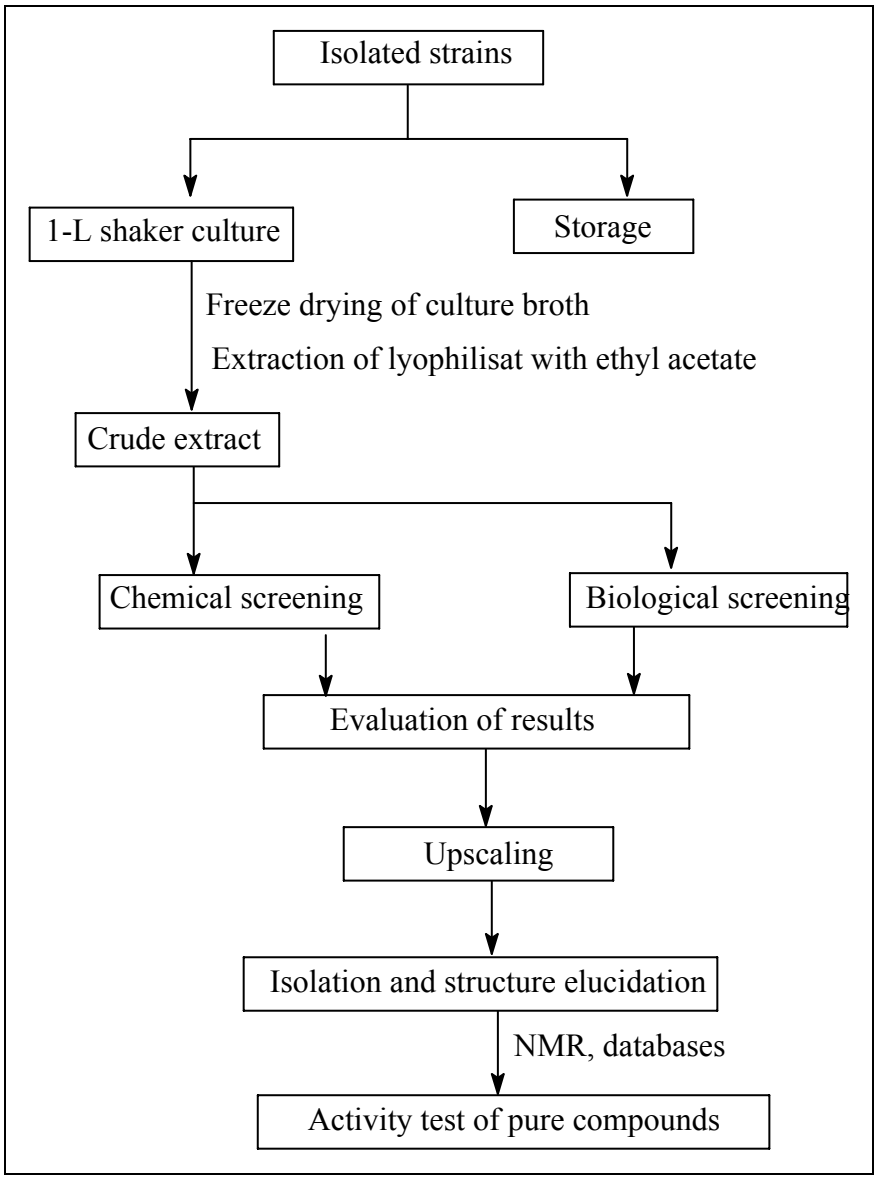


Figure 2: General screening of the selected strains

3.4 Pharmacological and Biological Assays

It is evident that in order to screen a crude extract for bioactive substances, an appropriate test is need. Many screening programs have been developed in natural product chemistry, and are usually divided into two groups: general screening bioassays and specialized screens. These screening programmes will be different, whether they are organized by a pharmaceutical company, or university research groups. In both cases, all bioassays should have high capacity, sensitive, low cost, and must give rapid answers. There are two types of screening: the *vertical screening* mostly used

in industry shows high selectivity and narrow results (1:10.000-1:20.000). The *horizontal screening* used in our group exhibits low selectivity, however, broad results (1:3-1:100) and gives therefore a quick overview. In our group the crude extract is screened using the agar diffusion test with bacteria (Gram-positive, Gram-negative), fungi, plants and higher organisms, the latter for cytotoxicity. Our crude extract are tested against *Escherichia coli*, *Bacillus subtilis, Mucor miehei*, *Candida albicans*, *Streptomyces viridochromogenes* (Tü 57), and *Staphylococcus aureus* as well as the 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. The bio-autography on TLC gives simultaneously more information about an unknown bioactive component in the crude extract. This is readily seen with antimicrobial compounds. The pharmacological tests in our group were carried out at bioLeads (Heidelberg). Chemical and biological screening complements each other and allows us to choose some strains for the scale-up.

3.5 Cultivation and scale-up

The cultivation and scale-up steps are carried out only after a primary screening. An optimisation of the culture conditions may sometimes be done in order to choose the best medium, improve the yield or comparison of produced secondary metabolites. The optimisation is almost applied when the amount of active substances obtained is very small.

There were two possibilities available for the culture of bacteria: the fermentation in shaking flaks or in a fermentor. For the latter, a pre-culture of 2 or 5 L is to be used for the inoculation.

After harvesting, the culture broth is mixed with Celite and filtered under pressure. The water phase can be submitted to extraction with ethyl acetate, but it is highly recommend to use a solid phase extraction with XAD resin due to the fact that the latter extracts also more polar compounds, is not harmful and reduces considerably the costs for solvents. The mycelium is extracted with ethyl acetate and acetone. The organic phases are evaporated to dryness and the remaining crude extract used for separations.

3.6 Isolation methods

The separation methods depend on the amount of the crude extract and the polarity of the compounds of interest. Generally, the crude extract is first defatted using cyclohexane, than subjected to silica gel chromatography using a gradient of increasing polarity with various solvent systems (CH_2Cl_2/MeOH or cyclohexane/ethyl acetate etc.). Size-exclusion chromatography (Sephadex LH-20) offers the advantage of a higher recovery rate and minimizes the destruction of compounds. It is used preferentially when the amount of the crude extract is < 4 mg. Further methods like PTLC and HPLC are also used for some final purification.

3.7 Partial identification and dereplication

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. Methods have been developed to recognize known compounds at the earliest stages of the purification or from a partially purified mixture; these complementary processes for rapid identification of known compounds or the elucidation of a partial structure of an unknown compound to prioritise or conclude an isolation have come to be termed "dereplication".[47]

Nowadays there are database available where NMR derived sub-structures or physico-chemical properties can be searched using computers.[48] The most useful and comprehensive tools for our purpose are the data collection AntiBase[49] and the Dictionary of Natural Products (DNP).[50] The latter allows also the dereplication of plant metabolites, however, AntiBase is much easier to use. These databases are also important tools in the identification of new metabolites with respect to compounds classes and chromophores.

Dereplication is also done with new methods like the combination of liquid chromatography and detection methods such as NMR spectroscopy (HPLC-NMR) and the tandem mass spectroscopy (HPLC-MS/MS), by which biological matrices, e.g. extracts from marine microorganisms,[51] extracts from plants[52] are screened to obtain most possible information about known constituents with minimum amount of material. Because most compounds of interest are thermally labile, HPLC-ESI-MS/MS is

the method of choice to identify known molecules from multi-component mixtures with high selectivity and sensitivity.[53] Due to the nonchiral character of NMR spectroscopy and mass techniques, no information concerning the full absolute 3-dimentional structure is available by HPLC NMR or HPLC-MS/MS techniques. The CD (circular dichroism) spectroscopy[54] is widely used for the attribution of the *absolute* configuration by comparison of the experimental data from structurally related compounds, if available.

4 Terrestrial *Streptomyces*

4.1 Terrestrial *Streptomyces* sp. GW 2/1332.

The terrestrial *Streptomyces* sp. GW 2/1332 grew on agar with a white aerial mycelium and a red agar colouration. The crude extract of the strain revealed activity against *Streptomyces viridochromogenes* (Tü 57), *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*. The chemical screening by TLC depicted a major intensive red spot, which turned violet with dilute sodium hydroxide, a characteristic colour reaction of *peri*-hydroxy quinones. From the crude extract from a 15-L shaker culture, griseorhodin A (30 mg) (**22**) was isolated after the chromatographic separation and precipitation from dichloromethane by addition of methanol.

4.1.1 Griseorhodin A

The compound SR1 (**22**) was obtained as a red solid, sparingly soluble in organic solvents like dichloromethane and methanol. The molar mass of 508 Dalton was established by ESI MS experiments. The ^{1}H NMR spectrum in [D_6]DMSO displayed three H/D exchangeable 1H singlets at δ 13.28, 11.76 and 10.76, attributed to *peri*-hydroxy groups of a quinone. An additional acidic proton signal was observed at δ 6.91. The sp^2 region of the spectrum delivered three singlets at δ 7.26, 6.58 and 6.42. Three aliphatic methine signals at δ 5.30 (d), 4.44 (br s) and 4.30 (d) were counted. Furthermore, two singlets each of intensity 3 at δ 3.90 and 2.23 assigned to a methoxy group and an aromatic or olefinic methyl, respectively, were noticed. The ^{13}C NMR spectrum showed 25 signals, whereby those at δ 186.1 and 180.3 could be assigned to quinone carbonyls. Assisted by the substructure search in AntiBase with the NMR data and molecular weight (Figure 3), the compound was identified as griseorhodin A (**22**) and confirmed by comparison of the measured NMR data with the literature.[55]

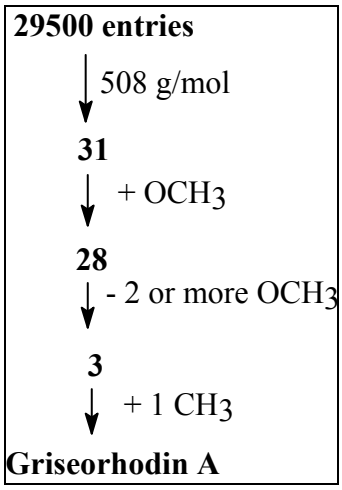
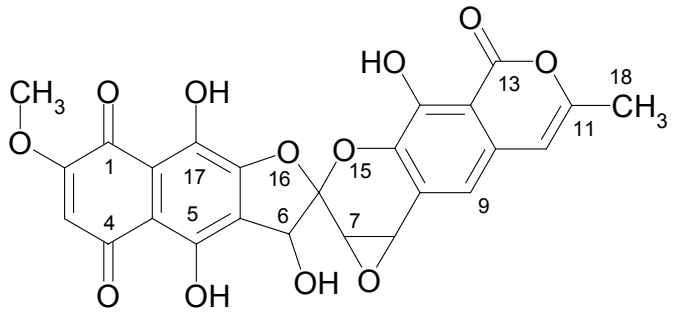


Figure 3: Substructure search of griseorhodin A (**22)** in AntiBase



22

Griseorhodin (**22**) was isolated from *Streptomyces californicus*[56] for the first time and reported to inhibit the growth of Gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus*. It is also reported to exhibit cytotoxicity against human tumour cell lines FCRC-57-G and FCRC-57-U.[57] Compound **22** has been previously isolated also in our group by Schiebel[58] and was found to be active against the tumor cell lines MCF (mamma carcinoma), HMO2 (stomach carcinoma) and Hep-G2 (liver carcinoma).

4.2 Terrestrial *Streptomyces* sp. GW 15/1817

The interest in the extract of the strain GW 15/1817 owes to pronounced antibacterial activity against *Bacillus subtilis,* C*andida albicans* and *Staphylococcus aureus* and phytotoxicity against *Chlorella vulgaris* and *Chlorella sorokiniana*. Additionally the yellow spots on TLC, which remained unchanged with dilute sodium hydroxide,

turned from brown to black with concentrated sulphuric acid and strengthened our further interest. From the extract from a 15 L-culture, 50 mg of the polyene antibiotic, roxaticin (**23**), was purified by chromatographic methods.

4.2.1 Roxaticin

The compound Poly b (**23**) was obtained as yellow amorphous power with the molecular weight of 606 Dalton determined from the *pseudo*-molecular ion $[M-H]^-$ in (-)-ESI MS. It turned brown to black on TLC with concentrated sulphuric acid, however, remained unchanged with dilute sodium hydroxide showing the polyene nature of this compound. The ^{1}H NMR spectrum of **23** showed two *trans* coupling signals at δ 7.25 (dd, ^3J = 15.8, 11.4 Hz) and 5.83 (d, ^3J = 15.8 Hz) characteristic for a polyene conjugated with a carbonyl group. Furthermore, six doublets of doublets each of intensity 1 and a multiplet of four protons were observed in the olefinic region. The aliphatic region presented several multiplets for methine and methylene groups between δ 4.24-1.10, of which those at δ 4.24-3.66 (1H) could be assigned to oxygenated methine groups. Additionally four methyl doublets were observed at δ 1.10, 1.07, 0.94 and 0.90.

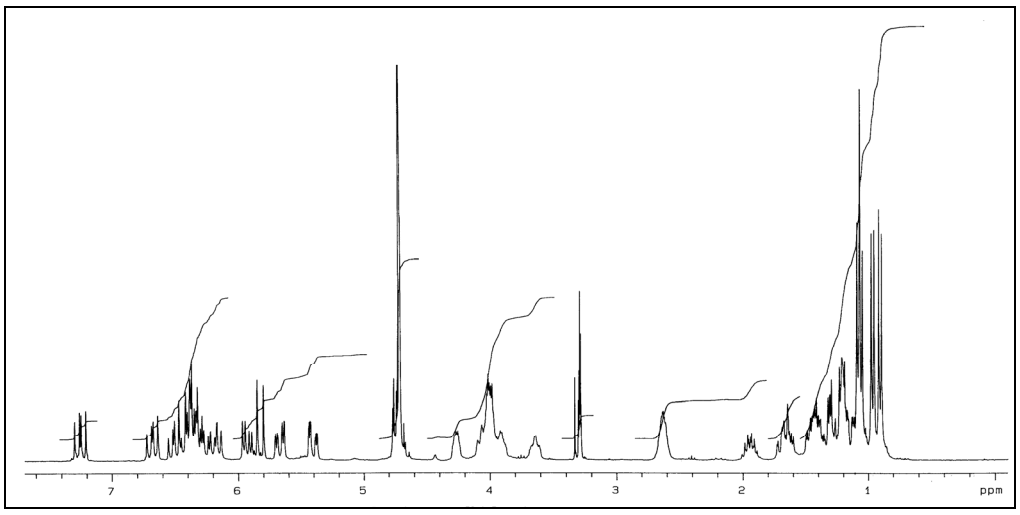


Figure 4: ^{1}H NMR spectrum (300 MHz) of roxaticin (**23**) in [D_4]MeOH

In the ^{13}C NMR spectrum, 34 carbon signals were seen. The signal at δ 169.1 could be attributed to a carbonyl of a lactone, amide, acid or ester, in addition to signals of 12 sp^2 hybridised methine carbons in the range of δ 146.9-121.4. The aliphatic region

showed 11 methine signals, of which 8 were connected to oxygen. Additionally, 5 methylene and 4 methyl carbons were also detected.

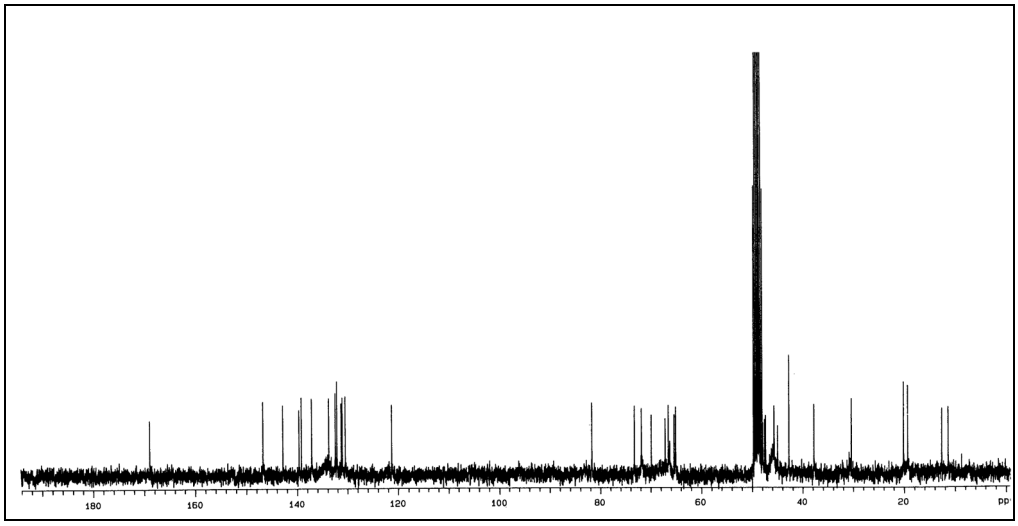
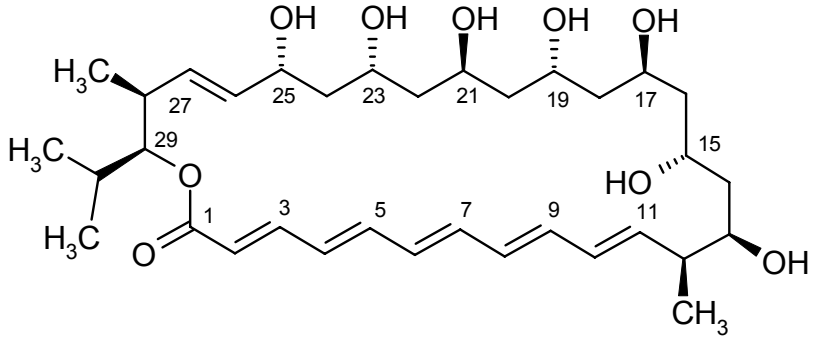


Figure 5: ^{1}H NMR spectrum (300 MHz) of roxaticin (**23)** in [D_4]MeOH

The compound under investigation was identified with the help of a sub-structure search in AntiBase and by comparison of the NMR data[59] as a polyene macrolide, roxaticin (**23**), which was also synthesized by Evans et *al*.[60]



23

Oxopentaenes such as mycoticins A and B[61], flavofungins[62] and roflamycoin[63] are polyene macrolides containing a polyene segment in conjugation with the carbonyl group. They are very large macrocyclic lactones with 22 to 44 membered rings, which are additionally characterised by a large segment with methylene and hydroxylated methine groups in alteration. The polyenic section usually incorporates 4 to 8 conjugated double bonds.[64] Polyene macrolides are belonging to an important class of clinically valuable natural products, which are used in treating systemic fun-

gal infections.[65] Over 200 polyene macrolides are known, they are derived from a variety of sources, including marine organisms, fungi and plants, but the complete stereochemistry has been determined only in a few cases.[65] The mycoticins were the only oxo-polyenes with established configuration. Roxacitin (**23**) was previously isolated in 1985 from an unidentified *Streptomyces* sp.[59] Its structure was solved by X-ray diffraction as the first example of its class. Its antimicrobial activity is limited to fungi; however, due to the toxic nature, **23** has found no application.

4.3 Terrestrial *Streptomyces* sp. GW 3/1122

The crude extract from a 1 L shaker culture of the terrestrial *Streptomycete* isolate GW 3/1122 exhibited strong antimicrobial activity against *Streptomyces viridochromogenes* (Tü 57), *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans* and *Mucor miehei*. On TLC, it depicted several yellow spots, which except one turned dark orange on spraying with anisaldehyde/sulphuric acid. The same colour reaction with anisaldehyde/sulphuric acid suggested them to be actinomycins, which was supported by co-chromatography with authentic actinomycin C_2 and D. The only yellow zone, which did not show any colour change with anisaldehyde/sulphuric acid, drew our attention. The strain was thus scaled-up to yield 1.5 g of dark orange crude extract. Chromatographical purification of the extract resulted in the isolation of the furanoquinone **24** from the band of interest.

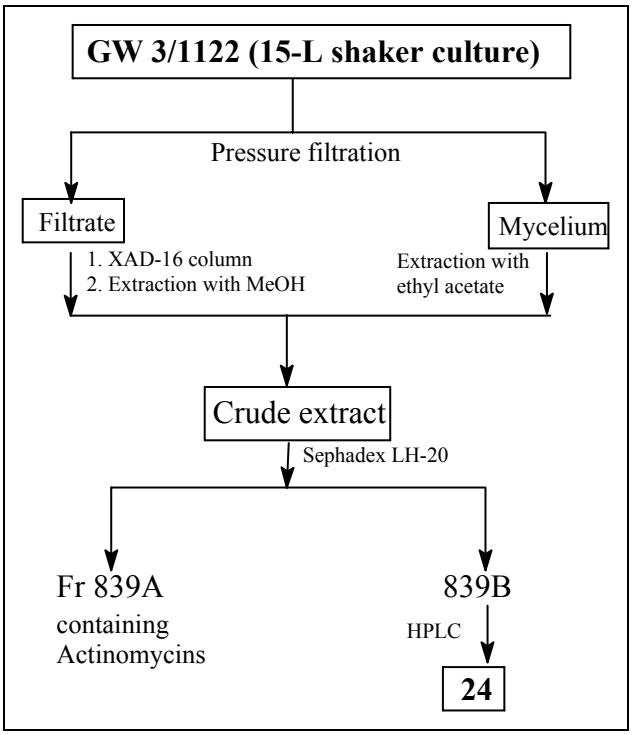


Figure 6: Work-up of the strain *Streptomyces* sp. GW 3/1122

4.3.1 Furanonaphthoquinone

Preparative HPLC of the fraction 839B led to the isolation of SF839a (**24**) as a yellow solid. The ^{1}H NMR spectrum showed an aromatic methine singlet at δ 7.15, an acidic proton signal at δ 6.50 (1 H) and a multiplet of an olefinic proton at δ 5.06. A methine quartet at δ 4.83 and a methyl doublet at δ 1.43 indicated a CH_3CH-O fragment. Additionally, a methoxy group at δ 3.99, an aromatic methyl singlet at δ 2.04, two double bond methyl singlets at δ 1.62 and 1.52 and an aliphatic methyl singlet at δ 1.23 were observed. Finally, two methylene groups in the range of δ 1.98-1.70 were also detected in the spectrum.

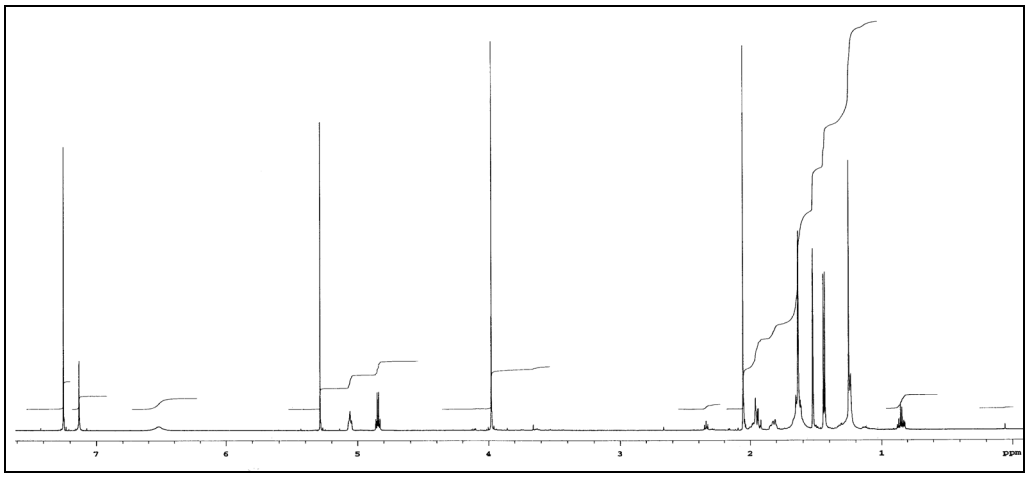
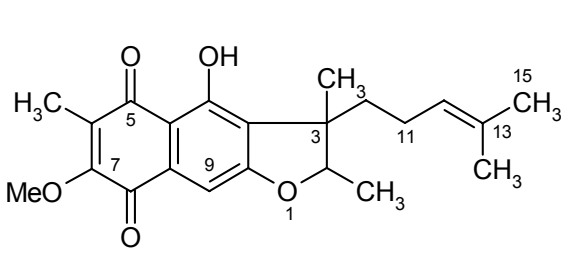


Figure 7: ^{1}H NMR spectrum (300 MHz) of the furanoquinone **24** in $CDCl_3$.

The ^{13}C and APT NMR spectra indicated ten aliphatic carbon signals for a methoxy, a methine, five methyl and two methylene groups and a quaternary carbon atom. Additionally, twelve *sp*^{2} carbons were counted: two at δ 184.2 and 181.3 of a quinone system, two methine groups and eight quaternary carbons (three are bearing oxygen). The (+)- and (-)-ESI mass spectra indicated *quasi*-molecular ions at *m/z* 393 $[M+Na]^+$ and 369 $[M-H]^-$ leading to the molecular weight of 370 Dalton. The substructure search in AntiBase using molecular weight and NMR data led to the furanoquinone[66] **24** and the isomeric furaquinocin C[67] (**25**) as the most probable structures.



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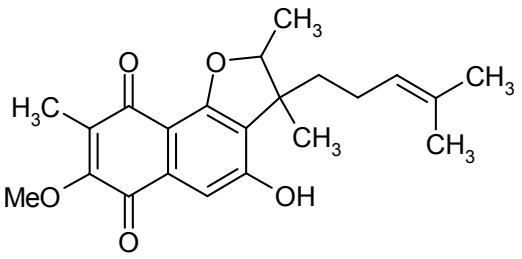

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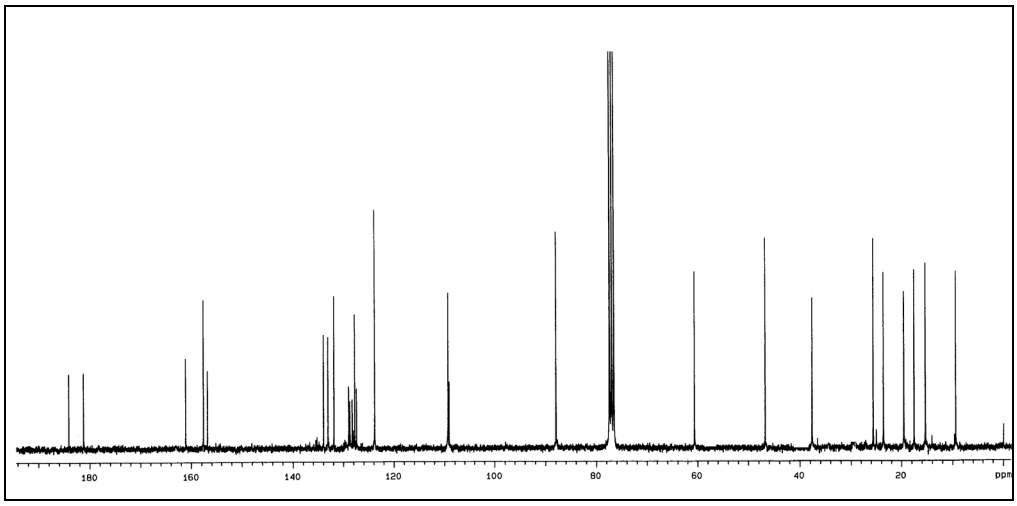
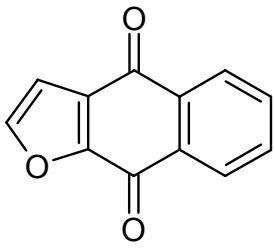


Figure 8: ^{13}C NMR spectrum (75.5 MHz) of the furanoquinone (**24**) in $CDCl_3$

Comparison of the NMR data of SF839a with the reported values for 2**5** and **24** assigned the structure of SF839a as the linear structure **24.** The major difference are noticed in the ^{13}C NMR shift of C-2 and the 3-methyl carbon, which appeared in **25** at δ 91.7 and 22.7, respectively, while they appeared in **24** at higher field (δ 88.1 and 19.6).



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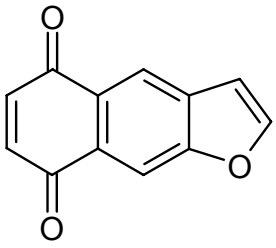
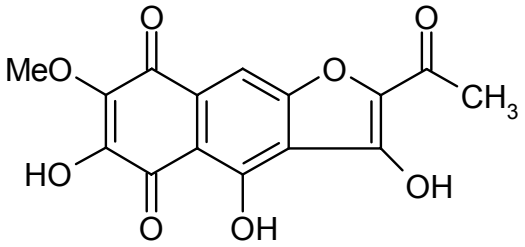


27

Furanonaphthoquinones are compounds contanining either the chromophore **26** or **27**. They are abundant in plants e.g rhodocladonic acid (**28**) isolated from *Mycoblastus sanguinarius* and cribrarione A[68] (**29**) reported from myxomycete *Cribraria purpurea*. They are rare in microorganisms and there is still no report about their biosynthesis.



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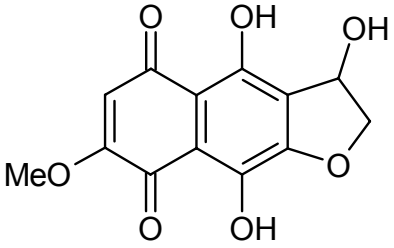


29

Furanonaphthoquinones are reported to exhibit antimicrobial[68] as well as significant cytotoxicity against HeLa cell lines.[69] Compound **24** has not been tested so far.

4.4 Terrestrial *Streptomyces* sp. GW 24/1229

The crude extract of the terrestrial *Streptomyces* sp. GW 25/1229 showed moderate activity in biological screening against the test organisms *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli,* and a good activity against *Streptomyces viridochromogenes*. The TLC of the crude extract indicated the presence of red spots, which turned to violet with 2N sodium hydroxide. The culture on agar plates showed after four days a red pigmentation. The 10-L culture on a rotating shaker took three days and after extraction gave 2 g of red crude extract, which was worked up following Figure 9.

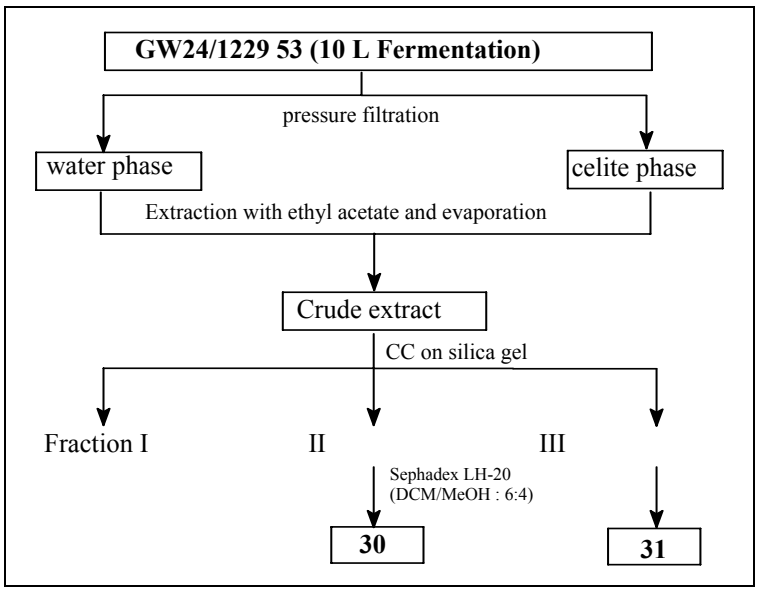


Figure 9: Work-up of the crude extract of *Streptomyces sp*. GW 24/1229

4.4.1 β-Rubromycin

TLC of fraction II showed a major red compound, which did not change on spraying with anisaldehyde/sulphuric acid. By purification on Sephadex LH-20, a compound FM1 (**30**) was obtained as a red powder. The (+)-ESI mass spectrum indicated an $[M+Na]^+$ ion at *m/z* 559. In the ^1H NMR spectrum of **30**, two broad singlets of chelated hydroxyl groups were observed at δ 12.50 and 10.66, three aromatic 1H singlets were found at δ 7.60, 7.22 and 7.04, three singlets in the range of δ 4.00-3.80 were attributed to three methoxy groups, three methylene groups were seen in the aliphatic region.

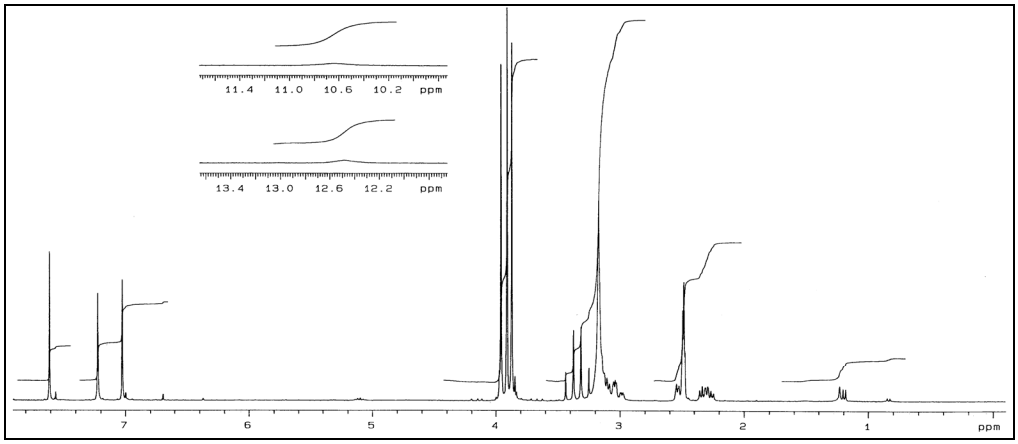
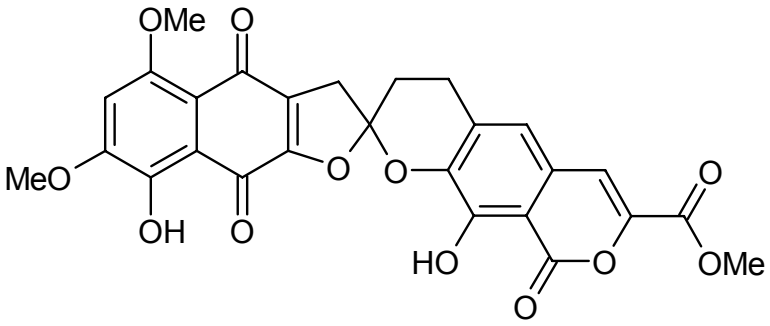


Figure 10: ^1H NMR spectrum (300 MHz) of β-rubromycin (**30**) in $[D_6]$DMSO

A search with this information in AntiBase led to the identification of FM1 as β-rubromycin (**30**), which was confirmed by comparison with an authentic sample and spectra from our collection.

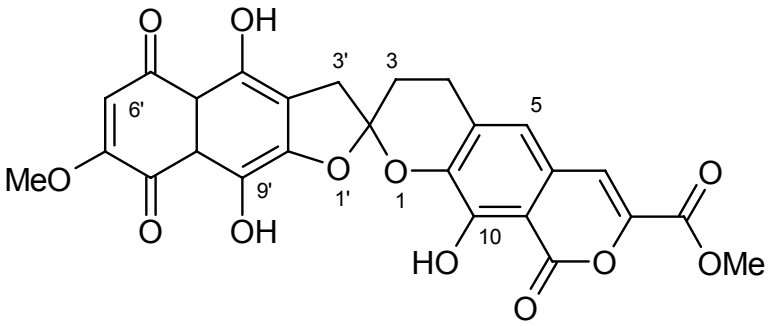


30

The red antibiotic β-rubromycin (**30**) was isolated as a mixture with γ-rubromycin by Brockmann *et al*. from *Streptomyces collinus*.[70] Its structure was determined by degradation reactions, and the two carbonyl groups were initially placed in *ortho* position. Further structural and biosynthetic investigations led to the revision of its structure into **30**.[71]

4.4.2 γ-Rubromycin

A second quinone FM2 (**31**) was also obtained as a red powder, which exhibited similar characteristics like FM1 (**30**). The EI mass spectrum indicated a molecular weight of *m/z* 522 and the mass difference of *Δm* = 15 between **30** and **31** indicated the loss of one methyl group. The ^{1}H NMR spectrum was very similar to that of β-rubromycin (**30**), but in addition to the two chelated hydroxyl groups, a further one appeared at δ 13.14. In the aliphatic region the major difference is the presence of only two methoxy signals. The compound FM2 was identified as γ-rubromycin (**31**), which always occurs in mixture with β-rubromycin (**30**).



31

γ-Rubromycin (**31**) and β-rubromycin (**30)** have been reported to show a marked growth inhibition of *Bacillus subtilis, Staphylococcus aureus, Staphylococcus albus, Escherichia coli* and are active against other Gram positive germs.[70]

4.5 Terrestrial *Streptomyces* sp. GW 22/1326

The terrestrial *Streptomyces sp*. GW 22/1326 was obtained from the strain collection of bioLeads. The ethyl acetate extract showed in the agar diffusion test an activity against *Streptomyces viridochromogenes* (Tü 57), *Mucor miehei*, *Chlorella so-*

rokiniana and *Scenedesmus subspicatus*. It also showed in pharmacological tests an interesting inhibition of anti-interferon-gamma activity. TLC of the crude extract exhibited some red bands, which turned violet with 2N NaOH indicating their quinonoid nature, and some pink spots after spraying with anisaldehyde/sulphuric acid. The fermentation of the strain on a 20 L scale using M_2 medium during four days resulted in a brown culture broth, which was filtered and extracted with ethyl acetate. Flash chromatography of the crude extract resulted in five fractions (fig. 11).

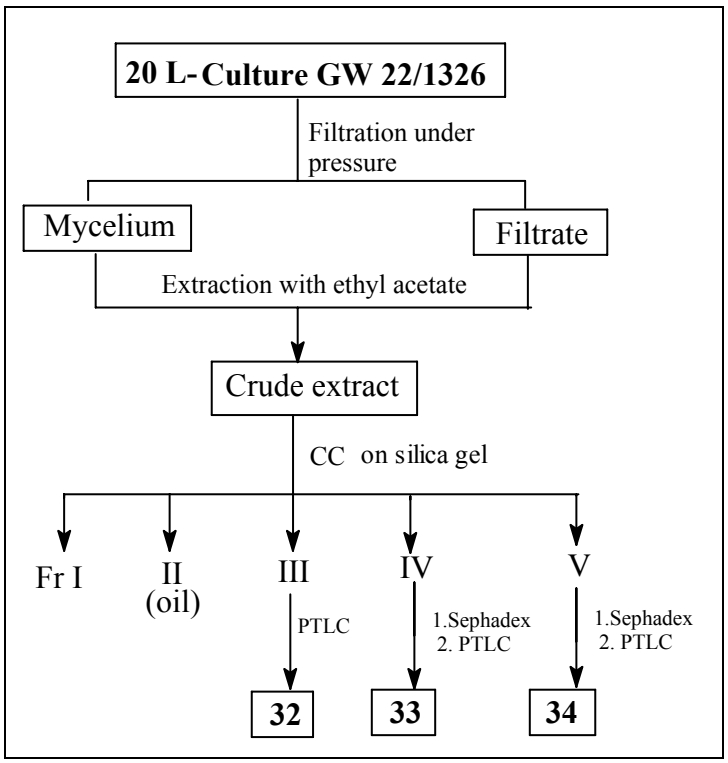


Figure 11: Isolation and purification of compounds from the *Streptomyces* sp. GW 28/1818

4.5.1 ε–Rhodomycinone

PTLC purification of fraction III gave the pure compound AVA3 (**32**) as a red solid, which showed an orange fluorescence under UV 366 nm. The red spot on TLC turned from pink to orange on spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum presented three singlets at δ 13.38, 12.80 and 12.00, which is characteristic for chelated hydroxy groups. The aromatic region displayed signals of three protons, one doublet centred at δ 7.80, a triplet at δ 7.64 and another doublet at δ 7.26 were attributed to a 1,2,3-trisubstituted aromatic ring. In addition, two methines

connected to oxygen appeared at δ 5.37 (m), 4.23 (s) and one methoxy group at δ 3.74. Furthermore, multiplets attributed to two methylene groups appeared at δ 2.26, the other gave two sextet signals at δ 1.80 and 1.58. Finally a triplet at δ 1.17 (J = 7 Hz) was attributed to a methyl group.

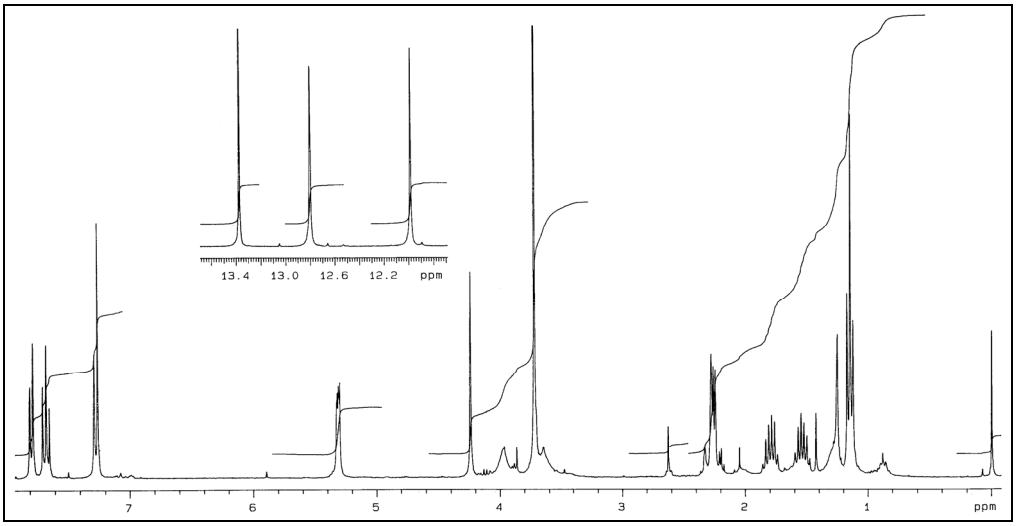
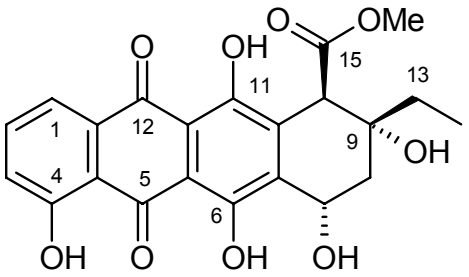


Figure 12: ^{1}H NMR spectrum of ε-rhodomycinone (**32**) in $CDCl_3$

The ^{13}C spectrum indicated 22 signals, of which according to the APT spectrum, 13 represented quaternary carbon atoms. It depicted resonances of quaternary carbons at δ 190.6, 185.9 and 171.3 two, of which were assigned to be carbonyls of a quinone system. The signal at δ 171.3 pointed to a carbonyl of an acid or ester. Furthermore, two methylene signals at δ 34.3 and 32.6, five methine, one methoxy and methyl groups were also seen. ESI MS indicated an $[M+Na]^+$ ion at *m/z* 451, and the molecular weight of 428 Dalton delivered ε-rhodomycinone (**32**). [72,73]



32

ε-Rhodomycinone is a widespread anthracylinone from streptomycetes, it was isolated in 1955 by Brockmann *et al.*,[74] and the structure was elucidated in 1961 by Brockmann and Brockmann jr.[75]. ε-Rhodomycinone is also occurring as an intermediate in the biosynthesis of anthracyclines with the same chromophore through the elimination of the carbomethoxy group at C-10.[76]

4.5.2 Dihydrotetrodecamycin

Fraction IV showed on TLC a strong absorption at 254 nm and a pink colour on spraying with anisaldehyde/sulphuric acid. The purification on Sephadex LH-20 followed by PTLC yielded 100 mg of a white solid indexed AVA 1a (**33**). The ^{1}H NMR spectrum in [D_6]DMSO of **33** exhibited two doublets at δ 5.58 and 3.42, which disappeared in [D_4]MeOH and were attributed to exchangeable protons of NH or OH groups. The presence of a CH_3CH-O fragment was characterised by a quartet and a doublet at δ 4.96 and 0.83, respectively. The aliphatic region exhibited also two methine protons connected probably to an oxygen atom, which appeared at δ 4.76 and 3.42 as a doublet of doublet and a doublet. In addition, a 1H multiplet at δ 2.70, a broad doublet at 1.98, two multiplets in the range of δ 1.50-1.00, and finally two methyl groups appeared as a doublet at δ 1.40 and a singlet at δ 1.04, respectively.

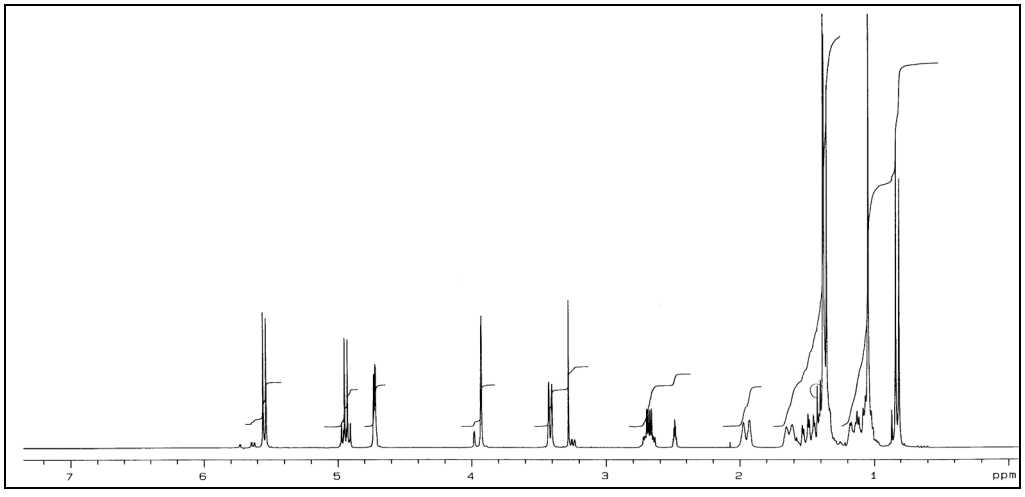


Figure 13: ^{1}H NMR spectrum (300 MHz) of dihydrotetrodecamycin (**33**) in [D_6]DMSO.

The ^{13}C spectrum of **33** revealed the presence of 18 carbons signals, which were sorted by a APT experiment into 6 quaternary, 5 methine, 4 methylene and 3 methyl groups. The carbon signals at δ 197.6, 183.1 and 171.5 indicated two conjugated ketones and the carbonyl of an ester or lactone, but it was found that the signal at δ 183.1 belonged to a double bond sp^2 carbon atom connected to oxygen. The molecular weight was deduced from ESI and EI mass spectra to be 336 Dalton.

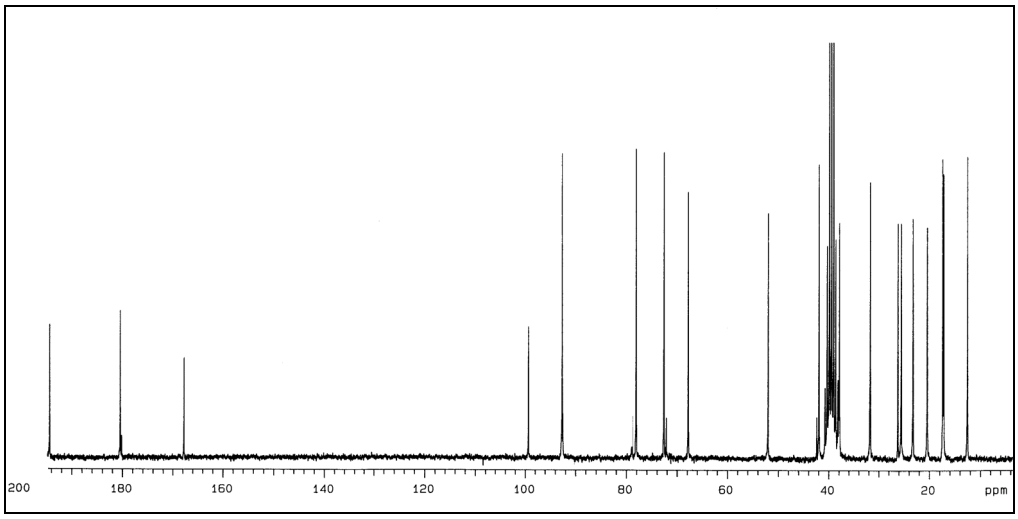
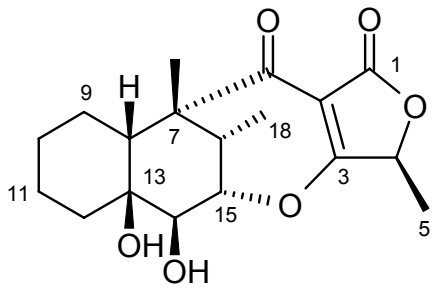


Figure 14: ^{1}H NMR spectrum (300 MHz) of dihydrotetrodecamycin (**33**) in $[D_4]$MeOH

The search in AntiBase led to the antimicrobial antibiotic dihydrotetrodecamycin (**33**) isolated from *Streptomyces nashvillensis* MJ885-mF8 by Toshio *et al*.[77] Dihydrodecamycin (**33**) was found to be active against *Pasteurella piscicida*, which causes pseudotuberculosis in cultured yellow tail, *Seriola quinqueradiata*.[78]



33

4.5.3 Cosmomycin A (Rhodilunancin A)

Chromatography of the polar fraction V on Sephadex LH-20 led to a sub-fraction Va, and the final purification by PTLC gave the pure compound AVAF (**34**). The (+)- and (-)-ESI mass spectra permitted to fix the molecular weight as *m/z* 755. The ^{1}H NMR spectra indicated three chelated hydroxyl groups, and three aromatic protons as doublet, triplet and doublet for a 1,2,3-trisubstituted aromatic ring. The presence of these structural elements similar as in ε-rhodomycinone (**34**) suggested an anthracylinone skeleton. In addition, three doublets at δ 5.40, 4.98 and 4.80 and a multiplet in the range of δ 3.40-4.42 were seen and attributed to three sugar moieties. The small coupling constants (^{3}J = 2.5 Hz) indicated that all glycosidic bonds had the α-configuration. The aliphatic region delivered a complex multiplet at δ 2.90 (2 H) and in the range of δ 2.10-1.50, further six methyl groups were observed, two as a broad singlet at δ 2.20, three doublets and one triplet.

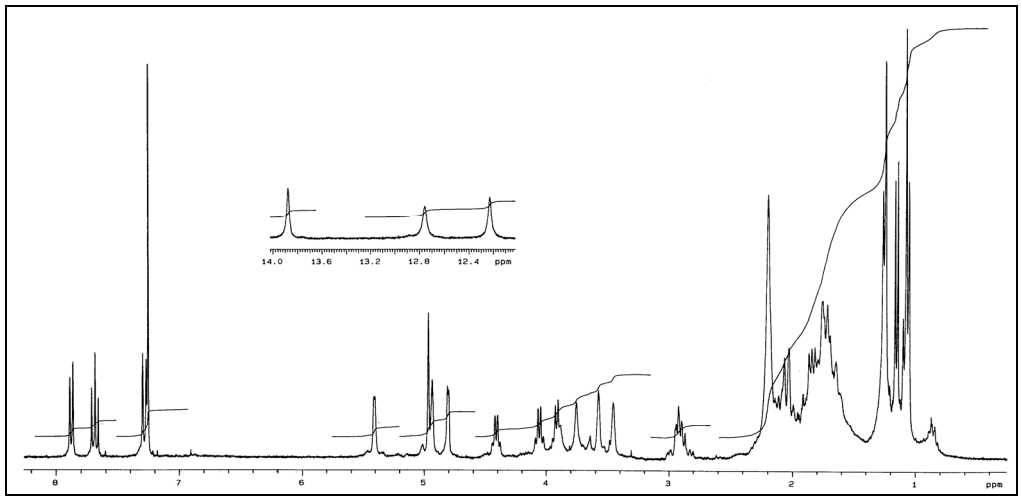
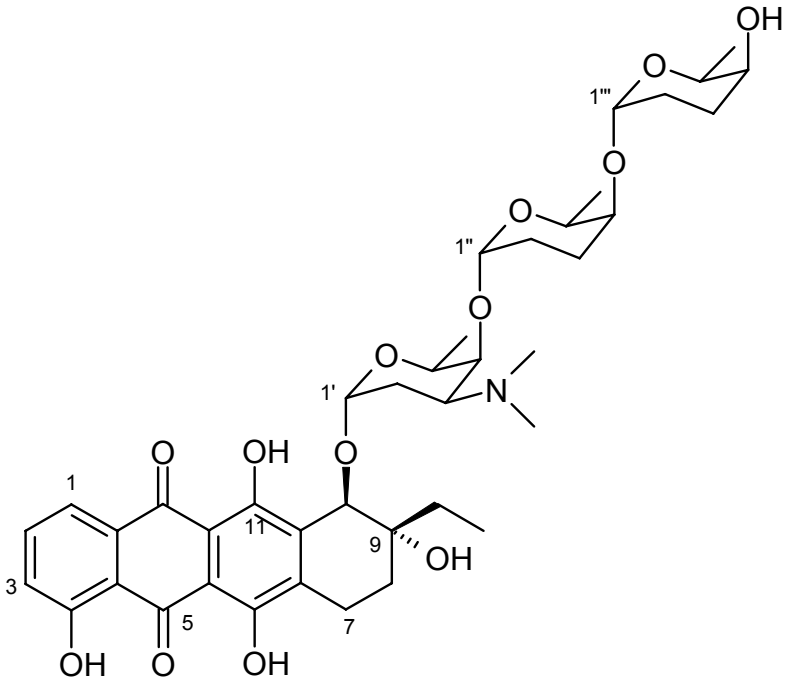


Figure 15: ^{1}H NMR spectrum (300 MHz) of cosmomycin A (**34**) in $CDCl_3$

The AntiBase search yielded cosmomycin A (**34**), which contains three sugar units, two rhodinoses and one rhodosamine. Comparison of the proton spectrum with that from our collection and with published information[79] confirmed the result. Compound **34** shows antimicrobial activity against Gram-positive bacteria and inhibits the DNA synthesis of P_{388} leukemia cells *in vitro*[79] with an IC_{50} value of 9.2 μg/ml.



34

4.6 Terrestrial S*treptomyces* sp. GW 28/1828

The terrestrial actinomycete GW 28/1828 was collected in Malta by bioLeads GmbH, Heidelberg. In our biological screening, the crude extract inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* and *Mucor miehei* and was also found to have an activity against *Artemia salina*. The chemical screening indicated, in addition to a yellow spot of a *peri*-hydroxy quinone, UV-absorbing bands. Some of them turned red on spraying with anisaldehyde/sulphuric acid. The 25 L culture on M_2 medium delivered 2.5 g of a dark brown crude extract after standard work-up, which was subjected to chromatographic separation as described in the following scheme.

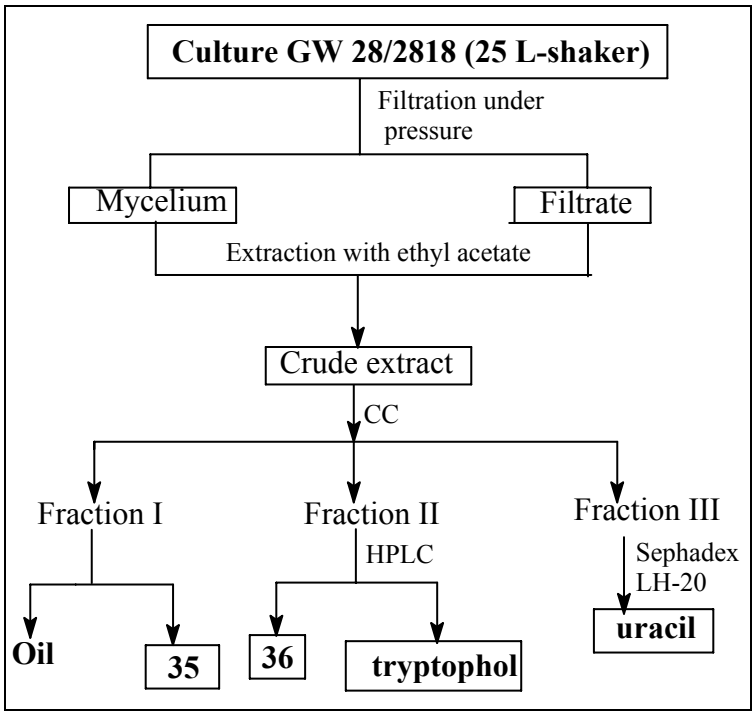
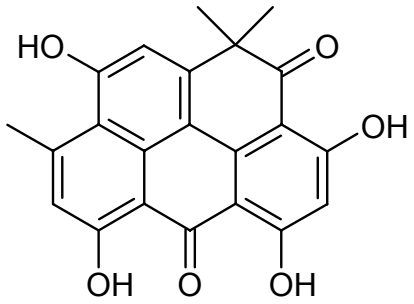


Figure 16: work-up scheme of the Strain GW 18/2818

4.6.1 Resistomycin

On dissolution in dichloromethane, the yellow coloured fraction I gave 25 mg of a yellow precipitate of FSRM2 (**35**), which showed on TLC an orange fluorescence under 366 nm and gave a red colour reaction with dilute sodium hydroxide presuming a *peri*-hydroxy quinone. The ^{1}H NMR spectrum exhibited three singlets at δ 14.54, 14.34 and 14.02 characteristic for chelated acidic protons. Three 1H singlets at δ 7.25, 7.05 and 6.38 were assigned to an electron-rich aromatic system. Furthermore, an aromatic methyl signal at δ 2.90 and a singlet of intensity 6 at δ 1.58 attributed to geminal methyl groups were visible. The ESI mass spectra delivered a $[M+Na]^{+}$ signal at *m/z* 399 and an $[M-H]^{-}$ signal at *m/z* 375, respectively, in positive and negative modes fixing the molecular weight at 376 Dalton. A substructure search in AntiBase supported by the ^{1}H NMR data and molecular weight led to resistomycin (**35**) as possible solution, which has already been isolated in the group. Final confirmation was done by the direct comparison of the ^{1}H NMR spectrum with the authentic one.[80]



35

Resistomycin (**35**) was reported for the first time in 1951 from *Streptomyces resistomycificus* and is reported to possess activity against Gram-positive bacteria .[81] It has an unique chromophore, which occurs only four times in nature.

4.6.2 N-Acetyldopamine

The fraction II obtained from size exclusion chromatography on Sepahdex-20 contained two major colourless UV-active bands, which turned red on spraying with anisaldehyde/sulphuric acid. Purification of this fraction by preparative HPLC delivered SF5 (**36**) and SF3a as low melting solids. By ESI MS experiments, the molecular weight of SF5 (**36**) was determined as 195 Dalton. The aliphatic region of ^{1}H NMR spectrum showed a methyl singlet of an acetyl group at δ 1.83, a methylene triplet at δ 2.60 and a triplet of doublet of a methylene group bearing NH at δ 3.30. The aromatic region showed signals for ABC protons of an electron-rich benzene ring at δ 6.50 (dd), 6.70 (d) and 6.74 (d). Additionally two broad singlets of acidic protons at δ 7.85 (2 H), 7.20 (1 H) and attributed to OH and/or NH groups were seen.

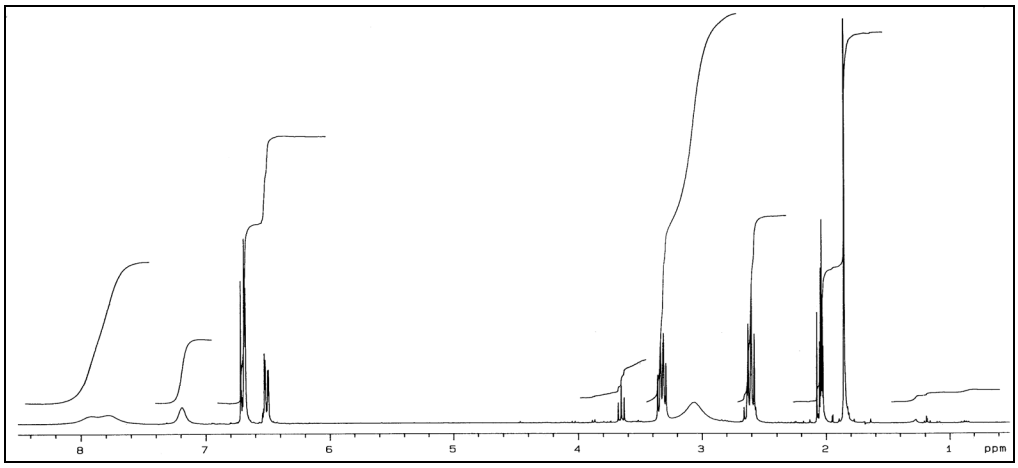


Figure 17: ^{1}H NMR spectrum (300 MHz) of N-acetyl dopamine (**36**) in $[D_6]$acetone

The ^{13}C NMR spectrum revealed ten carbon signals, which were identified by an APT spectrum as the carbonyl of an acid derivative (δ 170.7), a methyl, two methylene, three aromatic methines and three quaternary aromatic signals.

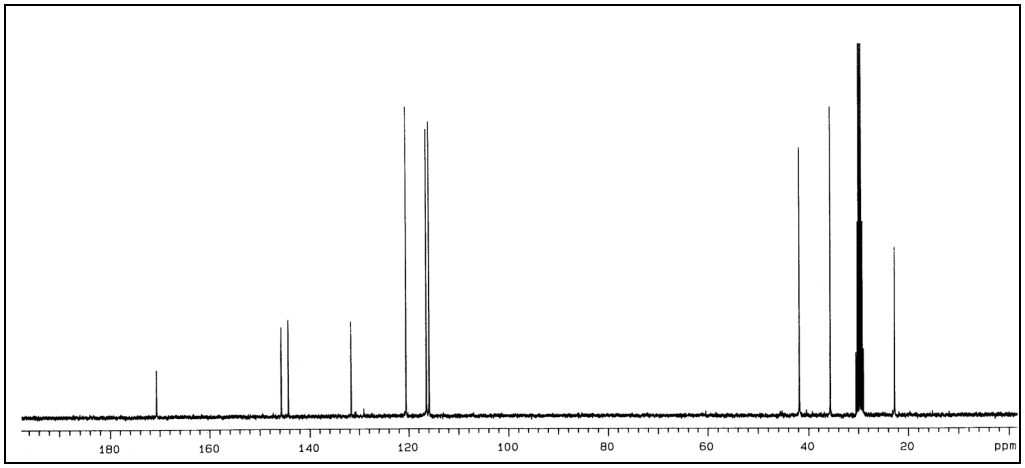
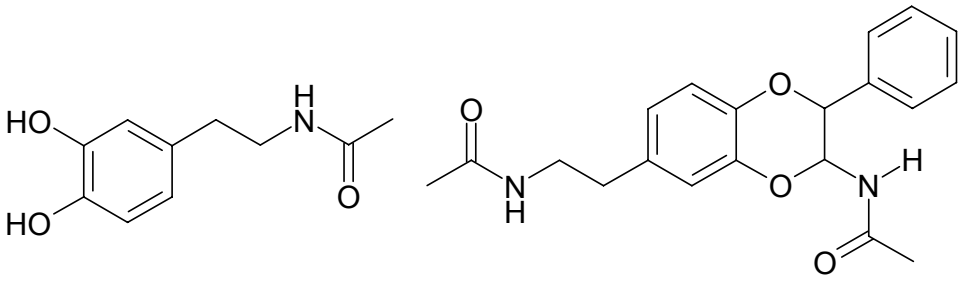


Figure 18: ^{13}C NMR spectrum (300 MHz) of N-acetyl dopamine (**36**) in $[D_6]$acetone

The search in AntiBase was negative, however the search in the Dictionary of Natural Products led to N-acetyldopamine, which has previously been isolated from *Cryptotympana* sp. and *Platylomia* sp[82] (Cicadidae). This is the first report of this compound from bacteria.



36 **37**

Dimers of N-acteyldopamine, e.g. **37** isolated from the same plant displayed high affinity for the 5-HT receptor and were shown to have neuroleptic activity suggesting that they are predictive tools for novel anxiolytic agents.[83] Tryptophol (3-Indolylethanol) firstly reported from fermentation of amino acids by yeast by F. Ehrlich,[84] was also isolated and easily identified from this strain. It is a very common metabolite of plants and fungi[85] and it is reported to inhibit the growth of Gram-positive bacteria and *Candida albicans*.[82]

4.7 Terrestrial *Streptomyces* sp. GW 10/580

The crude extract of the terrestrial *Streptomycetes* sp. GW 10/580 obtained from a 1 L culture exhibited biological activity against *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Candida albicans* and *Mucor miehei*. TLC of the crude extract showed a relatively non-polar light yellow spot, however on spraying with anisaldehyde/sulphuric acid, several colourless zones without UV absorption became visible as brown and violet spots. A 25 shaker culture in M_2 medium became brown after four days of growth and delivered on work-up 3.0 g of a dark brown crude extract, which after defatting was chromatographed on silica gel and pre-separated into four fractions under TLC control.

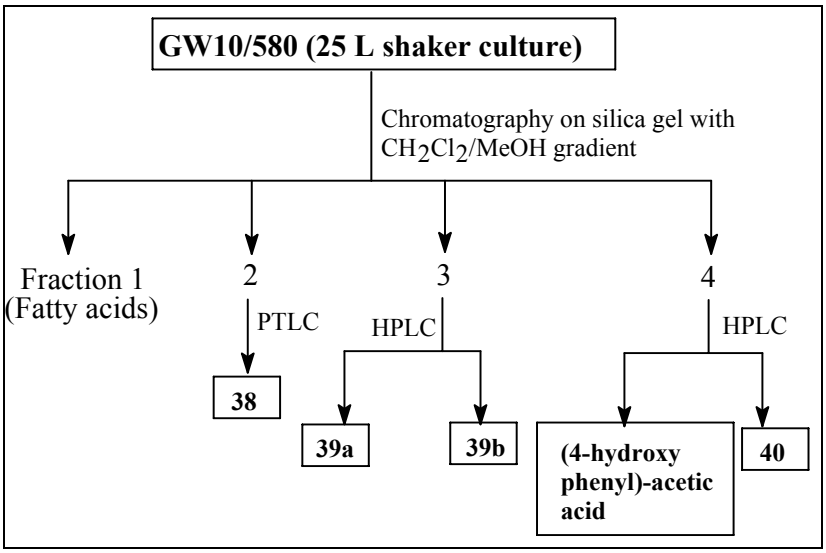
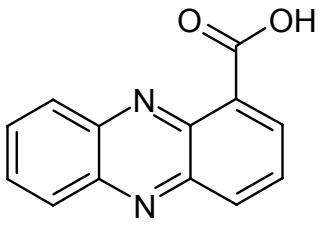


Figure 19: Work-up scheme of the strain GW10/580

The compound TJ3 was also isolated from fraction 4 as a colourless solid and was identified through the 1H and mass spectra as (4-hydroxyphenyl)-acetic acid.

4.7.1 Phenazin-1-carboxylic acid

TJ1 (**38**) was obtained as a light yellow solid from fraction 2 after purification by PTLC. The ^{1}H NMR spectrum depicted a broad signal of an acidic proton at δ 15.60, which could be attributed to a carboxylic acid or chelated hydroxyl group. The aromatic region of the spectrum exhibited signals for seven CH protons: two doublets of doublets at δ 9.00 (1 H) and 8.58 (1 H) and two multiplets between δ 8.40-8.20 (2 H) and 8.10-7.98 (3 H). The EI mass spectrum exhibited the molecular signal at *m/z* 224 and a fragment peak at *m/z* 180 corresponding to $[M\text{-}COO]^+$, which indicated the presence of a carboxylic group in the molecule. The search in AntiBase with the information from ^{1}H NMR and EI mass spectra led to phenazin-1-carboxylic acid (**38**), which was confirmed by comparison of the ^{1}H NMR data with the literature.[86]



38

Phenazin-1-carboxylic (**38**) acid also known as tubermycin B was isolated previously from microorganisms *Pseudomonas*, *Streptomyces cinnamonensis*, *Streptomyces misakiensis* and *Actinomadura dassonvillei*. It exhibits a weak activity against Gram-positive bacteria, and a moderate activity against both *Mycobacterium tuberculosis* BCG and *Mycobacterium tuberculosis* $H_{37}Rv$ (streptomycin resistant).[87]

4.7.2 Feigrisolide B

TLC of fraction 3 exhibited a colourless spots, which turned violet on spraying with anisaldehyde/sulphuric acid. Purification resulted in TJ2 (**39a**) and TJ3 as colourless oily compounds. (+)-ESI MS of compound TJ2 showed a signal at *m*/*z* 239, which could be interpreted as $[M+Na]^+$ signal in combination with the information obtained from the EI MS and ultimately gave the molecular weight of 216 Dalton. In addition to other signals, the EI mass spectrum delivered signals at *m/z* 198 $[M-H_2O]^+$, 168 $[M-H_2O-C_2H_5]^+$, and 125 $[M-H_2O-C_4H_9O]^+$. The ^{1}H NMR spectrum indicated signals for 21 protons all localised in the aliphatic region of the spectrum, of which two represented acidic protons. It contained two methyl groups as a triplet and a doublet, respectively, at δ 0.92 and δ 1.18. Additional multiplets appeared at δ 1.50 (1 H), 1.60-1.75 (4 H), 2.00 (2 H), 3.79 (1 H) and 4.22 (1 H), where the latter two represented methine groups bearing oxygen. Furthermore, a methine signal as quartet of doublet at δ 2.50, a quartet at δ 4.00 (1 H) and a broad singlet of two acidic protons at δ 6.40 were visible.

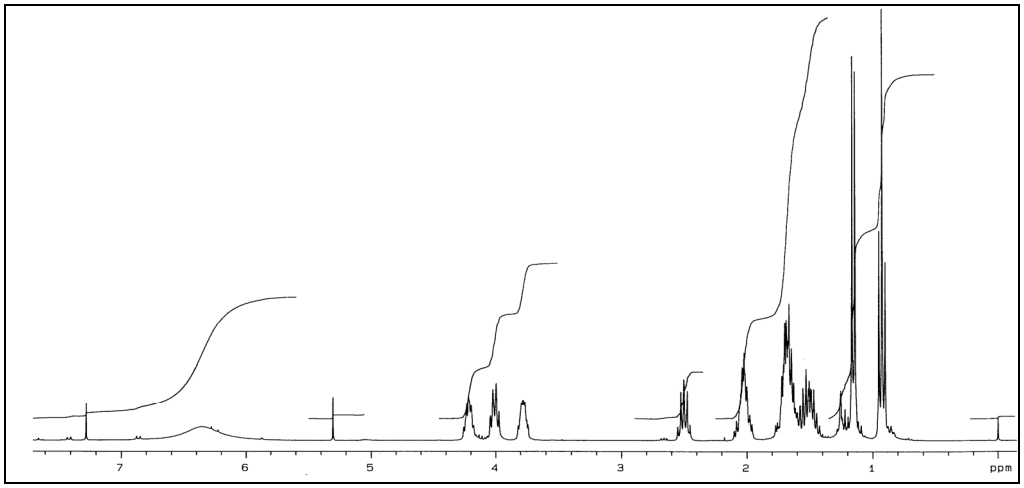


Figure 20: ^{1}H NMR spectrum (300 MHz) of feigrisolide B (**39a**) in $CDCl_3$

The ^{13}C NMR and APT spectra indicated eleven carbon signals: four methine, four methylene and two methyl groups and a carbonyl group at δ 178.2, which was assigned to an ester, lactone or carboxylic acid. The substructure search with the available data in AntiBase resulted in homononactic acid derivatives[88] and feigrisolide B (**39a**).

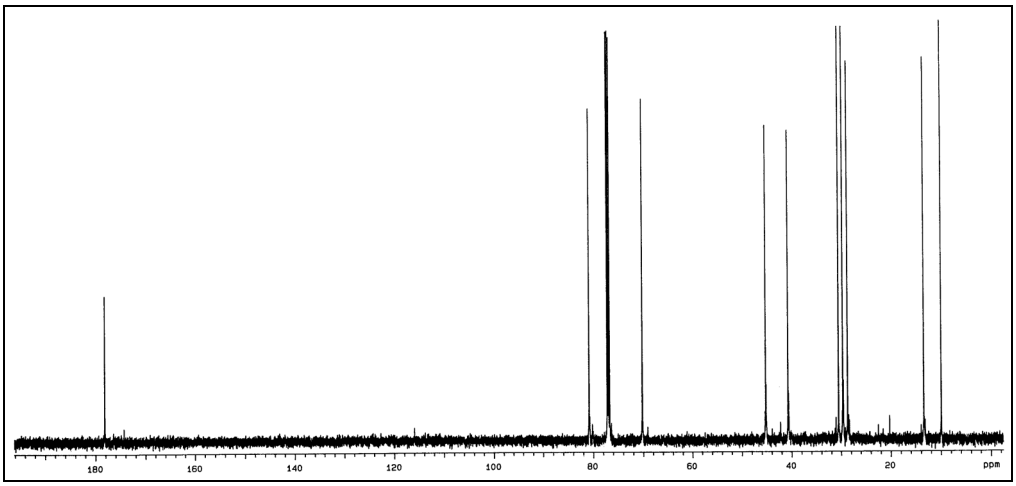


Figure 21: ^{13}C NMR spectrum (300 MHz) of feigrisolide B (**39a**) in $CDCl_3$

The IR spectrum of the compound **39a** showed an absorption band at ν = 1720 cm^{-1} of an ester or lactone functionality, which is present only in the structure of feigrisolide B (**39a**). Compound (**39a**) was finally identified by comparison of the NMR data as feigrisolide B (**39a**), previously isolated from *Streptomyces griseus*.[89] Feigrisolide B (**39a**) exhibits antibacterial activity against *Sporobolomyces salmonicolor* SBUG 549 and moderate activity against coxsackie virus B3. The synthesis of **39a** showed different stereochemistry as those of the natural product.

4.7.3 Feigrisolide A

The ^{1}H NMR spectrum of the colourless oily TJ4b (**39b**) exhibited close similarity to that of feigrisolide B (**39a**). The major difference was the displacement of the methyl triplet in **40a** by a second methyl doublet in **39b** and missing signals for a methylene group in the ^{1}H spectrum of **39b**. The analysis of the (+)-ESI mass spectrum delivered a molecular weight of 202 Dalton, i.e. 14 Dalton (CH_2) smaller than that of feigrisolide B (**39a**).

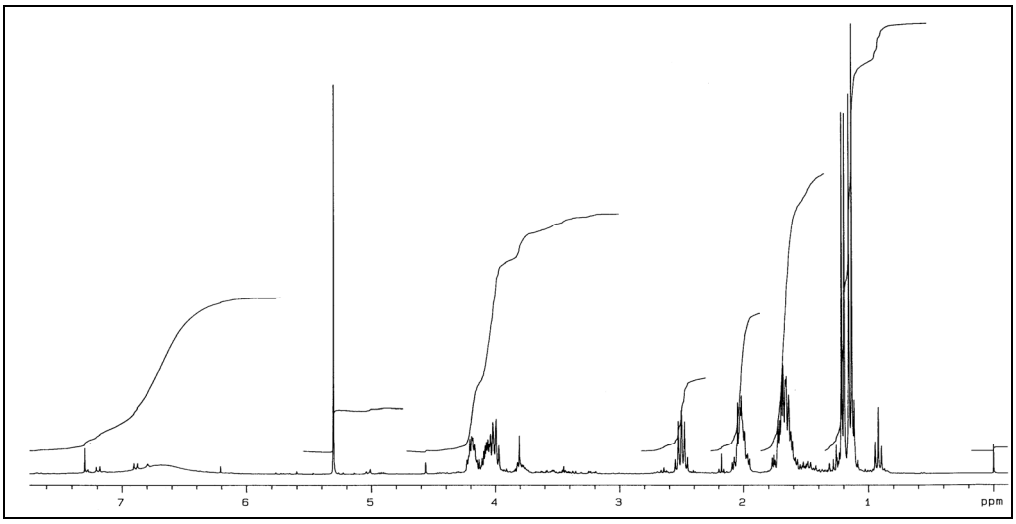
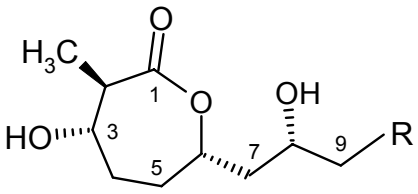


Figure 22: ^{1}H NMR spectrum (300 MHz) of feigrisolide A (**39b**) in $CDCl_3$

The NMR data and the molecular weight indicated the compound under investigation to be the lower homologue of **39a**, probably feigrisolide A (**39b**), which was confirmed by comparison of the ^{1}H NMR data with literature values.[89]



39a: R = CH_3
39b: R = H

A hypothetic biosynthesic pathway of feigrisolide A (**39b**) and B (**39a**) may start from acetate, propionate or succinate to give an open long chain hydroxylated carboxylic acid, which through different cyclisation size delivered homononactic acid derivates or feigrisolides as mention in the figure below.

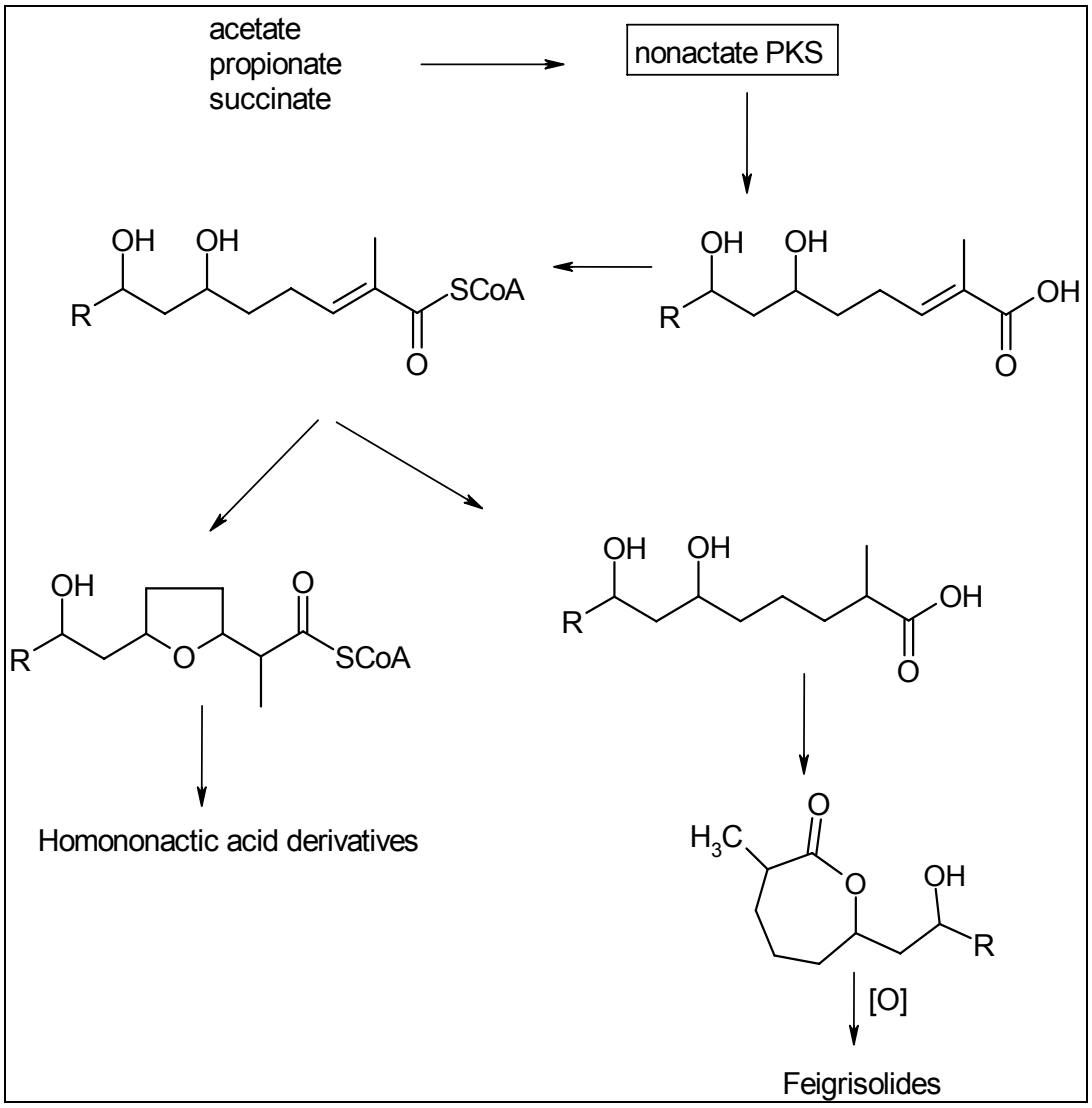


Figure 23: Biosynthesis of feigrisolides.

4.7.4 Feigrisolide C

Compound TJ6a (**40**), purified from fraction 4, was also obtained as colourless oil with a molecular weight of 400 Dalton [by (+)-ESI MS]. It was expected to have similarities in its structure with those of **39a** and **39b** due to the similar physical characteristics, colour reaction with anisaldehyde/sulphuric acid and the ^{1}H NMR spectra. The signals in the ^{1}H NMR spectrum were concentrated only in the aliphatic region as in **39a** and **39b**, however had a higher complexity. It exhibited four methyl signals as a triplet and doublets, respectively, at δ 0.92, 1.15, 1.10 and 1.22. Seven multiplets were observed in the range of δ 1.80-1.40 (13 H), 2.00 (4 H), 2.50 (2 H), 3.78 (1 H), 4.00 (3 H), 4.18 (1 H) and 5.02 (1 H).

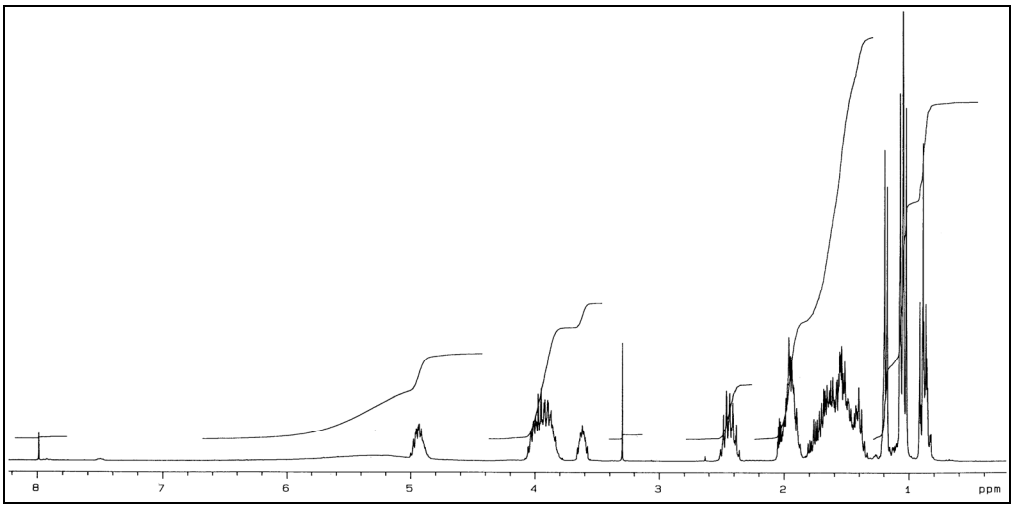


Figure 24: ^{1}H NMR spectrum (300 MHz) of feigrisolide C (**40**) in $CDCl_3$

The ^{13}C NMR spectrum showed signals of 21 carbon atoms, which were sorted by APT as two carbonyl carbons at δ 174.4 and 176.0, eight methine groups, seven methylene groups and four methyl groups. Comparison of the molecular weight, ^{1}H and ^{13} C NMR data of TJ4 with those of **39a** and **39b** suggested the latter two to be the building blocks of TJ6a, resulting in feigrisolide C (**40**) as the most probable structure.

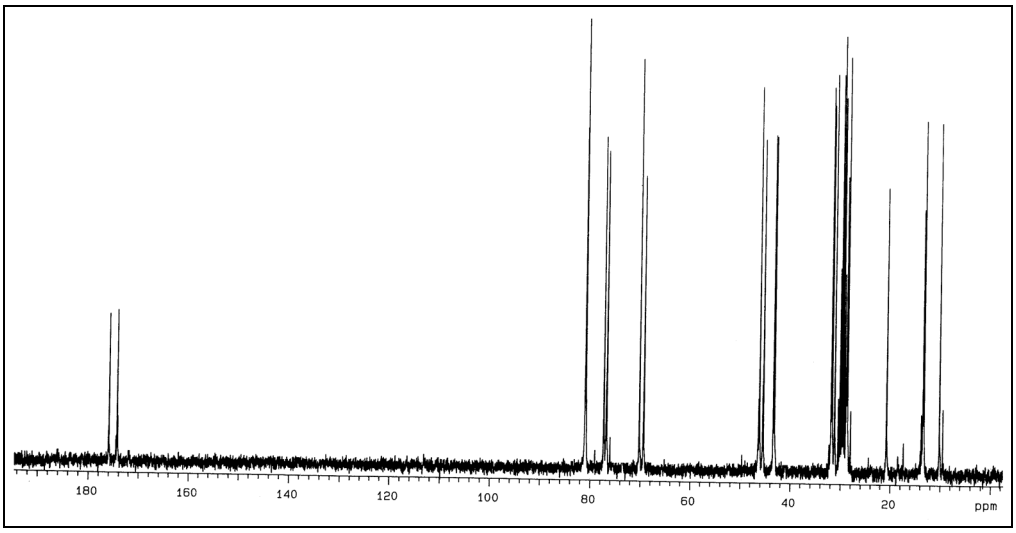
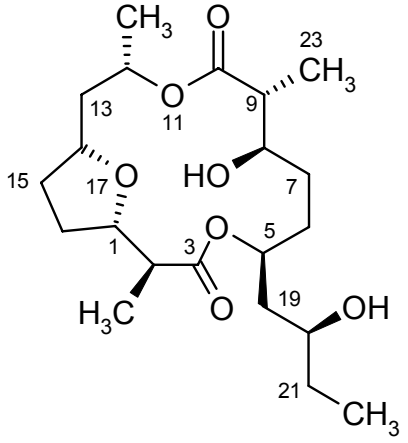
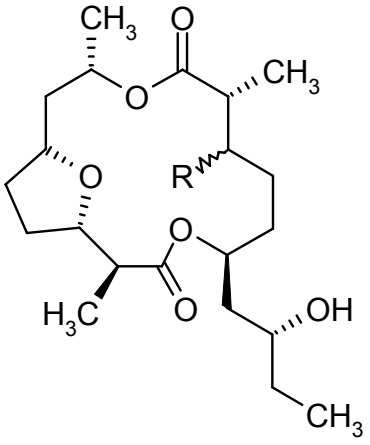


Figure 25: ^{13}C NMR spectrum (300 MHz) of feigrisolide C (**40**) in $CDCl_3$

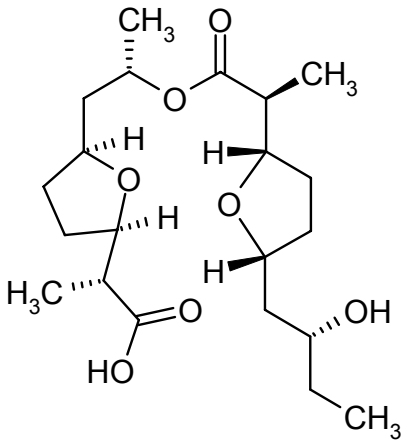


40

The idea was confirmed by the identity of the NMR data of our compound with the reported values of feigrisolide C (**40**) isolated from *Streptomyces griseus*.[89] Feigrisolide C (**40**) shows moderate inhibition of the 3α-hydroxysteroid dehydrogenase and moderate cytotoxicity against L-929, K562 and HeLa cell lines. A synthesis of feigrisolide C carried by Woo et *al.*[90] delivered three possible structures of feigrisolide C (**41a**), (**41b**) and (**42**), whose data did not match with the natural sample **40**. The difference may be due to the stereochemistry at position C-8 and C-20 as in **41a** and **41b**, or may be as postulated the free acid nonactylhomononactaoate (**42**) which, however, is less probable, due to the fact that a free acid is easily differenciate from a lactone by methylation. The structure of feigrisolide C (**40**) is therefore still under discussion.



41a: R = α-OH
41b: R = β-OH



42

4.8 Terrestrial Streptomyces GW 9/2335

The terrestrial strain *Streptomyces sp*. GW9/2335 showed strong activity against *Echerichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Mucor miehei*, *Chlorella vulgaris* and *Scenedesmus subspicatus*. TLC analysis of the extract exhibited many middle polar colourless bands, which became brown on spraying with anisaldehyde/sulphuric acid.

For the isolation of the secondary metabolites, the strain was cultivated in a 25 L scale, and worked up to afford 4.0 g of brown extract. Pre-separation of the crude extract by column chromatography on silica gel followed by a final purification by preparative HPLC (RP-18 silica gel) and Sephadex LH-20 resulted in six secondary metabolites.

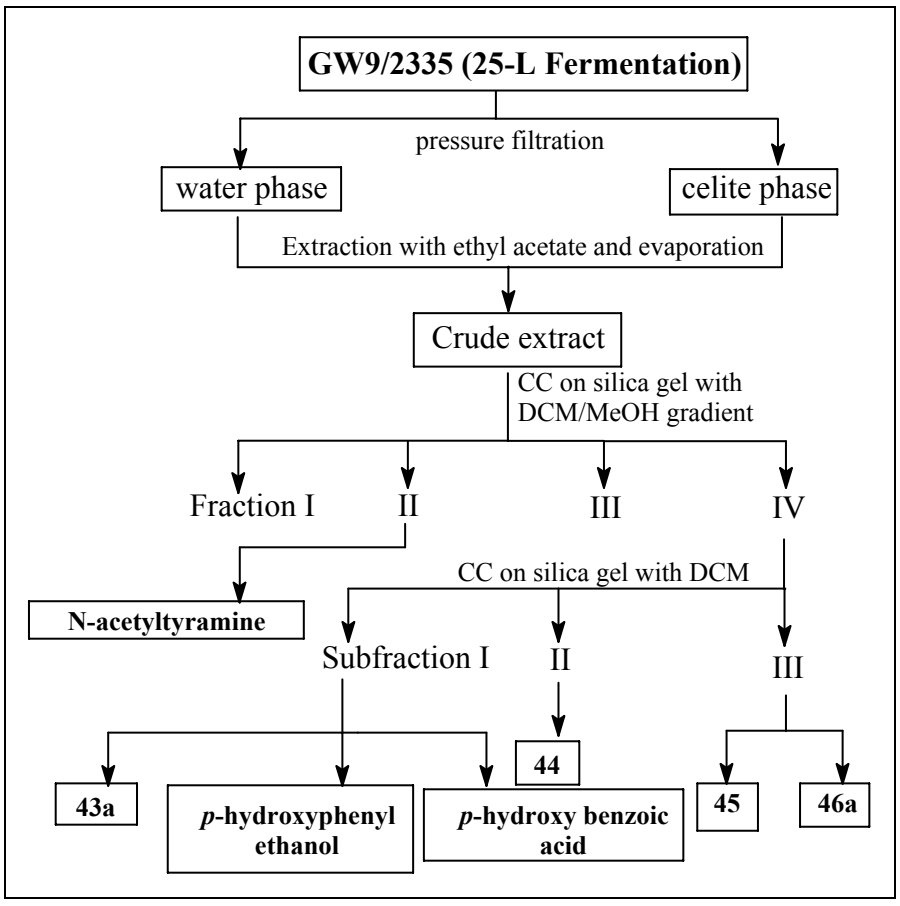


Figure 26: Work-up scheme of the strain *Streptomyces sp*. GW9/2335

4.8.1 5-Deoxy-enterocin

The ESI and EI MS experiments with the colourless solid MOYOB (**43a**) delivered the molecular weight 428 Dalton. The ^{1}H NMR spectrum showed signals of five aromatic protons at δ 7.82 (d, ^{3}J = 8.0 Hz, 2H), 7.58 (t, ^{3}J = 7.9 Hz, 1H) and 7.44 (t, ^{3}J = 7.9 Hz, 2H), which could clearly be assigned to a phenyl residue. Two olefinic 1H doublets with a long-range coupling (^{4}J = 2.0 Hz) were seen at δ 6.30 and 5.44. The aliphatic region exhibited two broad 1H doublets at δ 2.18 and 1.82 and two doublets of doublets each integrating one proton at δ 2.78 and 2.54. Furthermore, a methoxy signal at δ 3.79 and four 1H singlets at δ 5.30, 4.84, 4.30 and 3.99 were detected.

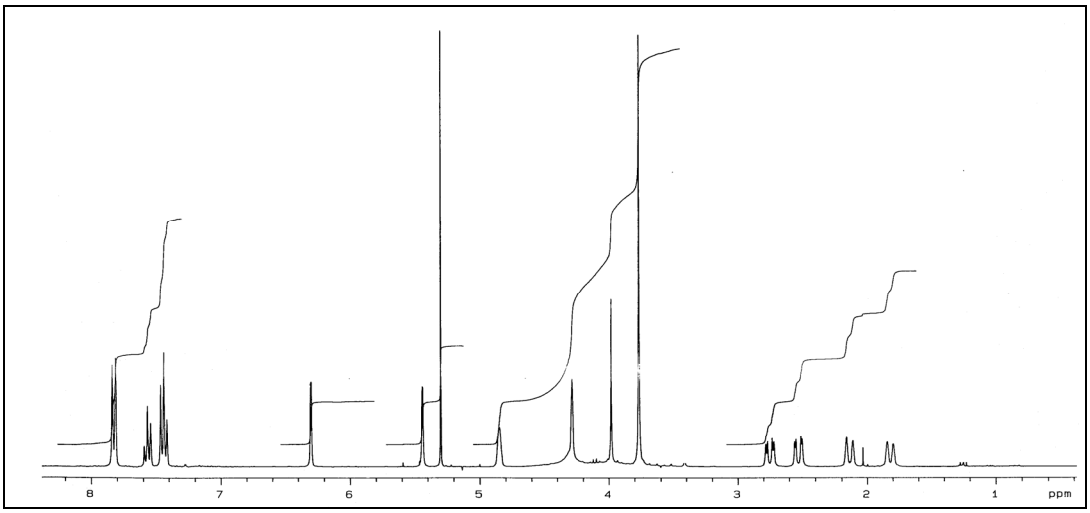
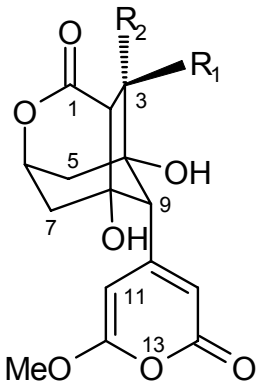


Figure 27: ^{1}H NMR spectrum (300 MHz) of 5-deoxy-enterocin (**43a**) in $CDCl_3$

The substructure search in AntiBase with the NMR data and molecular weight led to 5-deoxy-enterocin (**43a**) and 3-epi-5-deoxy-enterocin (**43b**) already reported from a marine derived actinomycete[91] and a marine ascidian of the genus *Didemnum*[92] as possible structures. The compound under investigation was finally identified as **43a** by comparison of the measured NMR data with the literature.[91,92]



43a: R_1 = COPh R_2 = H
43b: R_1 = H R_2 = COPh

The biosynthese of deoxyenterocin 5-deoxy-enterocin (**43a**) may start from benzoic acid co-enzyme A and propane dioic acid follow by cyclisation as proposed in the figure below

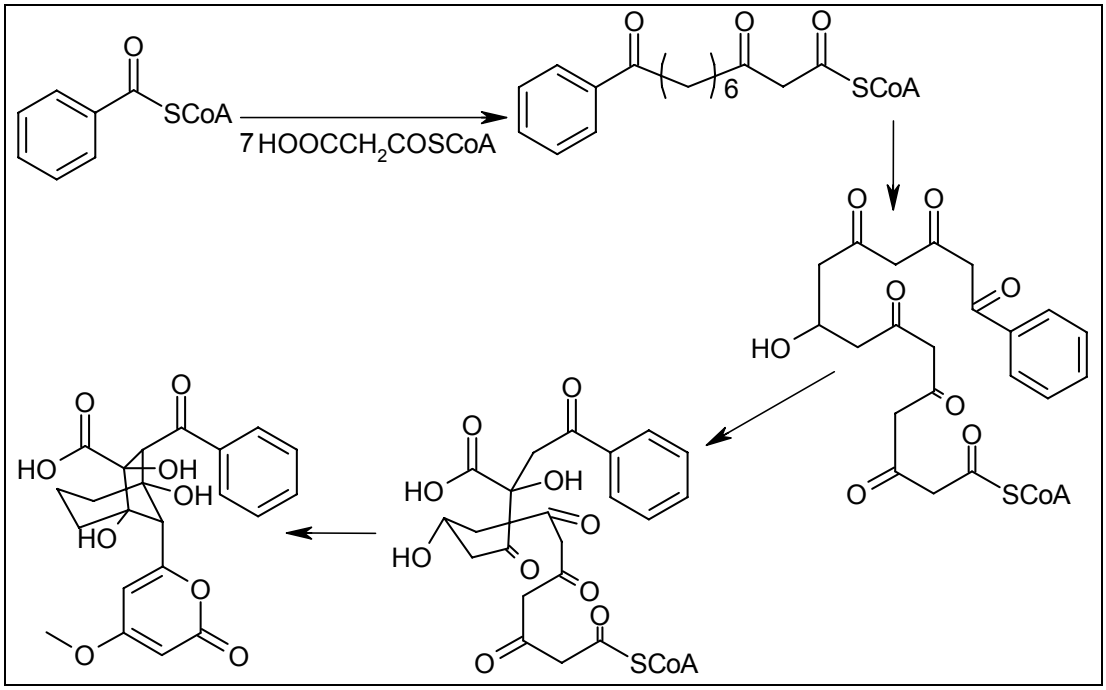


Figure 28: Hypothetic biosynthesis of 5-deoxyenterocin (**43a**)

Compound Moyo2 and Mo was obtained as a colourless solid and gave an orange red colour reaction with anisaldehyde/sulphuric acid and was identified respectively as 4-hydroxyphenylethanol and N-acetyltyramine, previously reported from leaves of *Aristolochia cucurbitifolia*.[93]

4.8.2 Irumamycin

^{1}H NMR spectrum of MoyoA (**44**) was rich in aliphatic proton signals with only four 1H-olefinic protons identified as two doublet of doublet at δ 5.56 and 5.22, two multiplets. In addition to other signals, a triplet at δ 1.00, five doublets at δ 1.28, 1.02, 0.88, 0.84 and 0.80 and three singlets at δ 1.48, 1.42 and 1.38 of altogether nine methyl groups were observed.

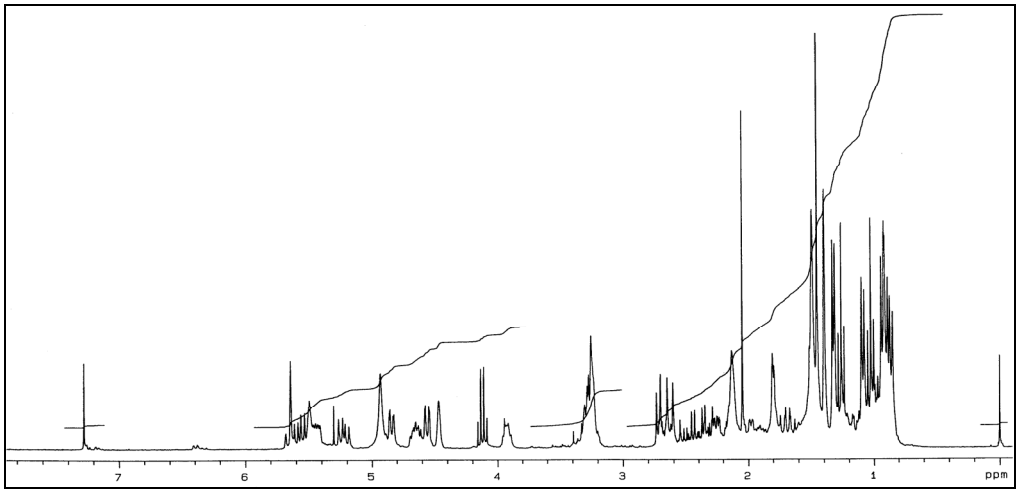


Figure 29: ^{1}H NMR spectrum (300 MHz) of irumamycin (**44**) in $CDCl_3$

The ^{13}C/APT NMR spectra displayed 41 signals for nine methyl, eight methylene, thirteen methine groups (nine bearing oxygen) and an acetal (δ 98.3), an hemiketal (δ 94.0), six olefinic at δ 134.9, 134.3, 134.1, 132.9, 129.3, 116.8 a carbamoyl (δ 157.6), a lactone or carboxylic acid (δ 173.3) and a ketone (δ 210.9) carbon. The presence of the acetal carbon indicated it probably to contain a sugar residue.

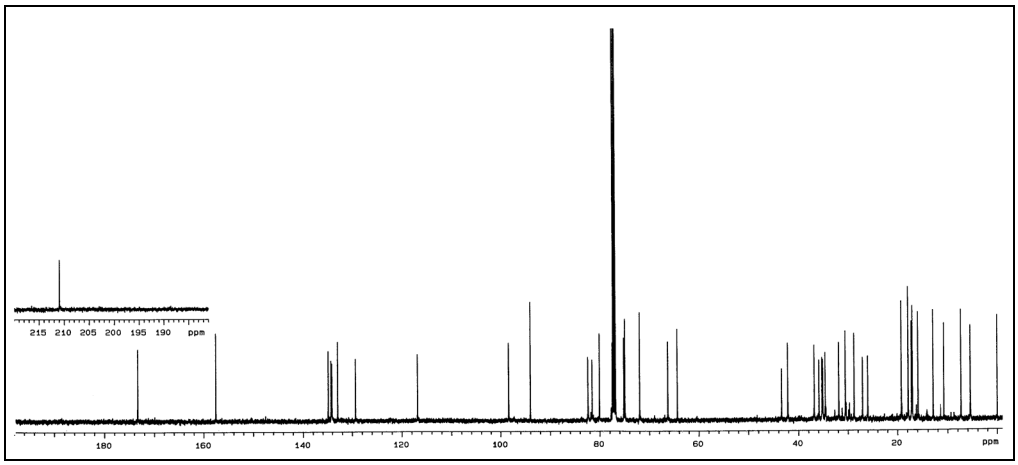
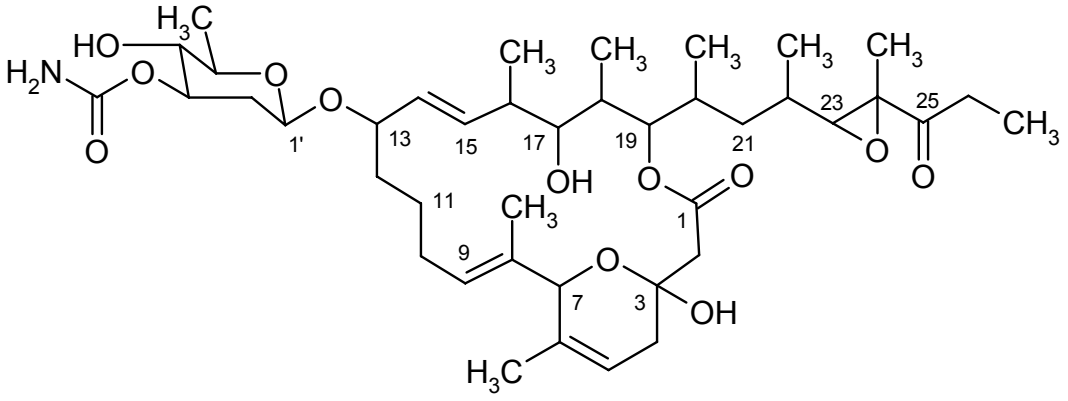


Figure 30: ^{13}C NMR spectrum (300 MHz) of irumamycin (**44**) in $CDCl_3$

With the help of ESI MS, the molecular weight was fixed at 763 Dalton; the ESI HRMS delivered the molecular formula $C_{41}H_{65}NO_{12}$. The substructure search in AntiBase supported by the NMR data and the molecular weight led to irumamycin (**44**), which has previously been isolated from *Streptomyces subflavus* by Ômura *et al*.[94] Irumamycin (**44**) is a 20-membered macrolide with a neutral sugar attached to the epoxidic aglycone. The gross structure resembles those of venturicidins,[95] although the latter compounds possess no epoxide group. Irumamycin (**44**) is reported as the first antifungal drug active against the phytopathogens *Piricularia oryzae, Sclerotinia cinerea* and *Botrytis cinerea*[94] and finds use in agriculture.[96]

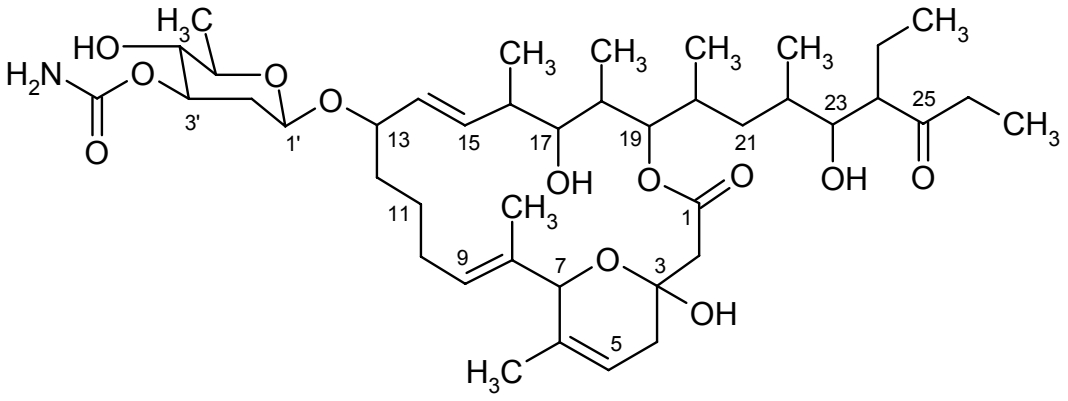


44

4.8.3 X-14952B

The TLC characteristic and the similarities in ^{1}H and ^{13}C NMR spectra with those of **44** indicated structural similarity of compound M_1 (**45**) with irumamycin (**44**). The

^{1}H NMR spectrum indicated also 9 methyl groups, however, the methyl singlet at δ 1.42 in **44** was replaced by an additional methyl triplet at δ 0.84 in **45**. The ^{13}C NMR spectrum delivered 42 signals, the chemical shift of the sugar residue and the lactone part of the aglycone moiety in **45** were nearly identical to those of **44** suggesting that **45** possessed the carbon skeleton of the aglycone of irumamycin (**44**). The carbon signals of the epoxy ring in **44** at δ 66.2 (C_q) and 64.2 (CH) were replaced by methine signals in **45** at δ 77.0 and 55.2. This spectral evidence indicated also the presence of the same 20-membered lactone ring in **45** as in **44** and the changes must have taken only in the side chain. ESI MS delivered the molecular weight of 779 Dalton, which is *Δm* 16 units higher than that of **44**. A substructure search in AntiBase suggested **45** as a possible structure, which was confirmed by comparison of the NMR data.[97]



45

The synthesis and biosynthesis of irumamycin (**44**) and X-14952B (**45**) were not reported.

4.8.4 KSM-2690 B

The compound Moyopo (**46a**) was obtained as a yellow to brown power (15 mg). The ^{1}H NMR spectrum of **46a** showed six 3H signals attributed to methyl groups at δ 1.72 (s), 1.71 (d), 1.11 (s), 1.04 (d), 0.98 (s) and 0.85 (d). Two additional methyl singlets bearing oxygen and nitrogen were, respectively, observed at δ 3.17 and 2.78. A 1H quartet was detected at δ 2.37. Complex signals of at least 10 protons between δ 6.80-5.60 revealed the presence of double bonds in **46a**. The aromatic region deliv-

ered only two signals: a 1H singlet at δ 8.22 and a triplet at δ 7.63. By ESI mass spectrometry, the molecular weight was deduced to be 669 Dalton.

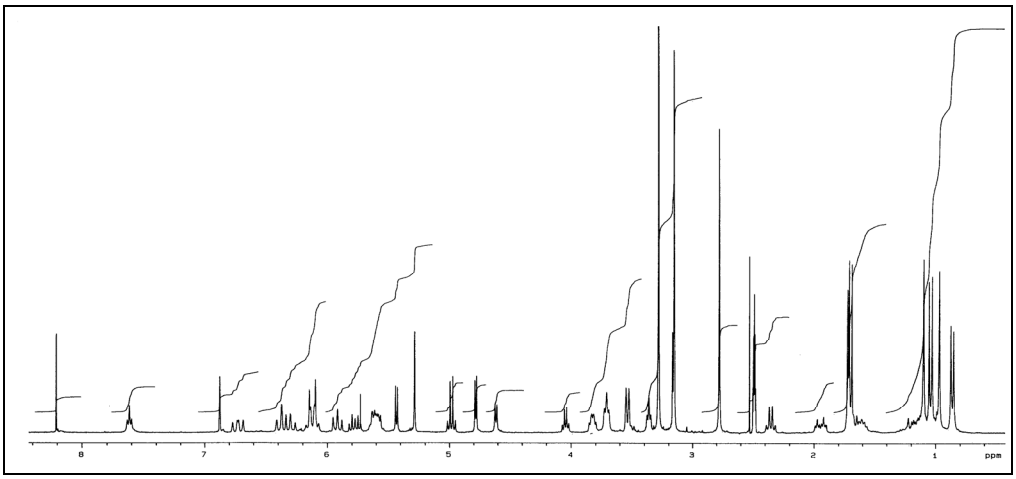


Figure 31: ^{1}H NMR spectrum (300 MHz) of KSM 2690 B (**46a**) in [D_6]DMSO

The ^{13}C/APT NMR spectra indicated 36 carbon atoms, where those appearing at δ 176.0, 174.3, and 170.0 could be assigned to carbonyl carbons of acid, ester, amide or lactone. Besides 13 further sp^2 atoms, 6 aliphatic methines at δ 82.6, 77.2, 75.1, 73.1, 43.5 and 36.7 with the three former probably connected to oxygen, three methylenes at δ 32.0, 40.3 and 28.2, 8 methyl and 3 aliphatic quaternary carbons at δ 83.5, 45.7, and 80.7 were identified.

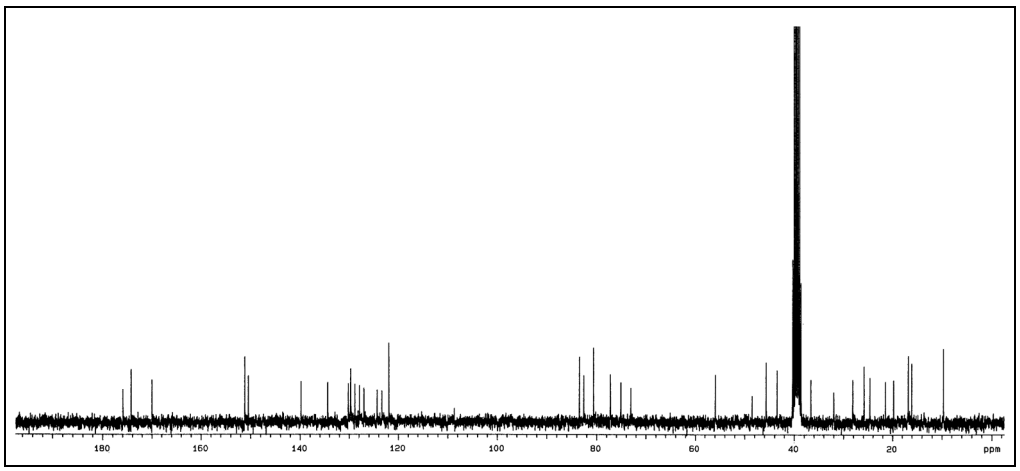


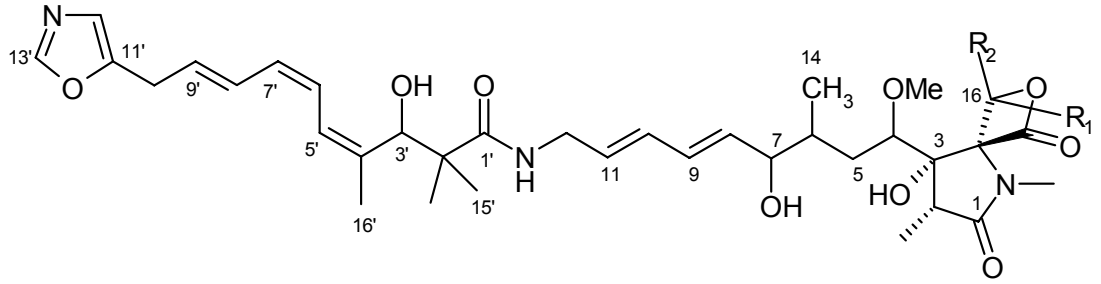
Figure 32: ^{13}C NMR spectrum (300 MHz) of KSM-2690 B (**46a**) in [D_6]DMSO

The search on AntiBase with the NMR data and molecular weight furnished three diastereomeric structures: KSM-2690 B (**46a**), KSM-2690 C reported from *Strepto-*

myces sp. KSM-2690 by Otani et *al*.[98] and 16-methyloxazolomycin (**46b**) isolated by Rhu et *al*.[99], as possibilities. By comparison of the experimental data with the literature values, it was finally identified as **46a,** which possess a triene moiety in the configuration 4'Z, 6'Z, and 8'E, while KSM-2690C presents 4'Z, 6'E, and 8'E. KSM-2690 B (**46a**) is active against human bladder carcinoma T24 cells with IC_{50} value of 10 μg/ml. Till now only some fragments of the oxazolomycin have been synthesized.[100,101] The biosynthetic pathway of oxazolomycin antibiotics have been studied by feeding experiments.[102] It reveals the following evidence

- That both the (E,E)-diene and the (Z,Z,E)-triene chains are built up from acetate *via* the polyketide pathway, each starting with a glycine-derived building block.
- The use of glycine indicated an uncommon biosynthetic pathway leading to oxazole rings in actinomycetes.
- The spiro-ring system in I is formed from acetate, glycine, and a C3 unit of unknown origin.

Another approach of the biosynthesis suggests the use of nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs). This hybrid NRPS-PKS system will allow the production of novel metabolites by incorporating both amino acids and carboxylic acids, greatly expanding the size and structural diversity. The oxazolomycin antibiotics are known to exhibit weak antimicrobial activity against only a limited range of bacteria such as *B. subtilis, Micrococcus luteus* and *Agrobacterium tumefaciens,* and to have cytotoxic activity against P388 leukemia cells.[99,103]



46a: R_1= H R_2 = CH_3
46b: R_1 = CH_3 R_2 = H

4.8.5 P-371-A2

The sub-fraction III of the fraction 4 showed an orange spot on TLC, which changed to dark brown on spraying with anisaldehyde/sulphuric acid. Its purification by HPLC afforded MOYOE (**47**) as an orange powder with the characteristic NaOH colour reaction of a *peri*-hydroxy quinone.

Compound **47** delivered a complex ^{1}H NMR spectrum. It presented a hydrogen bridged proton at δ 12.46 and two 1H doublets at δ 7.85 (^{3}J = 7.8 Hz) and 7.65 (^{3}J = 7.8 Hz), which could be assigned to *ortho*-aromatic protons. In addition, many signals of oxygenated methine protons appeared at δ 6.00 (d), 5.81 (d), 5.35 (d), 5.02 (dd), 4.84 (d) and in the range of δ 4.80-4.30. These signals as well as multiplets in the range of δ 3.80-3.10 suggested at least three sugar moieties. Furthermore, signals due to a methoxy group (δ 3.28), two acetyl residues (δ 2.30 and 2.18), and six methyl groups gave signals at δ 1.78, 1.42, 1.30, 1.25, 1.24 and 1.21, of which the first appeared to be connected to an sp^2 carbon atom.

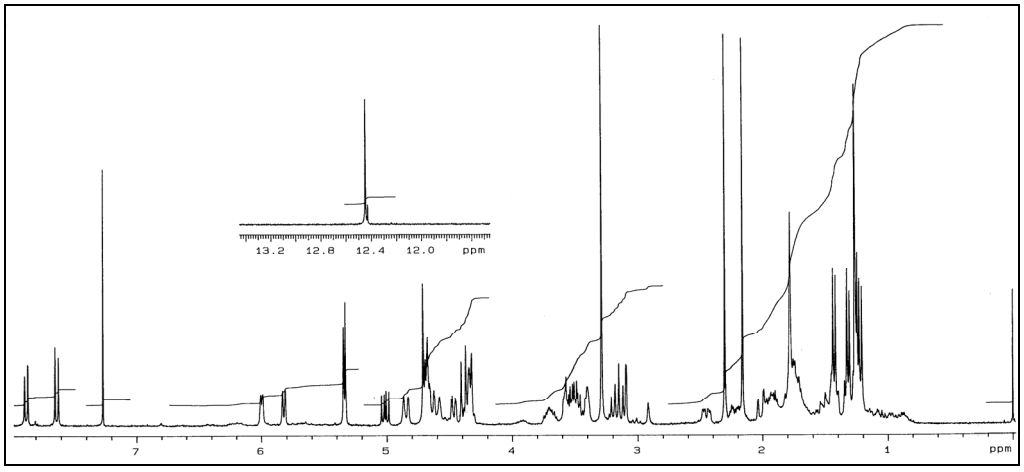


Figure 33: ^{1}H NMR spectrum (300 MHz) of P-371-A2 (**47**) in $CDCl_3$

The ^{13}C NMR spectrum indicated 50 carbon signals. As suggested by the NaOH test, it exhibited signals of two quinone carbonyls at δ 188.3 and 187.1. Further carbonyl signals were observed at δ 171.1 and 170.9 and were assigned to ester carbonyls. The signals at δ 158.4 and 155.7 could be attributed to amide or sp^2 oxygenated carbons. The APT spectrum indicated also signals at δ 103.1, 99.6 and 99.3 attributed to ano-

meric carbon atoms, confirming the assumption of ^{1}H NMR spectrum and indicating at least three sugar residues in the molecule **47**.

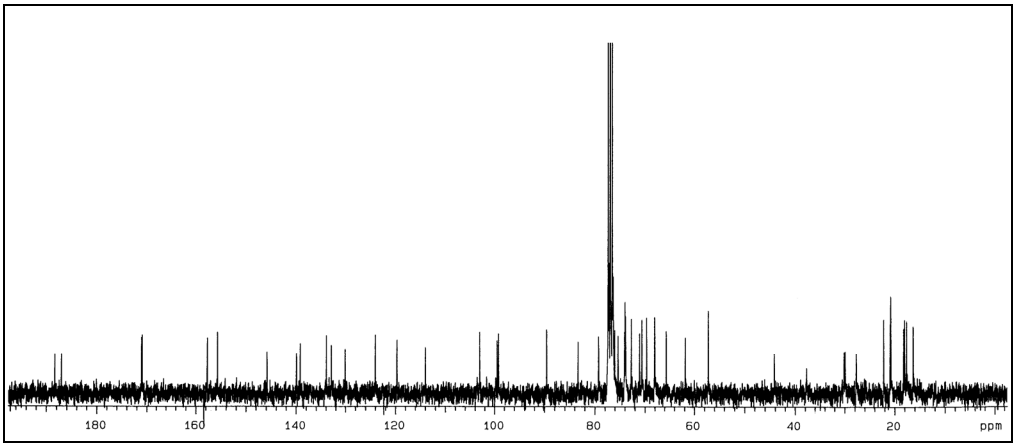
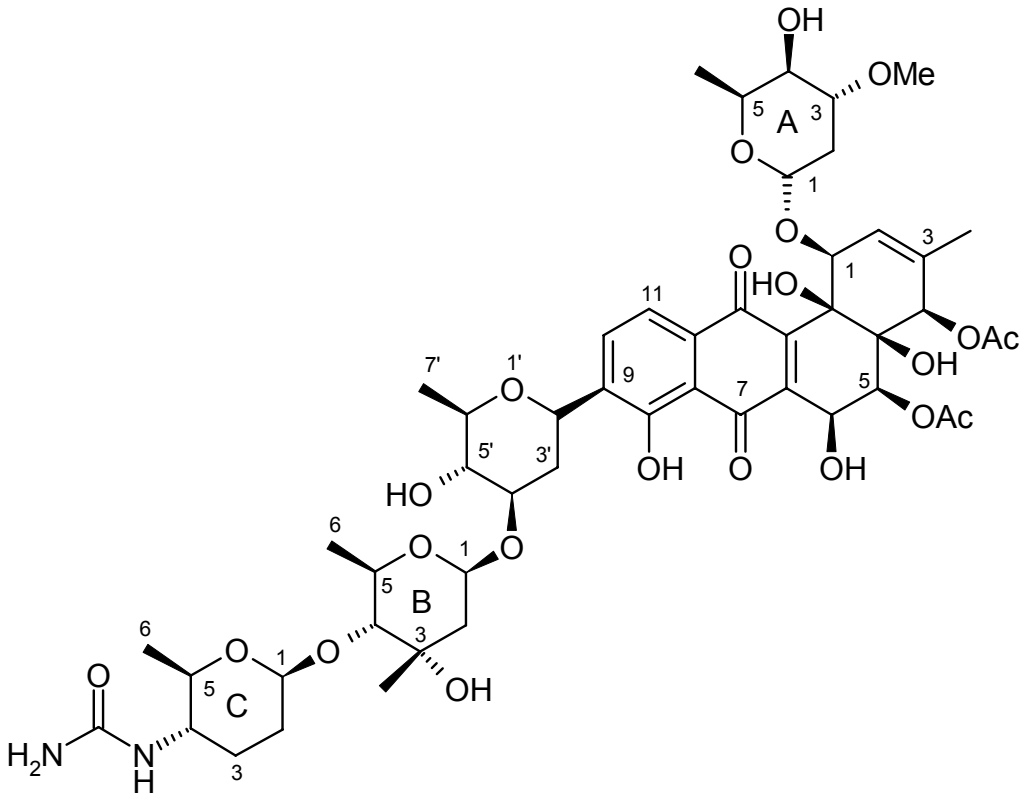


Figure 34: ^{13}C NMR spectrum (300 MHz) of P-371-A2 (**47**) in $CDCl_3$

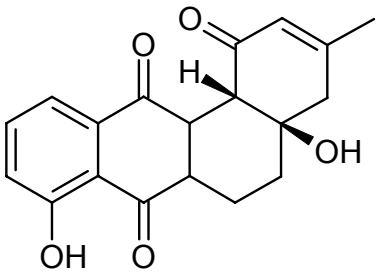


47

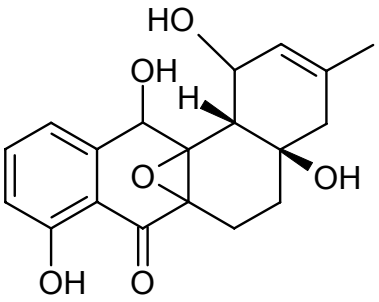
The molecular weight of 1048 Dalton was determined by ESI MS experiments, where the signals at *m/z* 1072 in positive and 1047 in negative mode, respectively, could be assigned to the $[M+Na]^+$ and $[M-H]^-$ ions. A substructure search in Anti-

Base furnished P-371-A2 (**47**) as possibility structure, which could be confirmed by comparison of the measured NMR data with the literature values. P-371-A2 (**47**) has been previously isolated from *Streptomyces sp.* P371[104] and was reported to have gastric antisecretory activity.[105]

The angular and reduced quinone chromophore of P-371-A2 (**47**) is very rare in natural products: The only related compounds SF2315A (**48**) and B (**49**) are produced by *Nocardia* sp., and *Excellospora viridilutea.* They are weakly active against Gram-positive bacteria and inhibited reverse transcriptase of avian myeloblastosis virus and show also immunosuppressant and antitumor activity.[106, 107]



48



49

4.9 Terrestrial *Streptomyces* sp.US80

The fungus *Fusarium oxysporum* sp. *albedinis* (Foa) has caused destruction of a large number of palms in the oases of Algeria and Morocco,[108, 109] but not in Tunisia. This surprising fact could be due to the physico-chemical characteristics of Tunisian oasis soil, and/or to the presence of antagonistic microorganisms, which might inhibit Foa development and dissemination. Hence screening of new strains, possessing antifungal activities, from the soil of Tunisian oases could be advantageous in this field. The strain US80 was collected from Tunisian oasis soil during this study in cooperation with a group of L.Mallouli in Sfax.

Chromatography of the methanolic derived crude extract on Sephadex LH-20 delivered three compounds, which were identified as irumamycin (**44**), X-14952B (**45**) and 17-hydroxy-venturicidin A (**50**); the first two had been previously identified and were also isolated from the terrestrial *Streptomyces* sp. GW 9/2235 (see p 52).

4.9.1 17-Hydroxyventuricidin A

Compound **50** showed the same physical properties as **44** and **45**. The ^{1}H NMR spectrum was very similar to that of **45** with only four olefinic protons but many signals in the aliphatic region, the major difference being the absence of the triplet at δ 0.84 in **45,** which was replaced by a doublet at δ 1.02 in **50**. The ESI mass spectrum indicated the *pseudo*-molecular ion at *m/z* 788 $[M+Na]^{+}$ and the molecular weight was deduced to be 765 Dalton. The molecular formula was determined by high resolution mass spectrum to be $C_{41}H_{67}NO_{12}$. The mass difference of Δm = 14 between **45** and **50** can be attributed to the loss of a methylene group. The ^{13}C NMR spectrum indicated the presence of only 41 carbon atoms. The comparison of the ^{13}C NMR data of **50** with those of **44** and **45** suggested that all possessed the same aglycone and sugar moiety, and that the differences were localized in the side chain. The carbon data of the side chain indicated the absence of the signal at δ 22.6 due to the missing methylene group of the ethyl connected at C-24 (24-CH_2CH_3). The structure **50** was finally confirmed by comparison of the ^{13}C NMR data with those of **45** which possesses the same side chain. Compound **50** was so far only reported in a Japanese patent as YP-02259L-C.[110] Compound **50** is related to venturicidin A[111] with the only difference being the additional 17-OH group: it is therefore 17-hydroxy-venturicidin A.

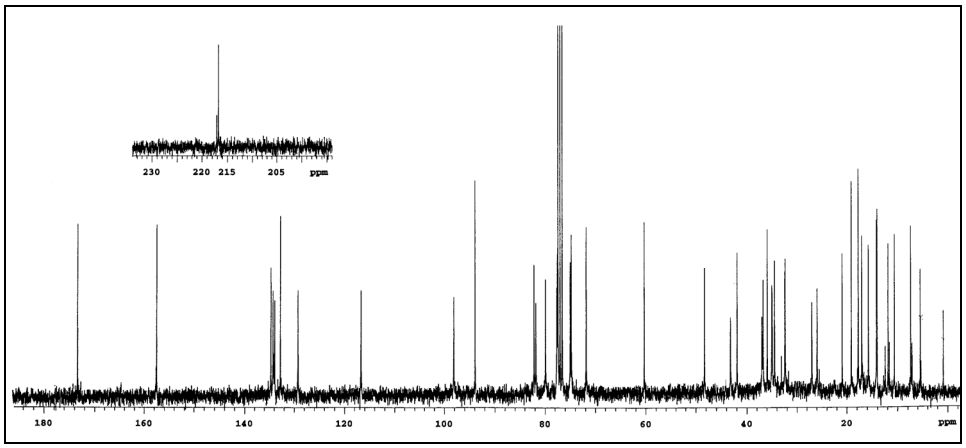
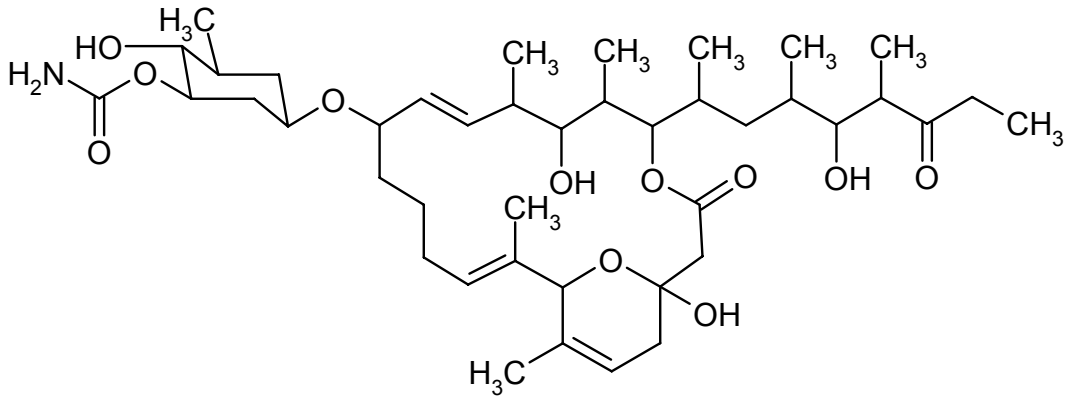


Figure 35: ^{13}C NMR spectrum (75.5 MHz) of 17-hydroxy-venturicidin A (**50**) in $CDCl_3$



50

Table 1: Comparison of ^{13}C NMR chemical shifts of X-14952B (**45**), 17-hydroxy-venturicidin A (**50**)

C No.	**45**	**50**	C No.	**45**	**50**
1	173.4	173.3	18-CH_3	5.5	5.5
2	43.3	43.2	19	81.9	81.8
3	94.0	93.9	20	33.4	33.5
4	35.1	35.0	20-CH_3	15.8	15.8
5	116.8	116.7	21	37.0	35.9
6	134.0	132.7	22	32.5	32.4
6-CH_3	19.1	19.1	22-CH_3	12.6	14.0
7	80.0	79.9	23	76.7	77.6
8	137.7	134.7	24	55.1	48.3
8- CH_3	10.7	10.6	24-CH_2CH_3	22.6	-
9	129.3	129.3	24-CH_2CH_3	11.7	14.1
10	27.0	27.0	25	217.2	216.8
11	26.0	25.9	26	37.9	35.9
12	35.0	34.5	27	7.2	7.4
13	82.2	82.2	1’	98.2	98.1
14	134.3	134.3	2’	37.0	36.8
15	134.0	133.9	3’	75.2	74.8
16	42.0	42.0	3’-$OCONH_2$	157.5	157.5
16-CH_3	17.1	17.1	4’	74.9	75.0
17	77.7	77.7	5’	71.9	71.9
18	34.5	34.5	5’-CH_3	17.8	17.7

Table 2: ^{1}H (300 MHz) NMR data of (**50**) in $CDCl_3$

H No.	δ_H	H No.	δ_H
1	-	18-CH_3	0.92
2	2.40~2.60	19	4.86 d
3	-	20	1.67
4	2.10~2.30	20-CH_3	0.83
5	5.52	21	1.1, 1.42
6	-	22	1.58
6-CH_3	1.48	22- CH_3	0.82
7	4.45	23	3.54
8-CH_3	1.40	24	2.68
9	5.45	24 -CH_3	1.02
10	1.84~2.10	25	-
11	1.2~1.5	26	2.50
12	1.50~1.60	27	1.26
13	3.93	1’	4.60
14	5.60	2’	1.62, 2.24
15	5.30	3’	4.66
16	2.40	4’	3.16
16- CH_3	1.08	5’	3.20
17	3.18	5’- CH_3	1.31
18	1.98		

Irumamycin (**44**), X-14952B (**45**) and 17-hydroxyventuricidin A (**50**) were found to inhibit the growth of the filamentous fungi *V. dahliae* and *Fusarium* sp. as well as *C. tropicalis* R2CIP203; the highest antifungal activity was obtained with irumamycin (**44**).

4.10 Terrestrial *Streptomyces* sp. GW 37/3236

The culture of the terrestrial *Streptomyce*s sp. GW 37/3236 gave an extract, which in the biological screening exhibited high activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Streptomyces viridochromogenes*, *Mucor miehei* and also the micro-algae. The chemical screening showed on TLC some red spots,

which turned violet to blue with sodium hydroxide, indicating *peri*-hydroxy quinones.

The 25 L fermentation of the strain was done on a linear shaker at 28 °C for four days followed by extraction with ethyl acetate. The resulting dark red crude extract was subjected to several purification steps like chromatography on silica gel, Sephadex LH-20, and preparative thin layer chromatography and 10 compounds were separated and elucidated as shown in Figure 36.

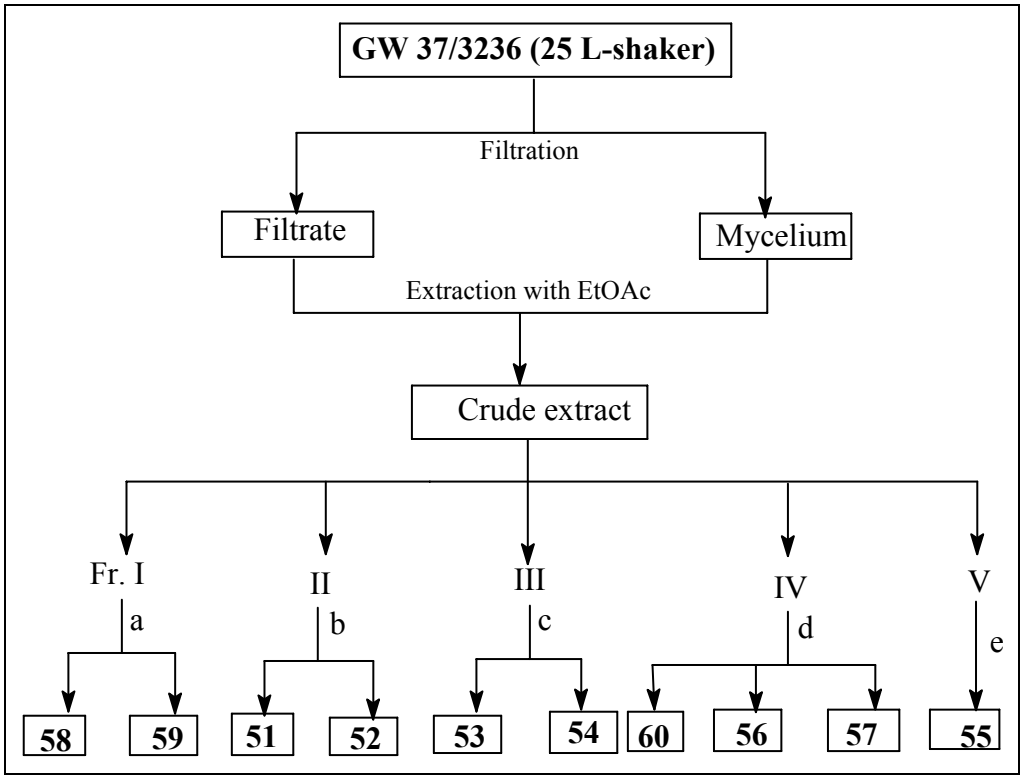


Figure 36: Work-up scheme of the strain *Streptomyces* sp. isolate GW 37/3236. a) PTLC with CH_2Cl_2; b) Crystallisation from CH_2Cl_2/MeOH and the PTLC with CH_2Cl_2/5% MeOH/0.1% TFA; c) Sephadex LH-20 with CH_2Cl_2/40% MeOH followed by PTLC with CH_2Cl_2/5% MeOH; d) Sephadex LH-20 with CH_2Cl_2/60% MeOH followed by PTLC with CH_2Cl_2/5% MeOH; e) PTLC with cyclohexane/85% ethyl acetate/0.1% TFA.

4.10.1 Bisanhydro-13-dihydrodaunomycinone

Purification of fraction II gave compound SM2b (**51**) as a red powder, which turned to violet with diluted sodium hydroxide and gave on TLC an orange fluorescent spot

under UV 366 nm. The ^{1}H NMR spectrum showed two exchangeable broad singlets between δ 16.60 and 15.64, which are typical for chelated hydroxyl groups of quinones. The red colour and the orange fluorescence were confirming this assumption. The ^{1}H NMR spectrum exhibited six aromatic protons divided into two groups. The first was constituted of a doublet of doublet, a triplet and a doublet at δ 8.07, 7.70 and 7.30, respectively, and indicated three consecutive protons. The other signals appeared as broad singlets and two doublets at δ 8.85, 8.58 and 8.18, suggesting an 1,2,4-trisubstitution pattern. Furthermore, a quartet of a methine proton bearing an oxygen atom at δ 5.37 and a methyl doublet at δ 1.70 attributed to the fragment CH_3CH-O, and a methoxy group at δ 3.98 were also seen.

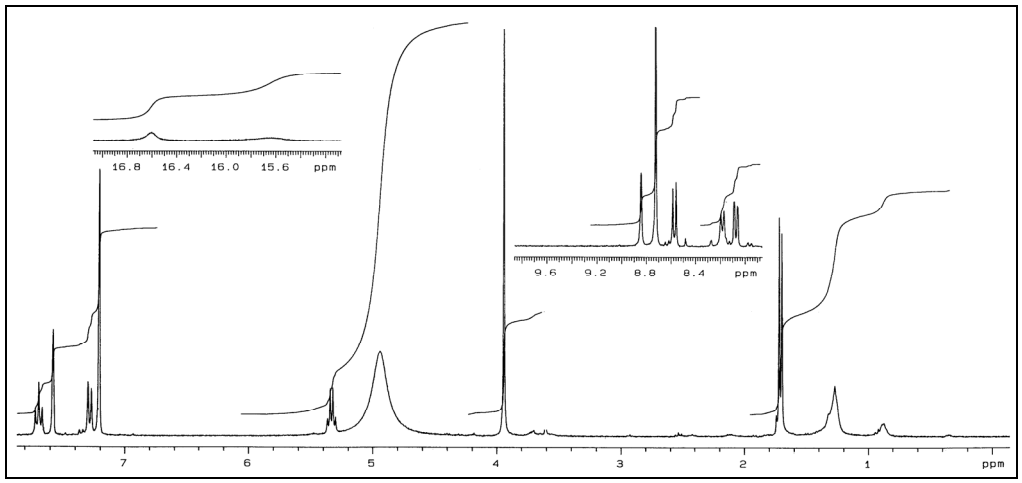
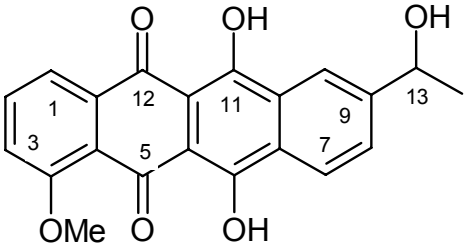


Figure 37: ^{1}H NMR spectrum (300 MHz) of bisanhydro-13-dihydro daunomycinone **51** in C_5D_5N

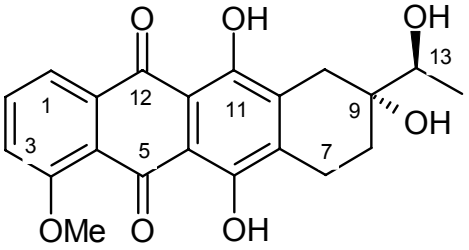
The ^{13}C NMR spectrum of **51** was in accord with the ^{1}H NMR spectrum and indicated the presence of 21 signals classified by APT as six aromatic methines, 8 quaternary sp^2 carbons, one methoxy signal (δ 56.5), one aliphatic methine, one methyl carbon signal. In addition four signals appeared at ca. δ 171, which may be due to a tautomerisation at C-6 and C-11. The EI and ESI mass spectra delivered the molecular ion at *m/z* 364. The search in AntiBase gave bisanhydro-13-dihydro aunomycinone (**51**) as possible structure. Bisanhydro-13-dihydrodaunomycinone is reported as a product of microbial conversion of daunorubicin.[112]



51

4.10.2 7-Deoxydaunomycinol

The compound SMa1 (**52**) was also obtained from fraction II as a red powder with the same characteristics as **51**. The ^{1}H NMR spectrum of **52** was very similar to that of **51**, with two chelated hydroxy groups at δ 14.48 and 13.86, the 1,2,3-trisubstituted aromatic ring, and the CH_3CH-O fragment. The only difference was the absence of signals of the 1,2,4-trisubstituted ring in **51** and the presence of 3 methylene groups instead in the range of 3.55-3.00 (4H) and 1.90-2.40 (2H). The ^{13}C and APT NMR spectra are in accordance with these observations and consisted of 21 carbon signals, among them two signals of quinone carbonyl groups at δ 186.8 and 186.4, three methylene groups at δ 32.4, 28.2 and 21.2, and a signal due to a quaternary aliphatic carbon atom bearing an oxygen at δ 71.5. The EI mass spectrum indicated the molecular ion at *m/z* 384 Dalton. The search in AntiBase using all this information followed by comparison with the literature[113] led to the identification as 7-deoxydaunomycinol (**51**).



52

4.10.3 Daunomycinol

Fraction III showed by TLC red spots with R_f = 0.6 (cyclohexane/15% ethyl acetate). PTLC yielded compound SMO2b (**53**) as major compound. The ^{1}H NMR spectrum was similar to that of **52**, however, it showed the presence of one methine connected

to oxygen, which appeared as triplet at δ 5.84, one singlet of two protons at δ 3.41 and two doublet of doublet of a methylene group at δ 2.84 and 2.58. The ^{13}C NMR spectrum matched with that of **52** showing 21 signals. The only difference was the presence of two methylenes instead of three in **52** and one additional methine at δ 64.7. The EI mass spectrum showed the molecular ion at *m/e* 400, the mass difference of Δm = 16 between **53** and **52** indicated only one additional hydroxyl group in compound **53**. Compound **53** was finally identified as daunomycinol (**53**) by comparison with the literature.[113]

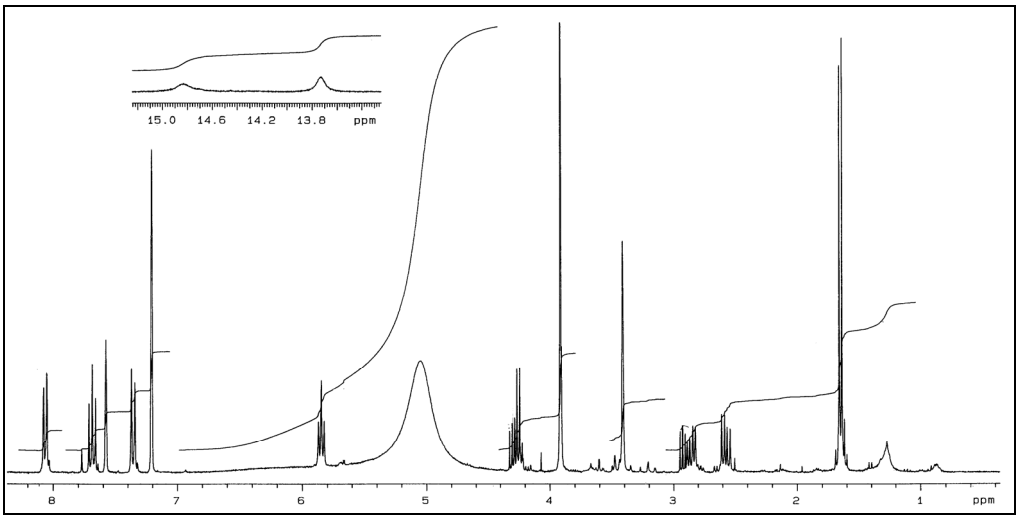
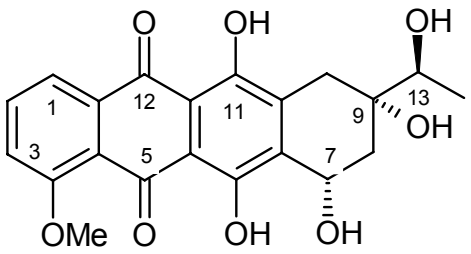


Figure 38: ^{1}H NMR spectrum (300 MHz) of daunomycinol (**53**) in C_5D_5N

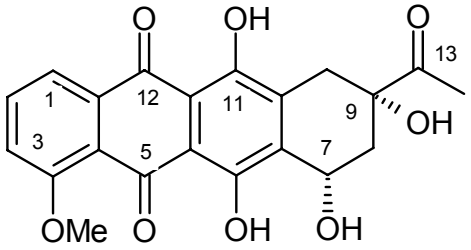


53

The 10-H_2 signal appeared in **53** as singlet instead of AB signals as in **52**, **54** and **55** may be attributed to a conformation change which allows these two protons to be equivalent as in cyclohexane or due to a rapid equilibrium.

4.10.4 Daunomycinone

Purification on Sephadex LH-20 followed by PTLC of fraction III delivered compound SMf (**54**) as a red powder with the same characteristics as **52** and **53**. Comparison of the ^{1}H NMR spectrum of **53** with that of **54** showed as the only difference the absence of the methine quartet at δ 4.23 in **53** and the presence of a 3H singlet at δ 2.50 in **54**, which could be assigned to an acetyl group. The EI mass spectrum indicated the molecular peak at *m/z* 398, a search using AntiBase identified SMf as daunomycinone (**54**)[113] which can be obtained from **53** by an oxidation of the secondary alcohol of the side chain.



54

4.10.5 Baumycin C1

The polar fraction V delivered after Sephadex LH-20 and PTLC with cyclohexane/ 85% ethyl acetate /0.1% TFA the red powdery compound SMO3a1 (**55**). In the aromatic region, the ^{1}H NMR spectrum showed the signals of the daunomycinone (**54**) moiety, however, an additional singlet at δ 8.08. A broad doublet at δ 5.50 in the ^{1}H NMR spectrum was attributed to an anomeric proton, a doublet of an H/D exchangeable proton at δ 6.16, two doublets of a methylene group at δ 3.20 and 1.90, a broad singlet of a methine at δ 3.62, a methine quartet and a methyl doublet at δ 1.29 indicated a desoxy hexose.

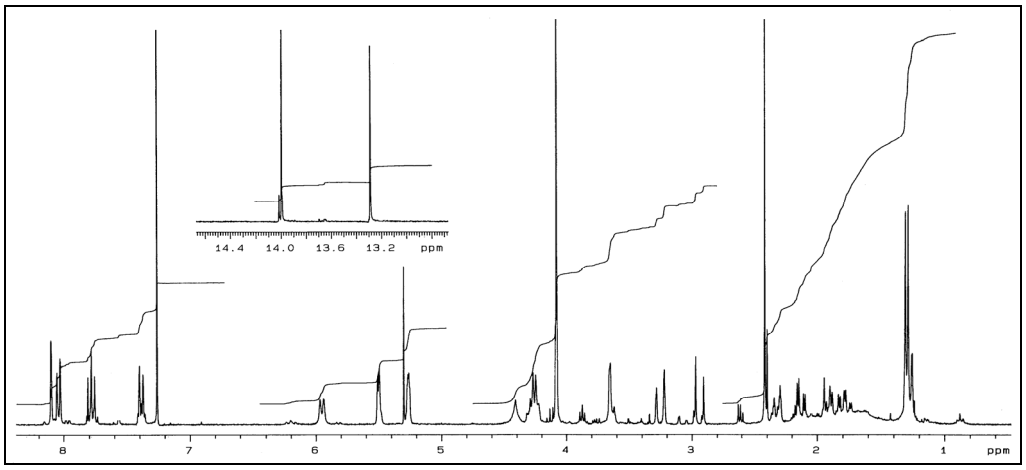


Figure 39: ^{1}H NMR spectrum (300 MHz) of baumycin C_1 (**55**) in $CDCl_3$

The ^{13}C NMR spectrum indicated the presence of 28 carbon atoms, among them three signals at δ 212.4, 187.3 and 186.9 assigned to the carbonyl of an aliphatic ketone and quinone groups, respectively. Another methine signal at δ 160.7 was attributed to an aldehyde group; two methyl, three methylene and four methine carbon signals were additionally observed.

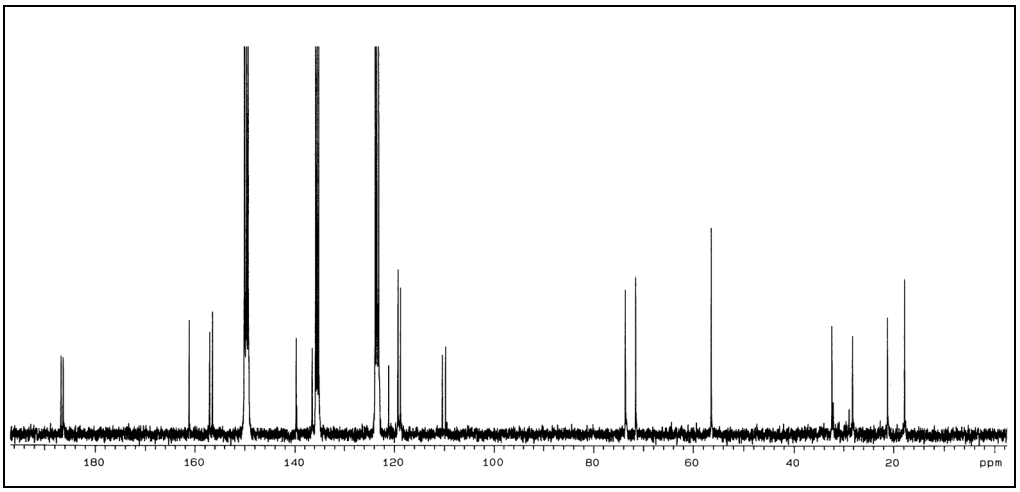
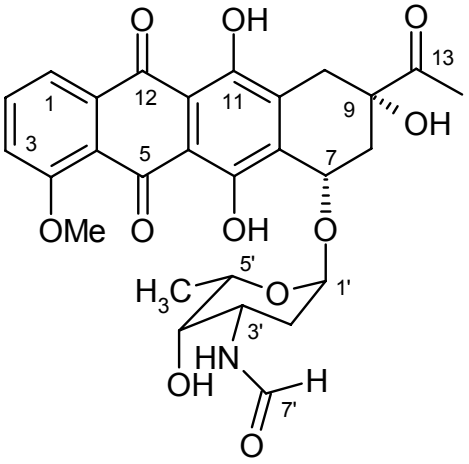


Figure 40: ^{13}C NMR spectrum (75 MHz) of baumycin C_1 (**55**) in C_5D_5N

The molecular weight was deduced from (+)-ESI MS, which indicated the *pseudo* molecular ion at *m/z* 578 $[M+Na]^+$. A search in AntiBase gave the daunosamine glycoside baumycin C_1 (**55**)[114] (N-formyldaunomycin), which is reported as a strong antibacterial and antifungal agent and also inhibits the nucleic acid synthesis of murine leukemia L1210 cells.[115]

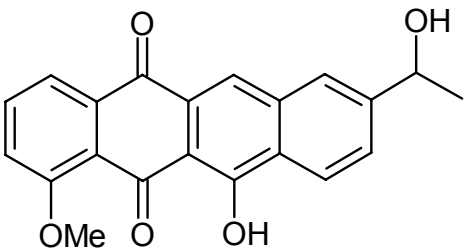


55

In the anthracycline antibiotics, the presence of the amino sugar residue is an important structural requirement for bioactivity as only few biological properties due to the aglycone alone have been reported.[116]

4.10.6 11-Deoxybisanhydro-13-dihydrodaunomycinone

The red compound SM5C1 (**56**) showed a ^{1}H NMR spectrum similar to that of bisanhydro-13-dihydrodaunomycinone (**51**), but in addition it gave one singlet in the aromatic region at δ 8.20 and only one chelated hydroxyl group δ 14.87 instead of two as in **51**. The EI mass spectrum indicated a molecular weight of *m/z* 348. The mass difference of Δm = 16 between **51** and **56** is in concordance with the ^{1}H NMR spectrum, where one chelated hydroxyl group was missing. With the help of Anti-Base, structure **56** was elucidated as 11-deoxybisanhydro-13-dihydrodaunomycinone (**56**), which was previously isolated from *Streptomyces coeruleorubidus* by Vanek *et al*.[117]



56

4.10.7 6,9,11-Trihydroxy-4-methoxy-5,12-naphthacenedione

Compound SM7a (**57**) was obtained from the fraction IV as brownish red powder. The aromatic region of the ^{1}H NMR spectrum of **57** was also very similar to that of **51**, but in the aliphatic region the signals of the side chain in **51** were absent in **57**.

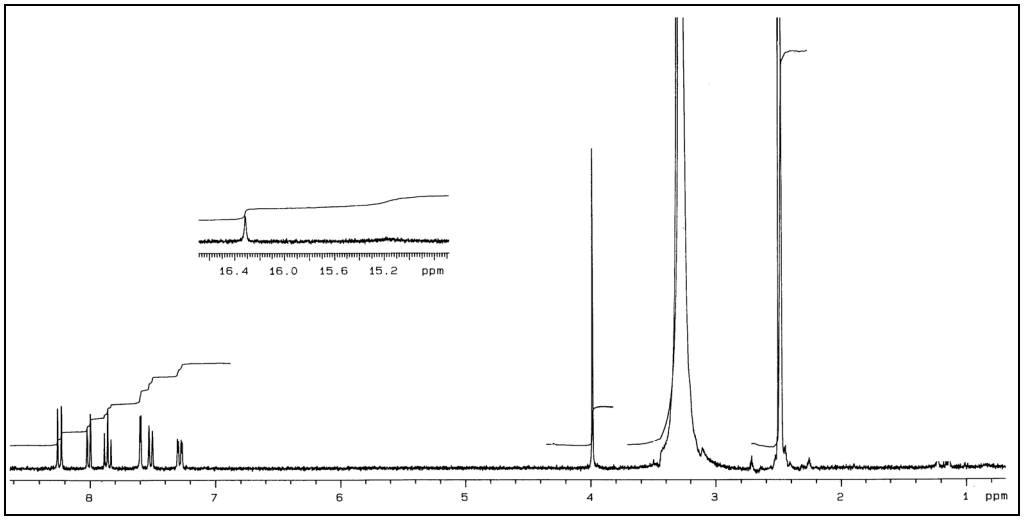
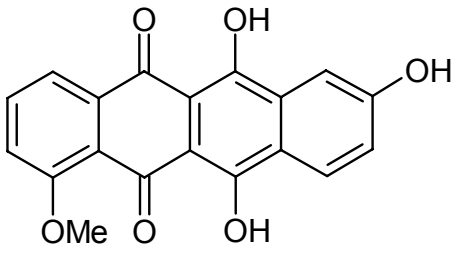


Figure 41: ^{1}H NMR spectrum (300 MHz) of anthracycline **57** in $CDCl_3$

The molecular weight was deduced from the ESI and EI mass spectra to be *m/z* 336. By a search in AntiBase with the NMR data and the molecular weight and comparison with the literature,[118] compound **57** was identified as 6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione. It was reported previously only as a degradation product of daunorubicin and is reported here now for the first time as natural product.



57

4.10.8 13-O-Acetyl-bisanhydro-13-dihydrodaunomycinone

Compound SMd (**58**) was obtained as a red powder. The IR spectrum showed a broad band for an OH-group at 3430 cm^{-1}; the signals for carbonyl groups at 1616 cm^{-1} indicated their chelation with hydroxyl groups, and other signals at 1737 and

1234 cm^{-1} were attributed to an ester carbonyl and a C-O bond, respectively. The UV spectrum in methanol showed a broad absorption in the region of 263-530 nm.

The ^1H NMR spectrum of **58** was very similar to that of bisanhydro-13-dihydrodaunomycinone (**51**). Here too, the spectrum depicted two sets of aromatic protons at δ 8.00, 7.65 and 7.20 for a 1,2,3-substituted (ring A) and at δ 8.35, 8.30 and 7.70 for a 1,2,4-substitued benzene ring (ring D). This assignment was confirmed by H,H COSY spectra. It also contained two chelated hydroxyl protons at δ 15.80 and 15.10, a methoxy signal at δ 4.00, a quartet at 5.95 for a methine group bearing an oxygen and a methyl doublet at δ 1.60 representing a CH_3CH-O fragment. Additionally it showed an acetate methyl signal at δ 2.10, which is absent in the spectrum of **51**. The difference in mass and molecular formula of *Δm* 42 Dalton and in the formula of C_2H_3O between **58** and **51** suggests **58** to be an acetate of **51**.

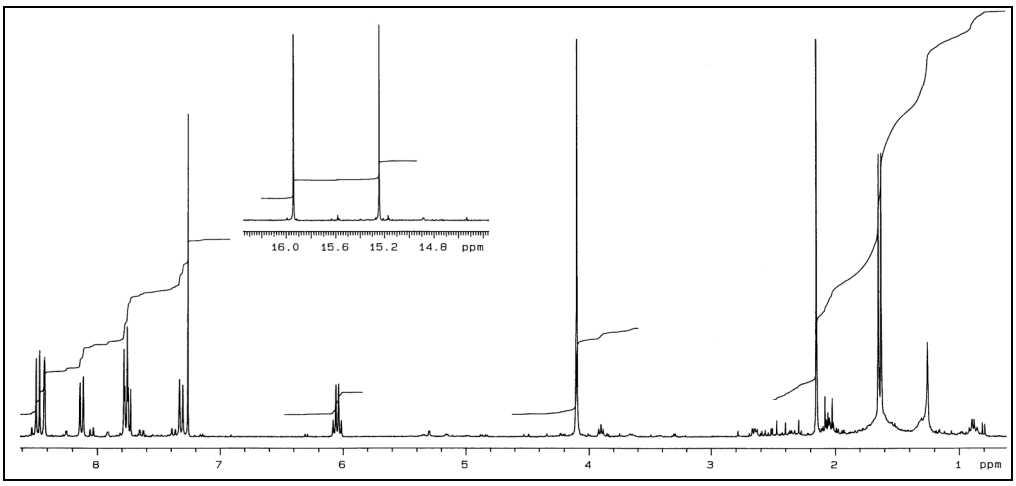


Figure 42: ^1H NMR spectrum (300 MHz) of anthracycline **58** in $CDCl_3$

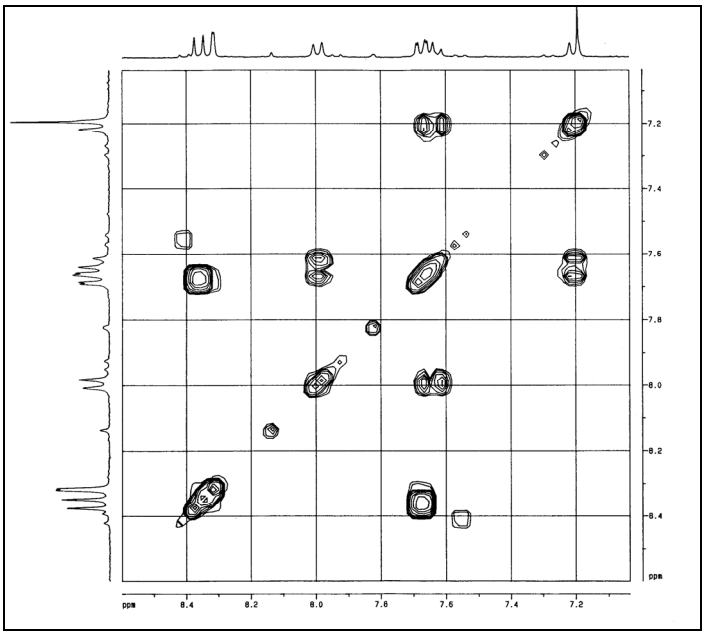


Figure 43: H,H COSY spectrum (300 MHz) of the aromatic region of anthracycline **58** in $CDCl_3$

The ^{13}C and APT NMR spectra possessed 23 signals as required by the molecular formula, including 3 signals of carbonyl groups at δ 177.8, 175.5 and 170. 2. The two first were attributed to the quinone system and the other to an ester group. The ^{13}C NMR spectrum indicated six aromatic methine carbons and an aliphatic methine bearing oxygen. Among the ten aromatic quaternary carbons, three were bearing oxygen.

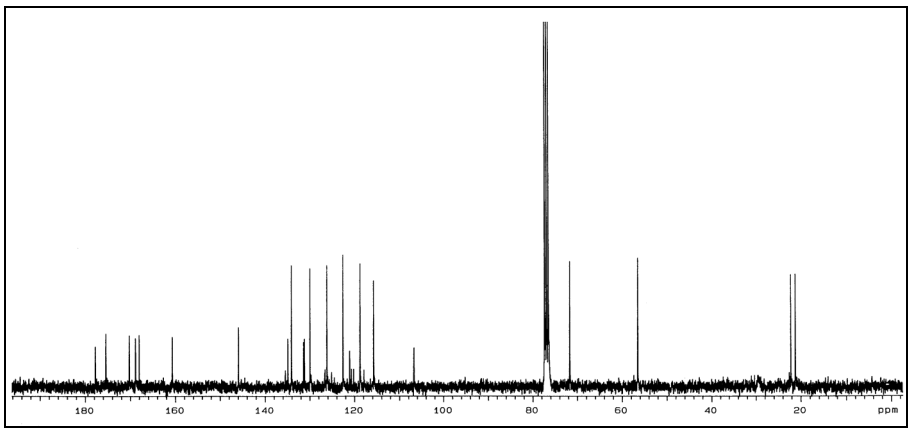


Figure 44: ^{13}C NMR spectrum (300 MHz) of anthracycline **58** in $CDCl_3$

The careful interpretation of the HMBC spectrum confirmed the presence of the two spins system constituted of ring A and ring D. In addition it indicated also a correlation of the methyl doublet to C-13 and the quaternary sp^2 C-9, the 11-OH and 6-OH indicated correlations to C-10a, C-11, C-11a and C-5a, C-6, C-6a respectively. Further interpretation of 2D NMR data in combination with the molecular formula led to 13-O-acetyl-bisanhydro-13-dihydro-daunomycinone (**58**), which is reported here for the first time.

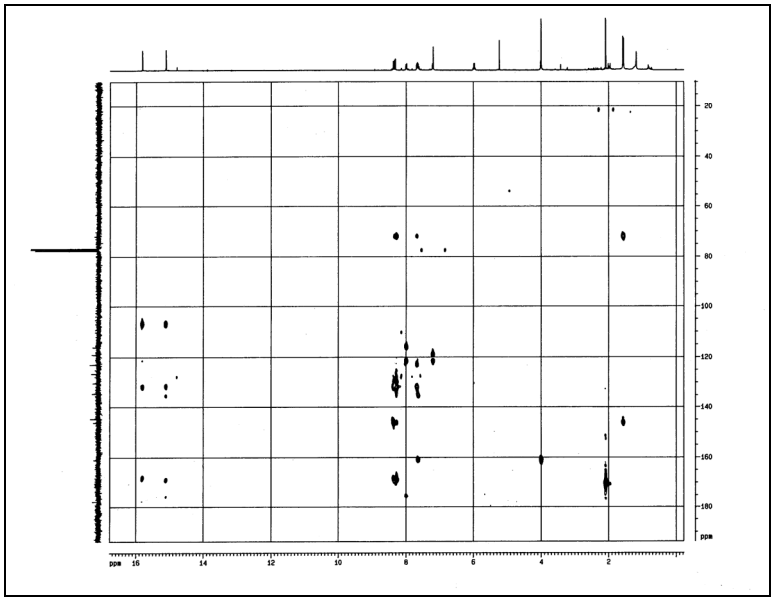
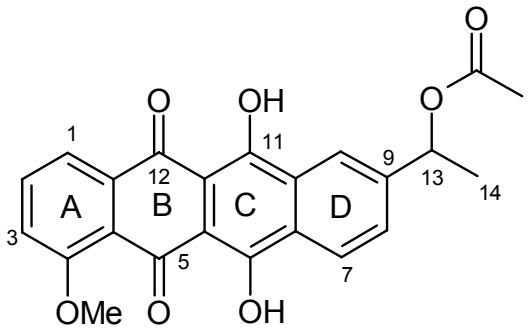


Figure 45: HMBC spectrum (300 MHz) of anthracycline **58** in $CDCl_3$

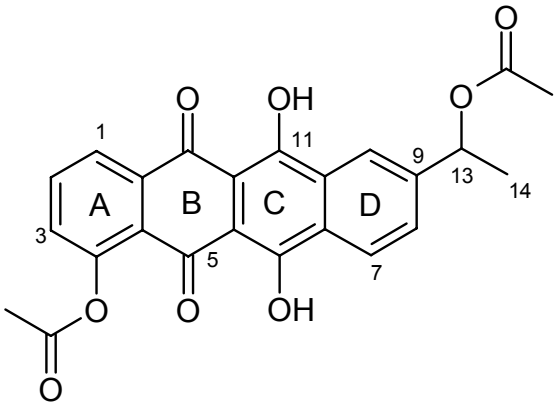


58

4.10.9 4,13-O-Diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone

Compound SMd1 (**59**) was also obtained from fraction I as red solid. The molecular weight of 434 Dalton was deduced from ESI and EI mass spectra. The ^{1}H NMR spectra of **59** and **58** showed a high similarity, the only difference being the replace-

ment of the methoxy signal in **58** with an acetate in **59**. The difference in mass and molecular formula *Δm* 28 (≡ CO) between these two compounds suggests that in **59** probably the methoxy group of **58** is replaced by an acetate group. The chelated hydroxy groups appeared at δ 15.61 and 15.20. Comparison of the ^{1}H NMR data of **58** and **59** indicated that the aromatic ring D and the side chain protons show nearly identical chemical shift. On the other hand, all proton signals of ring A were shifted to deeper field suggesting that a modification had occurred in that ring. On the basis of these facts, the structure SMd1 was assigned to be 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (**59**), which is also a new antibiotic.



59

The anthracycline family of antibiotics forms a unique group of red pigments[119] and is an important source for therapeutically useful antitumor agents. Adriamycin (doxorubicin) is now the most frequently used drug for the cancer treatment and has a broad antitumor spectrum with great therapeutic efficacy. Daunomycin[120] (daunorubicin) and aclacinomycin (aclarubicin)[121] are excellent therapeutic drugs for blood cancer treatment. However, further development of new anthracyclines is required to improve the therapeutic efficacy and to reduce side effects such as cardiotoxicity and bone marrow suppression.[122] Anthracyclines are present in nature in free form or as glycosides with one or more sugar residues. Their cytotoxic activity is usually due to intercalation into the DNA strand.

4.10.10 2-Acetylamino-3-hydroxy-benzamide

Fraction IV showed a strong blue fluorescent spot under 366 nm. Purification using Sephadex LH-20 and finally PTLC afforded compound SM7 (**60**) as colourless solid

with a molecular weight of 194 Dalton and a molecular formula of $C_9H_{10}N_2O_3$ (EI HRMS). The pattern of the ^1H NMR spectrum in the aromatic region suggested the presence of three protons, constituting a 1,2,3-trisubstituted aromatic ring. The spectrum also showed four exchangeable protons at δ 7.14, 7.79, 10.08, 11.83 and a signal for a methyl group linked to an sp^2 carbon at δ 2.24.

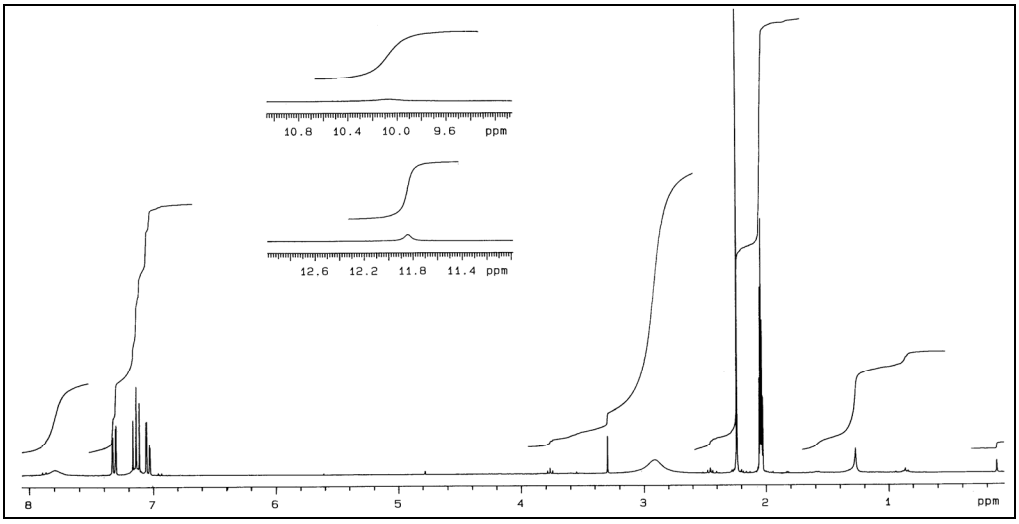
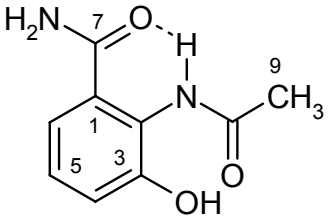
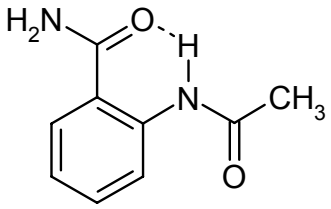


Figure 46: ^1H NMR spectrum (300 MHz) of 2-Acetylamino-3-hydroxy-benzamide (**60)** in $CDCl_3$

The ^{13}C NMR spectrum agreed well with the molecular formula and indicated the presence of nine signals containing two carbonyl groups of an acid, ester or amide at δ 170.1 and 169.6, the presence of a carbon signal at δ 151.2 indicated its connection to an oxygen atom. With the ^1H NMR data and the molecular formula, a search in AntiBase and the Dictionary of Natural Product gave no answer. The HMBC spectrum was in agreement with the 1,2,3-trisubstituted benzene ring and showed a coupling of 4-H to C-2, C-3 and C-6. Further correlations are seen between the 6-H and C-7, the methyl group and the carbonyl at C-8. Analysis of other HMBC correlations led to the identification of compound **60** as 2-acetylamino-3-hydroxy-benzamide. Compound **60** is a derivative of 2-acetylamino benzamide[123](**61**) isolated and reported from our group.



60



61

The free hydroxyanthranilic acid and several of their derivatives are common metabolites, whereas derivatives of the isomeric 3-aminosalicylic acid are unknown from microorganisms making this substituent pattern unlikely. The related 2-acetylaminobenzamide (**61**) is reported to have antifungal properties.[123]

4.11 Terrestrial *Streptomyces* sp. GW 4284

The culture filtrate of *Streptomyces* sp. GW 4284 was found to be biologically active only against *Escherichia coli* and *Staphylococcus aureus*. The chemical screening by TLC showed many yellow and red spots, which did not change on spraying with anisaldehyde/sulphuric acid. The variation of colouration from yellow or red to violet with diluted sodium hydroxide indicated that these compounds belonged to the family of quinones possessing a *peri*-hydroxy group. The fermentation of a 25 L shaker culture took four days at 28 °C and showed after three days an intensive yellow colour, which turned to intensive red on the fourth day. Filtration and extraction delivered 6 g of crude extract, which was chromatographed following the scheme in Figure 50.

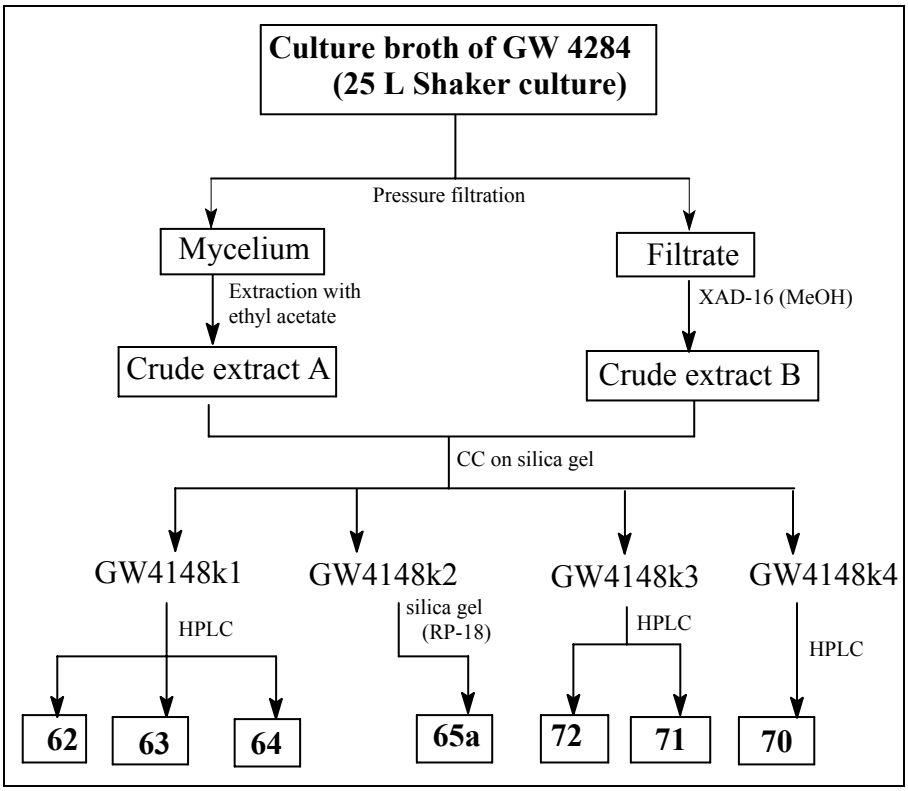


Figure 47: Isolation scheme of compounds from *Streptomyces* sp. GW 4284

4.11.1 Juglomycin A and B

The ^{1}H NMR spectrum of the yellow compound RMBSH2 (**62**) showed in the aromatic region a broad singlet of a chelated hydroxy group at δ 11.92 and proton signals of a 1,2,3-trisubstituted benzene ring at δ 7.80 (t), 7.60 (dd) and 7.38 (dd). The NMR spectrum exhibited also signals of a double bond or an aromatic proton at δ 6.96 (d), two methine protons probably connected to oxygen at δ 5.70 (dd) and 4.95 (m), and a broad singlet at δ 4.80 attributed to an H/D exchangeable proton. The aliphatic region contained only AB-signals of a methylene group at δ 3.20 (dd) and 2.50 (d).

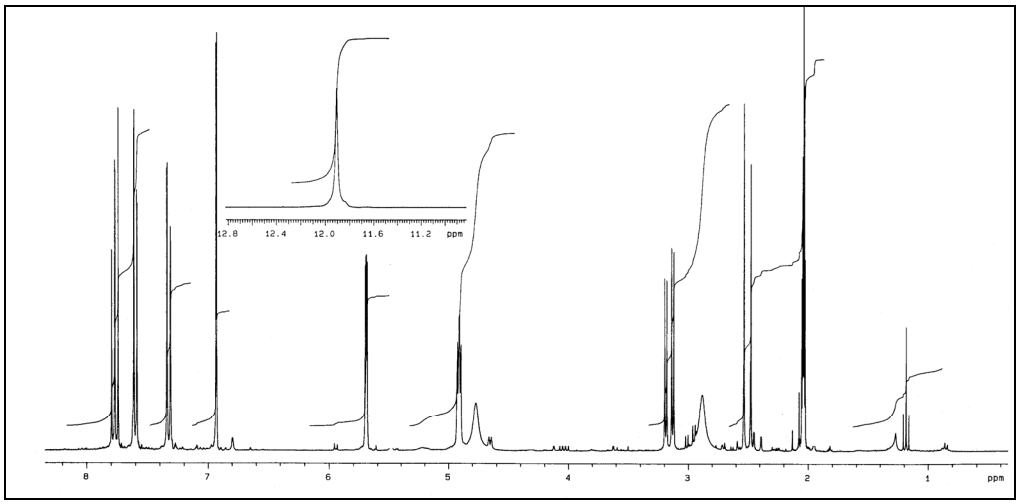


Figure 48: ^{1}H NMR spectrum (300 MHz) of juglomycin A (**62**) in [D_6]acetone

The EI MS indicated the molecular weight to be *m/z* 274. A search in AntiBase with the molecular weight and the 1,2,3-trisbustituted aromatic ring gave 3 solutions, where only juglomycin A (**62**) and B (**63)** were matching with the spectral data. Compound RMBSH2 was finally identified as Juglomycin A (**62**) by comparison with the literature data.[124]

Compound RMB156 identified as juglomycin B (**63**) was also isolated from the same fraction. The ^{1}H NMR spectrum was very similar to that of **62**. The major difference was observed for the chemical shift of the doublet and multiplet at δ 6. 96 (H-3) and 4.92 (H-3') in **62**, to 6.80 and 4.60 in **63**, respectively. The methine and methylene protons were all shifted upfield in **63** compared to **62**. Comparison of the CD spectra of **62** and **63** indicated a trough at 336 nm in **62**, while **63** exhibited an oppositely cotton effect to that of **62** concluding that **62** and **63** have different stereochemistry.

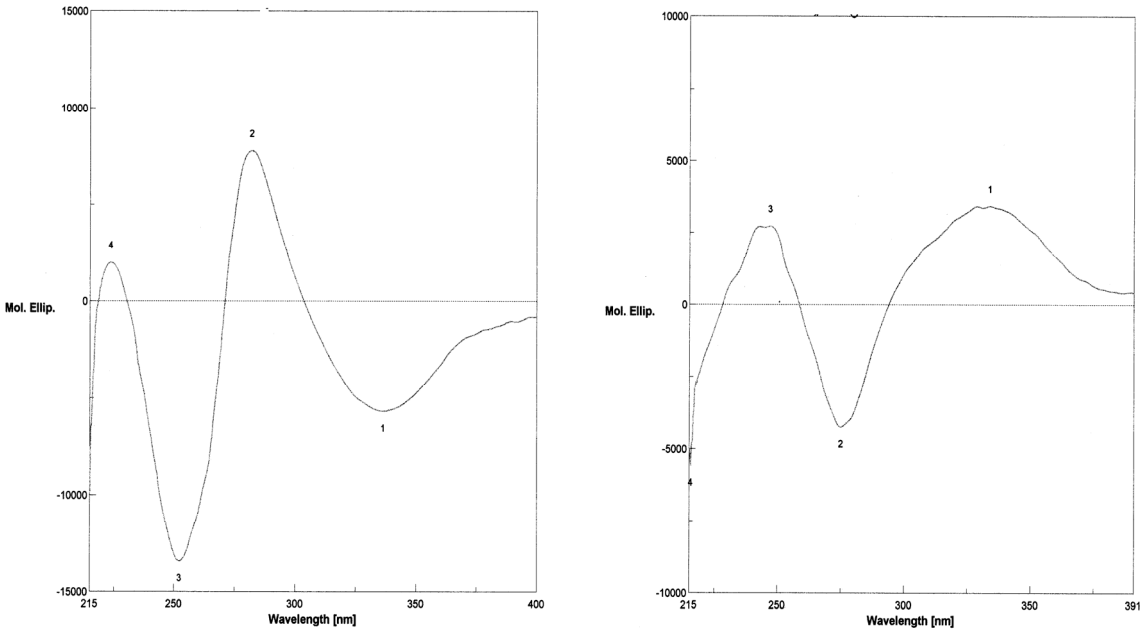
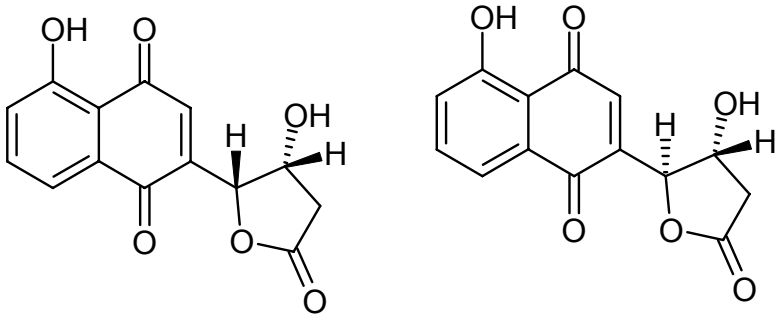


Figure 49: CD spectra of juglomycin A (**62**) and B (**63**) in MeOH



62 **63**

Jugloymycin A (**62**) and B (**63**) were firstly isolated by Ono *et al*.[125] from *Streptomyces* sp. 190-2. The absolute configuration of **62** and **63** was confirmed by single crystal X-ray determination.[126] Both isomers exhibited modest antitumor activity as well as antibacterial activity against both gram-positive and gram-negative bacteria.[125]

4.11.2 Juglomycin J

The compound RMBS1 (**64**) was obtained as yellow solid from the same fraction. The molecular weight was deduced from the (+)-ESI mass spectrum, which indicated

a *pseudo* molecular peak at *m/z* 327 $[M+Na]^+$; ESI HRMS gave the molecular formula $C_{15}H_{12}O_7$. The ^1H NMR spectrum in $[D_6]$acetone was very similar to that of juglomycin A (**62**) and B (**63**). The ^1H NMR spectrum delivered here also signals of a chelated OH group at δ 11.60, three consecutive aromatic protons, two methine groups bearing oxygen and a methylene group of the lactone ring like in juglomycin A (**62**) and B (**63**). The only significant difference was the substitution of the quinone 3-H in **62** and **63** by a methoxy group (δ 4.20) in **64**.

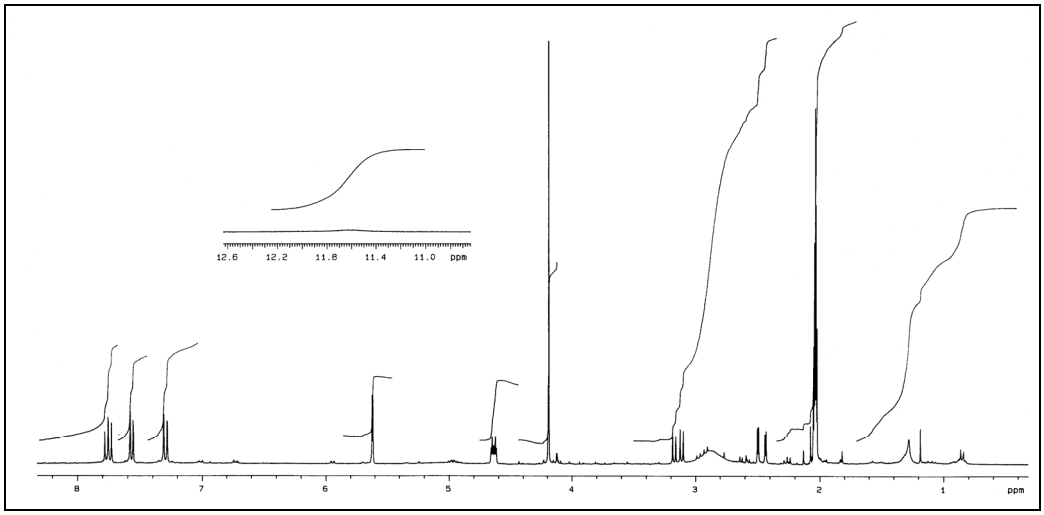


Figure 50: ^1H NMR spectrum (300 MHz) of juglomycin J (**64**) in $[D_6]$acetone

The ^{13}C NMR spectrum indicated the presence of 15 carbon signals as demanded by its molecular formula. Three aromatic methine carbons where observed at δ 138.0, 124.6 and 119.4. Additionally signals of five quaternary aromatic carbons, of which two at δ 162.2 and 159.6 were bearing oxygen, two aliphatic methine carbons bearing oxygen and a methylene group were identified. Furthermore a methoxy carbon signal at δ 62.9 was seen. A search in AntiBase with all these elements gave no result. The HSQC spectrum give all expected correlations. Comparison of the ^1H and ^{13}C data of **64** with those of **62** and **63** has led to the structure of the novel natural compound Juglomycin J (**64**) , which is therefore a 3-methoxy derivative of **62** and **63**.

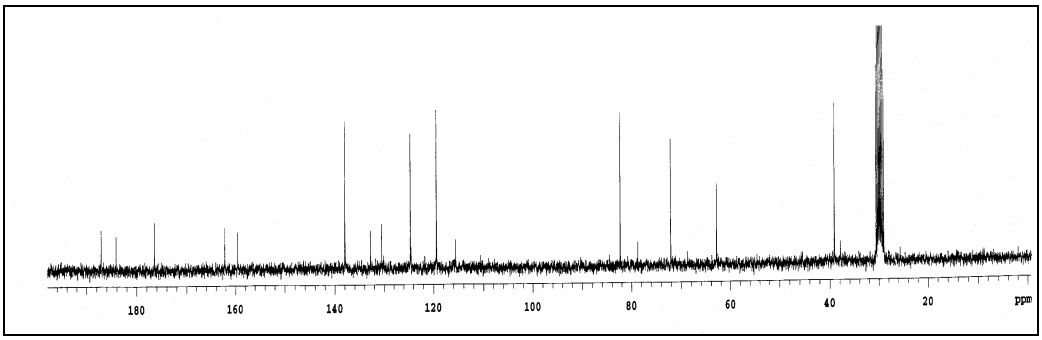


Figure 51: ^{13}C NMR spectrum (300 MHz) of juglomycin J (**64**) in [D_6]acetone

The CD spectrum of **64** showed a peak at 337 nm exactly as in juglomycin B (**63**), and permitted to fix the absolute configuration of **64** as identical to that of **63**.

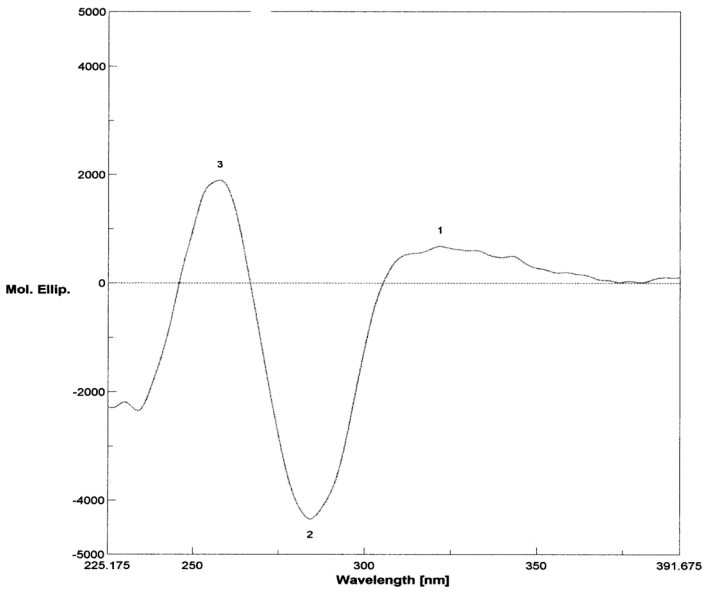
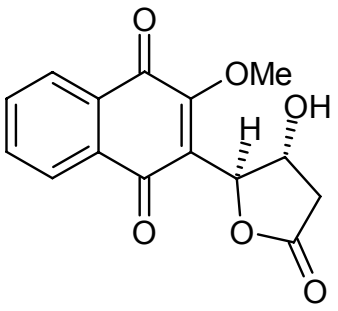


Figure 52: CD spectrum of juglomycin J (**64**) in MeOH



64

4.11.3 Oviedomycin

Chromatography of fraction II on Sephadex LH-20 and RP-18 silica gel gave an orange solid SFK1b (**65a**), slightly soluble in dichloromethane and fairly in dimethylsulfoxide. In the aromatic region of the ^{1}H NMR spectrum, ABC signals of three consecutive protons at δ 7.81, 7.49 and 7.38, a singlet at δ 7.61 and furthermore an acidic proton at δ 11.60 were indicated. The aliphatic region exhibited only one singlet at δ 1.90 attributed to a sp^2-bond methyl group.

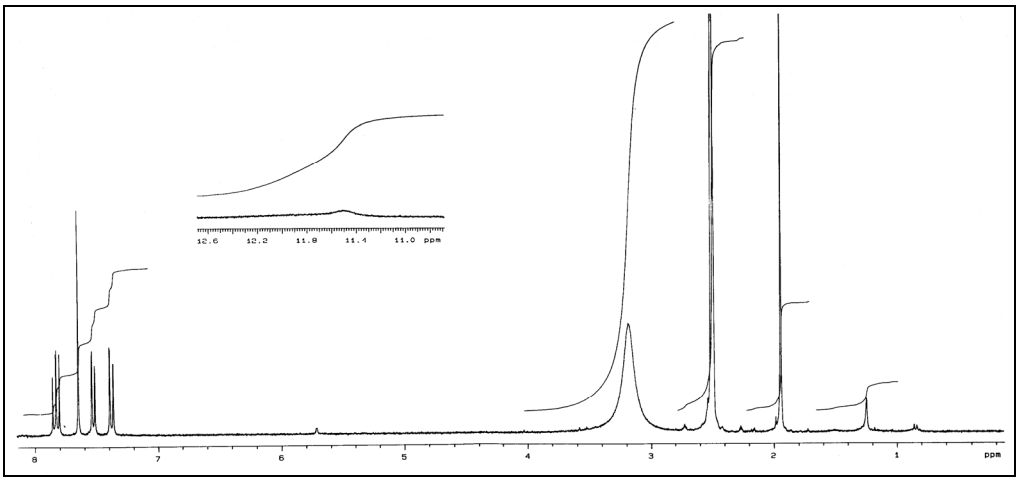
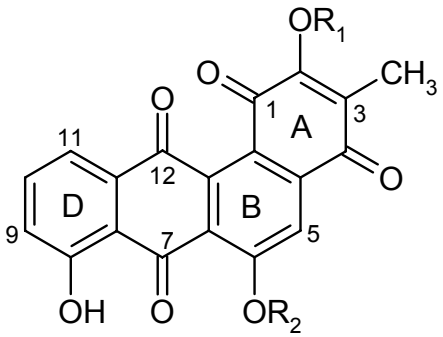


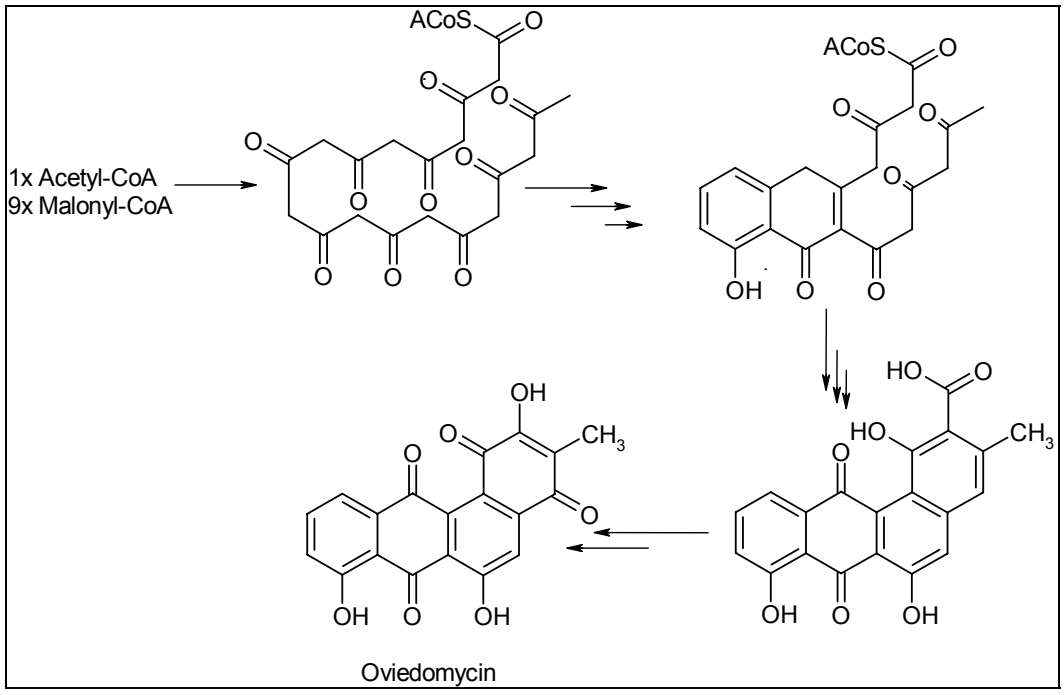
Figure 53: ^{1}H NMR spectrum (300 MHz) of oviedomycin (**65a**) in [D_6]DMSO

Among the 19 carbon signals present in the ^{13}C NMR spectrum, 14 were quaternary, including four quinone carbonyls at δ 190.0, 186.0, 183.1 and 178.5. The molecular weight was determined by an ESI mass spectrum to be *m/z* 350. The structure search using AntiBase led to the identification of compound SFK1b as oviedomycin (**65a**).



65a R_1= H R_2= H
65b R_1= OCH_3 R_2= H
65c R_1= OCH_3 R_2= OCH_3

Oviedomycin (**65a**) is an angucyclinone with novel structural features, particularly the two quinone systems and the unusually substituted ring A, and was first isolated by Rohr *et al*.[127] from *Streptomyces antibioticus* ATCC11891. The genetic organisation of the biosynthetic gene cluster for the antitumor angucycline oviedomycin (**65a**) has been reported.[128] It is derived from acetate in a polyketide biosynthesis as shown in the scheme below.



Scheme 1: Proposed biosynthetic steps of oviedomycin (**65a**)

It is also reported that enolization takes place in the oxygen rich ring A bearing two carbonyls, generating predominantly one more carbonyl at C-2. In our case, this tautomer was not observed. However, during the first fermentation, during PTLC purification on silica gel, the orange oviedomycin (**65a**) was completely converted into an intensive red solid **66**. This transformation was also observed in the presence of diluted sodium hydroxide. The red form was transformed back into yellow oviedomycin (**65a**) by treatment of the **66**-sodium salt with diluted HCl.

As **65a**, this compound did not deliver a pseudomolecular ion in the (+)-ESI MS mode, but on (-)-ESI MS, both exhibited *quasi*-molecular ions at *m/z* 349 $[M-H]^-$ and 721 $[2M+Na-2H]^-$.

The ^{1}H NMR spectrum of **66a** was very similar to that of **65a**, the only pronounced difference being the shift of the aromatic proton singlet of H-5 from δ 7.62 in **65a** to δ 6.98 in **66a**. In the ^{13}C NMR spectrum, the ring C and D signals of **66a** showed only minimal differences, which indicated that the structural changes must have occurred in ring A and/or B of **66a**.

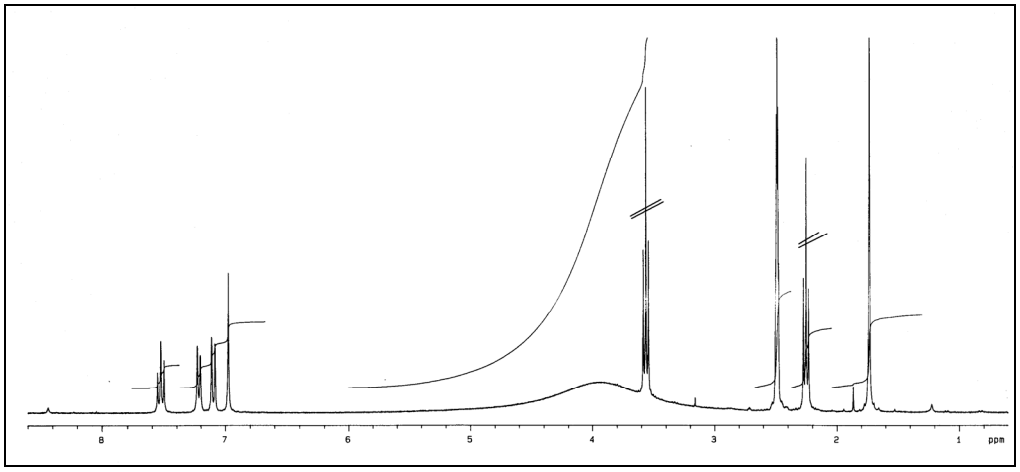
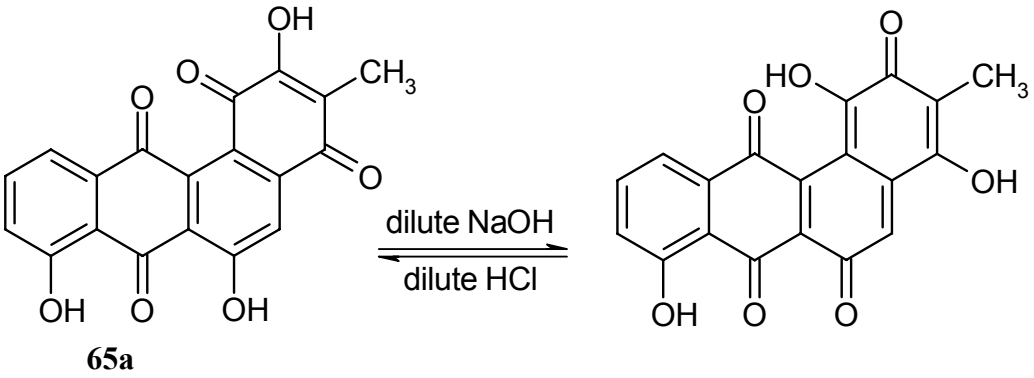


Figure 54: ^{1}H NMR spectrum (300 MHz) of oviedomycin B (**66a**) in [D_6]DMSO

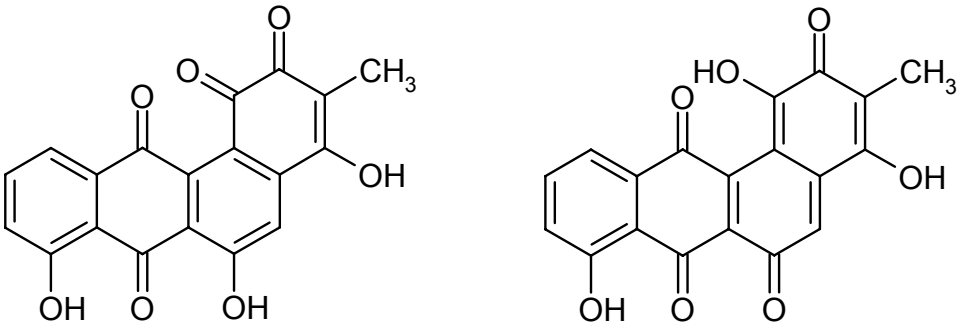
The shifts of the oxygen bearing carbon atoms in the red form **66a** were strongly modified and all three carbon values at δ 177.6, 176.6 and 174.6 can be assigned to carbonyl groups. Due to the identical molecular weight, the NMR spectra and the easy transformation of **65a** into **66a** and *vice versa,* we can conclude that the red product **66a** is a tautomer of **65a**.



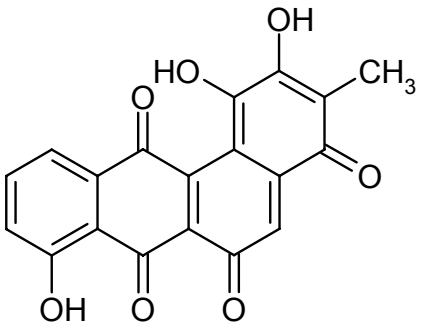
65a **66a**

Figure 55: Transformation of oviedomycin (**65a**) to **66a**

For ring A and B in **65a** there are three tautomeric quinonoid structures possible with carbonyls in 1,2- (**67**), 2,6-(**68**) and 4,6-position (**69**).



67 **68**

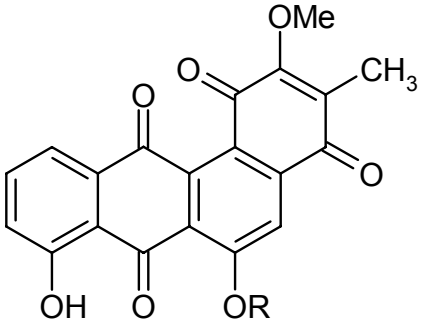


69

The 5-H value of the isomer **69** resembles that of the *peri*-protons in 1,4-naphthoquinones and is due to the influence of the carbonyl group in ring A. The up-field shift of 5-H in the red isomer **66a** indicates therefore that the former carbonyl

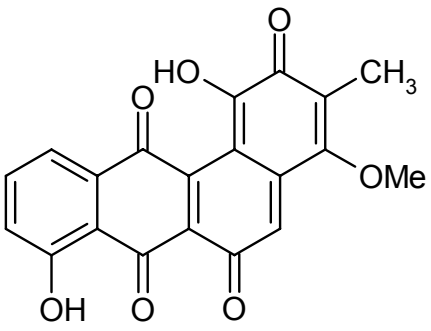
group in A was replaced by an OH group as in **67** and **68** in agreement with the lower shift of C-4 at δ 177.6. The extended 4,6-quinone **69** can be ruled out therefore and also the 1,2-quinone **67** can be excluded for two reasons: 5-H shows a weak ^{2}J or ^{4}J HMBC coupling on the carbonyl at δ 185.7. If the latter is located at position 1 as in **68**, C-6 would have a shift of δ 176.1, which does not agree with the expected value and the identical substructure as in oviedomycin (**65a**). The tautomer with the 1,2-quinone structure could also not explain the deep red colour of the compound **66a**. Obviously the red compound **66a** is the extended quinone **68**.

Tautomerism is a common process in hydroxyquinones, where the isomers are formed by migration of acidic protons and structures are in rapid equilibrium, but can be trapped by their methyl ethers.[129] Interestingly, methylation of **65a** and **66a** with diazomethane delivered only the methyl ethers **65b**/**65c** and **66b** of the respective isomer. We assume that the isomerisation of both forms is kinetically hindered and the activation energy must be higher than usually.



65b: R = H

65c: R = CH_3



66b

4.11.4 Juglorubin

The red compound RM5i (**70**) was responsible for the red colour of the culture broth and exhibited a violet coloration with 2N diluted sodium hydroxide indicating its *peri*-hydroxy quinonoid nature. The ^{1}H NMR spectrum of RM5i indicated a broad singlet of a chelated hydroxy group at δ 14.48 in [D_6]DMSO and two doublets of *trans*-olefinic protons at δ 8.02 and 6.09 (^{3}J = 16.8 Hz). The downfield chemical shift of the proton at δ 8.02 indicating that it is conjugated to a carbonyl group. Further, typical signals of two 1,2,3-trisubstituted aromatic systems appeared between

δ 7.44-6.90, and the aliphatic region presented only a methine proton connected to oxygen at δ 5.42 and signals of two methylene groups at δ 4.00 (1H, dd) and in the range of 2.70-2.50 (m, 3H).

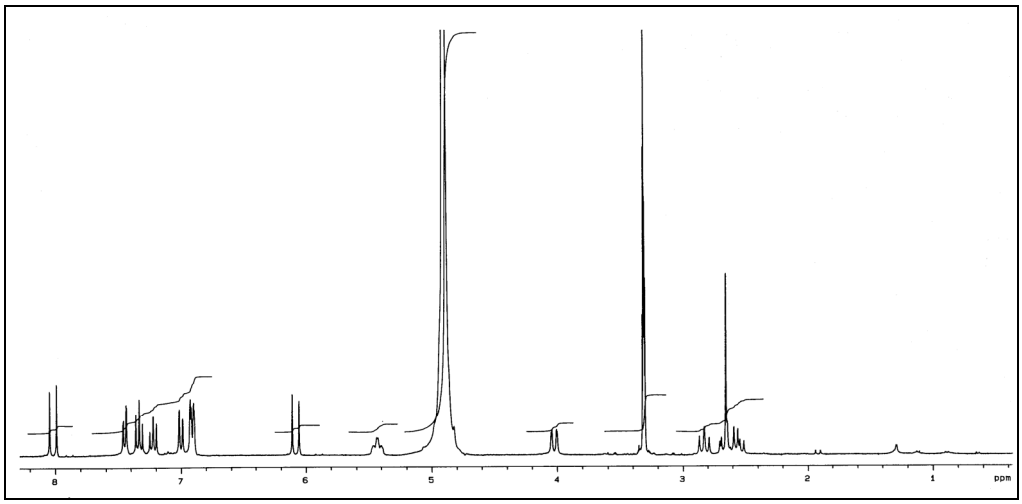


Figure 56: ^{1}H NMR spectrum (300 MHz) of juglorubin (**70**) in [D_4]MeOH

The ^{13}C NMR spectrum exhibited 28 signals comprising six carbonyl groups, and two oxygenated quaternary carbon atoms. The molecular weight was deduced to be 530 Dalton from the ESI mass spectrum. The substructure search using AntiBase and following the scheme of Figure 57 delivered 2 compounds, among which the spectroscopy data of one matched with those of juglorubin (**70**).

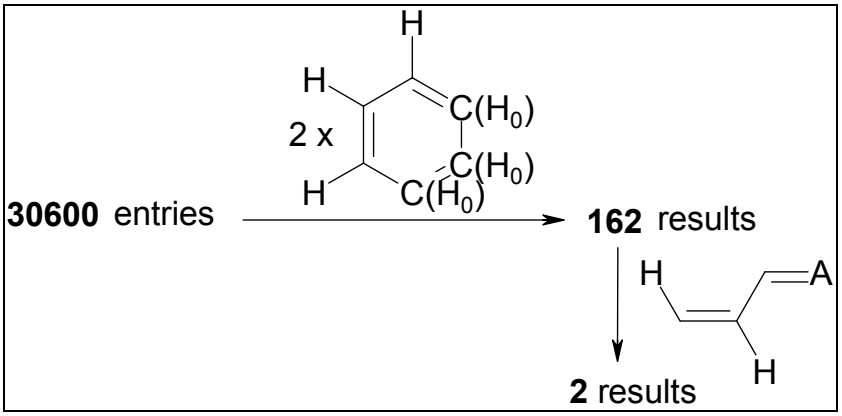
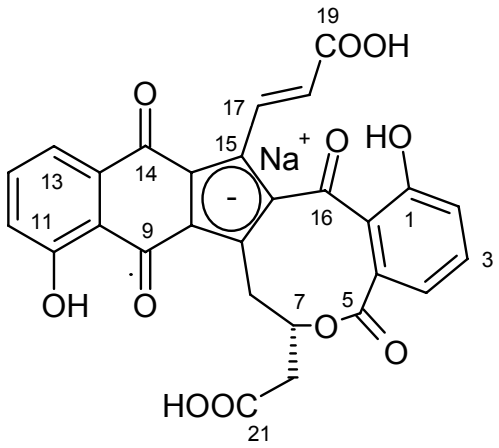


Figure 57: AntiBase search of juglorubin (**70**), A= any atom or group

Juglorubin (**70**) was previously isolated from a *Streptomycetes* sp. by Lackner *et al*.[130] and described as a unique, unusually ionic condensed ring system, which may be formed from the reaction of two juglomycin C units.



70

The condensation of the two juglomycin C (**I**, fig 63) units starts with the formation of a C-9/C-3' bond; comparable reactions are known from the literature.[131] The first one should be an addition of the extended enolate of juglomycin C (fig. 58) to C-3' of the partner, quite in parallel to the dimerisation of plumbagin (2-methyljuglone) to zeylanone.[131] Next, the resulting anion II should cyclize in a tandem Michael addition to form the C-3/C-2' bond and thus intermediate III. The hemiketal of the latter can be enzymatically oxidised (probably *via* the epoxide) to yield juglorescein (**71**). The pathway continues *via* the extended enole of IV to the intermediate V. This undergoes dehydratation and a C-1'/C-2' bond cleavage resulting in the formation of the nine-membered lactone ring, which, together with the conjugated benzindenylide-quinone system, forms the unique structure of juglorubin (**70**).

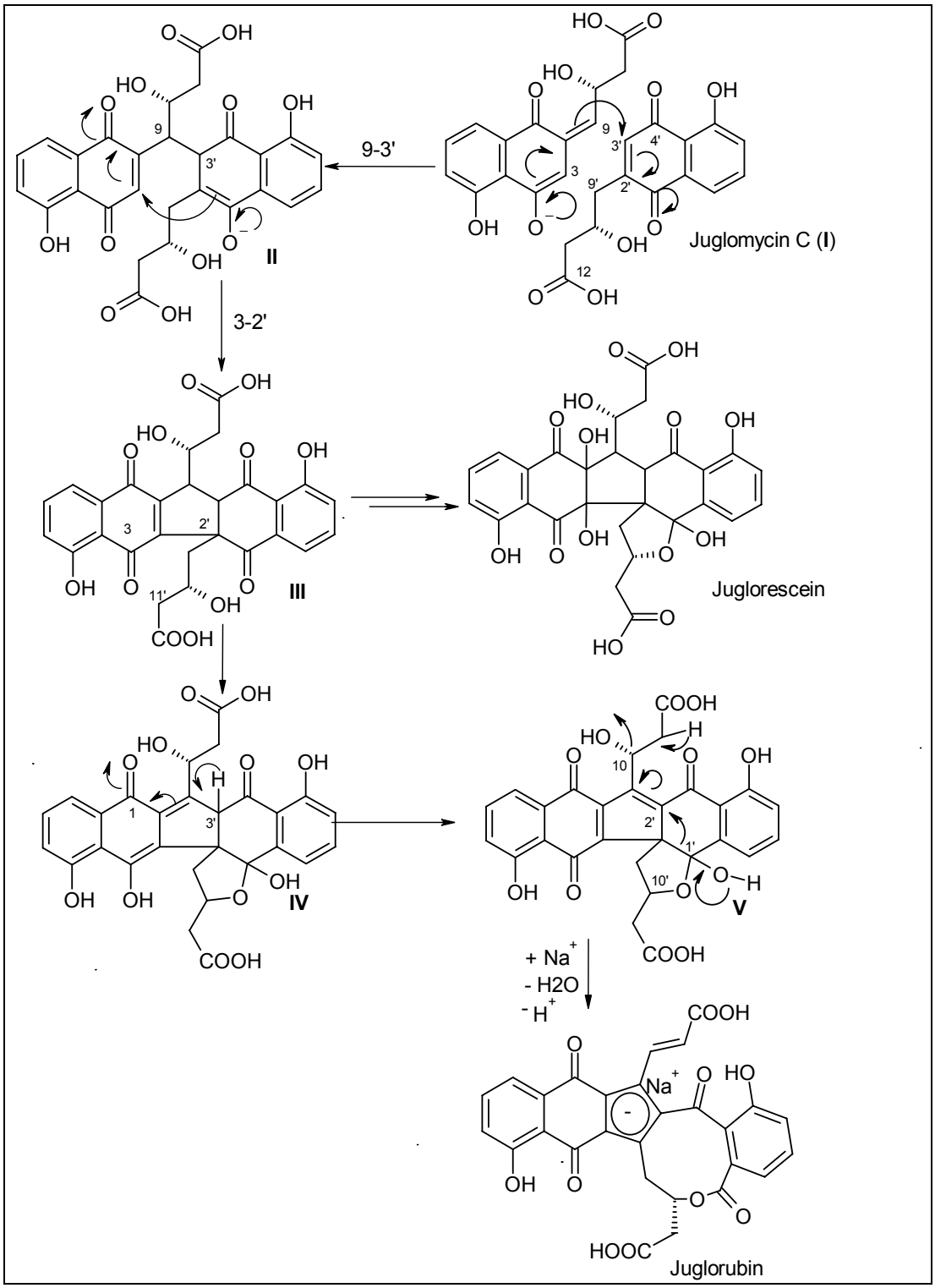


Figure 58 : Formation of juglorubin (**70**) and juglorescein (**71**) from juglomycin C

4.11.5 Juglorescein

Compound SFF8a (**71**) crystallized from $CHCl_3$/MeOH/C_6H_{12} as long colourless needles. The molecular weight *m/z* 584 was determined by ESI MS. The ESI HRMS

(*m/z* 585.12391 [M+H]) afforded the molecular formula $C_{28}H_{24}O_{14}$. The ^{1}H NMR spectrum of SFF8a indicated signals of two sets of consecutive aromatic ABC-protons at δ 7.68 (t), 7.54 (dd), 7.23 (dd) and 7.50 (t), 7.41 (dd) and 6.85 (dd), respectively. It also exhibited four methine protons at δ 3.52, 3.29, 4.58 and 4.82 the two last probably connected to oxygen, and three methylene proton signals in the range of δ 2.62-3.16.

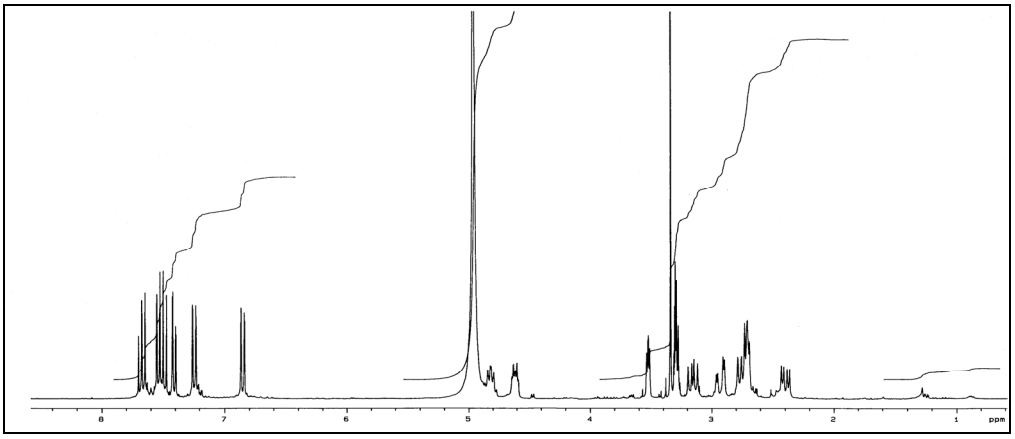


Figure 59: ^{1}H NMR spectrum (300 MHz) of juglorescein (**71**) in [D_4]MeOH

The ^{13}C NMR spectrum delivered 28 signals as expected for the molecular formula. The spectrum denoted only twelve aromatic carbon signals: six for methine and six for quaternary carbon atoms, two of the latter bearing oxygen. In addition, only three signals of conjugated ketones were visible at δ 206.6, 204.0 and 193.8. The presence of a ketal or hemiketal signal at δ 101.8 may be an indication for the existence of a fourth ketone group.

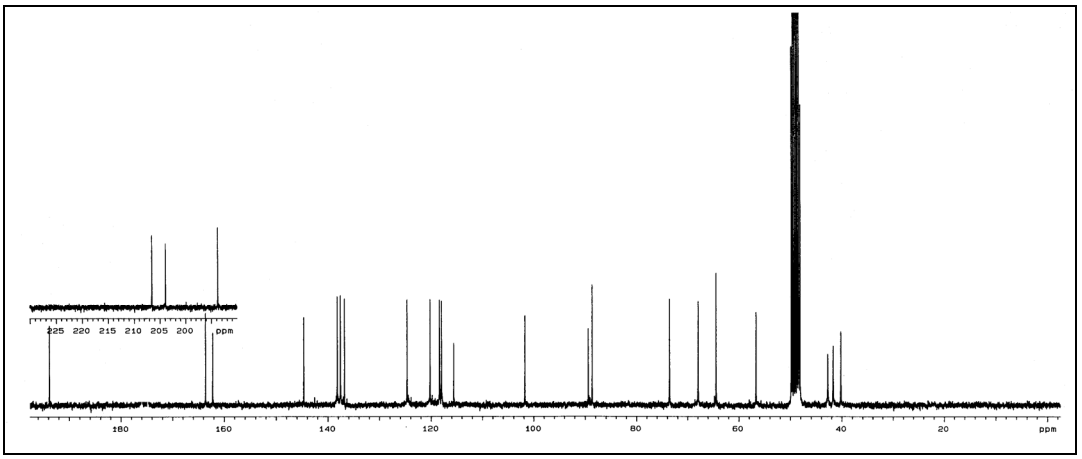
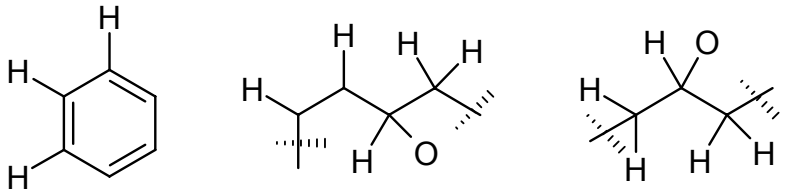


Figure 60: ^{13}C NMR spectrum (300 MHz) of juglorescein (**71**) in [D_4]MeOH

By interpretation of the COSY spectrum, four substructures were constructed, two 1,2,3-substituted benzene and two alkyl fragments (fig. 61).



I II III

Figure 61: Substructures of juglorescein (**71**)

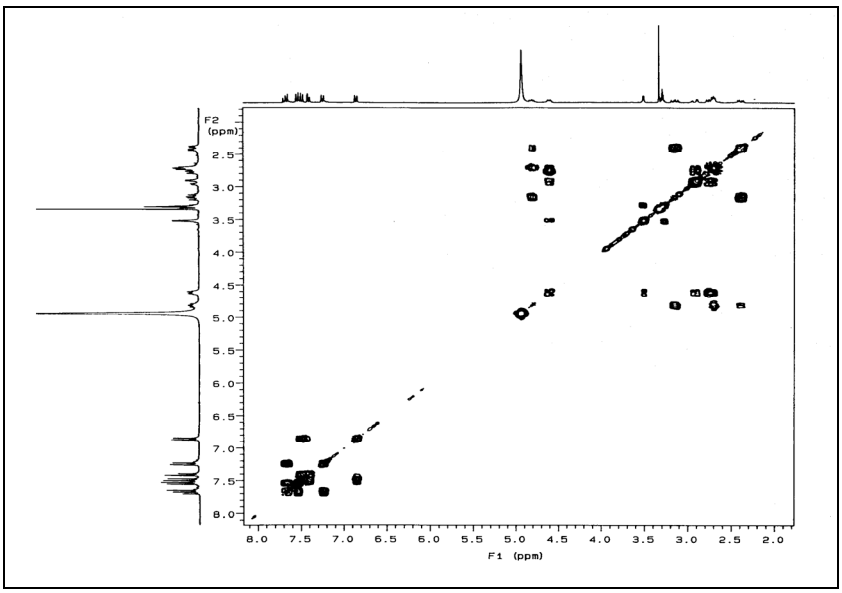


Figure 62: H,H COSY spectrum of (300 MHz) of juglorescein (**71**) in [D_4]MeOH

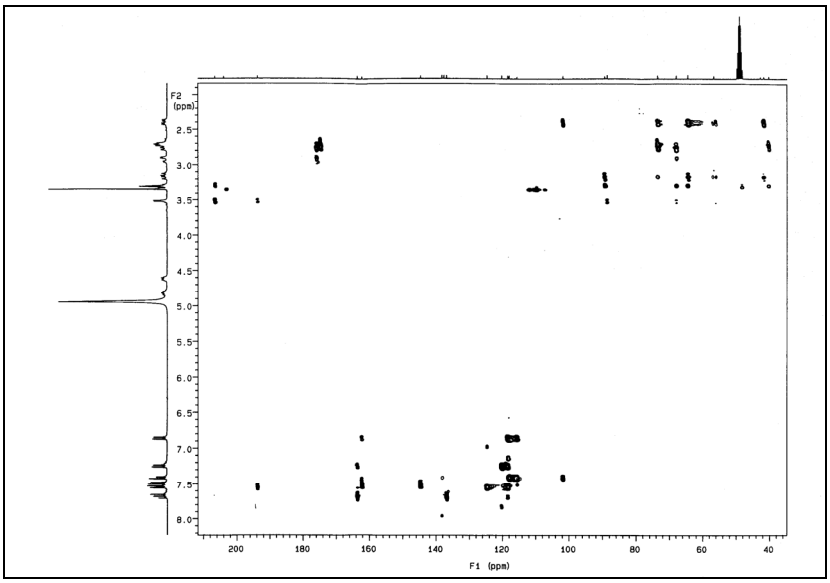
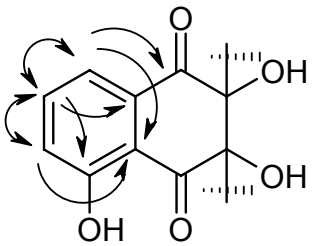
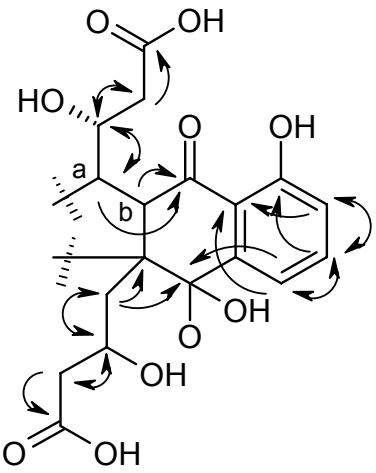


Figure 63: HMBC spectrum of (300 MHz) of juglorescein (**71**) in [D_4]MeOH

The HMQC spectrum delivered all $^1J_{C\text{-}H}$ correlations, and a careful interpretation of the HMBC spectrum indicated that both non-oxygenated methines of the fragment II showed couplings on the ketone carbonyl at δ 206.6. In addition, correlations were seen between the aromatic proton at δ 7.41 as well as the terminal methine of fragment II with the ketal or hemiketal carbon at δ 101.8. Further HMBC interpretation delivered the partial structures IV and V.



IV



V

These substructures were connected through the correlation of methines a and b and of the methylene at δ 3.16 of V with the quaternary carbon atoms at δ 88.7 and 89.4. The formation of an hemiketal could occur between CO-1' and one of

the four aliphatic OH groups. Due to missing couplings, the HMBC spectrum did not allow to distinguish between these alternatives. Because of the similarity in the ^{13}C NMR data of C-2 (δ 88.7) and C-3 (89.4), the ketalization *via* the alcoholic OH group at C-2 was ruled out. Accordingly, the ^{13}C NMR signal of C-10' (δ 73.6) was downfield shifted as compared with C-10 (δ 68.0), where the latter is similar as in Juglomycins C with open side chains. Therefore, it can be concluded that the ketalisation must have occurred between C-10' and C-1' giving the final structure **71** of juglorescein.

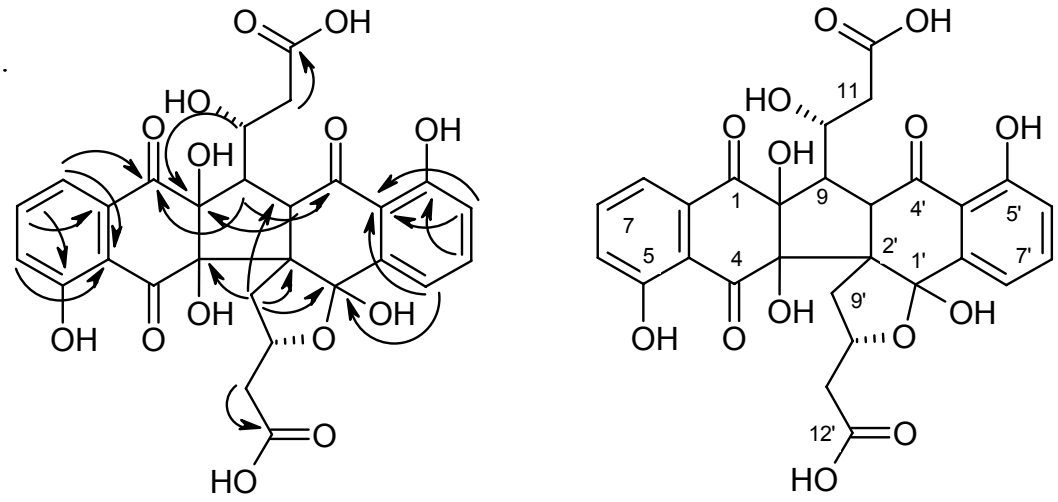
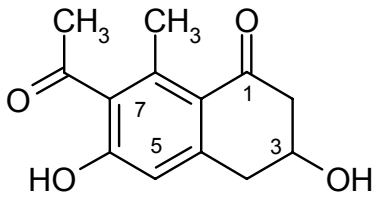


Figure 64: Structure of juglorescein (**71**) derived by H, H COSY (not shown), HMQC (not shown) and selected HMBC (→) couplings

Juglorescein (**71**) is a dimer of juglomycin C and may be formed as shown in figure 63. Only very few microbial metabolites display some structural similarity with **71**, *e.g.* momofulvenone A[132] or the microbial degradation products shikometabolin A and B.[133] The ebony constituent zeylanone[131] is, however, a clear analogue. During the formation of **71** from juglomycin C, up to six new stereocentres are generated. The determination of the stereochemistry of juglorescein (**71**) by interpretation of the CD spectrum was therefore not unambiguously possible.

4.11.6 GTRI-O2

The ESI mass spectrum of compound SFK8 (**72**) determined the molecular weight as 234 Dalton. The ^{1}H NMR spectrum exhibited a singlet at δ 6.62, which could be attributed to a proton of an acceptor substituted double bond or a donor substituted aromatic ring, and two singlets at δ 2.44 and 2.40, which could be assigned to aromatic methyl, acyl or N-methyl groups. Further signals for methines and methylenes appeared in the range of δ 3.20-2.50 and δ 4.23. The ^{13}C NMR spectrum indicated 13 signals, one aliphatic methine, two methylene, and six aromatic carbons between δ 112-160. The presence of two further signals at δ 208.3 and 199.3 in the range of keto carbonyl groups in conjugation with an aromatic rings or a double bond. The signals of two methyl groups appeared at δ 32.6 and 19.7. With all this information, a search in AntiBase gave GTRI-02 (**72**) and the data were confirmed by the reported values.[134]



72

GTRI-02 (**72**) is reported as free radical scavenger and an inhibitor of lipid peroxidation with an IC_{50} value of 1.89 μg/ml, which is about half of the value for vitamin E (0.91 μg/ml).

4.12 Terrestrial *Streptomyces* sp. GW 21/1313

The ethyl acetate extract of the terrestrial *Streptomyces* sp. isolate GW 21/1313 was found to be active against bacteria and fungi. The chemical screening indicated only the presence of actinomycins. After the variation of media and growth conditions, TLC showed a yellow zone, which on spraying with anisaldehyde/sulphuric acid and heating turned first to dark yellow, and then became brown and finally yellowish green. Additionally, some yellow fluorescent spots under 366 nm were seen. The

strain was cultivated twice on a 25 L and finally on a 50 L scale due to the presence of minor components not easily isolable.

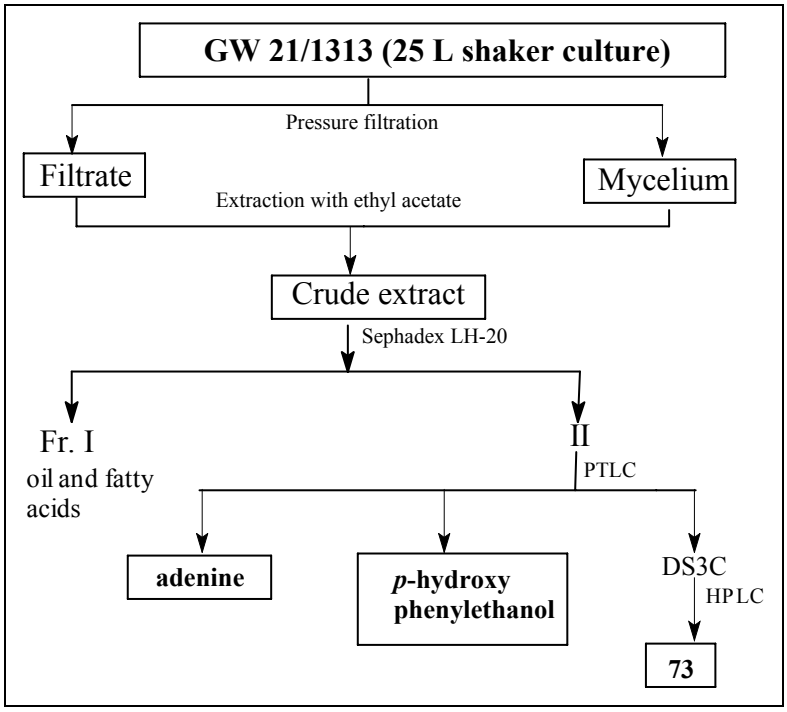


Figure 65: Work-up of the crude extract of *Streptomyces* sp. GW 21/1313

4.12.1 4-Hydroxy-2-(5-hydroxymethyl-furan-2-ylmethylene)-5-methyl-furan-3-one

Preparative HPLC of the sub-fraction DS3C delivered a pure compound D1301H (**73**) as an orange solid, which possessed the molecular weight *m/z* 222. The ^{1}H NMR spectrum depicted two doublets each with a coupling constant of ^{3}J = 3.4 Hz at δ 7.03 and 6.52, which could be assigned to a disubstituted heteroaromate like furan or pyrrole. Additionally, it contained a singlet of an olefinic methine at δ 6.61, a methylene bearing an oxygen at δ 4.59 and a methyl group attached to a sp^2 carbon at δ 2.30.

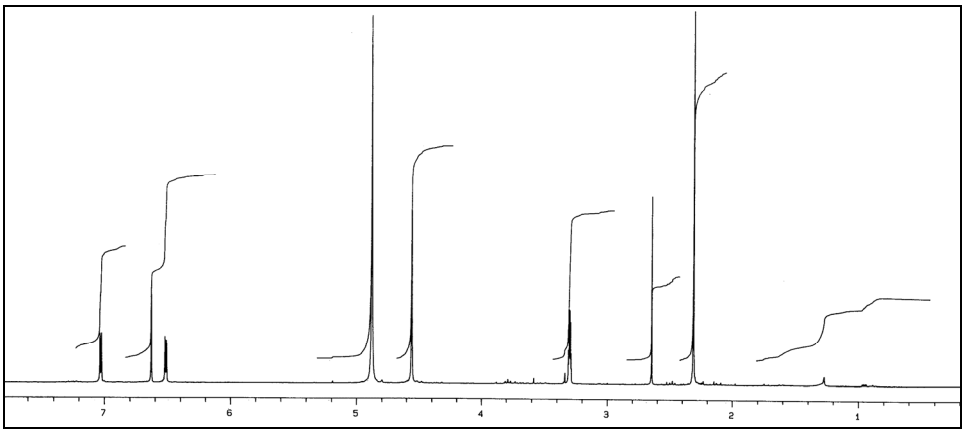


Figure 66: ^{1}H NMR spectrum (300 MHz) of furanone **73** in [D_4]MeOH

The ^{13}C APT NMR spectrum delivered signals of six quaternary and three methine carbons in the sp^2 region, of which the signal at δ 182.8 could be assigned to a carboxylic acid, ester carbonyl or a conjugated ketone. In the aliphatic region, only two signals of a methylene and a methyl group were observed at δ 57.5 and 12.2, respectively.

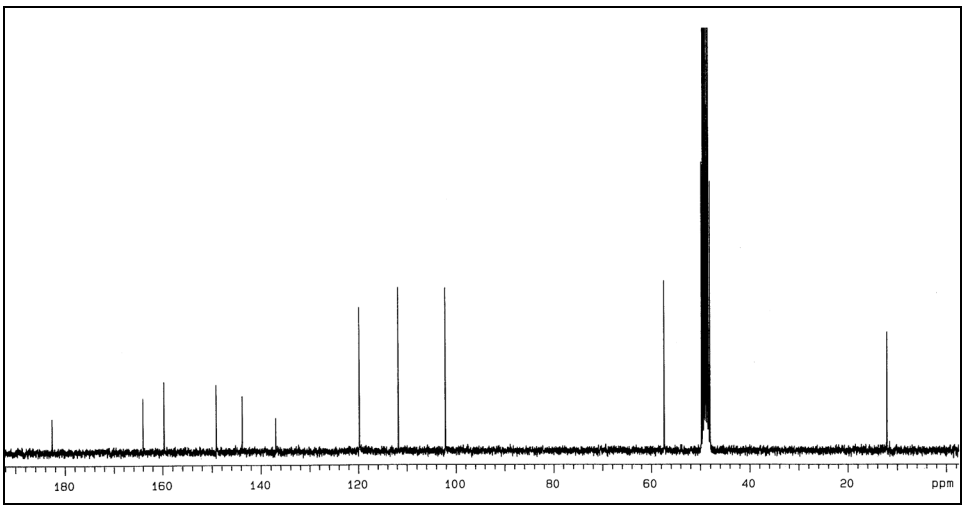


Figure 67: ^{1}H NMR spectrum (75.5 MHz) of furanone **73** in [D_4]MeOH

A search in AntiBase[49] and the Dictionary of Naturals Products (CD-ROM)[50] with the NMR data accompanied by the molecular weight delivered no known natural product. In the HMBC spectrum, the methyl group at δ 2.30 showed a correlation with signals at δ 164.2 and 137.2, while the singlet at δ 6.62 showed coupling with the carbonyl and carbon atoms at δ 149.3 and 144.0. It confirmed also the presence of the furan ring and indicated correlations of the the methylene group with furan

carbons at δ 159.9 and the methine carbon at δ 119.9, resulting in two fragments, which were connected by the correlation of the methine at δ 6.62 to the furan carbons at δ 144.0 and 111.9.

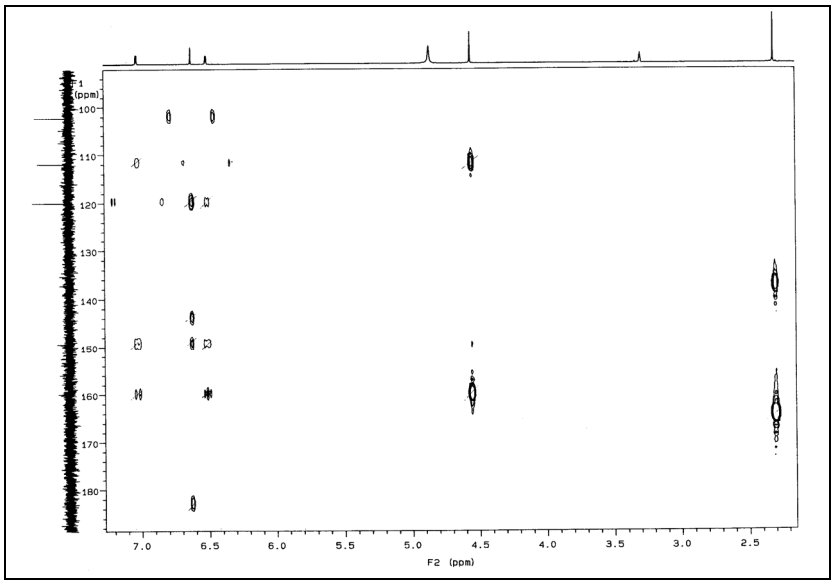


Figure 68: HMBC spectrum (300 MHz) of **73** in [D_6]acetone

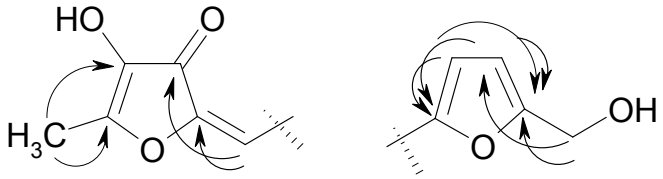


Figure 69: Fragments of Compound **73** resulting from the HMBC spectrum

The structure **73** derived by the connection of both fragments was identified as 4-hydroxy-2-(5-hydroxymethyl-furan-2-ylmethylene)-5-methyl-furan-3-one (**73**), a new natural product, which was, however, already obtained by Maillard reaction of pentose (e.g. D-Xylose) in water with isopropyl amine and acetic acid.[135]

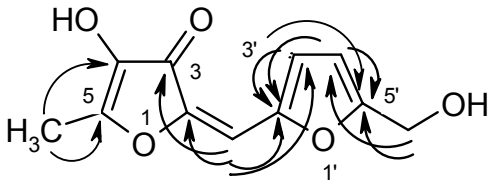
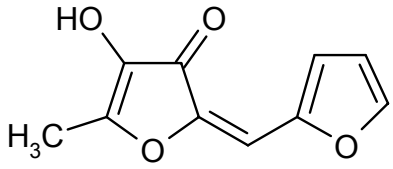


Figure 70: HMBC correlations in furanone **73**

Compounds possessing a similar chromophore as **73** are very rare in nature, but are describe in the literature as reaction products from furfural with 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one and also especially as Maillard reaction products, which are formed by heating sugar in presence of amino acids in aqueous solution.[136] It is reported that 2-furfurylidene-4-hydroxy-5-methyl-3(2H)-furanone (**74**) inhibits the growth of human tumor cells *in vitro* in the low micromolar range.[137] Compound **73** was tested against our test organisms but exhibited no activity.



74

4.12.2 Isolation from 50 L fermenter

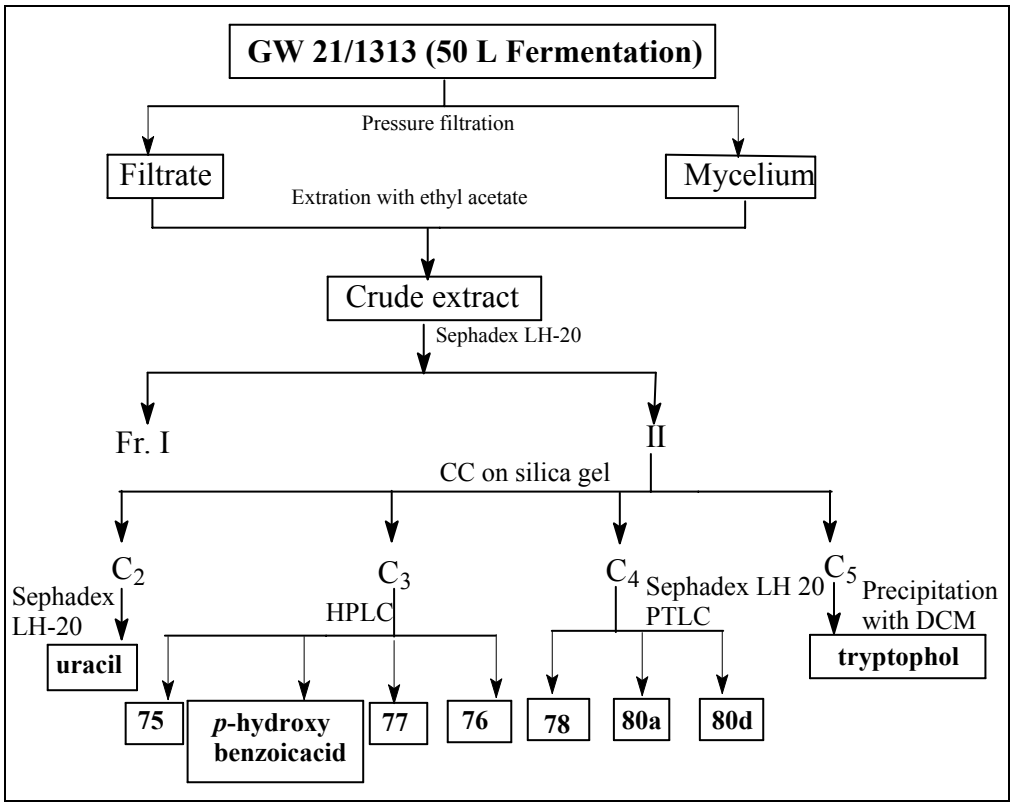
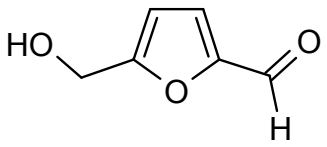


Figure 71 : Work-up of the terrestrial *Streptomyces* GW 21/1313.

Due to the presence of minor compounds which could not be isolated from the 25 L fermentation of the strain GW 21/1313, a second fermentation was carried out on a 50 L scale using the same medium. The work-up followed the above figure. Compound FM3d was obtained as colourless solid and identified as *p*-hydroxybenzoic acid by the ^{1}H NMR spectrum and by comparison with data from our collection.

4.12.3 5-Hydroxymethylfurfural

Preparative HPLC of fraction C_3 delivered FM3a (**75**) as light yellowish oil, which gave a dark grey colouration with anisaldehyde/sulphuric acid. The molecular weight *m/z* 126 was determined by ESI and EI mass spectrometry. The ^{1}H NMR spectrum of the compound depicted very few signals and contained a singlet at δ 9.58 of an aldehyde group and two aromatic doublets at δ 7.37 and 6.57, which, due to their coupling constants (^{3}J = 3.7 Hz), could be assigned to a 2,5-disubstituted furan or pyrrole. The latter can, however, be excluded due to the even value of the molecular weight. In addition, the spectrum delivered an H/D exchangeable doublet of doublet at δ 4.71 and a methylene doublet at δ 4.63 of a CH_2OH group. The ^{13}C NMR spectrum delivered signals of an aldehyde at δ 178.0, two quaternary carbons attached to oxygen at δ 162.8 and 153.3, two sp^2 methine groups at δ 123.8 and 110.2 and a methylene group at δ 57.5. With the molecular weight and the NMR data the structure 5-hydroxymethylfurfural (**75**) could be assigned easily and was confirmed by comparison of the NMR data with the literature.[138]



75

4.12.4 Krishnanone A

Compound FM3f (**76**) was also obtained as light yellowish oil and exhibited a colouration with anisaldehyde/sulphuric acid similar to that of **75**. ESI and EI MS delivered the molecular weight *m/z* 266 and EI HRMS the molecular formula $C_{13}H_{14}O_6$. The ^{1}H NMR spectrum possessed an aldehyde singlet like in **75**. It showed

four aromatic doublets, which in comparison with **75** could be assigned to two furan rings, a methine singlet at δ 5.59, which due to its downfield shift could be assigned to an acetal, signals of two types of methylene protons bearing oxygen, one forming an AB system and the other a singlet, and a methoxy group at δ 3.38.

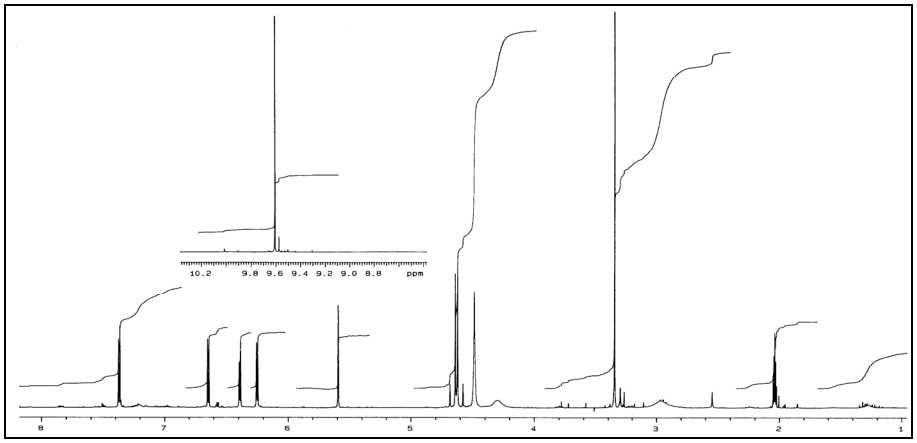


Figure 72: ^{1}H NMR spectrum (300 MHz) of krishnanone A (**76**) in [D_6]acetone

The ^{13}C NMR and APT spectra showed signals at δ 178.3 for a conjugated aldehyde group, four aromatic quaternary carbons, four sp^2 CH groups, two methylene groups connected to oxygen, an acetal methine at δ 97.8 and a methoxy group.

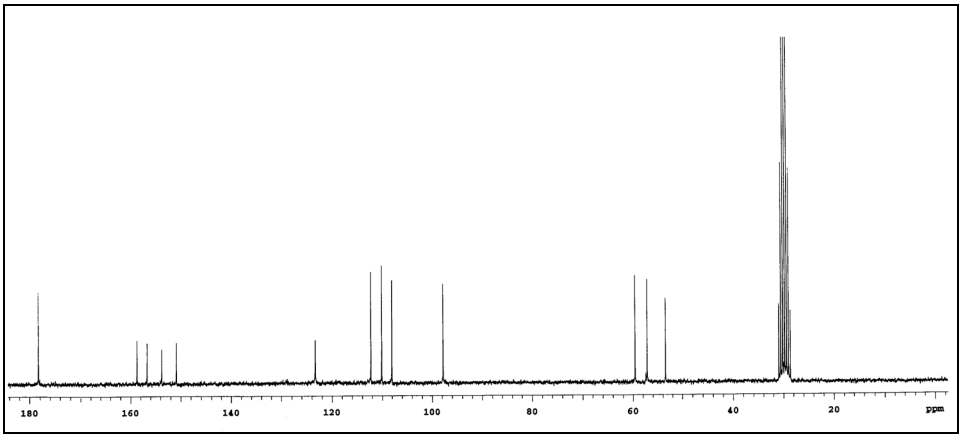


Figure 73: ^{13}C NMR spectrum (300 MHz) of krishnanone A (**76**) in [D_6]acetone

The molecular weight, ^{1}H and ^{13}C NMR data indicated this compound to be a dimer of 5-hydroxymethyl-furfural (**75**). It is also important to mention that **75** partially decomposed during chromatographic purification to give **75**, which also supports the dimerisation of **75** *via* an acetal bond. The H,H COSY spectrum permitted to locate

two pairs of doublets in different rings. In the HMBC spectrum two correlations were seen from the methylene singlet at δ 4.50 to the quaternary and methine carbon atoms at δ 156.7 and 108.1, respectively. The acetal methine coupled with the carbons at δ 150.9, 110.1 and the methylene of the AB system. The structure of **76** was fully confirmed by further couplings and by comparison with **75**; it is a new natural product.

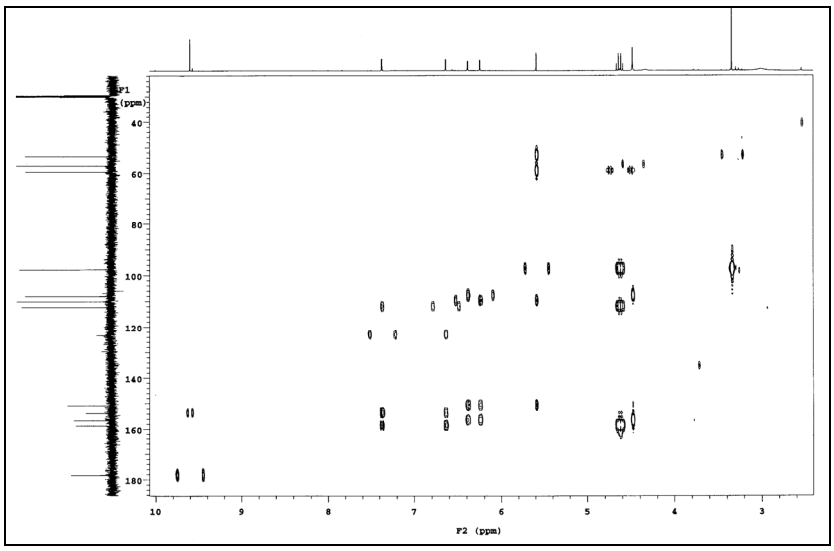


Figure 74: HMBC spectrum (300 MHz) of krishnanone A (**76**) in [D_6]acetone

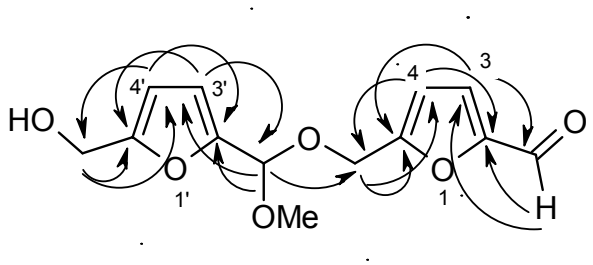


Figure 75: Selected HMBC coupling in krishnanone A (**76**)

4.12.5 Krishnanone B

The oily compound FM3g (**77**) was also obtained from the HLPC purification of fraction C_3. It exhibited the same colour reaction with anisaldehyde/sulphuric acid like **75** and **76** indicating that they may belong to the same class. The ESI mass spectrum indicated the *quasi*-molecular peak at *m/z* 429, and the EI HRMS delivered the molecular formula $C_{20}H_{22}O_9$. The ^{1}H NMR spectrum showed similarity with that of **76,** however, contained more signals. It indicated 3 pairs of doublets for three

2,5-disubstituted furan rings, three methylene groups, two singlets for two acetal protons at δ 5.62 and 5.52, two methoxy groups at δ 3.36 and 3.30 and an aldehyde proton at δ 9.60.

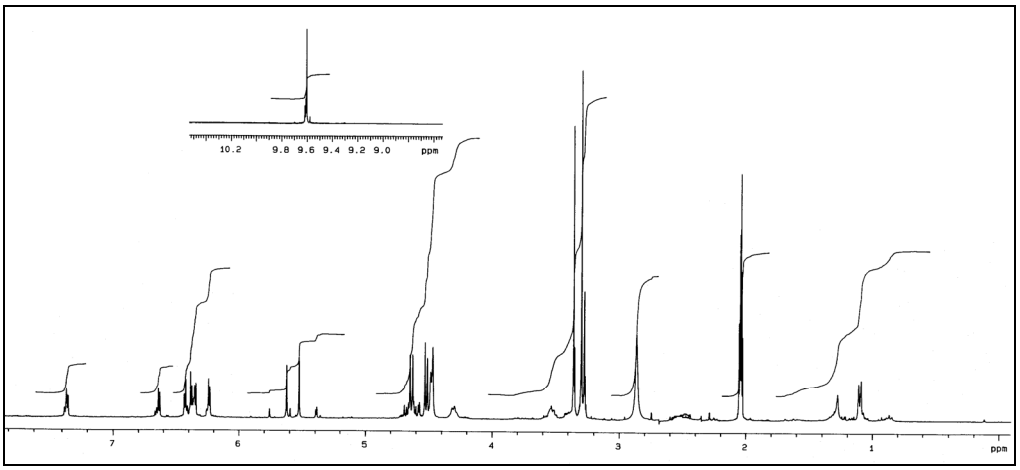


Figure 76: ^{1}H NMR spectrum (300 MHz) of krishnanone B (**77**) in [D_6]acetone

The ^{13}C NMR spectrum exhibited 20 signals for an aldehyde carbonyl, six *sp*^{2} quaternary carbons, six aromatic methines, three methylene groups, two acetal methine and two methoxy groups.

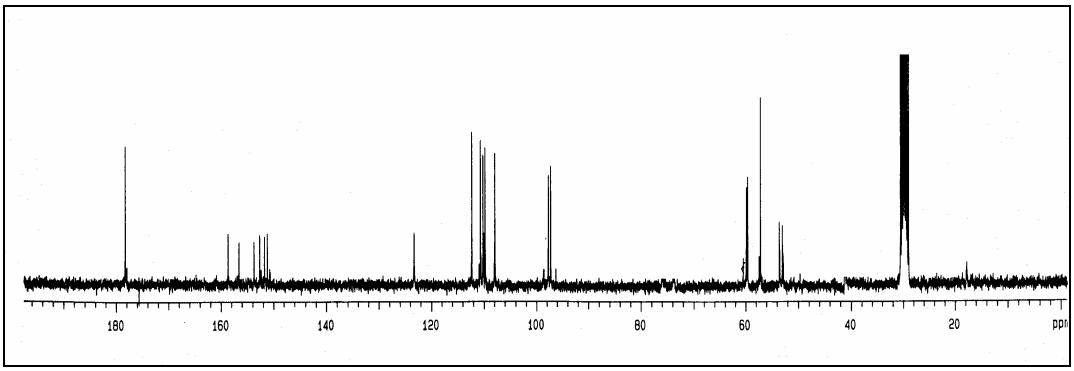
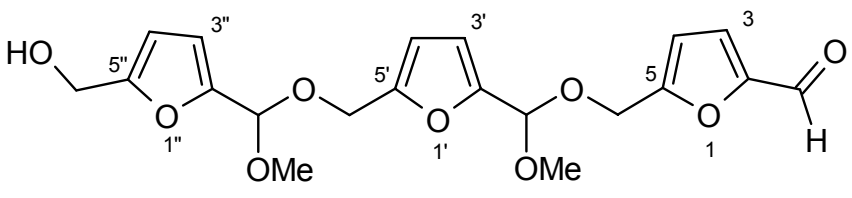


Figure 77: ^{13} C NMR spectrum (300 MHz) of krishnanone B (**77**) in [D_6]acetone

By comparison of the NMR data with those of **76**, the structure **77** was assigned for the new natural product and the name krishnanone B proposed.



77

Krishnanone A (**76**) and B (**77**) are a dimer and trimer, respectively of 5-hydroxymethylfurfural (**75**) and were firstly considered as artefacts, which may occur by polymerisation of **75** during work-up procedures. It was observed, however, that by keeping a mixture of 5-hydroxymethylfurfural (**75**) in methanol and *p*-toluenesulfonic acid to for 2 to 4 weeks no polymerisation occurred. Additionally, TLC of the crude extract exhibited already spots of krishnanone A (**76**) and B (**77**). The latter belongs to the large family of synthetic linear furanic polymers. Furane polymers derived from hexoses and pentoses (mono- and polysaccharides)[139] constitute a peculiar domain of macromolecules and may be exploitable as a source for novel renewable polymers.

4.12.6 Perlolyrin

The ^{1}H NMR spectrum of the yellow compound FM6a (**78**) from fraction C_4 contained signals of an acidic proton at δ 11.18, a singlet of a methylene group bearing oxygen at δ 4.68 and three sets of sp^2 protons for a 1,2-disubstituted benzene, a furan ring (due to the small coupling constant of (^3J = 3.4 Hz) and the shift values and two protons belonging to a third ring system. The molecular weight was deduced by the (+)-ESI mass spectrum, which exhibits the $[M+H]^+$ ion at *m/z* 265.

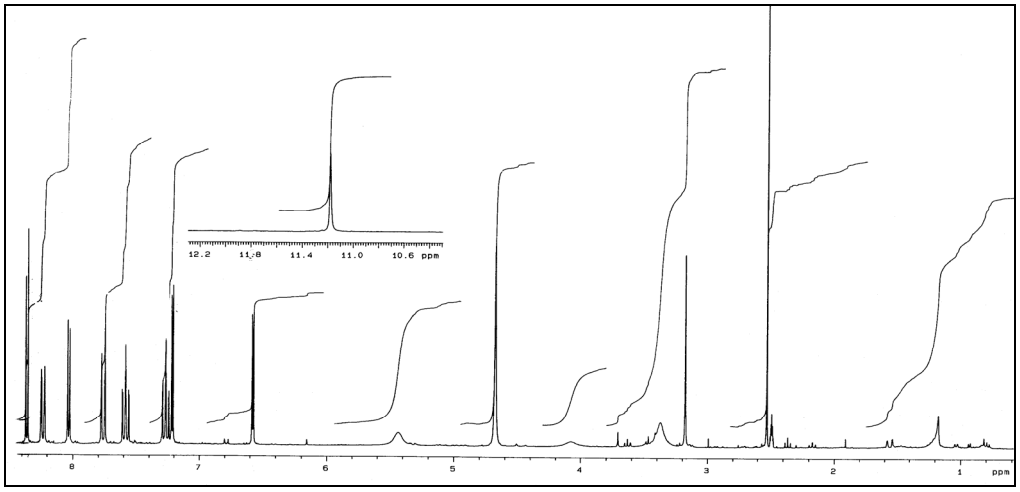


Figure 78: ^{1}H NMR spectrum (300 MHz) of perlolyrin (**78**) in [D_6]DMSO

The ^{13}C and APT spectra indicated the presence of 16 signals, eight methine carbon signals in the aromatic region, 7 quaternary and one methylene carbon.

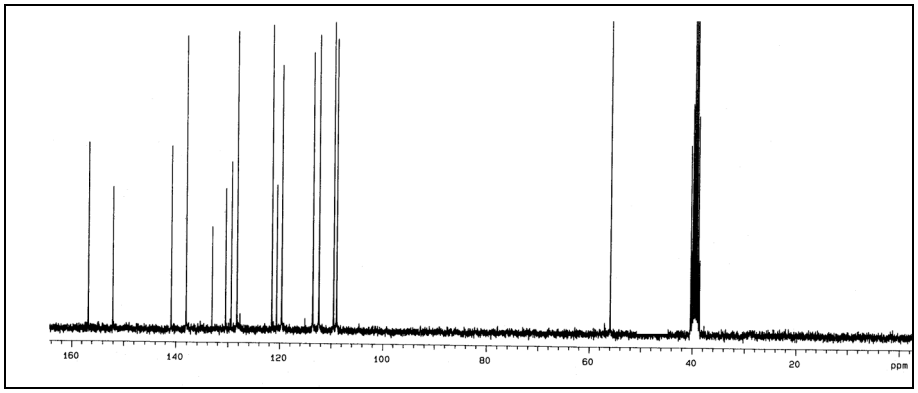
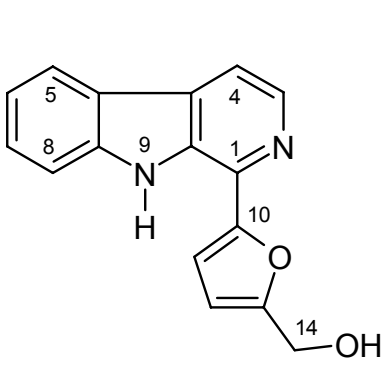
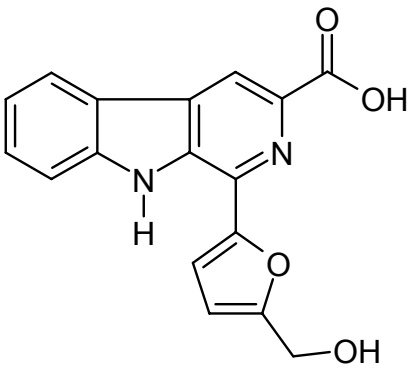


Figure 79: ^{13}C NMR spectrum (75.5 MHz) of perlolyrin (**78**) in [D_6]DMSO

The search in AntiBase using the NMR information and the mass delivered perlolyrin (**78**). The latter and its analogue flazin (**79**) were isolated previously as plant metabolite by Higashi from sake (Japanese rice wine),[140] Japanese rice vinegar, soy sauce[141] and miso (fermented soy bean paste). Structure **78** was previously isolated from microorganism in our group by Schröder.[142]



78



79

Perlolyrin (**78**) is known from different sources and possesses antiplatelet aggregation activity and antithrombotic effects.[143]

4.12.7 Rhadanone A

The ^{1}H NMR spectrum of the yellow solid FM6b (**80a**) exhibited a strong similarity with that of perlolyrin (**78**). Here too a set of four protons for a 1,2-disubstituted benzene ring, a set of two *ortho*-protons of the pyridine ring and an NH-proton were

observed for the 1-substituted β-carboline structure. The difference lies in the replacement of the furan and methylene proton signals in **78** by two protons in **80a** at δ 8.68 (d) and 6.93 (d), which due to the high coupling constant (^3J = 16.2 Hz) were assigned to a *trans*-substituted double bond in conjugation with a carbonyl group. In addition, a methoxy signal at δ 3.82 is also seen.

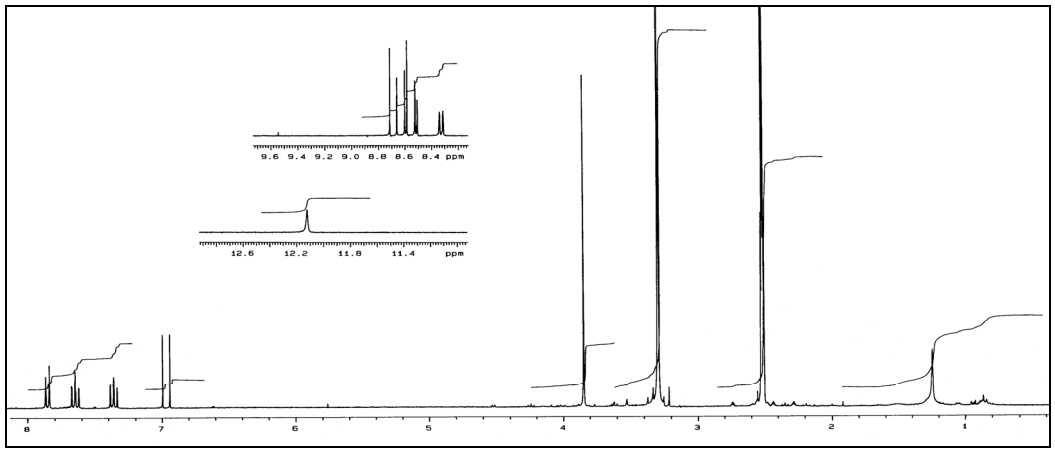


Figure 80: ^{1}H NMR spectrum (300 MHz) of rhadanone A (**80a**) in [D_6]DMSO

A search in AntiBase with the ^{1}H NMR data resulted in (*E*)-3-(β-carbolin-1-yl)-propenoic acid methyl ester (**80b**) isolated from the fungus *Cortinarius infractus*[144] as a possible structure. Comparison of the measured data with those of **80b**[145] showed similarities, which indicated that **80a** differs from **78** and **80b** only in the substituent at C-1. The molecular weight *m/z* 280 was determined by ESI and DCI MS and is by *Δm/z* = 28 higher than that of **80b**. The ^{13}C spectrum contained 16 carbon atoms and indicated the presence of two carbonyl groups at δ 189.2 and 165.5 attributed to an α,β-unsaturated ketone and an ester carbonyl.

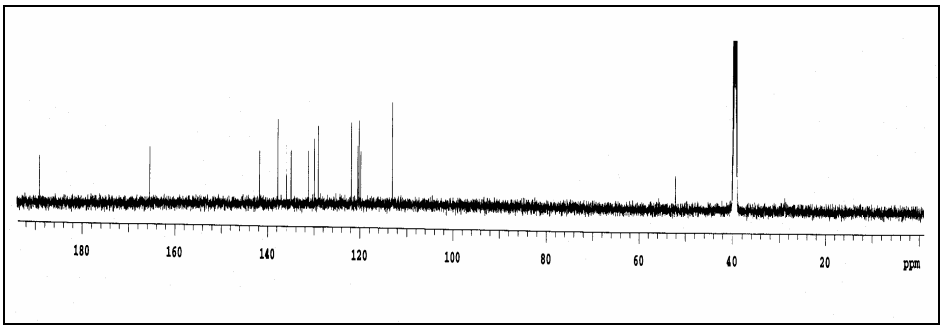
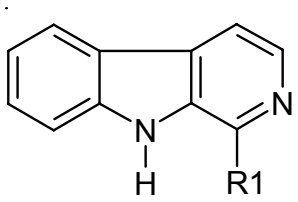
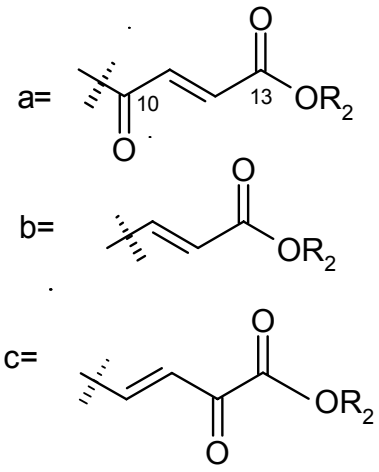


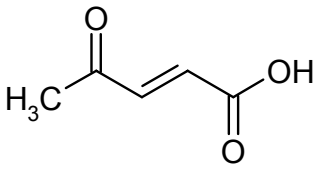
Figure 81: ^{13}C NMR spectrum (150.8 MHz) of rhadanone A (**80a**) in [D_6]DMSO

Compared to **80b**, this additional carbonyl signal at δ 189.2 allows two possible structures for the side chain: 2-keto-3-butenoi-4-yl acid methyl ester (c) and 4-keto-2-butenoi-4-yl acid methyl ester (a). Both side chains a and c in **80a** and **80c** were not reported in natural products so far. The ^{13}C NMR shifts of the side chain at δ 136.1, 130.0 for the double bond and the carbonyls at δ 189.2 and 165.5 are very similar to those of 4-oxo-pent-2-ene carboxylic acid [146] (**80e**) (δ 132.6/ 139.8, 166.8/198.5), so pointing to side chain a. The strong shift difference of the double bond protons in the side chain resembles, however, an α,β-unsaturated carbonyl as in **80e**. This may indicate, that the abnormal shift of the double bond protons is due to anisotropy effects.





80a: R_1 = a R_2 = CH_3
80b: R_1 = b R_2 = CH_3
80c: R_1 = c R_2 = CH_3
80d: R_1 = a R_2 = H



80e

The structure **80a** for radhanone A was finally established by the HMBC couplings (Figure 82), where both of the olefinic proton (δ 8.68 and 6.93) showed strong cross signals to both carbonyls (δ 189.2 and 165.5).

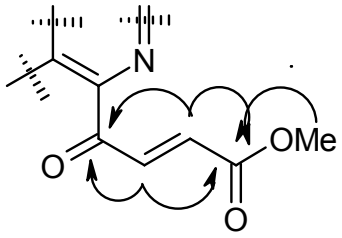


Figure 82: HMBC couplings in the side chain of radhanone A (**80a**)

In case of the 2-keto-3-butenoi-4-yl acid methyl ester (**80c**) side chain, the coupling of the signal at δ 8.68 to the ester carbon must be explained by a ^{4}J coupling, which is, however, expected to be weak. The strong coupling between the deep field olefinic proton and the ester carbonyl can be better explained as a ^{3}J coupling in a 4-keto-2-butenoi-4-yl acid methyl ester side chain delivering the structure of radhanone A (**80a**) as a new natural product.

4.12.8 Rhadanone B

The compound FM6d (**80d**) was also obtained as a yellow solid. The ^{1}H NMR spectra of **80a** and **80d** were very similar, except the methoxy signal at δ 3.82 of **80a,** which was missing in **80d**. The spectrum revealed here also a set of four protons of the 1,2-disubstitituted benzene ring, a set of two protons of the 2,3,4-trisubstituted pyridine system and an NH proton for the 1-substituted β-carboline chromophore. It also contained signals of two *trans*-protons double bond conjugated with carbonyl groups. The molecular weight of *m/z* 266 was determined by ESI and CIMS, which is *Δm/z* 14 (CH_2) smaller than that of **80a**, giving the final structure of radhanone B as free acid **80d**.

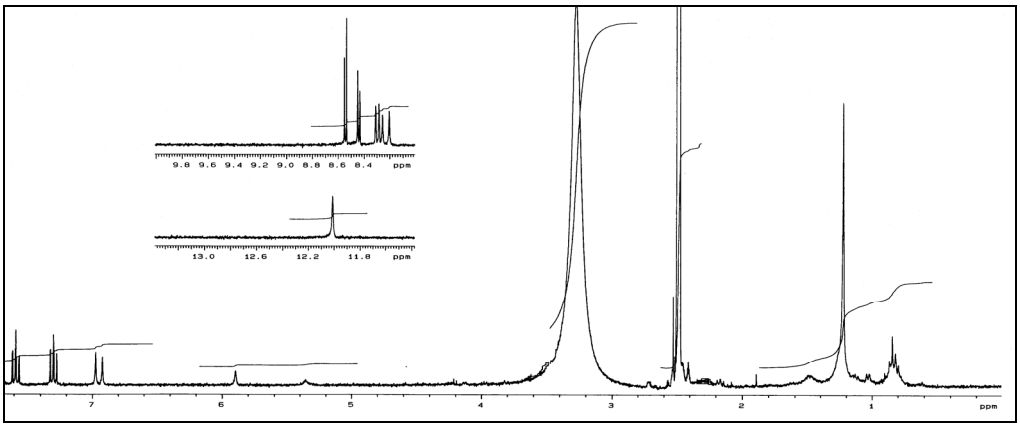
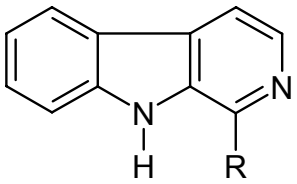


Figure 83: ^{1}H NMR spectrum (300 MHz) of rhadanone B (**80d**) in [D_6]DMSO



81

ß-Carbolins possessing the basic structure **81** are metabolites from plants, bacteria and fungi, many of them show pharmacological effects[147] e.g are enzyme inhibitors or antiviral agents. [148] ß-Carbolins are also used as herbicides and fungicidal agents;[149] of especial interests is their affinity to the benzodiazepine-receptor.[147]

They can be synthetically prepared from tryptophan or tryptamine by Bischler-Napieralski or Pictet-Spengler cyclisation.[147] Perlolyrin (**78**) is formed from the acid catalysed reaction of tryptophan with 5-hydroymethylfurfural (**75**) followed by de-carboxylation.

4.13 Terrestrial *Streptomyces* sp. GW 99/1572

The ethyl acetate extract of the *Streptomyces* isolate GW99/1572 was found to exhibit biological activity against bacteria, fungi and microalgae. In the chemical screening a complex mixture of yellow polar polyene antibiotics was identified by the brown colour reaction with concentrated sulphuric acid on TLC and by ESI MS. More interesting was a colourless weakly UV absorbing band, which gave a violet colouration with anisaldehyde/sulphuric acid and a highly fluorescent spot under 366

nm. The 25 L fermentation of the strain using M_2 medium was performed as a shaker culture during four days, followed by extraction with ethyl acetate.

4.13.1 Kettapeptin

The PTLC purification of fraction II followed by the chromatography on Sephadex LH-20 delivered SIMO (**82a**) as colourless solid. The colour reaction with the chlorine/tolidine reagent indicated it to be a peptide antibiotic, and the missing colour reaction with nihydrin indicated it to be N-terminal blocked. The (+)- and (-)-ESI spectra showed *quasi*-molecular ion peaks at *m/z* 1029 $[M+Na]^+$ and 1005 $[M-H]^-$, respectively, which fixed the molecular weight at *m/z* 1006. HRMS delivered the molecular formula $C_{48}H_{78}N_8O_{15}$. The IR spectrum contained a weak absorption at ν = 1749 cm^{-1} owing to an ester carbonyl or a lactone and strong absorption bands at ν = 1667, 1651 and 1636 cm^{-1} attributed to the amide carbonyls. The ^1H NMR spectrum was rich in signals and delivered eight acidic proton signals and methine protons between δ 9.90-4.30 indicating that **82a** contains 6 α-amino acids. Two 3H singlets at δ 3.37 and 3.06 were assigned to two heteroatom bound methyls. The aliphatic region presented 3 signals of methyl groups connected to double bonds, seven further methyl signals appeared as doublets in the aliphatic region.

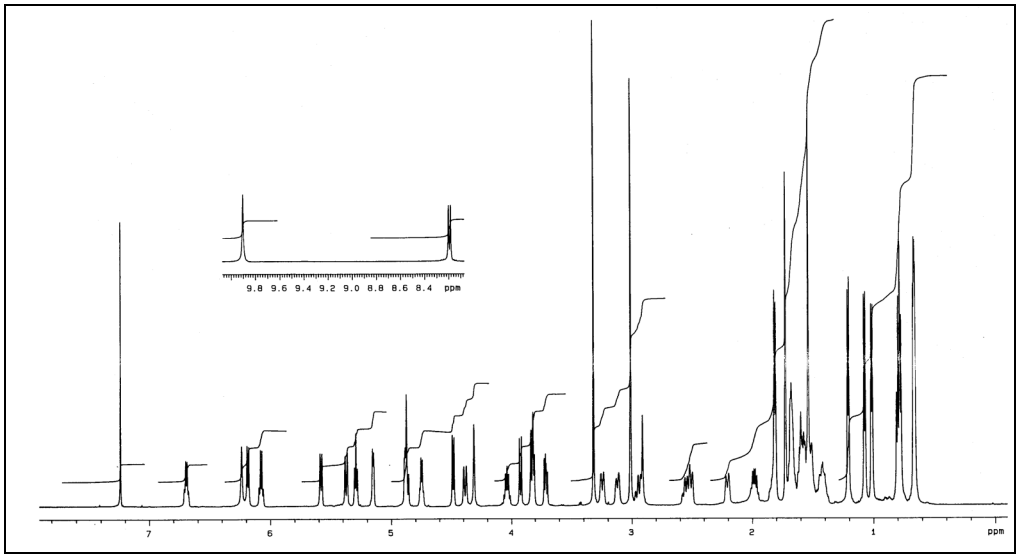


Figure 84: ^1H NMR spectrum (300 MHz) of kettapeptin (**82a**) in $CDCl_3$

The ^{13}C and APT NMR spectrum depicted 48 carbon signals. It contained, in addition to a signal at δ 203.0 of a conjugated ketone, seven closely placed amide or ester

carbonyl signals between δ 175-170. In the aromatic region, two quaternary carbons at δ 137.5 and 132.8, and two methine carbon signals at δ 136.8 and 129.3 were detected. Signals for a quaternary acetal carbon at δ 99.6 and a quaternary carbon bearing oxygen at δ 80.1 were visible. Ten methylene signals, one at δ 68.5 with an oxygen atom and two at δ 48.0 and 45.6 bearing nitrogen were also observed. The spectrum also contained the expected 6 α-methine carbons of amino acids between δ 56.2- 47.7, three signals for CH-O groups and three further methine signals at δ 38.2, 32.5 and 29.2. In addition, nine C-methyl were also visible. The presence of 7 carboxyl signals but only 6 α-amino acid signals suggests that one is due to a normal acid.

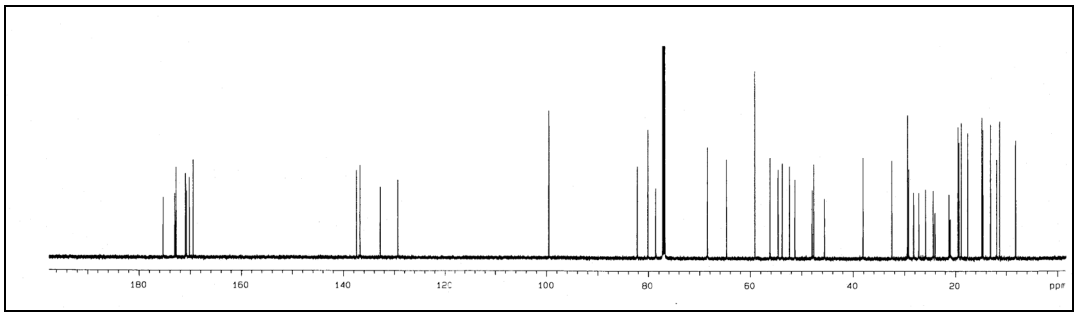
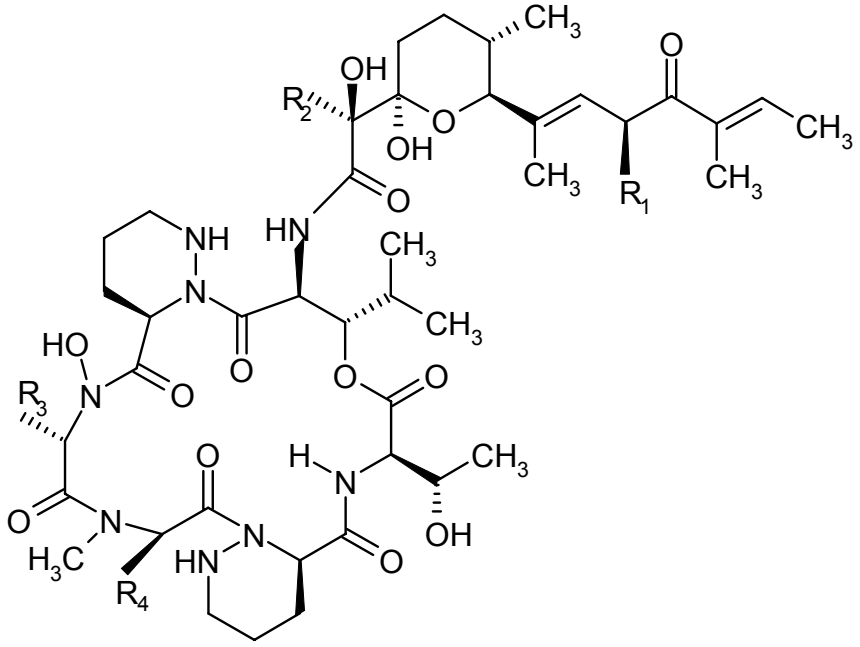


Figure 85: ^{13}C NMR spectrum (150.8 MHz) of kettapeptin (**82a**) in $CDCl_3$

The search in AntiBase using ^{1}H and ^{13}C information following the scheme above led to 5 results with less or bigger mass than that of compound **82a**, indicating it to be a novel natural product; But among these results it was observed that the data of azinothricin[150] (**82b**) and derivatives[151-153] were similar to that of compound **82a**. Among all these structurally related compounds (**82c-e**), it was found that azinothricin (**82b**) and citropeptin (**82e**) were the only, which possess two methyl groups connected to a heteroatom as in **82a**.



82a:	R_1= CH_3	R_2= CH_2CH_3	R_3= CH_2OCH3	R_4= CH_3
82b:	R_1= CH_2CH_3	R_2= CH_2CH_3	R_3= CH_2OCH_3	R_4 = CH_3
82c:	R_1= CH_3	R_2= CH_2CH_3	R_3= CH_3	R_4= CH_3
82d:	R_1= CH_3	R_2 = CH_3	R_3= CH_3	R_4= $CH_2CH(CH_3)_2$
82e:	R_1= CH_3	R_2 = CH_3	R_3= CH_2OCH_3	R_4 = $CH_2CH(CH_3)_2$

The structure of **82a** was established by 2D NMR measurements. The interpretation of the COSY and HMBC spectra led to the construction of the peptide (Figure 86) and the acyl residues (Figure **89**) of compound **82a**. The location of the N-OH was unambigously done as nearly all the acidic protons showed a H,H COSY and HMBC correlations, only the acid proton of the hydroxamic acid did not exhibited neither a COSY nor a HMBC coupling, but later in the NOSY spectrum it exhibited a correlation to the C-20 methylene and the C-19 methine.

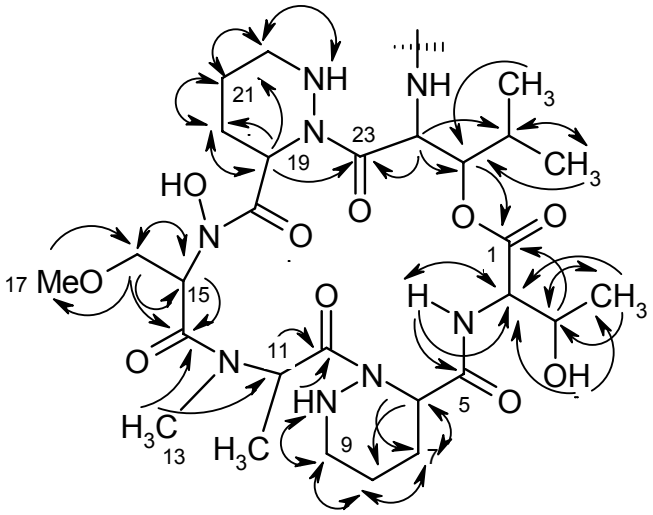


Figure 86: Structure of the peptide core of the kettapeptin (**82a**) derived by H,H COSY (↔) and HMBC (→) correlations.

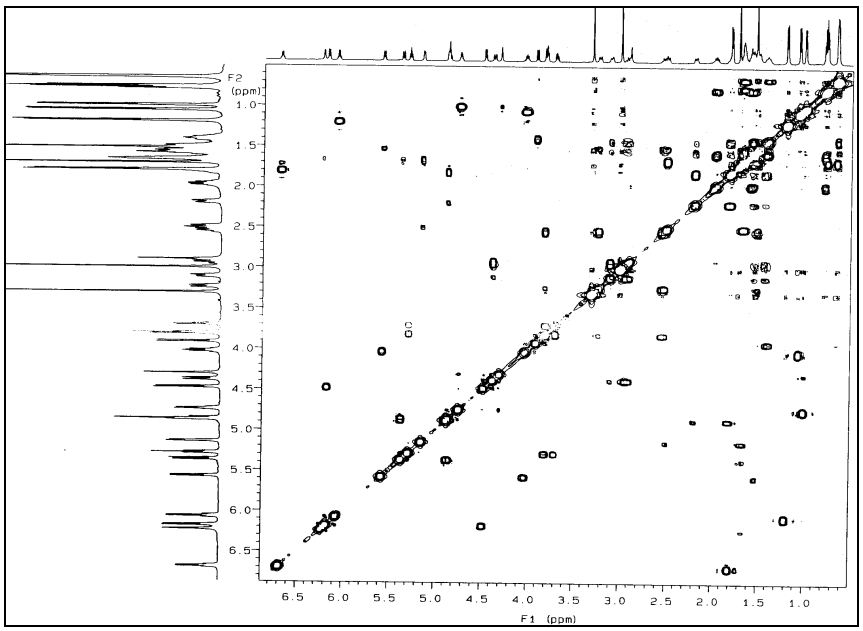


Figure 87: H,H COSY spectrum (300 MHz) of kettapeptin (**82a**) in $CDCl_3$

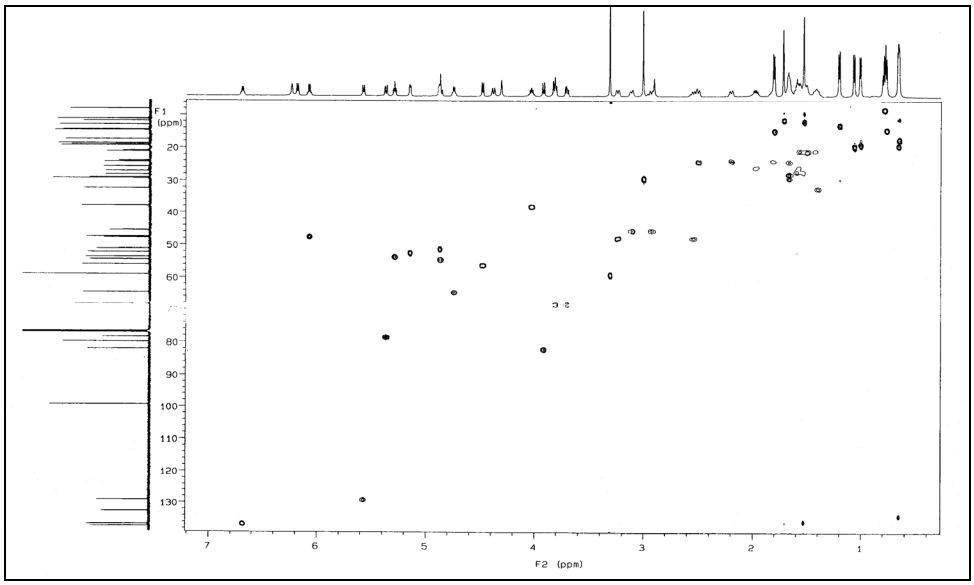


Figure 88: HMQC spectrum (300 MHz) of kettapeptin (**82a**) in $CDCl_3$

The peptide core forms a 19-membered ring (Figure 86) which, was found to be identical to that of azinothricin (**82b**) and similar to A83586C (**82c**), GE3 (**82d**) and citropeptin (**82e**). The ß-hydroxyleucine provides the hydroxy group for the lactone linkage and the amino group for the linkage of the side chain.

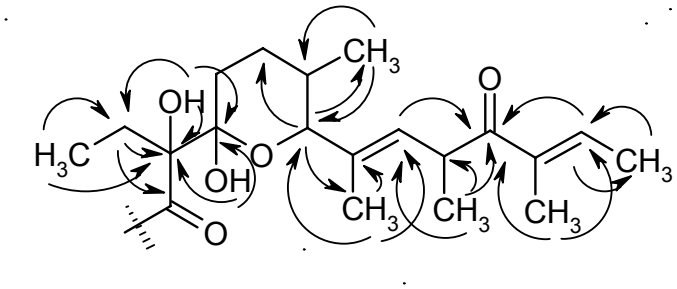


Figure 89: Structure of the acyl residue of kettapeptin (**82a**) derived by H,H COSY (not shown) and HMBC (→) correlation.

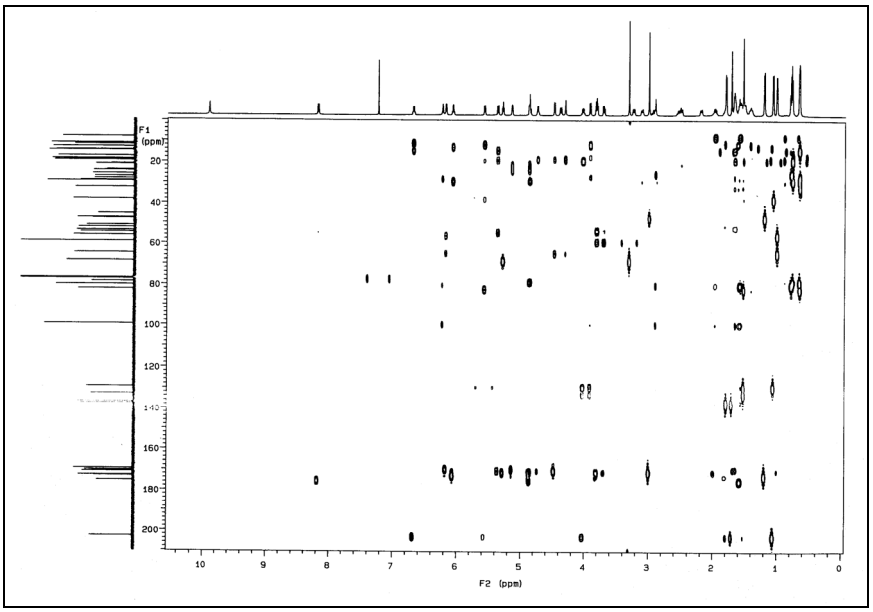
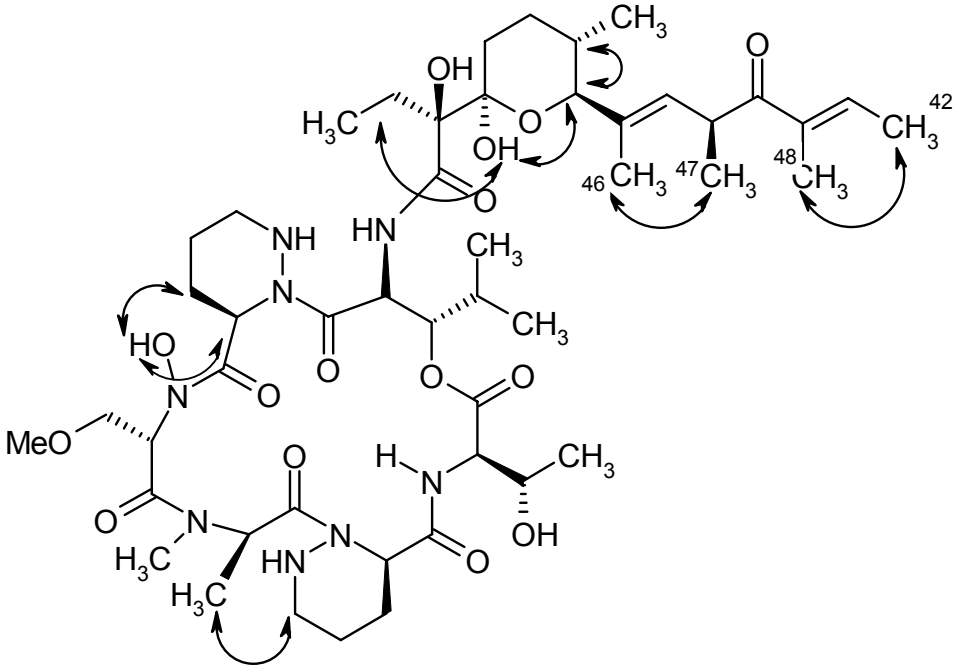


Figure 90: HMBC spectrum (300 MHz) of kettapeptin (**82a**) in $CDCl_3$

The acyl residue of compound **82a** is identical to that of A83586C (**82c**), and differs to that of azinothricin (**82b**) by the ethyl substituent at C-10, while **82a** possesses a methyl group. This assumption is also confirmed by the mass difference of Δm = 14 (CH_2) between **82b** and **82a**. The correlation signal between the proton at δ 8.24 (24-NH) as well as the methine at δ 4.92 (24-H) and the carbonyl at δ 175.5 in the HMBC spectrum allows the connection of the peptide and acyl residue resulting in **82a**, for which the name kettapeptin (**82a**) is proposed.



82a

4.13.2 Crystal Structure Analysis

As kettapeptin (**82a**) crystallised easily from acetone, a crystal structure analysis was performed (**82a**, Figure 91) which indeed confirmed our assumption.

The crystal structure was solved by Madhumati Sevvana from the departement of Inorganic chemistry. The needle-like crystals were grown by evaporation from acetone solution. For data collection, the crystal was shock frozen in a cold nitrogen stream using perfluoropolyether oil as cryoprotectant. Kettapeptin crystallized in the orthorhombic space group $P2_12_12_1$ with a single molecule in the asymmetric unit.

The data reduction was performed using SAINT[154] and the data were corrected *semi*empirically for absorption and other effects with SADABS.[155] The phase problem was solved by conventional direct methods using SHELXS[156] and the model was refined against F^2 on all data by full-matrix least-squares with SHELXL.[157] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included at geometrically calculated positions.

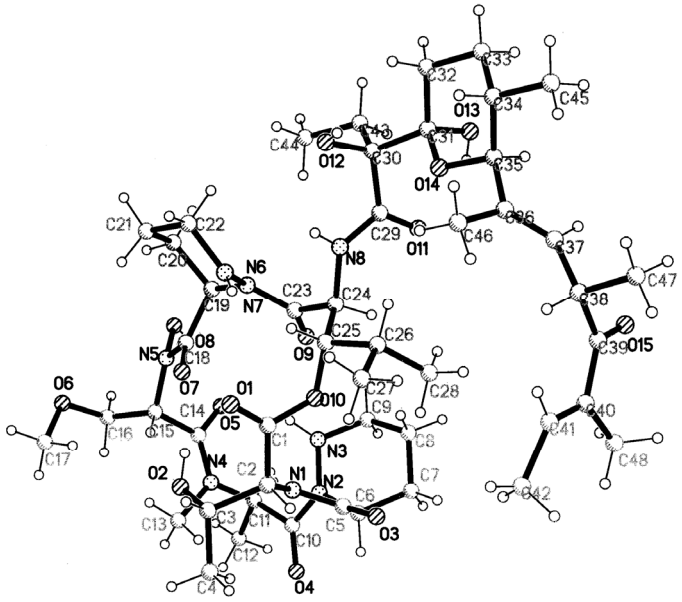


Figure 91: X-ray structure of kettapeptin (**82a**)

Based on the similar specific rotation ($[\alpha]^{20}_D$ +111.1°, $_C$ 1.0, $CDCl_3$) and spectroscopic data of kettapeptin (**82a**) to those of azinothricin (**82b**) ($[\alpha]^{20}_D$ +117.65°, c 0.6, $CHCl_3$),[150] GE3 (**80d**)[152] ($[\alpha]^{20}_D$ +111.5°, c 0.08, $CHCl_3$) and A83586C (**82c**)[151] ($[\alpha]^{20}_D$ +116.1°, $_C$ 0.1, $CHCl_3$), the stereochemistry including the absolute configuration of kettapeptin (**82a**) was deduced from the X-Ray structure and is probably identical with those of **82b**, **82c** and **82d**. Kettapeptin (**82a**) incorporates the six unusual amino acids ß-hydroxyleucine, D-threonine, L-piperazic acid, N-methyl-D-alanine, N-hydroxy-O-methyl-L-serine and D-piperazic acid, which due to their chemical shift similarities in the ^1H and ^{13}C NMR spectra with those of the above mentioned compounds were deduced to possess the same configuration. The configurations at C-8 and C-12 were determined to be both *E* by the cross peaks between protons of 46-Me/47-Me and 42-Me/48-Me in the NOE spectrum; other cross-peaks are shown in Figure 92:

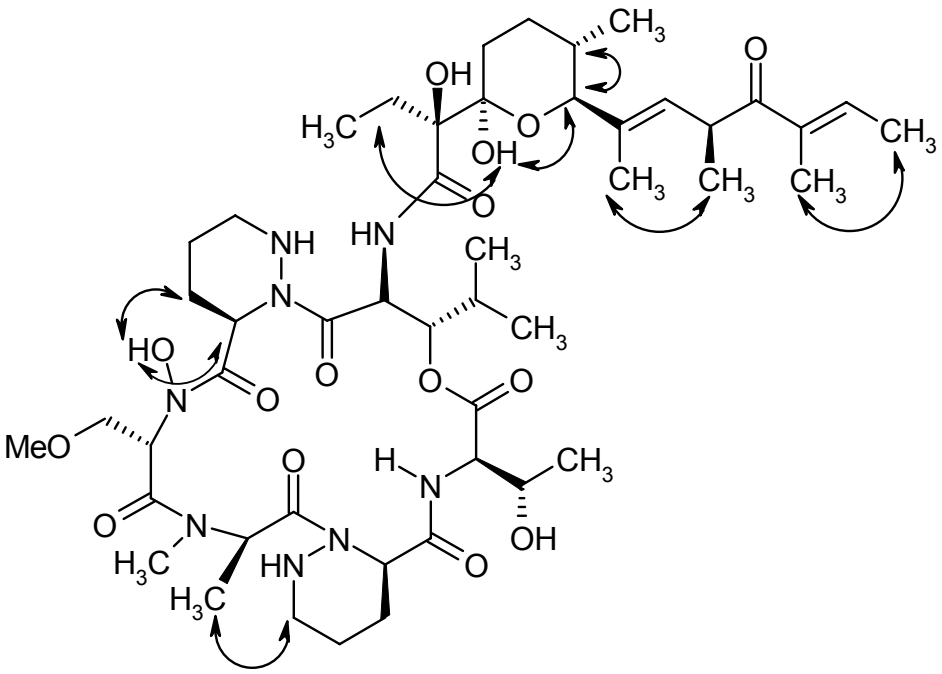


Figure 92: Structure of kettapeptin (**82a**) with important NOE couplings (↔)

The antibiotics azinothricin (**82b**) from *Streptomyces* X-14950, [150] A83586C (**82c**) from *Streptomyces karnatakensis*, [151] GE3[152] (**82d**) and citropeptin[153] (**82e**) exhibit strong antitumor and antibacterial activity against Gram-positive bacteria [158-160] and show activity against peptic and duodenal ulcer.[161] Additionally, they are known to promote the healing of wounds[162] and show anti-inflammatory activity.[163] A83586C (**82c**) exhibits an IC_{50} of 0.0135 µg/ml against CCRF-CEM cells, a human T-cell leukaemia line.[164] Kettapeptin (**82a**) exhibited activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus* and *Escherichia coli* which proved to be much stronger as compared to bacitracin A (table 23). The compound showed no activity against *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*. In addition to the antibacterial activity, kettapeptin (**82a**) was also found to be 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.

4.14 Terrestrial *Streptomyces* sp. GW 32/698

The crude extract of the terrestrial *Streptomyces* sp. GW 32/698 exhibited in the biological screening pronounced antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. The TLC screening revealed several non polar yellow to orange zones which changed to violet with diluted sodium hydroxide (2N) indicating the quinonoid nature of these compounds. The strain was cultivated on M_2 medium in the scale of 25 L during three days. The yellow culture broth was mixed with Celite and filtered through a filter press, and both water and mycelium phase were extracted with ethyl acetate and the organic phase brough to dryness. The dark yellow oily crude extract was defatted using cyclohexane and submitted in column chromatography on silica gel using CH_2Cl_2/MeOH gradient following the Figure 93.

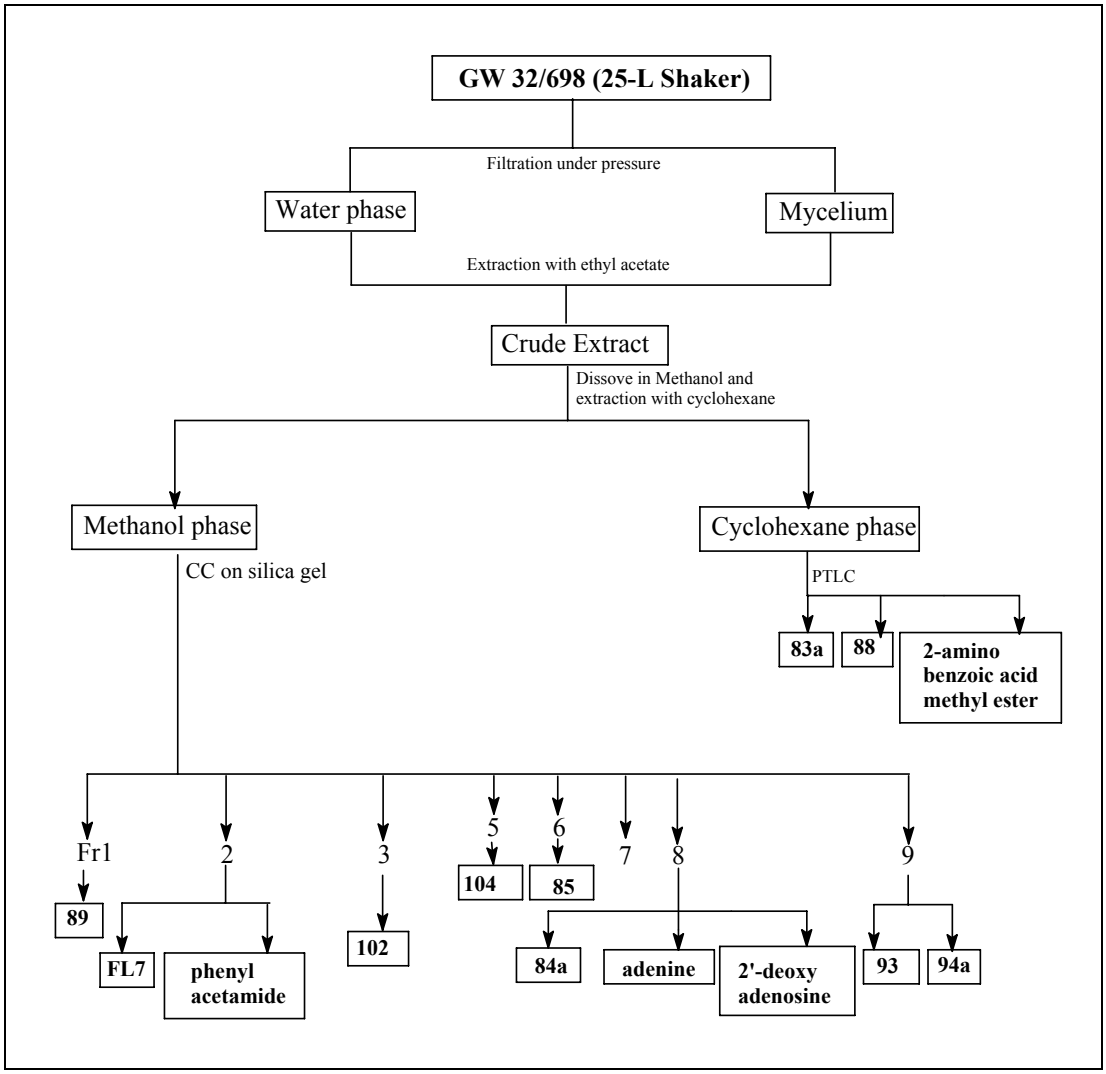


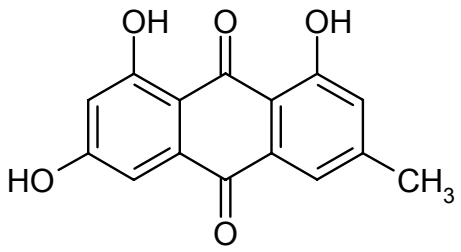
Figure 93: Work-up scheme of the terrestrial *Streptomyces* sp. GW 32/698

Compound FL3 was identified in view of the ^{1}H NMR and mass spectra as phenylacetamide. Compounds FL8 and FL8a gave a strong fluorescence under 254 nm and showed a light blue colour on spraying with anisaldehyde/sulphuric acid. They were identified as adenine and 2'-deoxyadenosine, which are commonly isolated from bacteria.[165]

4.14.1 Chrysophanol

PTLC of the cyclohexane phase gave 2-aminobenzoic acid methyl ester and a second compound FLO1 (**83a**) as a yellow powder. The yellow spot on TLC remained unchanged on spraying with anisaldehyde/sulphuric acid but gave a violet colour reaction with diluted alkali.[166] The ^{1}H NMR spectrum showed two chelated hydroxy groups at δ 12.09 and 11.90. The aromatic region presented five protons, which could be assigned to a 1,2,3-trisubstituted and a 1,3,5,6-tetrasubstituted aromatic ring. Furthermore, an aromatic methyl group was observed at δ 2.44.

The EI mass spectrum indicated a molecular weight of *m/z* 254. These facts and comparison with an authentic sample identified FLO1 as chrysophanol (**83a**), which is a common plant constituent.[167]



83a

4.14.2 3,8-Dihydroxy-1-methylanthraquinone-2-carboxylic acid

Compound FL4 (**84a**) was obtained from the precipitation of fraction 8 in contact with dichloromethane, and Centrifugation delivered FL4 as an orange powder. The quinone nature of **84a** was confirmed by the violet colour reaction with 2 N sodium hydroxide. The aromatic region of the ^{1}H NMR spectrum exhibited only four aromatic protons, of which three indicated a 1,2,3-trisubstitution pattern and a singlet at δ 7.22. Furthermore a chelated hydroxyl group appeared as a singlet at δ 13.40. The

aliphatic region exhibited only a singlet of intensity three at δ 3.08 attributed to an aromatic methyl.

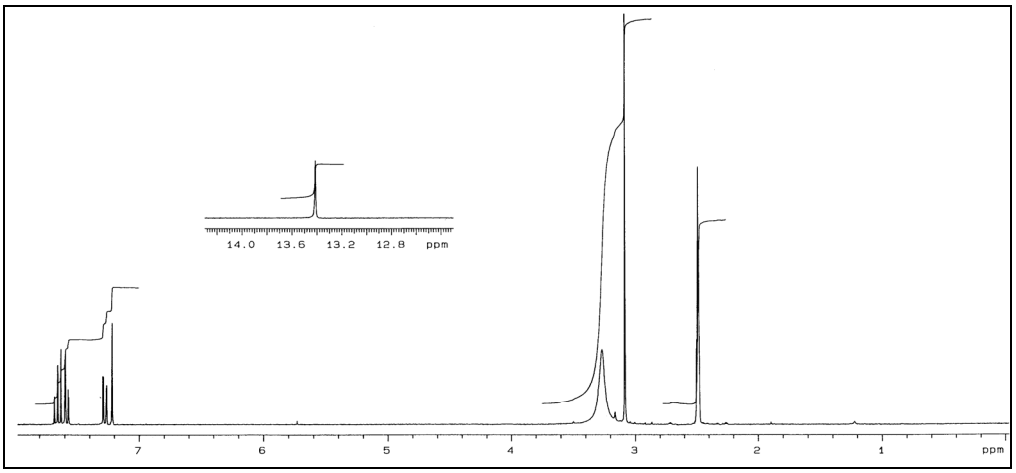
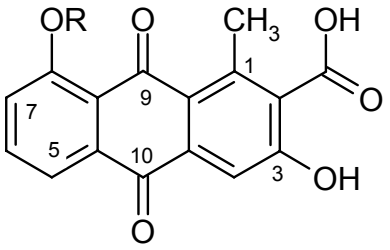


Figure 94 ^{1}H NMR spectrum (300 MHz) of anthraquinone **84a** in [D_6]DMSO.

The ^{13}C NMR spectrum revealed additionally to the signals expected for the quinone carbonyl at δ 188.8 and 182.9, one carbonyl of an acid or ester at δ 172.1, eight quaternary sp^2-hybridised carbon atoms and finally a methyl signal at δ 20.3. EI MS indicated the molecular ion at *m/z* 298, and EI HRMS gave the molecular formula $C_{16}H_{10}O_6$. The search in AntiBase with this information gave as the only solution 3,8-dihydroxy-1-methyl-anthraquinone-2-carboxylic acid (**84a**), previously isolated from *Streptomyces* sp. by Lackner *et al*.[168] Comparison of the proton and ^{13}C NMR data with the literature showed, however, significant differences especially for the proton singlet, which is reported for **84a** at δ 7.54 (4-H), but found in FL4 at δ 7.22 as well as the carbon shift C-1, which appear in **84a** at δ 142.1 and in FL4 at δ 149.8. As **84a** and FL4 seemed to be isomers, the latter was converted into its acetate (**84b**) and a NOESY spectrum was measured, however, without any useful result. The HMBC spectrum exhibited correlations both of the methyl and the 1H singlet to the quarternary carbons C-2 and C-9a, while the methyl and the singlet showed correlations, respectivelly, to carbonyls at δ 168.8 and 182.9 leading to the previously cited compound. The spectroscopic differences may be due to various reasons, however, as the authentic sample and spectra were not accessible for direct comparison, this question remains open.



84a: R = H
84b: R = OAc

4.14.3 Aloesaponarin II

The yellow solid FLIII (**85**) obtained by HPLC purification of fraction 6 gave also a positive test for *peri*-hydroxyquinones. The molecular ion was found by EI MS to be 254 Dalton. The ^{1}H NMR data exhibited in the aromatic region a pattern for a 1,2,3-trisubstituted aromatic ring. Two further 1H doublets showed a *meta* coupling, and a chelated hydroxyl signal and an aromatic methyl group appeared at δ 13.06 and 2.72, respectively.

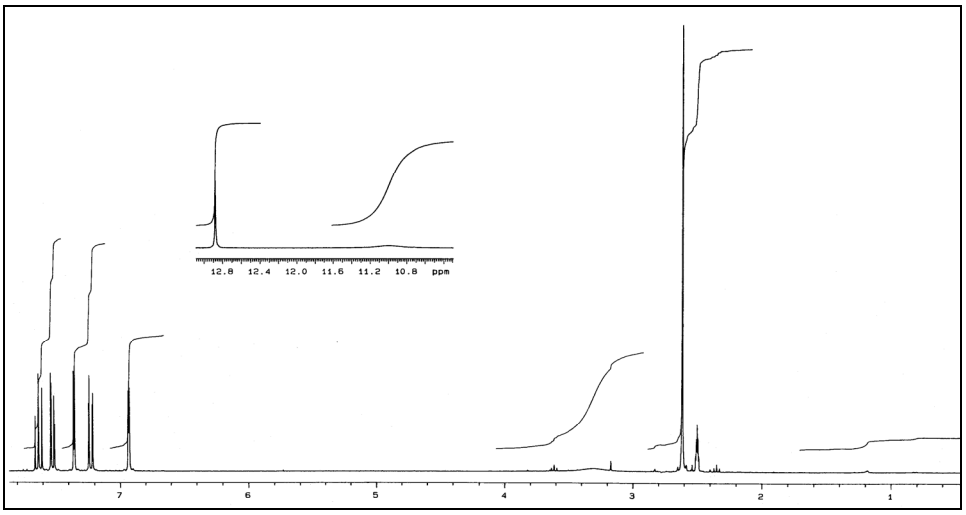
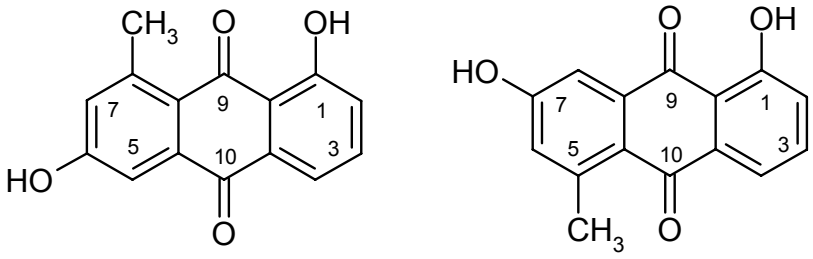


Figure 95: ^{1}H NMR spectrum (300 MHz) of aloesaponarin II (**85**) in [D_6]DMSO.

With this information two compounds can be constructed, aloesaponarin II (**85**) and 1,7-dihydroxy-5-methylanthraquinone (**86**)



85 **86**

The small quantity of the compound did not permit further measurements. But a comparison of the ^{1}H NMR spectrum with that of aloesponarin II (**85**) previously isolated in our group by M. Schiebel[58] identify clearly confirmed the identity. This compound was firstly isolated in 1974 from the plant *Aloe saponaria*[170] and its biosynthesis studies showed that compounds **84a** and **85** are polyketides with eighth acetate units.

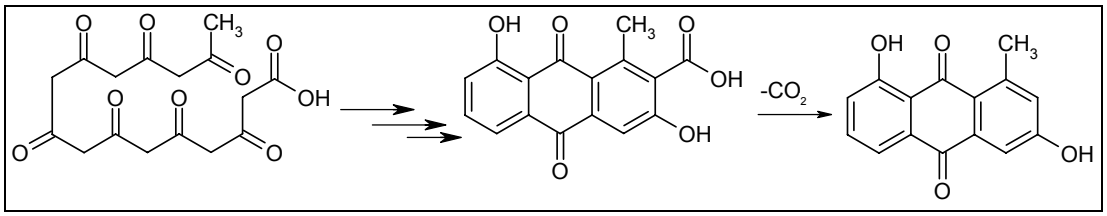
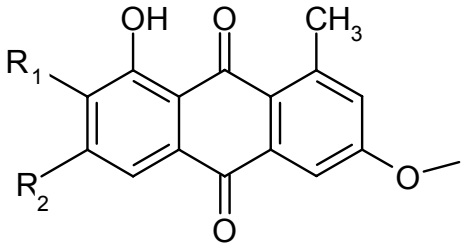


Figure 96: C_{16}-hypothetic polyketide biosynthetic way of compound **84a** and **85**

3,8-Dihydroxy-1-methylanthraquinone-2-carboxylic acid (**84a**) and aloesaponarin II (**85**) are rare naturally occurring α-methylanthraquinones. Native compounds of this group became known only as insect pigments[169] and later as constituents of a few plants.[170] Further examples are 1-hydroxy-6-methoxy-8-methyl anthraquinone[58] (**87a**), deoxyerythrolaccin-dimethyl ether (**87b**), flavomarine A (**87c**) and B (**87d**)[171], which have been recently isolated from bacteria in our group.



87a:	$R_1 = H$	$R_2 = H$
87b:	$R_1 = H$	$R_2 = OCH_3$
87c:	$R_1 = CH_2OCH_3$	$R_2 = OCH_3$
87d:	$R_1 = CH_2OH$	$R_2 = OCH_3$

4.14.4 Bhimamycin A

Compound FL2 (**88**) was obtained from the cyclohexane phase as a brown solid, which was a *peri*-hydroxy quinone due to the violet colour reaction with sodium hydroxide. After spraying with anisaldehyde/sulphuric acid, the colour of the compound changed from yellow to grey. The EI and CI mass spectra indicated the molecular weight to be 272, and EI HRMS led to the molecular formula $C_{15}H_{12}O_5$. The ^{1}H NMR spectrum indicated a methyl doublet at δ 1.61 and a quartet of one proton at δ 5.10 suggesting the presence of a CH_3CH-O-fragment. The aliphatic region delivered two singlets at δ 2.79 and 5.25 for an aromatic bound methyl group and an acidic proton, respectively. Furthermore, the spectrum exhibited an ABC pattern of three adjacent aromatic protons and the signal of a chelated hydroxyl group at δ 12.7.

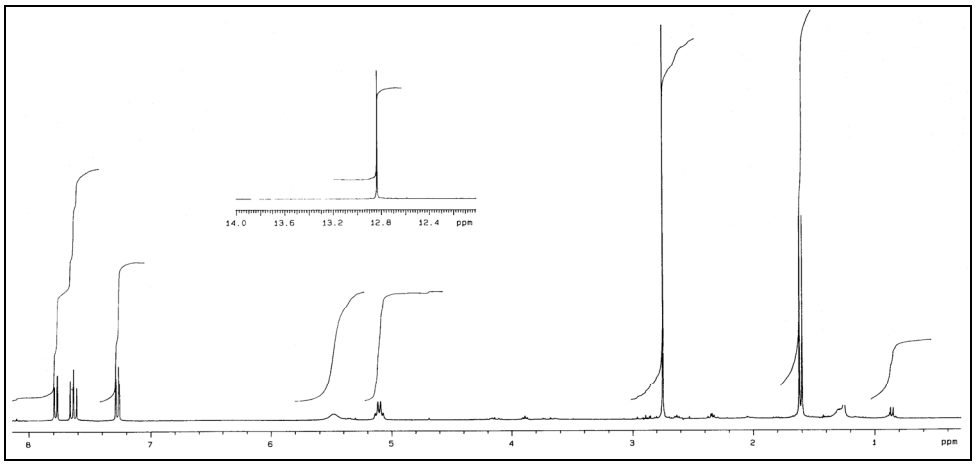


Figure 97: ^{1}H NMR spectrum (300 MHz) of bhimamycin A (**88**) in $CDCl_3$.

The ^{13}C NMR and APT spectra are in accord with all the proposed fragments and interpretations: They showed 15 signals including two quinone carbonyls at δ 186.1 and 180.7, aromatic and aliphatic methyls at δ 21.6 and 13.7, respectively, and the signal at 63.9 was attributed to a methine carbon connected to oxygen.

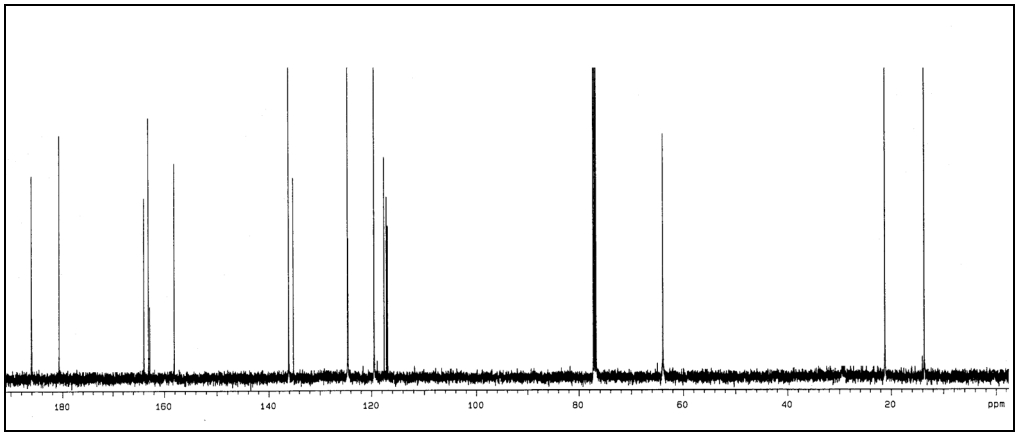
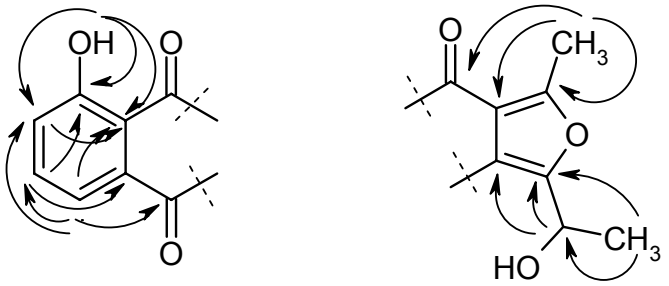


Figure 98: ^{13}C **NMR** spectrum (75.5 MHz) of bhimamycin A (**88**) in $CDCl_3$.

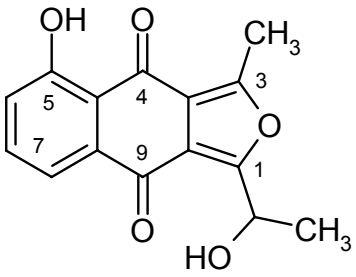
According to a database search, Bhimamycin is a new compound. Interpretation of the H,H COSY and HMQC spectra resulted in two fragments I and II having a common carbonyl group.



I II

Figure 99: Selected HMBC-couplings for fragment I and II

These substructures can be connected in two different manners, however, as in the HMBC spectrum, the methyl singlet at δ 2.78 showed a weak ^4J-coupling with the signal of the chelated carbonyl group at δ 186.6 and the *peri*-proton at δ 7.76 a ^3J-coupling to the non chelated carbonyl at δ = 180.6, it results that the methyl group and chelated hydroxyl group must be placed *syn-peri*planar resulting in **88** as the only possible structure for bhimamycin A.



88

4.14.5 Bhimamycin B

Column chromatography of fraction 1 resulted in a brown solid FL1, which presented on TLC the same characteristics as bhimamycin A (**88**) and turned from yellow to blue-violet on spraying with anisaldehyde/sulphuric acid. The yellow fluorescence under 366 nm and the similarity of the UV/VIS spectrum with bhimamycin A (**88**) indicated that they probably possess the same chromophore.

The ^{1}H NMR spectrum showed again relatively few signals, which were similar to those of **88** in the deep field region. In the aliphatic region, instead of one singlet at δ 2.74 as in **88**, two at δ 2.86 and 2.87 for methyl groups attached to sp^2 carbons were visible. The signals for the CH_3CHO-fragment of bhimamycin A (**88**) were missing in the spectrum.

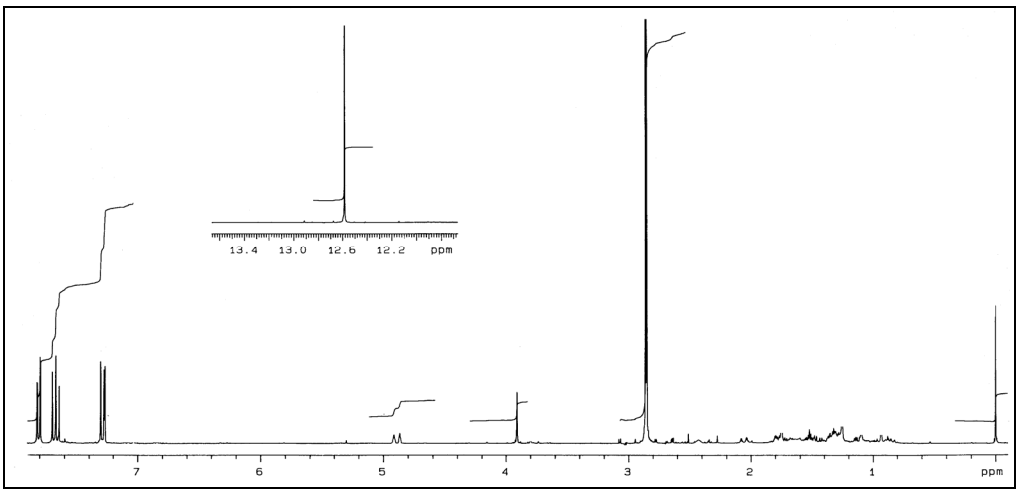


Figure 100: ^{1}H NMR spectrum (300 MHz) of bhimamycin B (**89**) in $CDCl_3$.

In the ^{13}C NMR spectrum, 15 signals were observed, which represent two methyls, three sp^2 methine groups, seven aromatic or olefinic quaternary carbon atoms, two

quinone carbonyls and an additional carbonyl signal at δ 187.2. The latter could be due to a lactone or conjugated ketone.

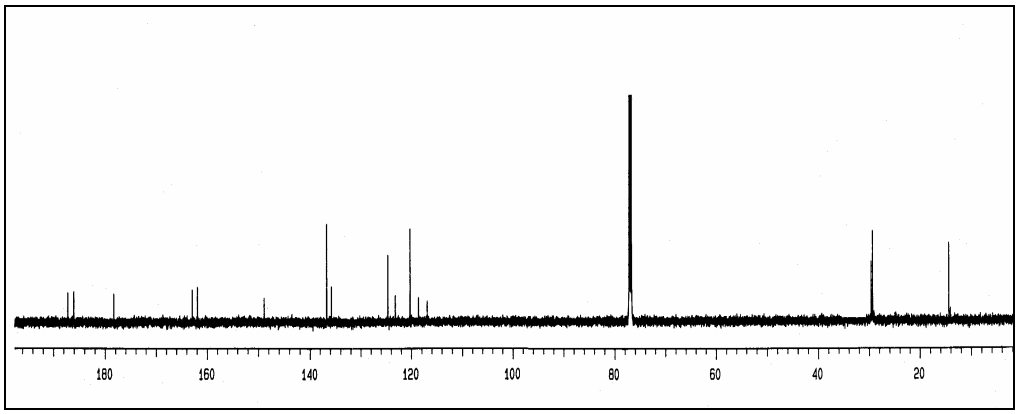
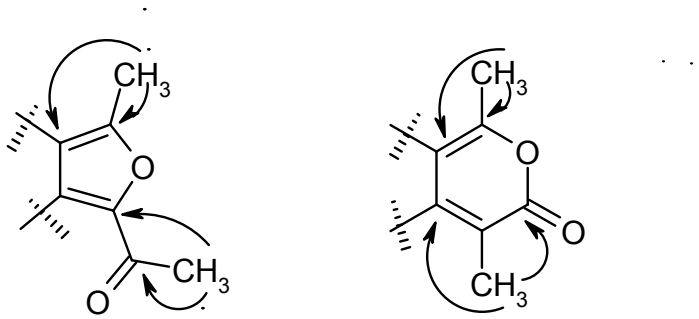


Figure 101: ^{13}C NMR spectrum (75.5 MHz) of bhimamycin B (**89**) in $CDCl_3$.

The EI and CI mass spectra showed a molecular peak at *m/z* 270 whose high resolution delivered the molecular formula $C_{15}H_{10}O_5$. Interpretation of the 2D spectra delivered two fragments I (Figure 99) and III or IV (Figure 102).



III IV

Figure 102: Selected HMBC-couplings for fragments III and IV

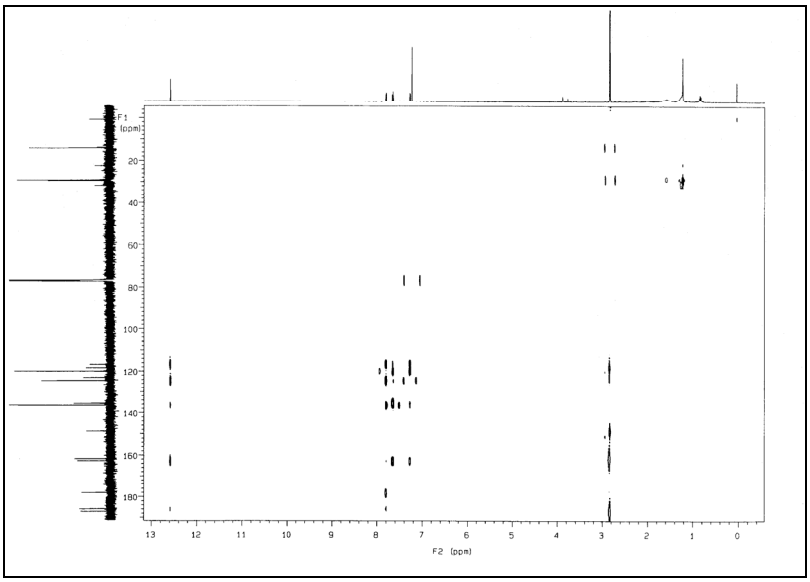
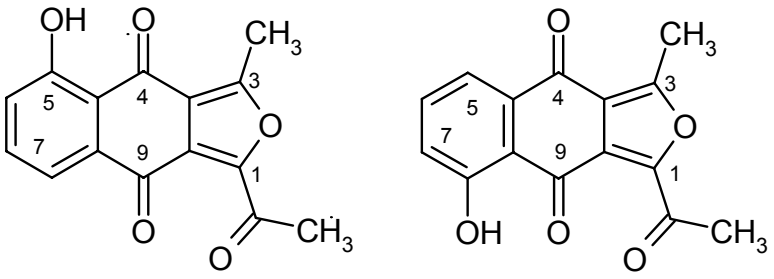


Figure 103: HMBC spectrum (300 MHz) of bhimamycin B (**89**) in $CDCl_3$.

Fragment IV with the lactone structure could be ruled out due to the absence of an IR band above 1700 cm^{-1}, which has been reported for anhydrofusarubin lactone[172] with similar structure. Therefore, only fragments I and III came into question. Their connection resulted in two structures with the chelated hydroxyl group at C-5 or 8 (**89, 90**), which were indistinguishable with the available NMR data.



89 **90**

Figure 104: Possible structures for bhimamycin B (**89**)

As the structurally related bhimamycin A (**88)** was isolated from the same strain, it can be assumed, however, that both compounds have the same biosynthetic pathway and with this presumption, the OH group must be at the C-5 resulting in the final structure 8**9** for Bhimamycin B, which was confirmed spectroscopically, indeed. The biosyhthesis parthway of isofuranonaphthoquinone are still not investigated, but it

seems that they may be derived from 8 acetates follow by decarboxylation and dehydratation as suggest in the following figure.

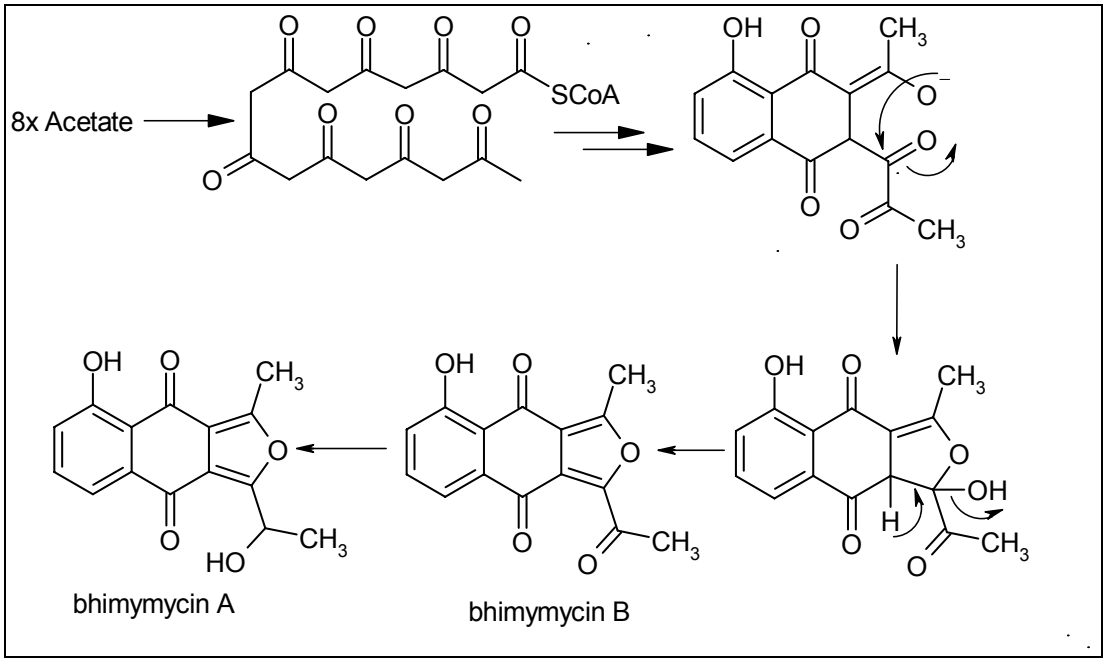
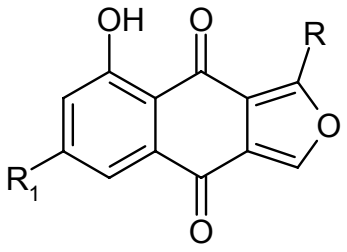


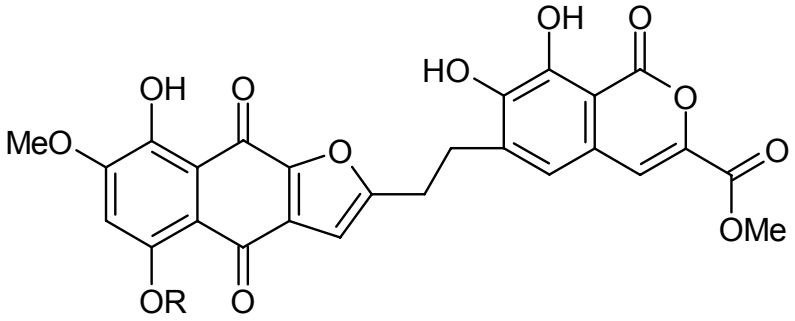
Figure 105: Hypothetic biosynthesis of bhimamycin A (**88**) and B (**89**)

Bhimamycin A (**88**) and B (8**9**) are the first compounds with the rare naphtho[2,3c]furan-4,9-dione chromophore, which have been isolated from bacteria so far. Closely related furanoquinones have, however, been isolated from plants e.g. the 5-hydroxy-3-methylnaphtho[2,3-c]furan-4,9-dione (**91a**) from *Bulbine capitata*[173] and *Aloe ferox*,[174] arthoniafurone A (**91b**) from cultures of the lichen *Arthonia cinnabarina* Wallr[175] and monosporascone (**91c**) from the fungus *Monosporascus cannonballus*.[176]



91a: R = CH_3, R_1 = H
91b: R = $COCH_3$ R_1 = H
91c: R = H R_1 = OCH_3

The only two antibiotics with the isomeric naphtho[2,3-b]furan-4,9-dione chromophore from bacteria, α-rubromycin (**92a**) and γ-isorubromycin (**92b**) have been reported from *Streptomyces collinus* and *Streptomyces antibioticus*.[177]



92a: R= Me
92b: R= H

4.14.6 Bhimamycin C

PTLC of fraction 9 gave the yellow, orange fluorescent FL5 (**93**), which turned pink with anisaldehyde/sulphuric acid. (+)-ESI MS indicated the $[M+Na]^+$ ion at *m/z* 338, and EI HRMS led to the molecular formula $C_{17}H_{17}NO_5$.

The ^1H NMR spectrum was again similar to that of bhimamycin A (**93**) showing an acidic proton signal for a chelated OH group at δ 13.12, three aromatic protons of a 1,2,3-substituted aromatic system, a quartet of an aliphatic methine proton connected to oxygen, a methyl doublet at δ 1.54 corresponding to the fragment CH_3CH-O, and a singlet at δ 2.70 of an aromatic methyl. The evident difference between both spectra was the appearance of two additional methylene multiplets at δ 4.19-3.99 and 3.90 indicating their connection with hetero atoms.

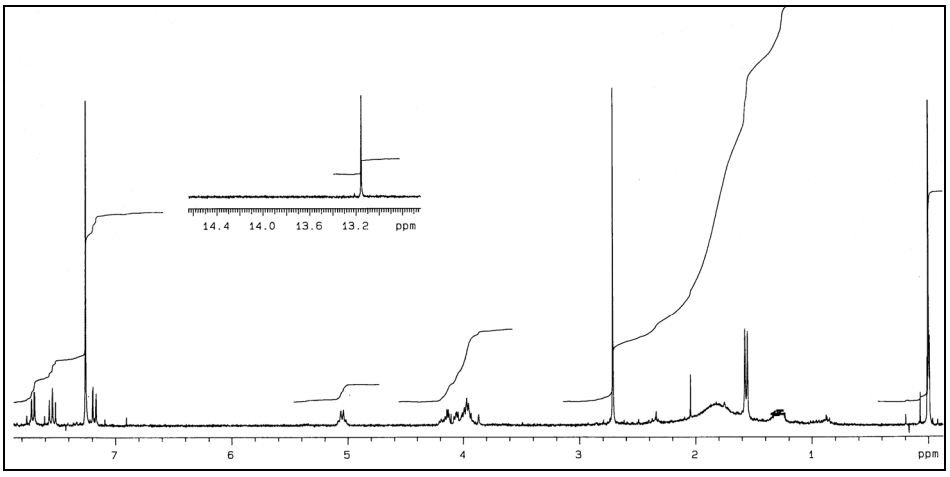


Figure 106: ^{1}H NMR spectrum (300 MHz) of bhimamycin C (**93**) in $CDCl_3$.

The ^{13}C and APT NMR spectra indicated the presence of 17 signals. Both methylene groups appeared at δ 61.9 and 47.9; in view of these values, the ethanediyl fragment should be connected with oxygen and nitrogen, respectively. Comparison of the sp^2 carbon signals with those of bhimamycin A (**88**) confirmed the close similarity except for the signals of C-1 and C-3, which were shifted from δ 163.9 and 158.1 in bhimamycin A (**88**) to δ 147.4 and 140.6, respectively. It followed that these carbon atoms are more likely connected to nitrogen and not to oxygen as in bhimamycin A (**88**).

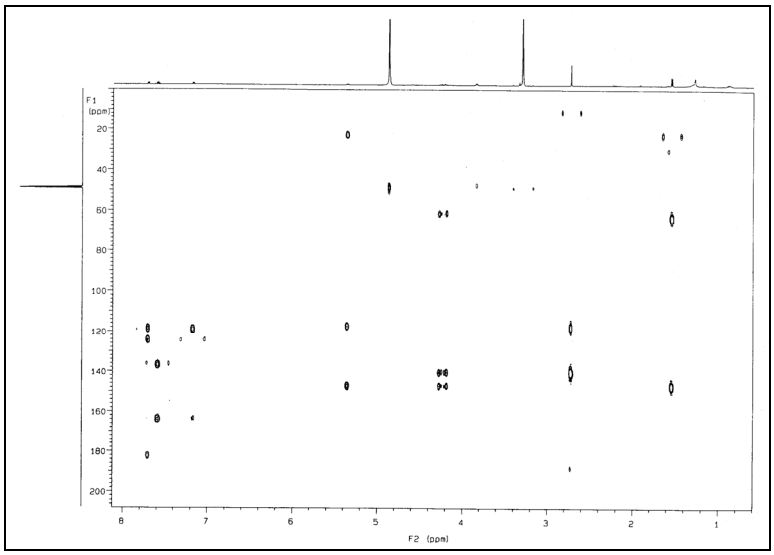


Figure 107: HMBC spectrum (300 MHz) of bhimamycin C (**93**) in $CDCl_3$.

Interpretation of the 1D and 2D NMR data in combination with the molecular formula delivered the fragments I (fig. 99) and V. Because of the cross peak of the methyl signal at δ 2.70 with the signal of the chelated carbonyl group at δ 188.8 and on the other hand the cross peak of the proton at δ 7.76 and the non-chelated carbonyl, these fragments could be connected only in one way forming structure **93** for bhimamycin C.

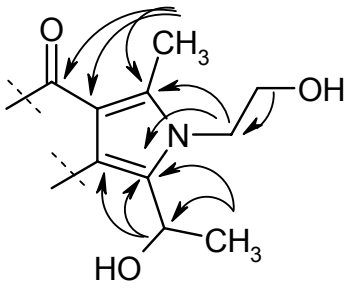
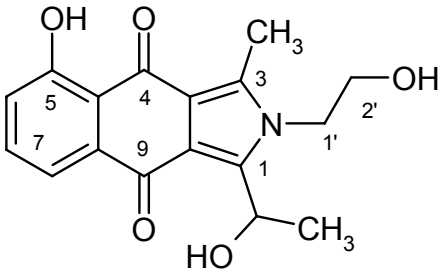


Figure 108: Selected HMBC couplings of fragment V



93

4.14.7 Bhimamycin D

Compound FL6 (**94a**) was obtained as yellow solid, which turned red-violet with anisaldehyde/sulphuric acid and showed an orange fluorescence under 366 nm like Bhimamycin C (**93**). The molecular weight was determined by EI MS as *m/z* 389; the molecular formula was deduced from the EI HRMS to be $C_{22}H_{17}NO_6$. The ^1H NMR spectrum showed 7 sp^2 protons, which could be assigned by H,H COSY data to three adjacent aromatic protons as in fragment I of bhimamycins A (**88**), B (**89**) and C (**93**), and additional 4 consecutive protons of an electron-rich aromatic system at δ 7.86, 6.90, 6.84 and 6.56. The spectrum exhibited further a signal of a chelated hydroxyl group at δ 13.45 and two methyl singlets at δ 2.80 and 2.20.

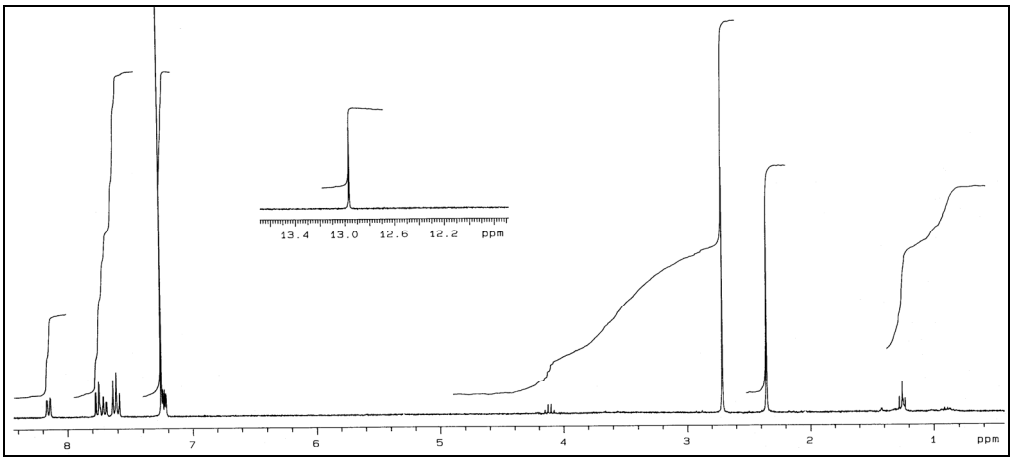
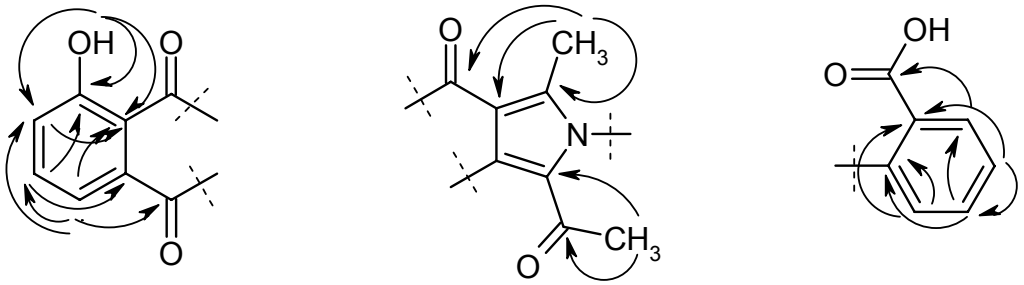


Figure 109: ^{1}H NMR spectrum (300 MHz) of bhimamycin D (**94a**) in $CDCl_3$.

The ^{13}C NMR spectrum displayed 20 sp^2 carbons and two methyls. Beside two quinone carbonyl groups at δ 187.5 and 179.9, two additional carbonyl signals were observed. The first one at δ 192.9 was assigned to an aromatic or α,β-unsaturated ketone, and a second signal at δ 167.8 may be due to an acid group, which was confirmed indeed by the facile formation of a methyl ester **94b** by treatment of **94a** with diazomethane. In the HMBC spectrum, the methyl group at δ 2.20 showed a coupling with the carbons at δ 117.2, 141.4 and a ^{4}J-coupling with the chelated carbonyl group at δ 187.5. On the other hand, the methyl group at δ 2.70 coupled with the α,β-unsaturated ketone and the carbon at δ 134.2. From this interpretation, fragment VI resulted, and the four-proton pattern, HMQC and HMBC data delivered the fragment VII.



I VI VII

Figure 110: Selected HMBC couplings of bhimamycin D (**94a**)

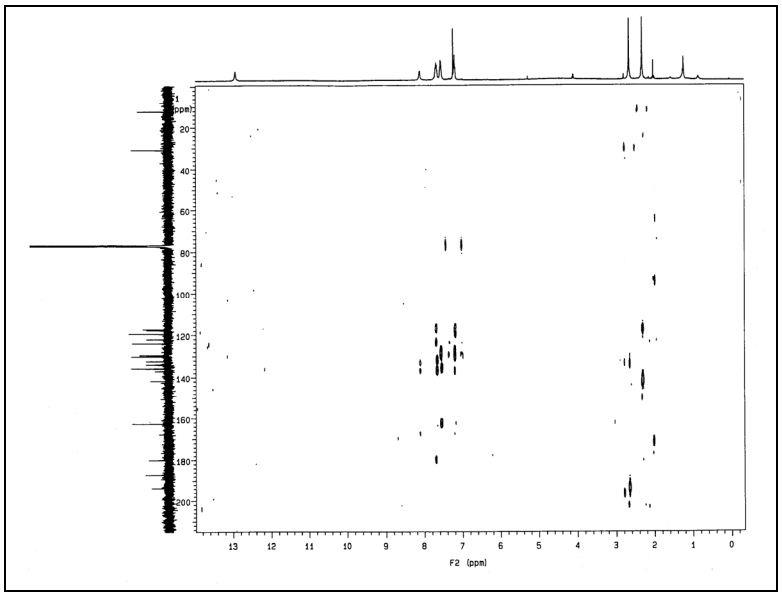
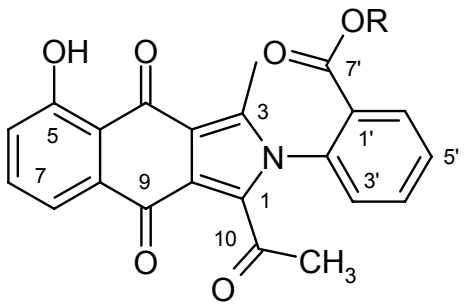


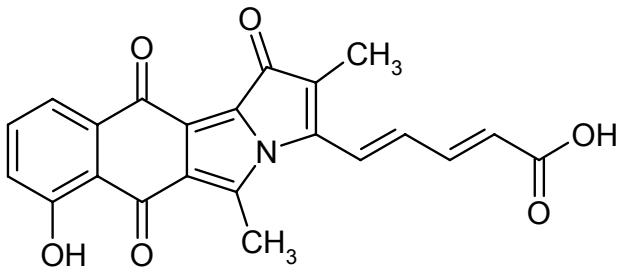
Figure 111: HMBC spectrum (300 MHz) of bhimamycin D (**94a**) in $CDCl_3$.

Connection of the three fragments resulted in bhimamycin D (**94a**), which is a new natural product possessing an isoindolequinone chromophore like bhimamycin C (**93**).

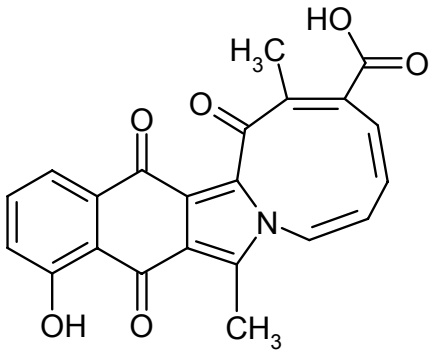


94a: R = H
94b: R =Me

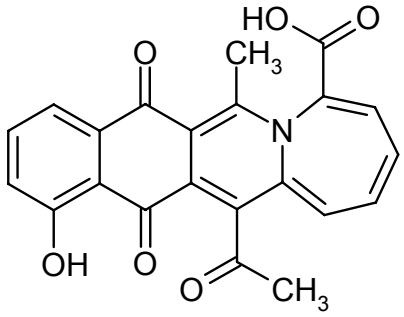
It should be noted that after the structure of bhimamycin D (**94a**) was supported by the spectra interpreter SESAMI. It should be emphasized that the latter delivered besides **94a** several further structures like **95**, **96**, **97**, which were in full agreement with the spectroscopic data and could be excluded only with additional arguments.



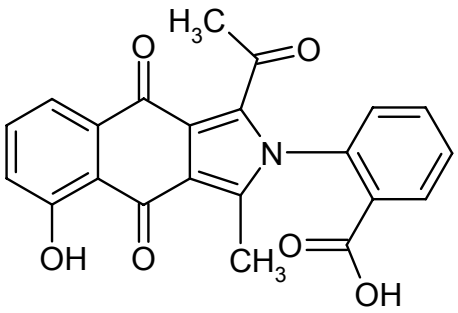
95



96



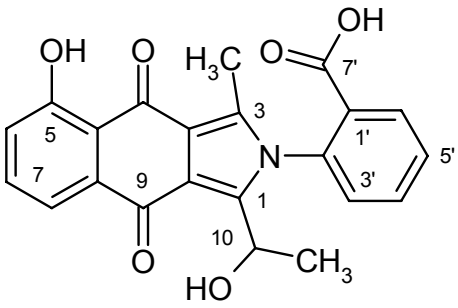
97



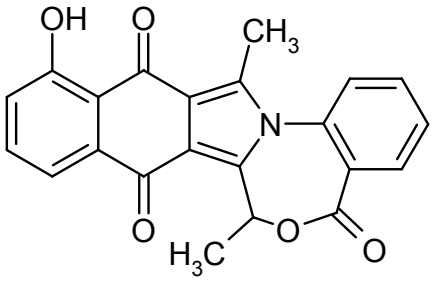
94a

Furans can be transformed easily into pyrroles by refluxing with ammonia or primary amines. It can be assumed therefore that bhimamycin C (**93**) originates from bhimamycin A (**88**) and ethanolamine, and in the same way bhimamycin D (**94a**) may be formed from bhimamycin B (**89**) and anthranilic acid.

As we had bhimamycin A (**88**) in larger amount, this was firstly refluxed with anthranilic acid in ethanol in a model reaction to see if the oxygen of the furan ring can be replaced also by aromatic amines. The reaction resulted indeed in a mixture of two new compounds named bhimamycin F (**98**) and G (**99**). Finally the structure of bhimamycin D (**94a**) was confirmed by the synthesis from bhimamycin B (**89**) and anthranilic acid in the same way. It should be mentioned that the ^{1}H NMR shifts of the aromatic protons in bhimamycin D (**94a**) depended strongly of the purity of the sample. Shifts differences up to $\Delta\delta$ 0.38 were observed between pure and impure products.

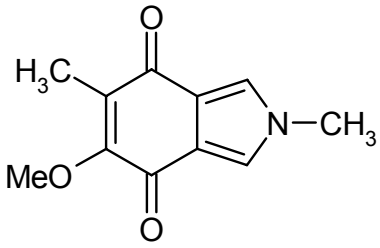


98

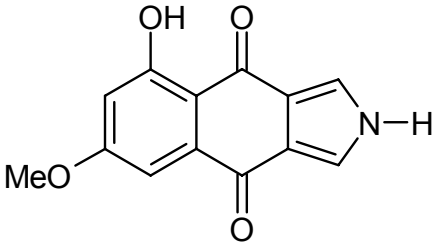


99

Compounds with 2*H*-benzo[f]isoindole-4,9-dione chromophore have not been reported as natural products, however, 2*H*-benzo[f]isoindole-4,9-dione[178] and 2-methyl-2*H*-benzo[f] isoindole-4,9-dione[179] are two compounds, which possess the same chromophore but are from synthetic origin. The only related natural metabolites reported so far are the antimicrobial 5-methoxy-2,6-dimethyl-2*H*-isoindole-4,7-dione (**100**) from the sponge *Reniera* sp.[180] and the recently reported azamonosporascone (**101**) from the fungus *Monosporascus cannonballus*.[176]



100



101

4.14.8 Bhimamycin E

Purification of fraction 3 on Sephadex LH-20 gave the yellow solid bhimamycin E (**102**) with a molecular weight of *m/z* 246 and a molecular formula of $C_{13}H_{10}O_5$ by EI HRMS. The ^{1}H NMR spectrum exhibited signals of three adjacent protons as in the other bhimamycins. Two acidic protons at δ 11.08 and 7.38 can be attributed to a chelated and a free hydroxyl group, respectively. A 2H singlet at δ 3.76 corresponds to a methylene group connected to oxygen, a second singlet at δ 2.34 is characteristic for the methyl of an acyl group.

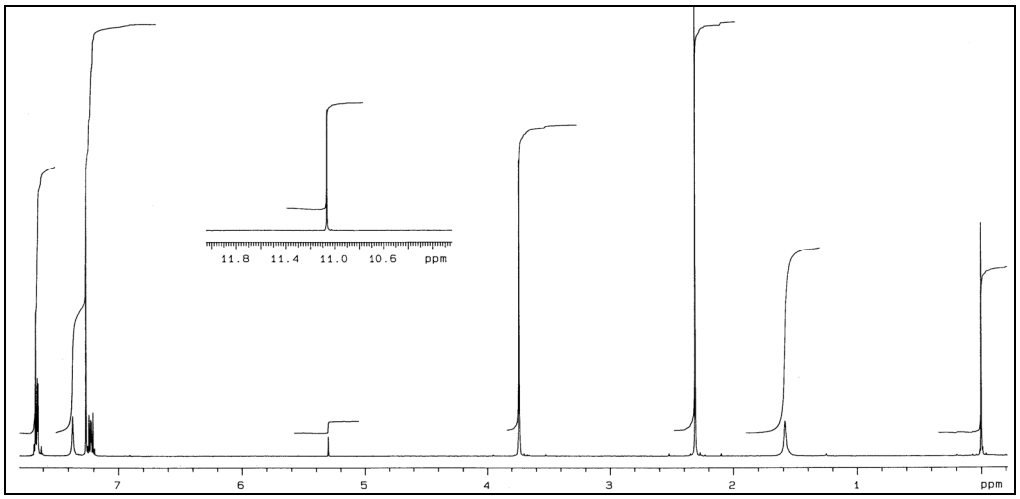
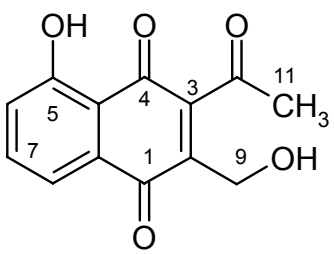
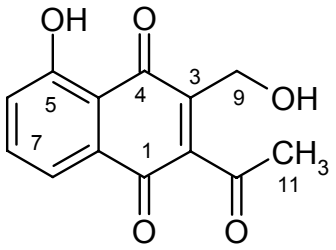


Figure 112: HMBC spectrum (300 MHz) of bhimamycin E (**102**) in $CDCl_3$

The ^{13}C NMR spectrum delivered 13 carbon signals as demanded by the molecular formula, two quinone carbonyls at δ 184.3 and 183.1, a conjugated ketone at δ 203.5, eight aromatic carbons, a methylene and a methyl signal at δ 38.1 and 30.1, respectively. This information led to the construction of two possible structures **102** and **103**.



102



103

The HMBC spectrum showed couplings of the methyl and methylene groups to the quaternary carbon C-3. Further couplings of the methylene group were observed with the carbon C-2 (δ 153.7) and to the non-chelated carbonyl group a δ 183.1, which permits to determine the structure of bhimamycin E as **102**.

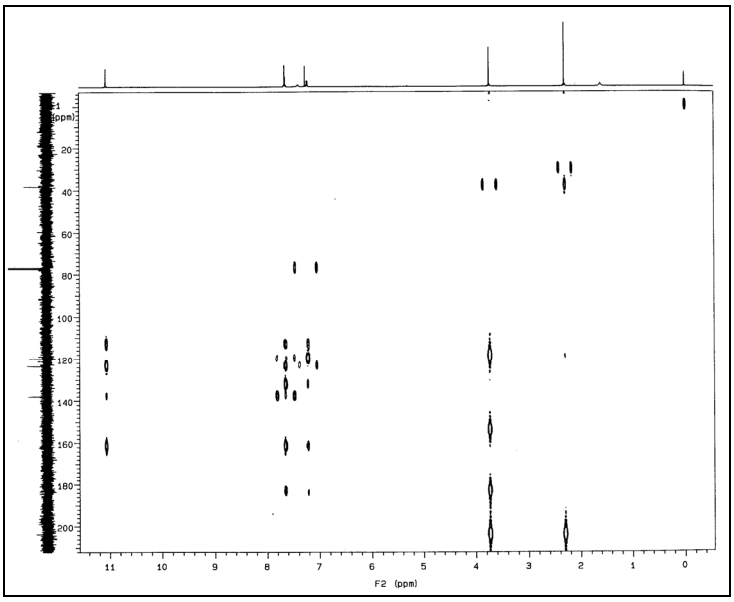


Figure 113: ^{13}C NMR spectrum (300 MHz) of bhimamycin E (**102**) in $CDCl_3$.

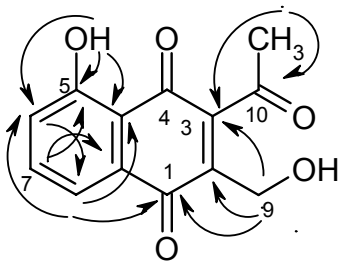


Figure 114: HMBC-couplings in bhimamycin E (**102**)

4.14.9 Bhimanone

Colourless needles of MS2 (**104**) were obtained by chromatography of fraction 5 on silica gel from a zone, which gave a light blue spot on spraying with anisaldehyde/sulphuric acid. The ^1H NMR spectrum contained signals for a 1,2,3-substituted benzene ring and two aliphatic methines, one bearing an oxygen (δ 5.05, t), the other appearing as a multiplet at δ 3.50. Further signals appeared in the aliphatic region as two doublets of doublets at δ 2.90 and 2.50 which were easily assigned to a methylene group, a 3H singlet at δ 2.15 corresponding to an acetyl group, a 2H multiplet at 2.10, and finally an acidic proton at δ 9.40.

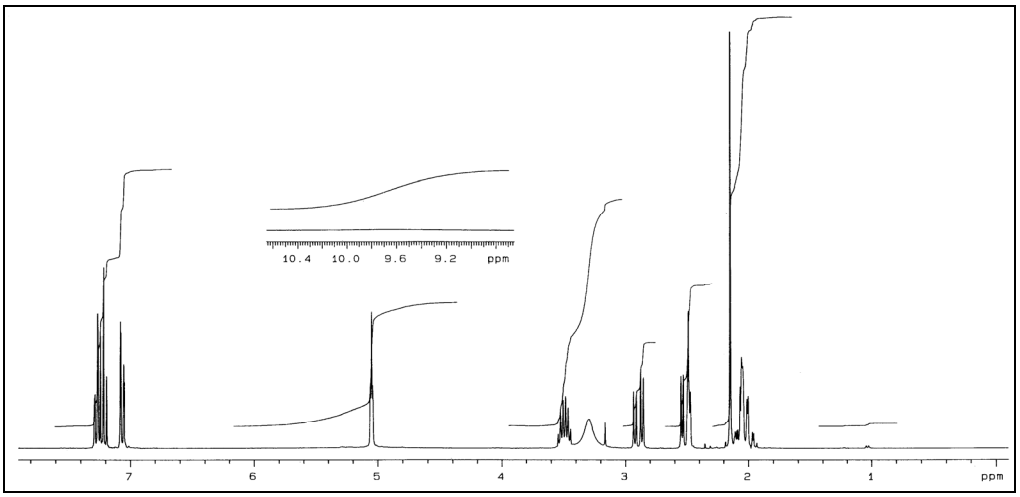


Figure 115: ^{1}H NMR spectrum (300 MHz) of bhimanone (**104**) in [D_6]DMSO

From the (+)-ESI and EI mass spectra, the molecular weight was deduced to be 234 Dalton, and EI HRMS delivered $C_{13}H_{14}O_4$ as molecular formula. The ^{13}C NMR spectrum showed two carbonyl signals at δ 206.7 and 199.0, assigned to a saturated and an α,β-unsaturated ketone group, respectively, two methine carbons - one connected to oxygen at δ 58.9, the other at δ 37.2 - two methylene carbons at δ 43.2 and 36.5 and a methyl group at δ 30.0.

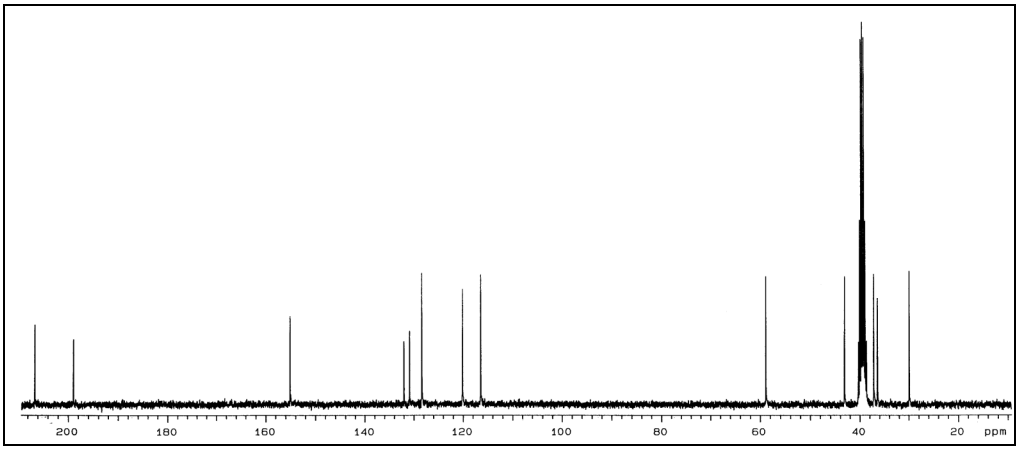


Figure 116: ^{13}C NMR spectrum (75.5 MHz) of bhimanone (**104**) in [D_6]DMSO

The H,H COSY spectrum confirmed the 1,2,3-substituted benzene ring (fragment I) and established a CH_2CH-CH_2CHOH-fragment.

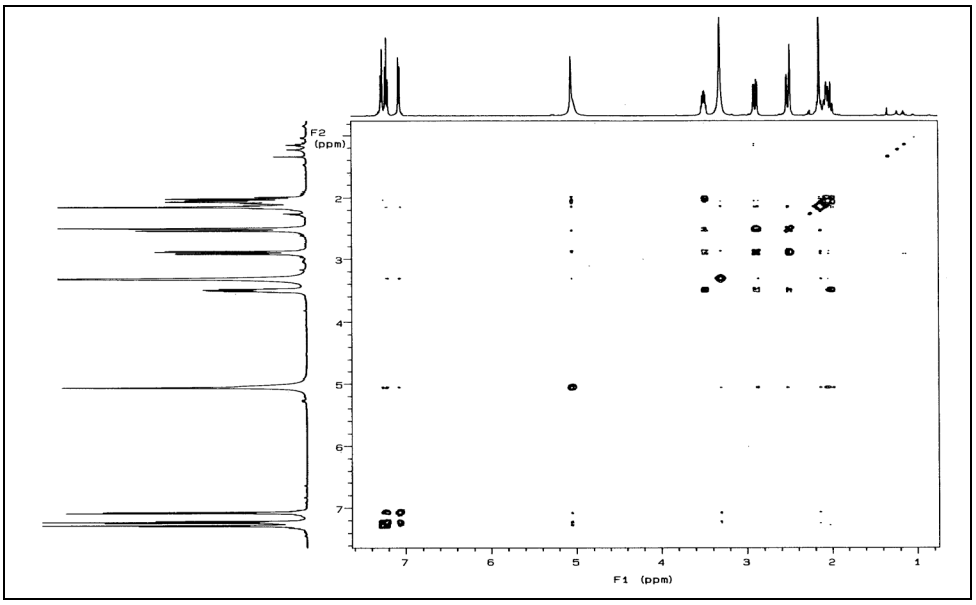


Figure 117: H,H COSY spectrum (300 MHz) of bhimanone (**104**) in [D_6]DMSO

The HMBC spectrum revealed a coupling of the methyl with the ketone group at δ 206.7 and the methylene at 43.2. The methylene signal at δ 2.90-2.50 and the methine group at δ 3.50 showed couplings on the two carbonyl groups, in addition the methine at δ 3.50 coupled also on the methine and methylene carbons at δ 58.9 and 43.2. Another coupling was seen between the aromatic proton at δ 7.27 and the α,β-unsaturated carbonyl group at δ 199.0. This information permits to join the fragments and obtain the structure **104** of bhimanone, a novel natural product.

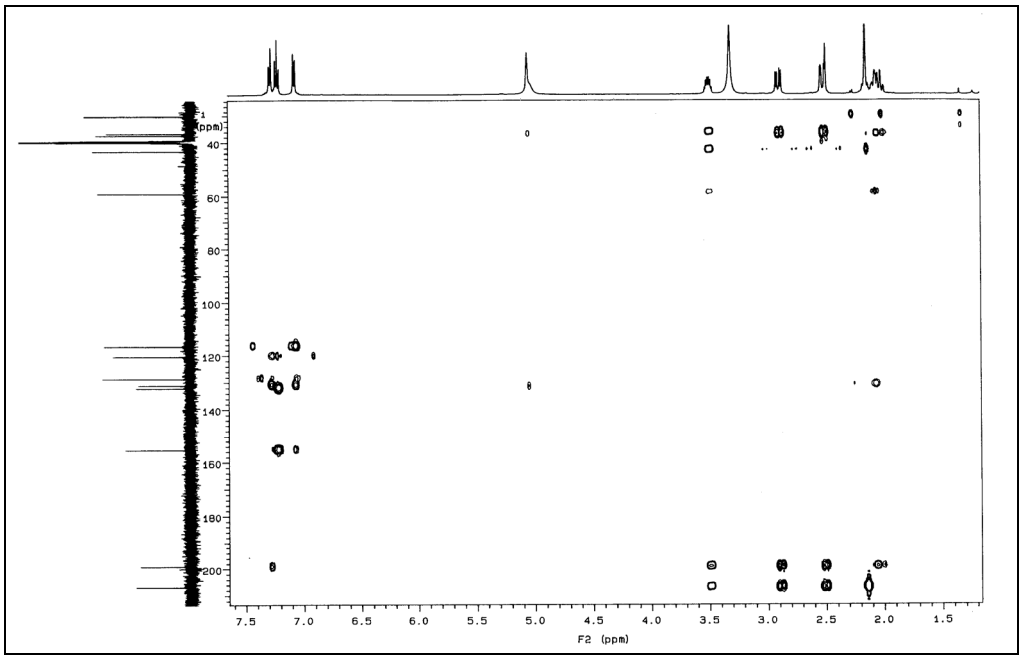


Figure 118: HMBC spectrum (300 MHz) of bhimanone (**104**) in [D_6]DMSO

In order to further confirm the position of the OH and the carbonyl groups in the ring, bhimanone (**104**) was converted into its diacetate **105**. The NOESY spectrum indicated a correlation between the two acetate residues, and a coupling between the 4-$COCH_3$ group and 9-H_2 was also seen, confirming the structure and the relative stereochemistry of bhimanone (**104**).

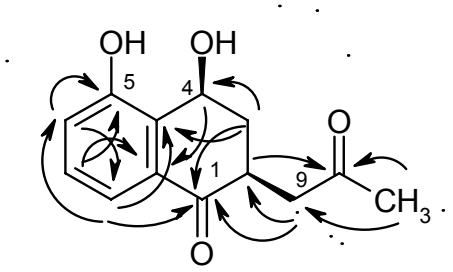


Figure 119: Selected HMBC couplings of bhimanone (**104**)

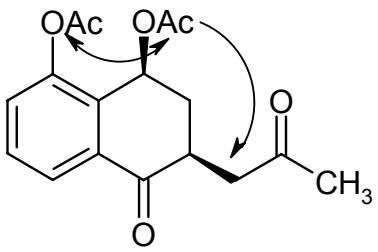


Figure 120: NOE correlations of bhimanone diacetate (**104**)

The rare naphthofurane dione chromophore of bhimamycin A (**88**) and B (**89**), can be obtained through a intramolecular cyclisation of the 9-OH and C-10 in bhimamycin E followed by dehydration. Bhimamycin A (**88**) may derived from bhimamycin B (**89**) by reduction of the keton group to the secondary alcohol. Bhimamycin C (**93**) is supposed to be formed from bhimamycin A (**88**) and ethanolamine, while bhimamycin D (**94a**) was formed from B (**89**) and anthranilic acid. Only bhimamycin A (**88**), B (**89**) and E (**102**) exhibited moderate antibacterial activities *Escherichia coli*, *Bacillus substilis* and *Staphylococcus aureus*.

4.15 Terrestrial *Streptomyces* sp. GW 29/1540

The terrestrial *Streptomycetes* GW 29/1540 showed on agar a white mycelium. In the agar diffusion test, the extract revealed good activity against microalgae. The 20 L fermentation of the strain was grown for 4 days and worked up in the usual manner. After defatting with cyclohexane, the remaining 2.1 g were chromatographed on Sephadex LH-20 and three fractions were obtained. Further chromatography of these fractions yielded four compounds as shown in the figure below.

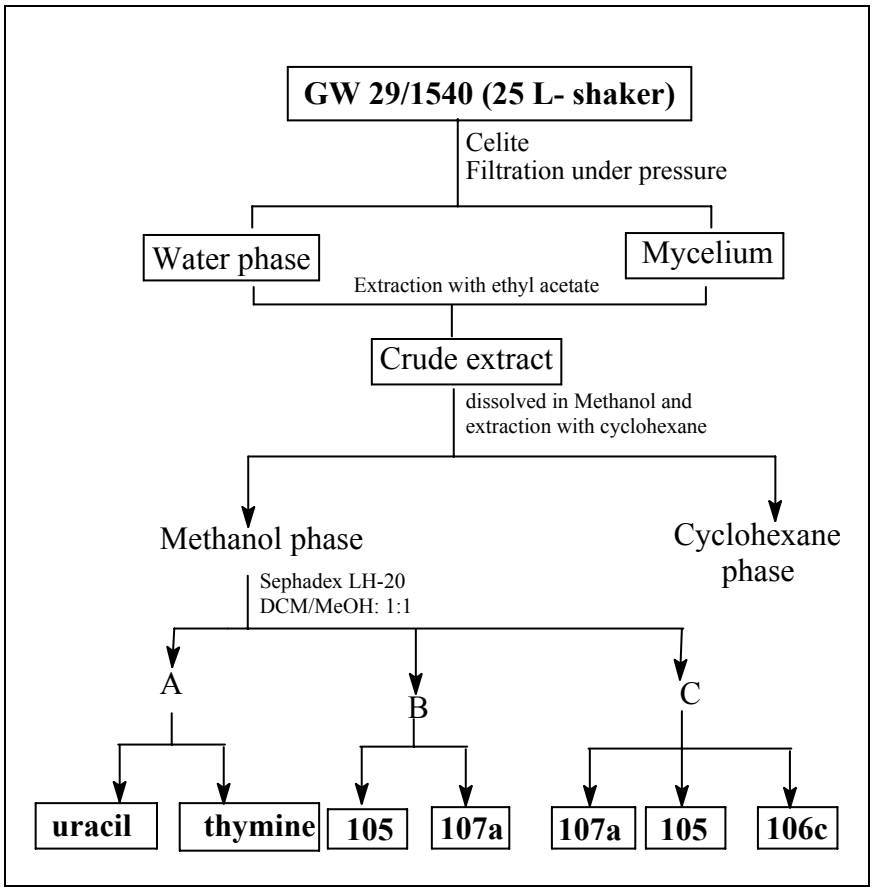


Figure 121: Work-up of the terrestrial *Streptomyces* GW 29/1570

The compounds FG2 and FG4 were obtained as white powder and gave a brown colour with anisaldehyde/sulphuric acid. They were easily identified as uracil and thymine.

4.15.1 Chloramphenicol

Compound **105** was obtained as colourless oil by HPLC of fraction B and C (FG10a). The (-)-ESI mass spectrum exhibited three isotope signals at *m/z* 321, 323 and 325 characteristic for a compound containing 2 chlorine atoms in view of the relative intensities (100, 80 and 10%); the molecular weight was deduced to be *m/z* 322. The ^{1}H NMR spectrum presented two 2H doublets at δ 8.17 (^{3}J = 9.1 Hz) and 7.63 (^{3}J = 9.0 Hz) suggesting a *para*-substituted benzene ring, and two 1H doublets of doublets at δ 3.80 and 3.60, respectively, attributed to a methylene group. In addition three methines appeared as a singlet, a doublet and a multiplet at δ 6.23, 5.15

and 4.13, respectively, which may be attributed to protons connected to double bonds or electron withdrawing groups.

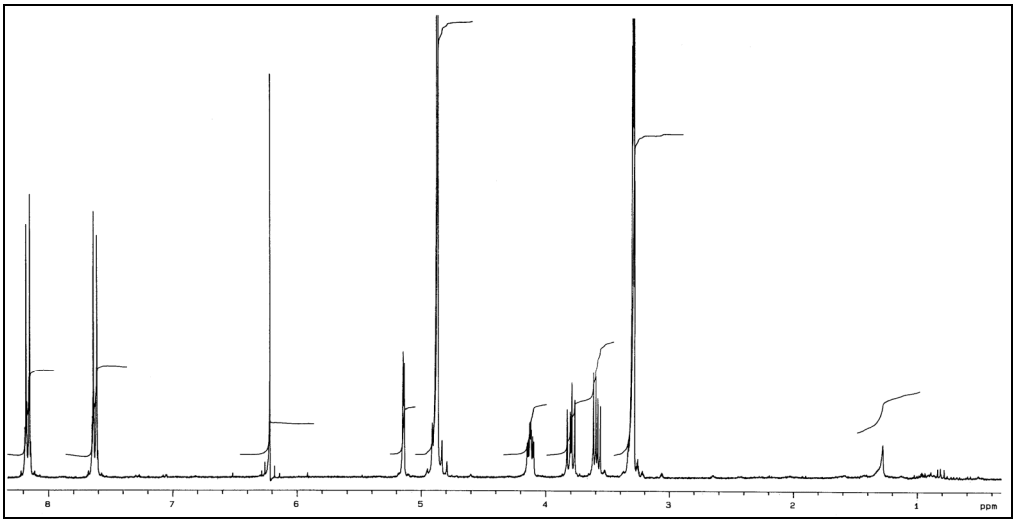
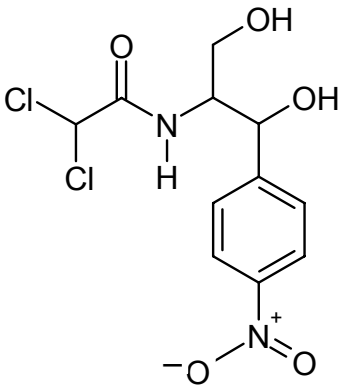


Figure 122: ^{1}H NMR spectrum (300 MHz) of chloramphenicol (**105**) in [D_4]MeOH

With all these information the search in AntiBase furnished chloramphenicol (**105**) with the molecular formula $C_{11}H_{12}N_2O_5Cl_2$ as the only possibility, which was confirmed by direct comparison with an authentic sample from our collection.



105

Chloramphenicol (**105**) is a bacteriostatic broad spectrum antibiotic, that binds to 50S ribosomal subunit of susceptible bacteria and inhibits bacterial protein synthesis.[181] Chloramphenicol (**105**) is still in use in a few countries; in Germany it was interdicted because of the induction of an irreversible bore narrow damages.

4.15.2 Corynecin-C

The separation of fraction C using Sephadex LH-20 and finally HPLC led to the isolation of a colourless pure compound FG10b (**106c**). The ^{1}H NMR spectrum of **106c** was very similar to that of **105**. The major difference in the ^{1}H NMR spectrum was the absence of the methine singlet at δ 6.23 in **105** and the presence of two methyl groups as doublet at δ 0.86 and δ 0.98 and a methine multiplet at δ 2.39, which are characteristic for an isopropyl group.

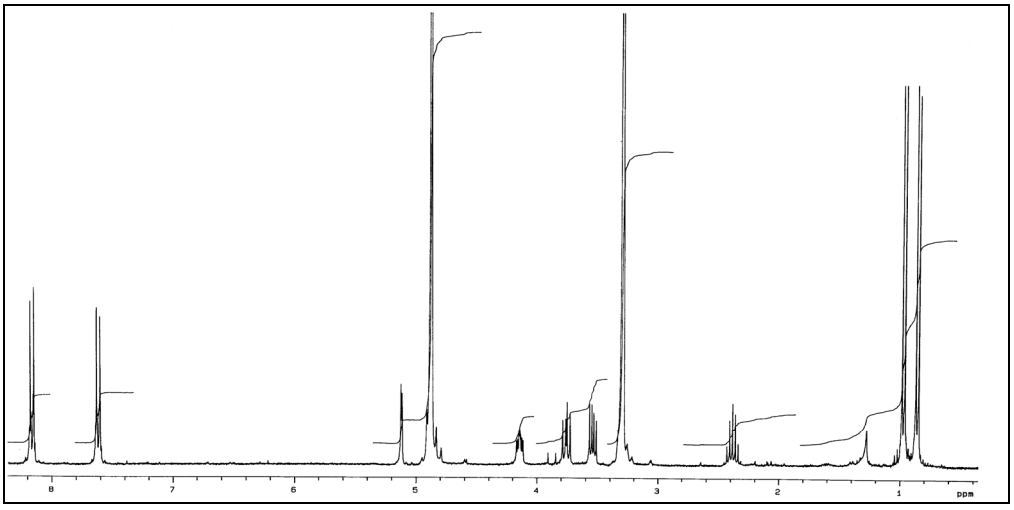
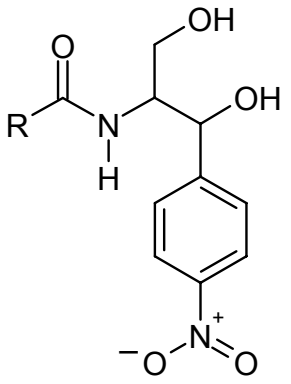


Figure 123: ^{1}H NMR spectrum (300 MHz) of corynecin C (**106c**) in [D_4]MeOH

ESI MS indicated an $[M+Na]^+$ ion at *m/z* 305 for a molecular weight ion of 282 Dalton. A sub-structure search in AntiBase with these data afforded corynecin C (**106c**).



106a: R = CH_3
106b: R = CH_2CH_3
106c: R = $CH(CH_3)_2$

Corynecin C (**106c**) is a chloramphenicol analogue. From *Corynebacterium hydrocarboclastus*, corynecin A (**106a**) and B (**106b**) were also reported.[182] Chloramphenicol (**105**) and **106c** are derived from the shikimate pathway *via* chorismic and 4-amino-4-deoxychorismic acid. Further reactions catalysed by the enzyme system arylamine synthase lead in a multistep synthesis to L-*p*-aminophenylalanine. The plausible sequence of functional group transformations proposed for conversion of *p*-aminophenylalanine to chloramphenicol (**105**) is supported by molecular-genetic evidence.[183]

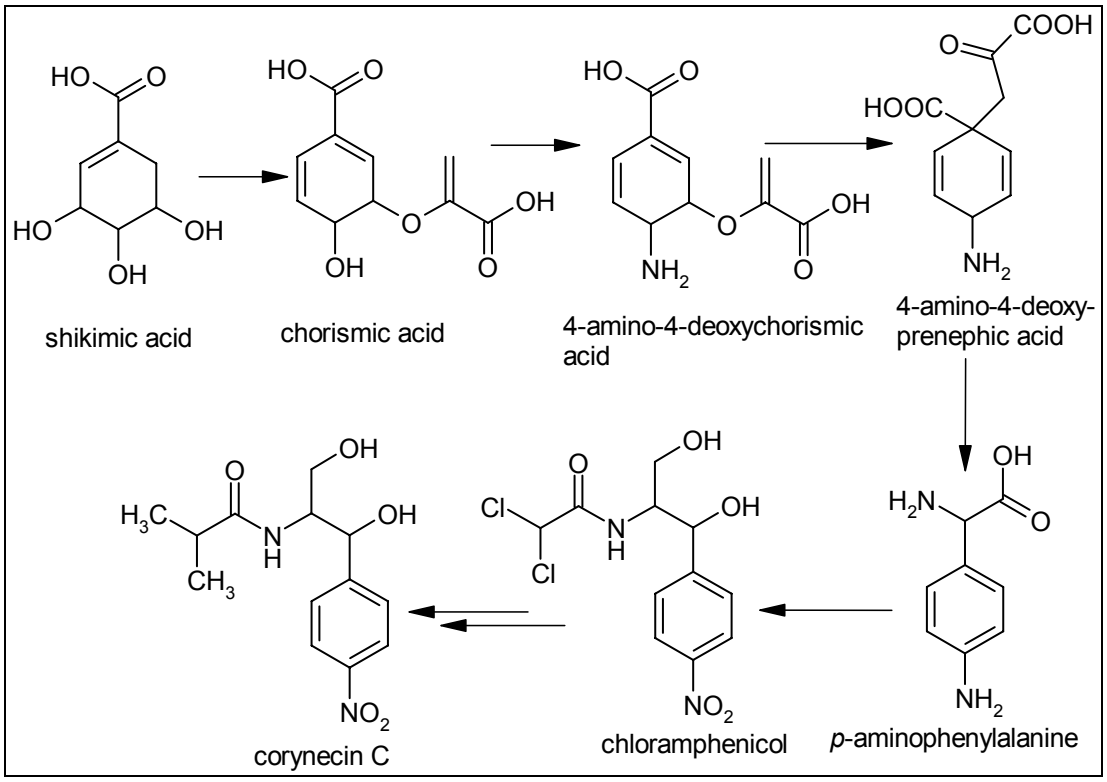


Figure 124: Biosynthetic pathway of chloramphenicol (**105**) and corynecin C (**106c**)

4.15.3 Staurosporine

PTLC of fraction C delivered compound FG9a (**107a**) as white, highly fluorescent (366 nm) powder. ESI MS showed in the positive mode an $[M+H]^+$ signal at *m/z* 467. The ^1H NMR spectrum of **107a** exhibited two singlets in the aliphatic region at δ 1.54 and 2.35 attributed to methyl groups connected to hetero atoms a or double bond. Besides two 1H multiplets at δ 2.40 and 2.74 of a methylene group, a methine

quartet at δ 3.34, and one singlet of a methoxy group at δ 3.41 are observed. Furthermore, one (probably oxygen-connected) methine group appeared as a doublet at δ 3.87, and a methylene singlet at δ 5.01 indicated its connection with an hetero atom. The aromatic region presents at δ 6.57 and 6.55 signals of two protons, which appeared as a singlet and a doublet and can be attributed to NH or OH and to a methine, respectively. Furthermore, a complex of 5H multiplet in the range of δ 7.20-7.50, three doublets of one proton each at δ 7.88, 7.93 and 9.43 were also seen.

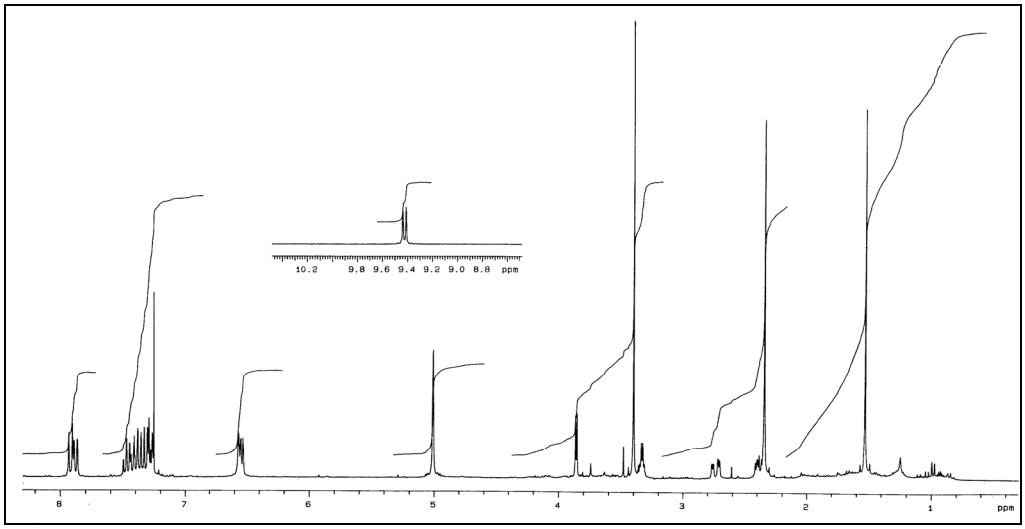


Figure 125: ^{1}H NMR spectrum (300 MHz) of staurosporine (**107a**) in $CDCl_3$

The ^{13}C and APT NMR spectra indicated the presence of 28 carbon signals including a carbonyl signal at δ 173.8, two methylenes at δ 46.0 and 30.2, signals of a methoxy group at δ 57.2, a methyl and an N-methyl signal at δ 30.0 and 33.3, respectively. Eight methines appeared in the aromatic region. The shifts of three aliphatic methines (δ 84.1, 80.1, and 50.1) indicated their connection with oxygen or nitrogen. Finally, 12 quaternary carbon signals were counted, among which the signal at δ 91.1 was assigned to an hemiketal carbon.

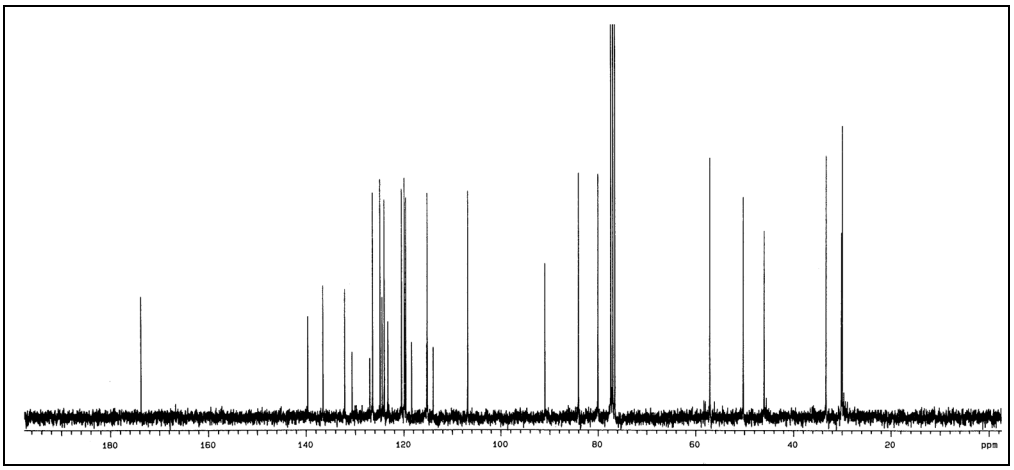
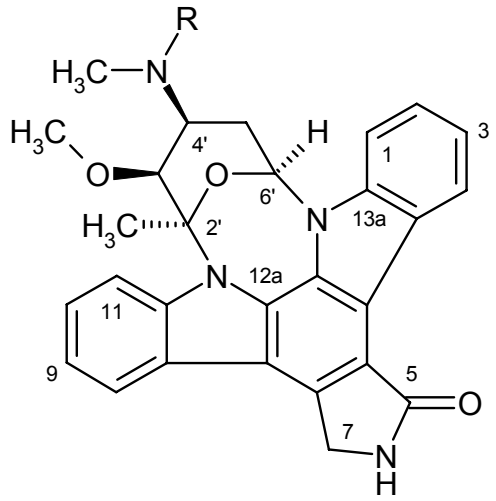


Figure 126: ^{13}C NMR spectrum (300 MHz) of staurosporine (**107a**) in $CDCl_3$

The sub-structure search in AntiBase with mass, proton and carbon data gave as solution staurosporine (**107a**), which was confirmed by literature data.[184]



107a: R = H
107b: R = CHO
107c: R = $CONH_2$

Staurosporine (**107a**) is an indolo[2,3-a]carbazole alkaloid and was first isolated from *Streptomyces staurosporeus* Awaya (AM-2282)[185] and subsequently from several other actinomycetes. According to Mekuriyen *et al*., staurosporine (**107a**) is formed from a tryptophane-derived aromatic unit and connected with the amino sugar stereospecifically by an unusual double N-glycosidic linkage.[186] Staurosporine (**107a**) possesses inhibitory activity against fungi and yeast but has no significant activity effects on bacteria.[186] It shows strong antihypertensive activity and

pronounced *in vitro* activity against a number of experimental tumours.[187] Most interesting is its potent inhibition of protein kinase C and the platelet aggregation.[188] The aglycone moiety was found to be important for the biological activity, strongly suggesting that other indolo[2,3-a]carbazole derivatives might also possess important biological activity, which is the case, indeed.

5 Marine-derived *Streptomyces* sp.

The marine *Streptomycetes* were obtained from different sources. Mei strains were obtained from the collection of Prof. Meiners (Emden) and were precultivated on M_2 100% seawater with additional calcium carbonate at 28 °C. The other marine *Streptomyces* sp. were mainly cultivated on the standard M_2^+ medium (= M_2 medium + 50% sea water) or fermented as stated otherwise.

5.1 Marine *Streptomyces* sp. QD518

The marine strain QD518 was isolated from the seashore of Qingdao, east China. It showed in the biological screening a high activity against fungi, the chemical screening exhibited on TLC fluorescent spots under UV light 366 nm, which turned light green on spraying with anisaldehyde/sulphuric acid. The strain was cultivated in meat extract medium for 7 days at 28 °C. The culture broth was filtered and extracted with XAD-16 resin, and the resulting crude extract was separated as indicated in Figure 126.

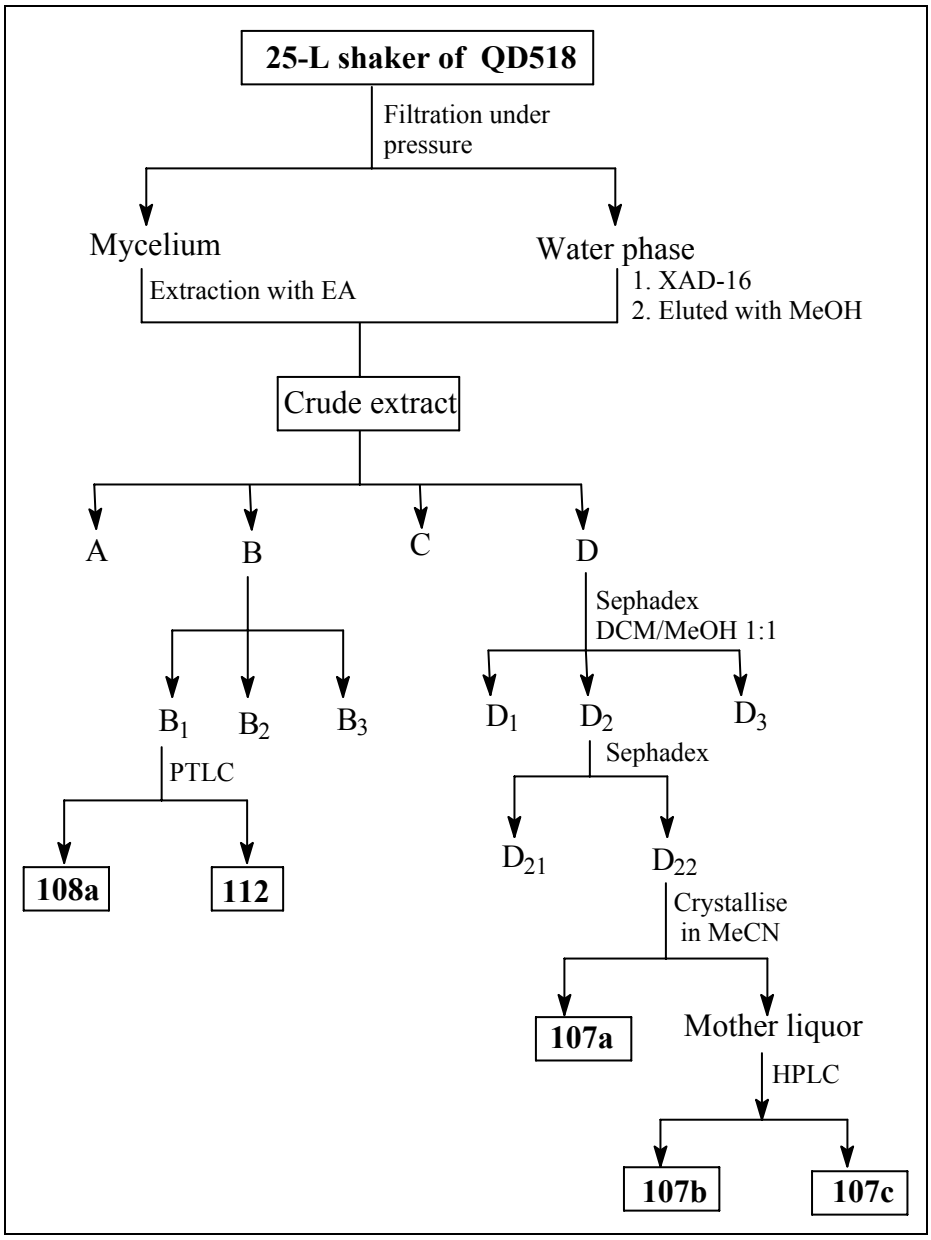


Figure 127: Simplified work-up scheme of the marine *Streptomyces* sp. QD518

Staurosporine (**107a**), N-Carboxamido-staurosporine (**107b**) and chartreusin were isolated and identified by comparison of spectral data from authentic samples from our collection and the literature.

5.1.1 N-Carboxamido-staurosporine

Compound **107c** was obtained as colourless crystals by preparative HPLC. It exhibited similar physico-chemical properties as **107a** and **107b** suggesting also the presence of the indolo[2,3-a]carbazole chromophore. This was confirmed by the UV

spectra in methanol, which exhibited characteristic absorptions[189] at 243, 292, 317, 333, 354 and 370 nm. The ^{1}H NMR spectrum in [D_6]DMSO was very similar to that of **107b**. The major difference was the absence of the aldehyde proton, which appears at δ 8.24 in **107b**, and the downfield shift of 4'-H from δ 3.18 in **107b** to δ 4.84 in **107b**. The ^{1}H NMR spectrum exhibited an additional 2H singlet at δ 6.02, which disappeared in D_2O indicating an NH_2 group. The aliphatic region remained unchanged as in **107c** with the methoxy group (δ 3.77), the NMe (δ 2.63) and the 2'-CH_3 signals (δ 2.34). (+)-ESI MS indicated a *pseudo*molecular ion at *m/z* 532 $[M+Na]^+$, and ESI HRMS delivered the molecular formula $C_{29}H_{27}N_5O_4$, which possesses one nitrogen atom more than **107a** and **107b**. The mass difference Δm = 16 between **107c** and **107b** could be attributed to the additional NH_2 group.

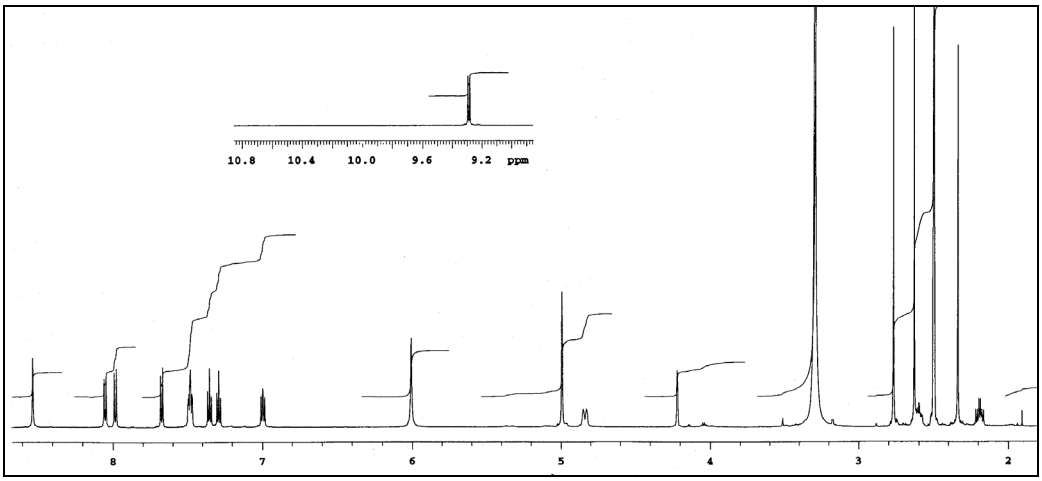


Figure 128: ^{1}H NMR spectrum (600MHz) of N-carboxamido-staurosporine (**107c**) in [D_6]DMSO

The ^{13}C NMR spectrum indicated the presence of 29 carbon signals as demanded by the high resolution mass and contained also two carbonyl carbon signals (δ 171.8 and 158.7) as that of N-Carboxamido-staurosporine (**107b**). A search in AntiBase delivered no answer.

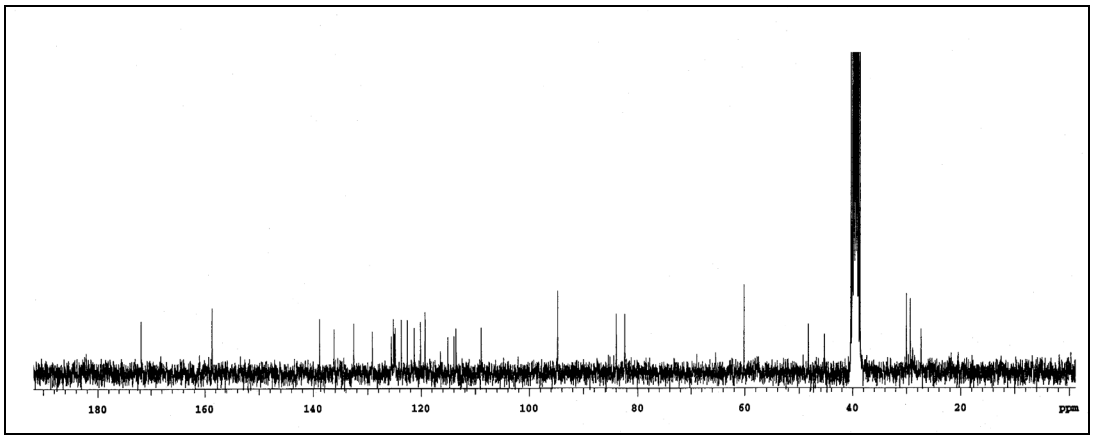


Figure 129: ^{13}C NMR spectrum (150 MHz) of N-carboxamido-staurosporine (**107c**) in [D_6]DMSO

The 2D spectra indicated that the substitution had taken place in the formyl residue, where the aldehyde proton was substituted by NH_2. This was confirmed by the correlation signals in the HMBC spectrum between the N-methyl and the carbonyl signal at δ 158.7. Compound **107c** was thus identified as N-carboxamido-staurosporine (**107c**), which is reported here as a new compound.

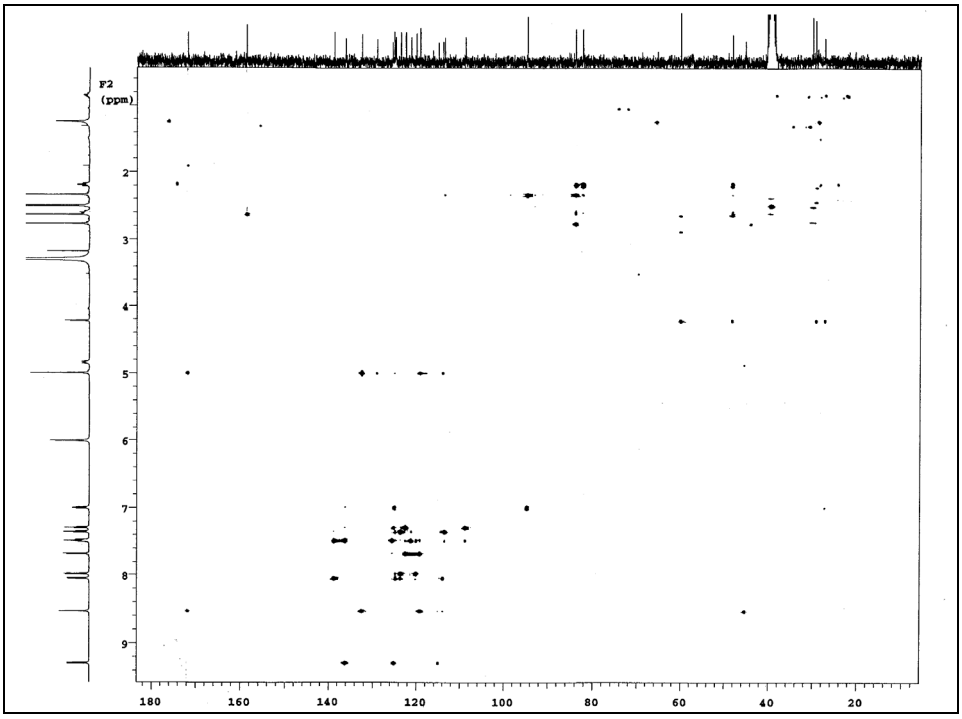
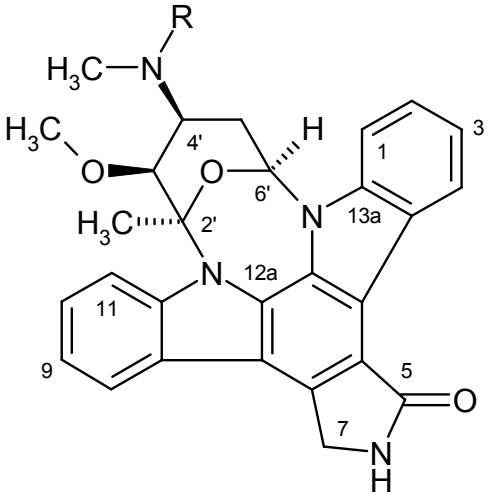


Figure 130: HMBC spectrum of N-carboxamido-staurosporine (**107c**) in [D_6]DMSO



107a: R = H
107b: R = CHO
107c: R = $CONH_2$

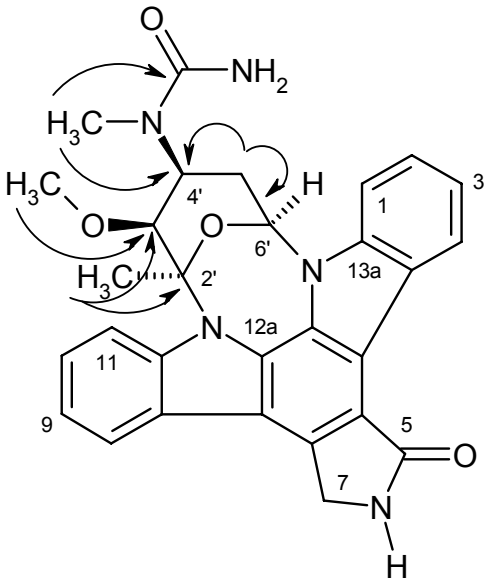


Figure 131: Selected HMBC correlations of the sugar moiety of N-carboxamido-staurosporine **(107c)**

Table 3: ^{13}C (600 MHz) and ^{1}H NMR (*J* in Hz) of **107c** in [D_6] DMSO

	C	^{1}H (300 MHz)	No.	C	^{1}H (300 MHz)
1	108.9	7.68 (d, 8.2)	10	124.8	7.47 (br t, 7.2)
2	125.0	7.47 (br t, 7.2)	11	113.6	8.05 (d, 8.6)
3	119.3	7.28 (t, 7.5)	11a	138.9	-
4	125.6	9.28 (d, 7.5)	12a	129.1	-
4a	122.6	-	12b	125.0	-
4b	115.2	-	13a	136.2	-
4c	132.5	-	2’	94.8	-
5	171.8	-	3’	84.0	4.22 (br s)
NH	-	8.55 (s)	4’	48.3	4.84 (m)
7	45.4	5.00 (s)	5’	27.4	2.18 (m), 2.63(m)
7a	119.3	-	6’	82.4	7.00 (t, 6.7)
7b	114.0	-	Me	29.3	2.34 (s)
7c	123.7	-	OMe	60.1	2.77 (s)
8	121.3	8.00 (d, 7.7)	NMe	30.1	2.63 (s)
9	120.1	7.34 (t, 7.6)	$CONH_2$	158.7	-
NH_2	-	6.02 (s)	$CONH_2$	158.7	-

5.1.2 Selina-4(14),7(11)-dien-8,9-diol

Compound **108a** was isolated as colourless oil, which gave no UV absorption and showed a violet colour after spraying with anisaldehyde/sulphuric acid. Instead of aromatic protons, four methine doublets appeared in the ^{1}H NMR spectrum of **108a** at δ 4.82, 4.80 and 3.22. The aliphatic region showed two multiplets of five and three protons in the range of δ 2.50-1.90 and δ 1.70-1.50, and finally a doublet of doublet at δ 1.20 and three methyl signals at δ 1.80 (d), 1.75 (d) and δ 0.99 (s) were visible.

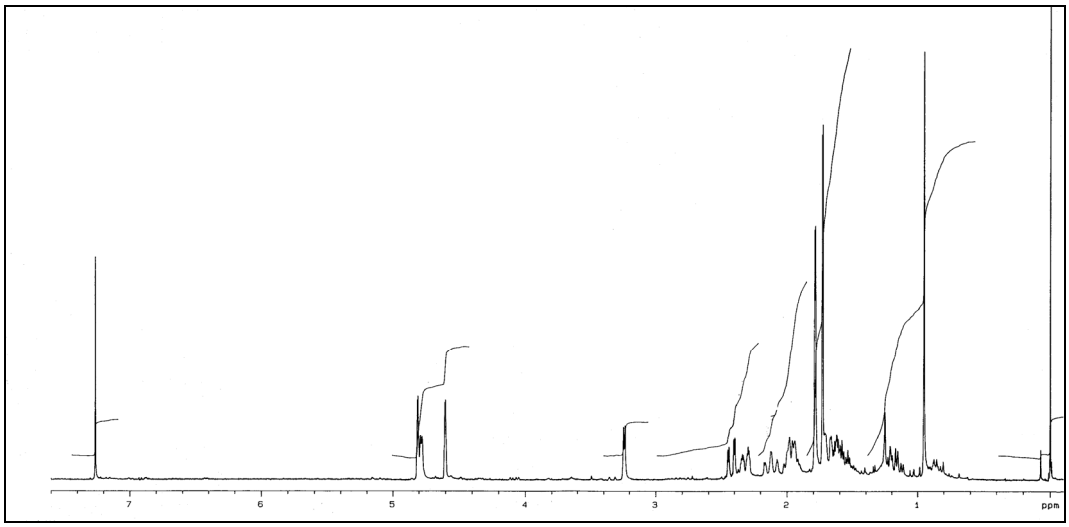


Figure 132: ^{1}H NMR spectrum (300 MHz) of compound **108a** in $CDCl_3$

The carbon spectrum indicated the presence of 15 signals, which were assigned by shifts, HMQC and APT spectra to 3 quaternary sp^2 and 1 quaternary sp^3 atoms, 3 methines, 1 olefinic and 4 aliphatic methylenes and finally 3 aliphatic methyl carbons. EI MS indicated the molecular ion at *m/z* 236 and EI HRMS afforded the molecular formula $C_{15}H_{24}O_2$. The search in AntiBase and DNP gave no hits indicating compound **108a** as a novel natural product. The careful interpretation of the HMBC spectrum exhibited a correlation between the exo methylene 14-H_2 and the 15-H_3 to the methine carbon C-5, in addition the 15-H_3, the 6-H_2 and the 8-H indicated couplings to the quaternary carbon C-10. Further correlations were seen from the 12- and 13-H_3 and 8-H to the C-7. These information combined with the H,H COSY data delivered structure **108a** which is selina-4(14),7(11)-dien-8,9-diol.

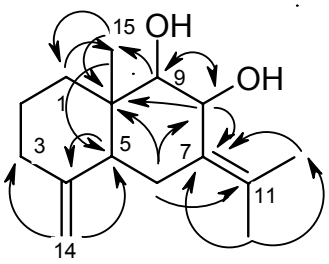
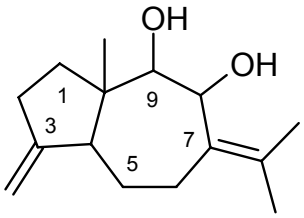
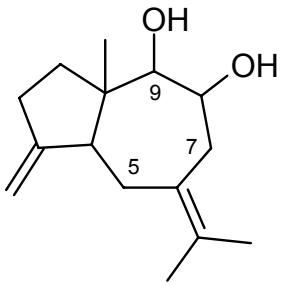


Figure 133: H,H COSY (↔) and HMBC (→) correlations of **108a**

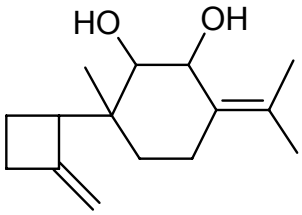
Due the absence of correlations in the HMBC spectrum of 2-H_2, the program COCON was additionally used for structure elucidation, which furnished further 3 structures:



109



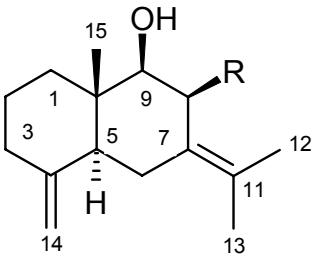
110



111

While compound **111** was ruled out due to the four member ring strain, compound **109** containing the guaiane skeleton was left out due to the absence of couplings between 5- and 6-H_2 in the H,H COSY spectrum, in addition only one methylene group exhibited correlation in HMBC spectrum to C-9, C-10 and C-11. For similar reasons compound **110** was not taken into consideration due to the missing coupling in COSY spectrum between 7-H_2 and 8-H as well as the missing correlation in the HMBC spectrum of 7-H_2 to the carbons C-6, C-8, C-9 and C-11. Furthermore, the unusual position of 15-H_3 at a bridge-head giving **109** and **110** a new skeleton, as well as the high carbon value of C-3 reported for guaiane (δ 155.6) compared to **108a** (δ 149.6).

Selina-4(14),7(11)-dien-8,9-diol **(108a**) belongs to the group of selinane sesquiterpenes and is closely related to selinadienol (**108b**) isolated from the cultures of *Streptomyces fradiae* IMRU3535[190]. The relative stereochemistry follows from the NOESY spectra: The missing NOE between 5-H and 15-H_3 indicated a *trans*-decalin system as in all other salinanes. Irradiation of the well-separated signal of 9-H (δ 3.24) showed a positive NOE to the signals at δ 4.78 (8-H), 1.70 (5-H) and one proton of 1-H_2 (δ 1.13). The relative stereochemistry of **108a** is therefore (5S,8S,9R).



108a: R = OH
108b: R = H

Sesquiterpenoids are common constituents of plants, fungi and algae, however, are rare in bacteria: Pentalenolactone was one of the first sesquiterpenes from bacteria,[191] less polar monoterpenes have been found recently as odour components in actinomycetes[190] and streptomycetes.[192]

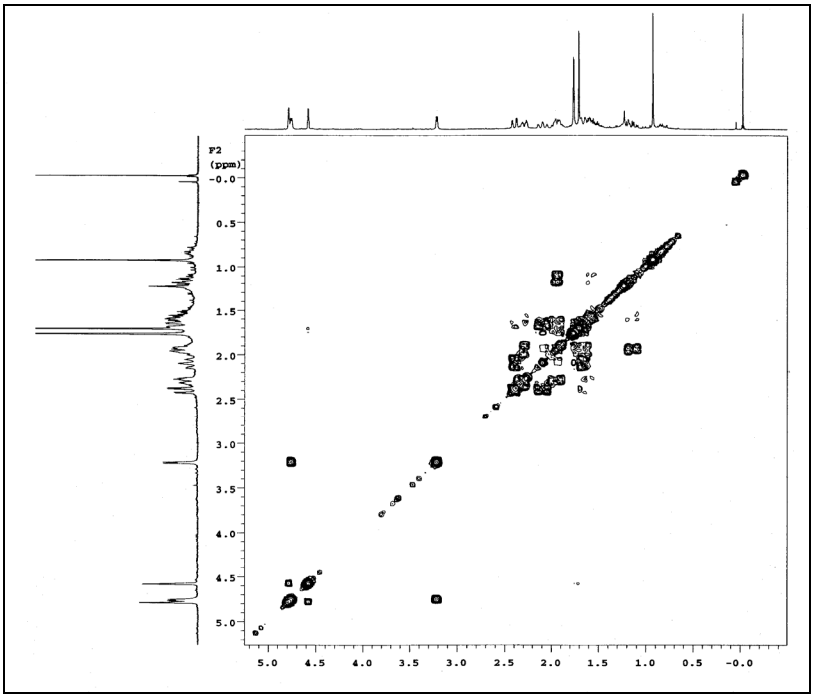


Figure 134: H,H COSY spectrum of the selinane **108a** in $CDCl_3$

5.1.3 (Z)-5,7-Dihydroxy-5,6,7,8-tetrahydroazocin-2(*1H*)-one

The oily compound **112** was obtained by preparative TLC and showed an UV absorbing spot, which turned green on spraying with anisaldehyde/sulphuric acid. The ^1H NMR spectrum of **112** in $CDCl_3$ was very simple and exhibited only two doublets of doublets in the aromatic region at δ 7.26 (5.8, 1.8 Hz) and 6.07 (5.8, 1.6 Hz) and

two methines connected to oxygen at δ 4.85 (m) and 4.77 (m). In addition two pairs of signals assigned to methylene groups appeared at δ 4.05 and 3.31, δ 2.39 and 1.64.

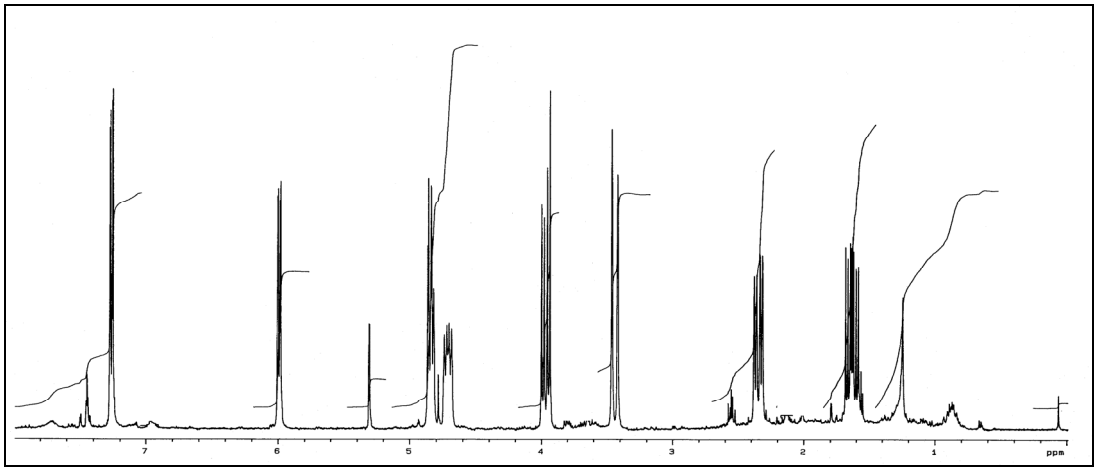


Figure 135: ^{1}H NMR spectrum (300 MHz) of compound **112** in $CDCl_3$

EI MS indicated a molecular weight of *m/z* 157 and the molecular formula $C_7H_{11}NO_3$ (EI HRMS) containing three degrees of unsaturation. The ^{13}C NMR spectrum showed in accordance with the molecular formula 7 carbon signals, which were assigned by the HMQC spectrum to one amide carbonyl at δ 175.6, two sp^2 methines at δ 149.2 and 128.6, two sp^3 oxygen bond methines at δ 65.6, 62.8 and two methylene carbons at δ 53.2 and 40.1.

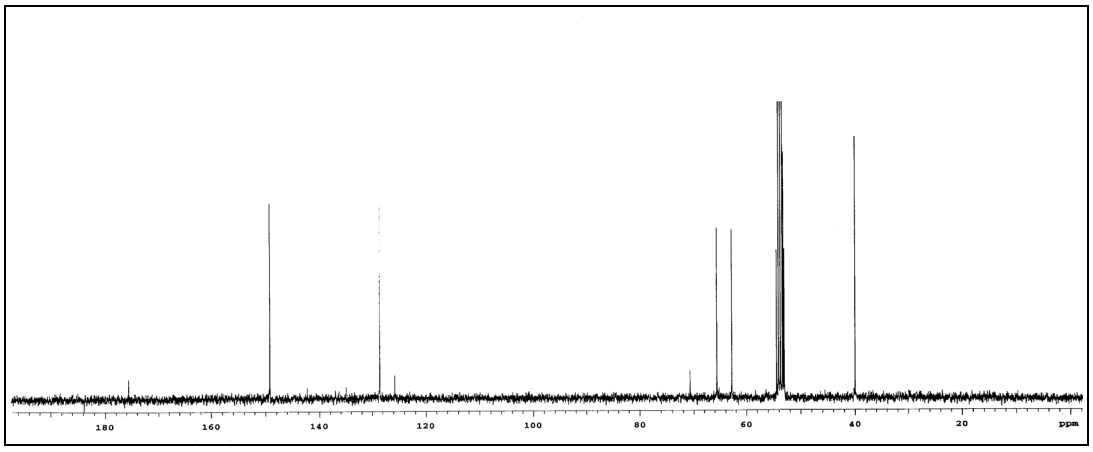


Figure 136: ^{13}C NMR spectrum (300 MHz) of compound **112** in $CDCl_3$

The systematic interpretation of the H,H COSY spectrum indicated a coupling between both olefinic proton, the 8-H_2 and 7-H as well as 5-H and 6-H_2. The HMBC spectrum of compound **112** indicated correlations of the sp^2 protons 3-H, 4-H and the

8-H_2 to the carbonyl group. Further correlations were seen from both olefinic protons to C-5, both methylene protons to C-7 as well as 8-H_2 to C-6. The structure of **112** was determined as (Z)-5,7-dihydroxy-5,6,7,8-tetrahydroazocin-2(*1H*)-one and is reported here for the first time.

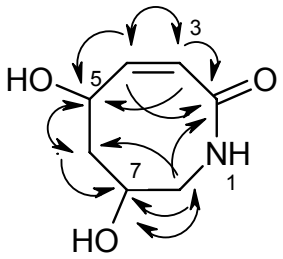
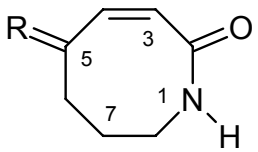


Figure 137: Selected H,H COSY (↔) and HMBC (→) correlations of **112**

Compound **112** belongs to the group of hexahydroazocin-2(*1H*)-ones, which are very rare in natural products. In microorganisms, no compound of this type has been reported so far, but homologous hexahydroazepin-2(*1H*)-one (caprolactams) like caprolactin A and B have been isolated and are cytotoxic against human epidermoid carcinoma cells and human colorectal adenocarcinoma (LoVo) cells and exhibit antiviral activity against the *Herpes simplex* type II virus.[193] Although the chemistry of hexahydroazepin-2(*1H*)-one (caprolactam) and its unsaturated derivatives has been extensively explored, very few reports have appeared regarding the eight-membered ring homologues, the azocin-2(*1H*)-one[194]. Damon *et al.*[194,195] have prepared many tetrahydroazocin-2(*1H*)-one derivates, including 5,6,7,8-tetrahydroazocin-2(*1H*)-one (**113a**).



113a: R= H, H
113b: R= H, OH
113c: R= O

Tetrahydroazocin-2(*1H*)-ones have been reported to possess an unusual reactivity, which was attributed to ring conformational effects: It appeared that in a preferred conformation, suitably substituted tetrahydroazocin-2(*1H*)-ones may undergo an intramolecular cyclisation.[195]

During the NMR measurements of compound **112**, a total decomposition was observed when it was allowed to stay in the NMR tube for some hour. TLC of the decomposed solution indicated the formation of two new compounds, which were purified by PTLC giving compounds **114** and **115**.

Compound **114** was obtained as minor component and showed in its ^{1}H NMR spectrum only four signals, three protons in an ABC system at δ 7.02 (dd, 2.9 Hz), 6.43 (t, 3.1Hz) and 5.94 (dd; 2.8 Hz), which due to the small coupling constant was assigned to the protons of a pyrrole or furan derivative, and a broad 4H singlet at δ 3.00 of two methylene groups.

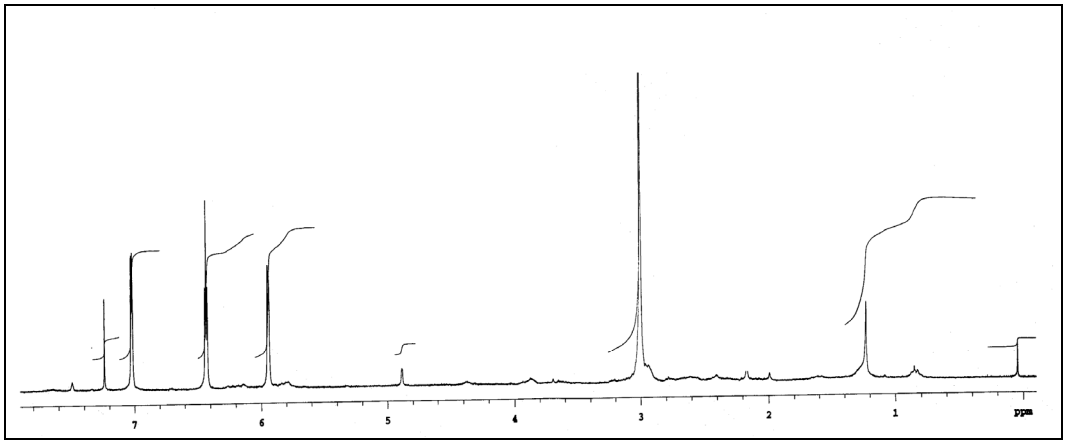
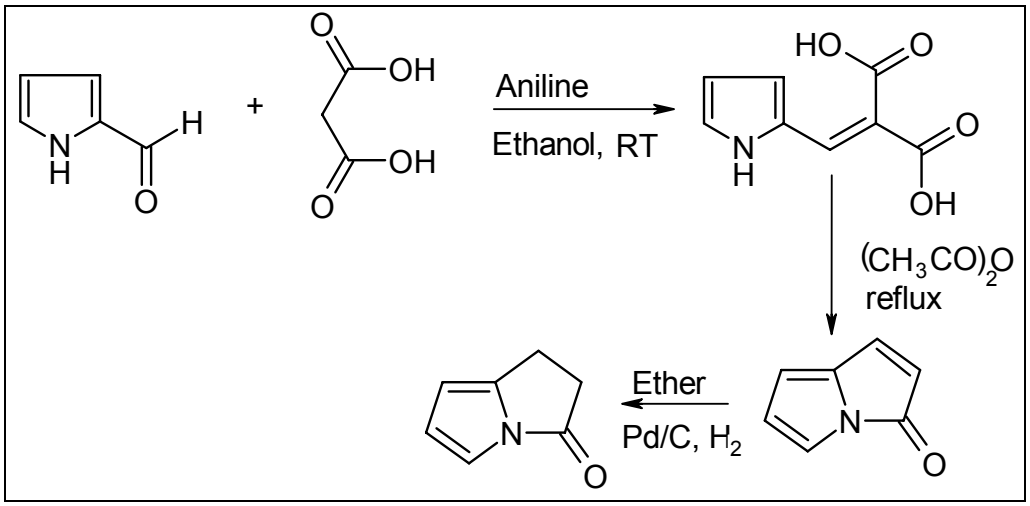


Figure 138: ^{1}H NMR spectrum (300 MHz) of the pyrrolizinone **114** in $CDCl_3$

The ^{13}C NMR spectrum of **114** indicated the presence of seven carbon atoms as in **112**. They were identified as one carbonyl at δ 175.1, one quaternary carbon at δ 139.6, three sp^2 methines at 119.0, 110.9, 104.5 and two methylene at 19.3 and 34.8. Interpretation of the HMBC spectrum revealed a ^{3}J coupling of 5-H, 2-H_2, and a ^{2}J correlation of 7-H to C-8. In addition a ^{3}J coupling of 1-H_2 to C-3 was also seen. Further examination of HMBC correlations led to 1,2-dihydro-pyrrolizin-3-one (**114**), which was previously reported as synthetic product by Flitsch *et al.*[196]

Figure 139: Selected HMBC coupling in compound **114**

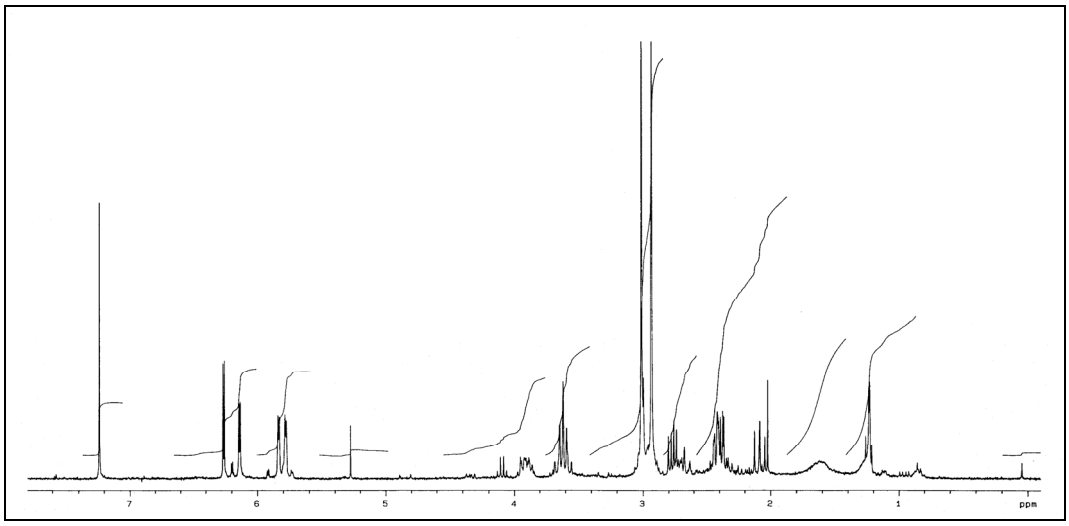
Due to the small amount of the decomposition product **114** for further measurements and the unexpected fact, that 1- and 2-CH_2 had the same shift, a synthesis using the Flitsch method was carried as indicated in the following scheme:



Scheme 2: Synthesis of 1,2-dihydro-pyrrolizin-3-one (**114**)

The synthetic product **114** was obtained by hydrogenation of the deep red pyrrolizine precursor and showed identical proton NMR spectrum as the natural compound.

The molecular formula of **115** was established by high-resolution ESI MS of the peak at *m/z* 364 $[M+H]^+$ and found to correspond to $C_{21}H_{23}N_3O_3$. EI MS displayed a fragment at *m/z* 242, which corresponds to [M-**114**] and may indicate that compound **115** was polymerised. The ^1H NMR spectrum of **115** showed similarities with that of **114** and revealed 21 protons including three pairs of doublets and one doublet for the olefinic protons at δ 6.26, 6.14, 5.84 and 5.78. In addition, a methine connected to a hetero atom at δ 3.90 and finally eight methylene groups, the latter two as broad singlets, appeared as in **114**.



F**igure 140:** NMR spectrum (300 MHz) of compound **115** in $CDCl_3$

The ^{13}C NMR spectrum exhibited signals of eight methylene groups in the range of δ 46.8-19.0, out of which the resonances at δ 46.8 and 44.6 may indicate a connection to nitrogen, a methine at δ 37.2, four sp^2 methines, four quaternary sp^2, one quaternary sp^3 carbons and three carbonyls of amides at δ 175.4, 172.6 and 172.4, suggesting **115** to be a trimer.

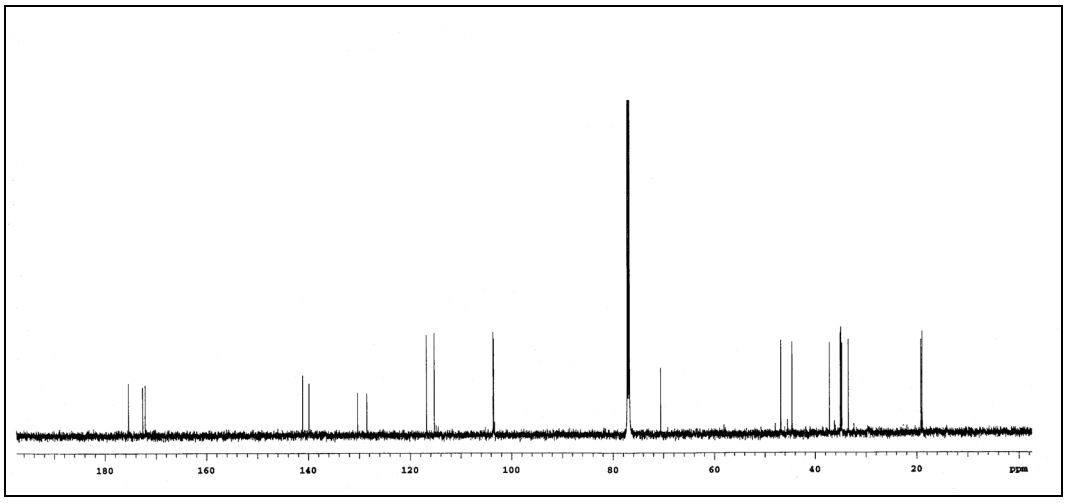


Figure 141: ^{13}C NMR spectrum (300 MHz) of compound **115** in $CDCl_3$

Based on comparison of the data with those of **115** followed by intensive interpretation of H,H COSY and HMBC spectra, three fragments were built.

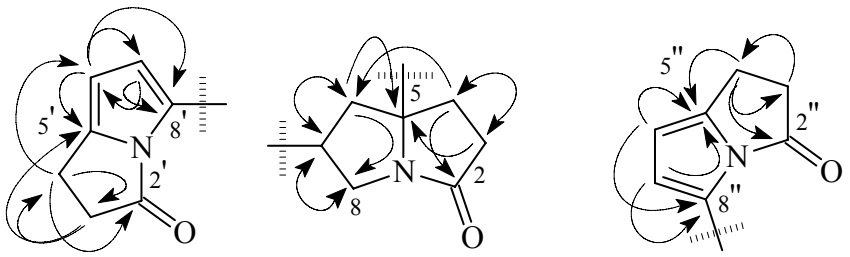


Figure 142: Fragments of **115** derived from H,H COSY (↔) and HMBC spectra (→)

Connection of the fragments is possible only in a single way and was further supported by HMBC correlation of the 7-methine at δ 3.90 (δ 37.2), the two methylene groups 6-H_2 and 8-H_2 at δ 3.60 (δ 46.8) and δ 2.80, 2.10 (δ 44.6), respectively, to the carbon atom C-8' at δ 128.5. As the 4- and 6-methylenes indicated cross-peaks to the quaternary carbon atoms C-5 (δ 70.6) and C-8" (δ 130.3), the trimeric structure was finally deduced as **115**.

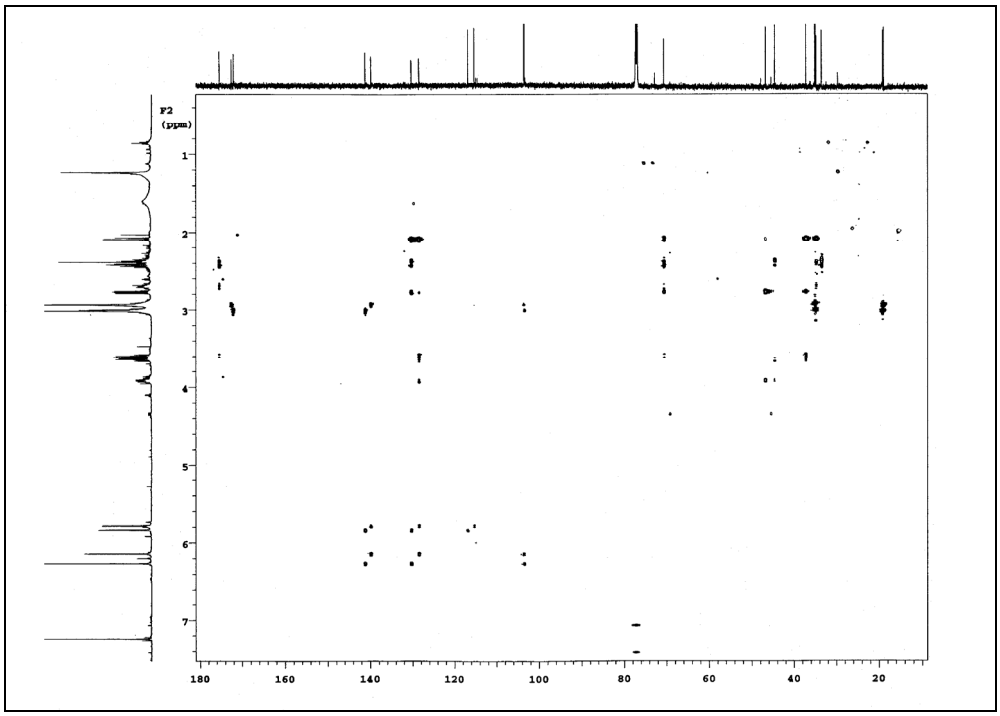


Figure 143: HMBC spectrum of compound **115** in $CDCl_3$

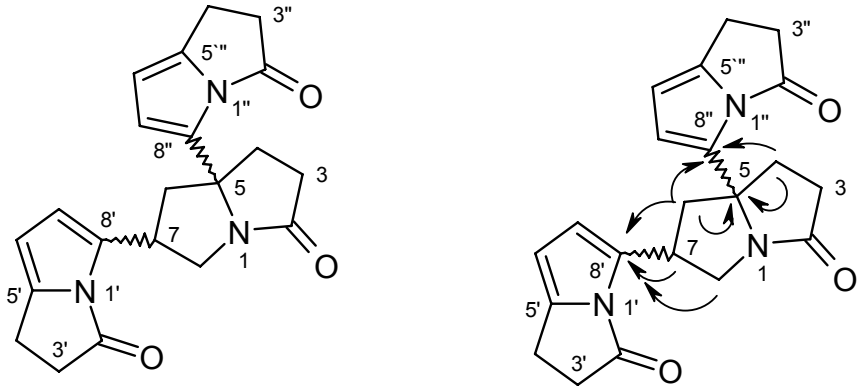
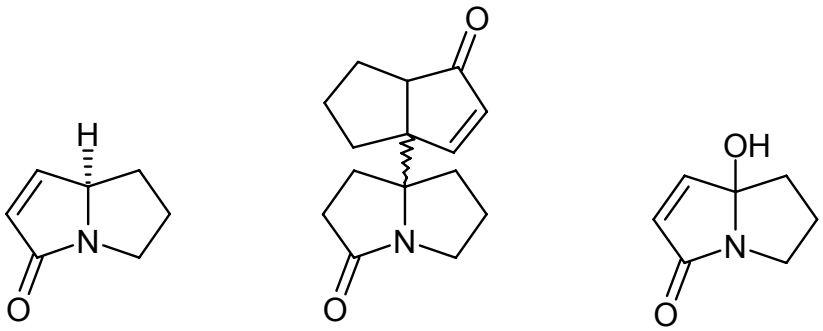


Figure 144: Structure of compound **115** and important HMBC correlations between the sub-structures

Compound **114** and its congener **115** belong to the 1-azabicyclo[3.3.0]octanes (pyrrolizidines), which have been found in plants[197] and insects[198] and serve as pheromones, defensive agents, or growth determinants. Mammals converte many of these pyrrolidizine alkaloids into dehydropyrrolizidines, which exhibit e.g. hepatotoxic, mutagenic, and carcinogenic activities[199]. The pyrrolams A (**116**) - D are the only compounds of this type from microorganisms and have been isolated from *Streptomyces olivaceus* by Zeeck et *al.*[200] Here too, a dimerisation (**117**) was observed when **116** was kept for 3 weeks in solution.



116 **117** **118**

It can be assumed that the cyclisation of **112** in alcohol-free chloroform was catalysed by an light-induced liberation of HCl: Protonation of 5-OH will allow an attack of the nitrogen atom on C-5 under cyclisation and elimination of water. A second loss of water and rearrangement of the double bond delivers **114**. It can be speculated

that the *Streptomyces* metabolite **116** is formed in a similar way from the hypothetical **113b**, and **118** originated from the corresponding ketone **113c**.

Based on the fact that **116** dimerized slowly in solution under formation of **117**,[200] an alcohol-free chloroform solution of **114** was put aside for a while, however, no reaction occurred. This observation lets us to assume that the trimer **115** may result from an intermolecular reaction of **112** or between **112** and **114**. Mechanistic details of the rearrangement of **112** are, however, still unknown.

5.2 Marine *Streptomyces* sp. QD491

The crude extract of the marine *Streptomyces* sp. QD491 from Qingdao coast (China) exhibited activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and showed a weak inhibition of *Streptomyces viridochromogenes* (Tü 57) in the biological screening. The chemical screening indicated non-polar zones, which showed no significant UV absorption, but turned violet to blue with anisaldehyde/sulphuric acid. Separation of the crude extract by silica gel chromatography led to the isolation of four sesquiterpenes, among which three were new and all were isolated from bacteria for the first time. Additionally, indole-3-carbaldehyde,[201] the isoflavones genistein[202] and prunetin,[203] the phenolic *o*-hydroxyacetanilide[204] and a macrolide, chalcomycin A[205], were obtained and identified by comparison with authentic sample from our collection.

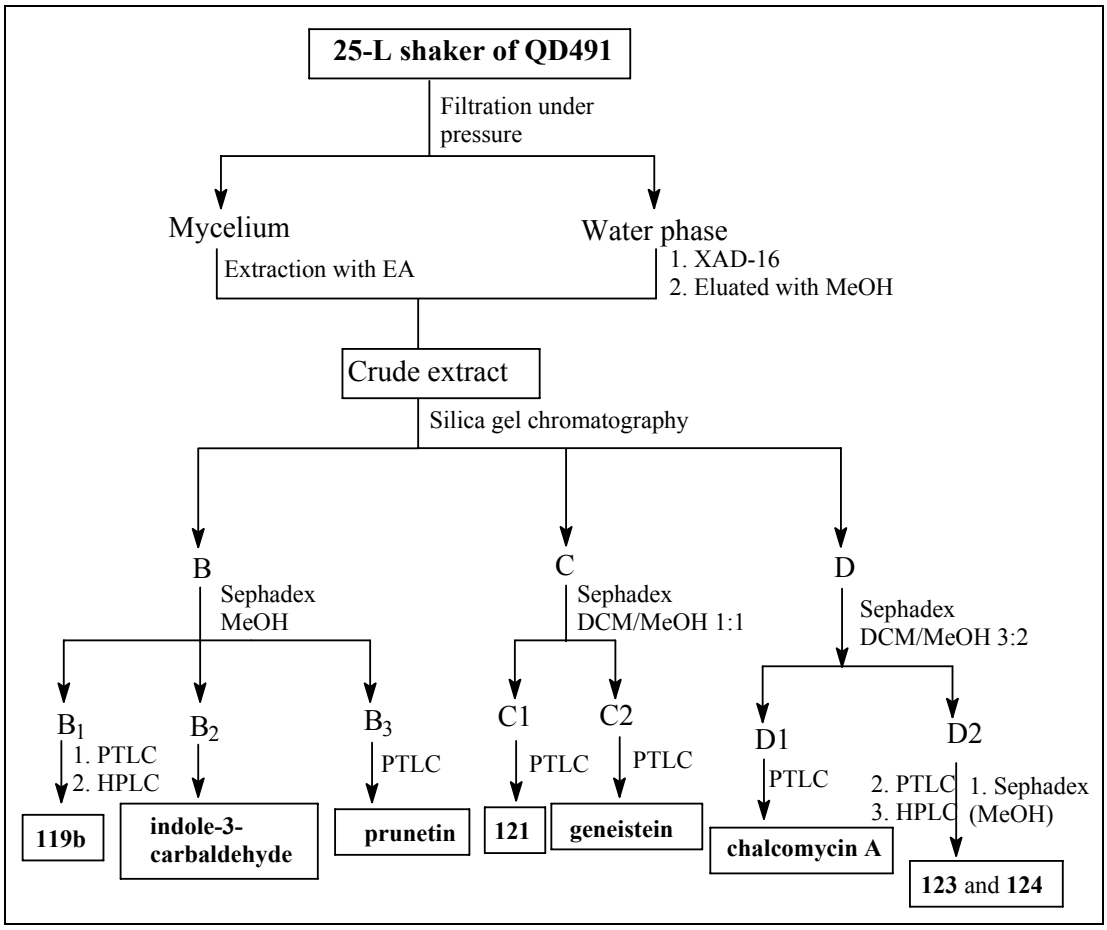


Figure 145: Work-up scheme of the marine *Streptomyces* sp. QD491

5.2.1 10β-Hydroxyamorph-4-en-3-one

Compound **119b** was isolated as colourless solid with the molecular formula $C_{15}H_{24}O_2$ (*m/z* 236.17763 by HREIMS). The ^{13}C NMR spectrum indicated 15 carbon signals, including those of a carbonyl group (δ 201.1) and a double bond (δ 153.2, 135.7), forming an α,β-unsaturated ketone fragment. In addition, four methines, three methylenes and four methyl groups were visible. The ^{1}H NMR spectrum exhibited only one olefinic proton as doublet of a quartet at δ 7.08 (5-H), which showed an allyl coupling with the methyl group at δ 1.79. The HMBC spectrum showed correlations of the methyl signal and 5-H to the carbonyl carbon at δ 201.1 confirming the presence of an α,β-unsaturated ketone. The aliphatic region showed the resonance of a methine proton as multiplet at δ 2.72, the AB signal of a methylene group at δ 2.50 and 2.31, signals of two methine groups as triplet of doublet and a multiplet at δ 2.10

and 1.90, respectively. Additionally, a 6H doublet at δ 0.92 for an isopropyl group, a methyl singlet and a multiplet at δ 1.50 were also observed.

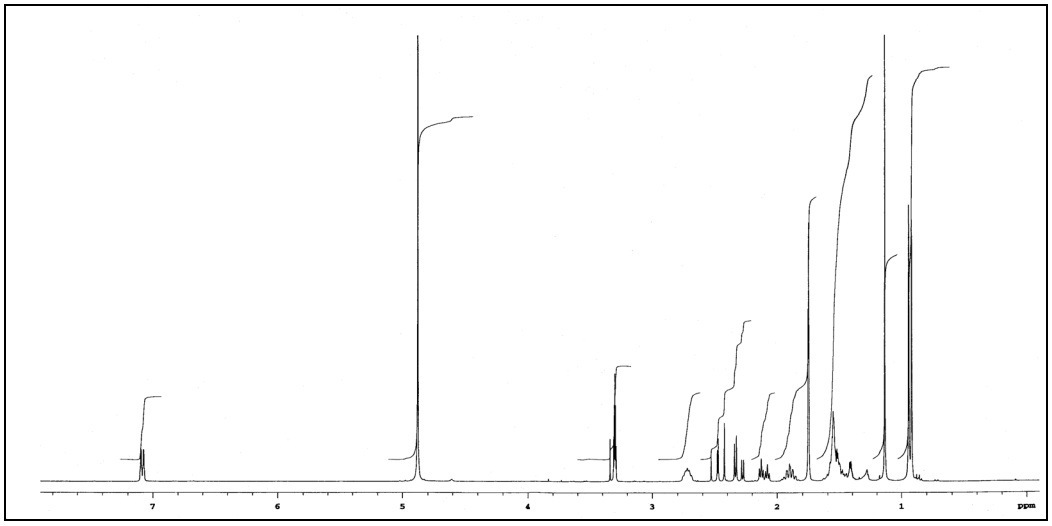


Figure 146: ^{1}H NMR spectrum (300 MHz) of compound **119b** in $CDCl_3$

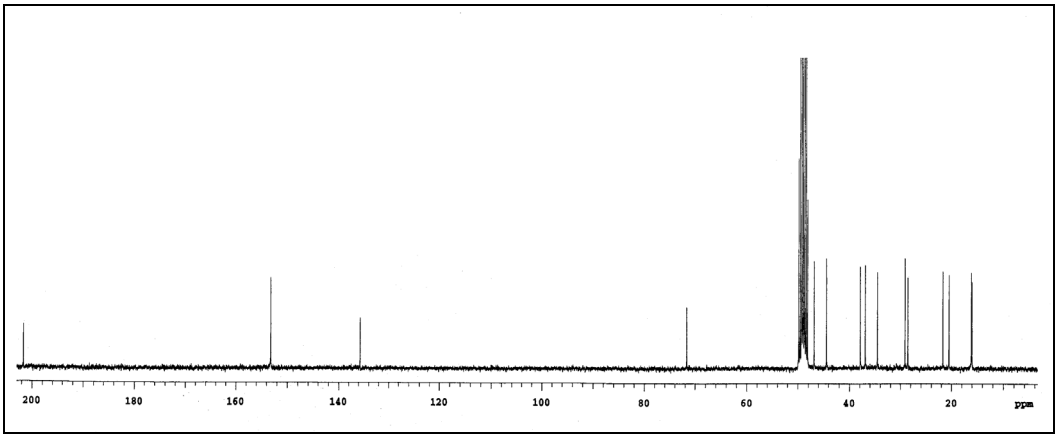
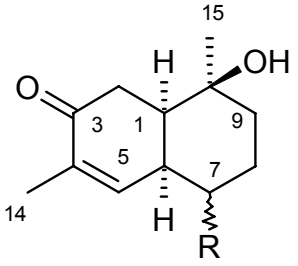
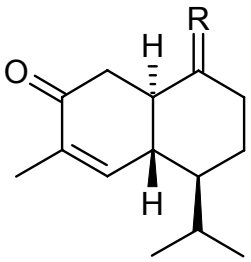


Figure 147: ^{13}C NMR spectrum (300 MHz) of compound **119b** in $CDCl_3$

After a detailed analysis of the 2D spectra, the *trans*-fused C-10 epimers (-)-3-oxo-cadinol (**120a**) and 10α-hydroxcadin-4-en-one (**120b**), and the *cis*-fused C-7 epimers (+)-10β-hydroxy-4-muurolen-3-one (**119a**) and 10β-hydroxyamorph-4-en-3-one (**119b**) were taken into account. The relative stereochemistry was deduced by interpretation of the NOESY spectrum, which indicated correlations between 15-H and 1-H and further correlations between 6-H, 7-H and 1-H. These data as well as published values indicated that both rings in the isolated compound should be *cis*-fused as **119a** and **119b**, because in the latter, the olefinic proton (5-H) showed a significant coupling (6.4 Hz) with the bridgehead proton 6-H,[206] whereas in the *trans*-

fused **120a** and **120c**,[207] the olefinic proton appears as a broad singlet. Based on these data and by comparison with the literature, compound **119b** was identified as 10α-hydroxyamorph-4-en-3-one. This compound is reported here for the first time from microorganisms, however, was previously isolated from *Taiwania cryptomerioides* Hayata. It exhibited moderate activity in the brine shrimp lethality test.[208]





119a: R = α-$CH(CH_3)_2$
119b: R = β-$CH(CH_3)_2$

120a: R = α- CH_3, β-OH
120b: R = β-CH_3, α-OH

5.2.2 10β,11-Dihydroxyamorph-4-en

Compound **121** was isolated as a white solid with similar properties and the molecular formula $C_{15}H_{26}O_2$. The difference in the ^{13}C NMR spectra of **119b** and **121** was the presence of an additional methylene group and a quaternary carbon signal at δ 76.6, instead of the ketone carbonyl and the isopropyl methine as in **119b**. The ^{1}H NMR spectrum showed similarities with that of **119b** as well and displayed an olefinic 1H signal now at δ 5.70 in agreement with the absent ketone carbonyl. Besides four multiplets of methine and methylene groups in the aliphatic region, four methyl singlets at δ 1.67, 1.22, 1.24 and 1.17 indicated that the isopropyl methine of **119b** was substituted.

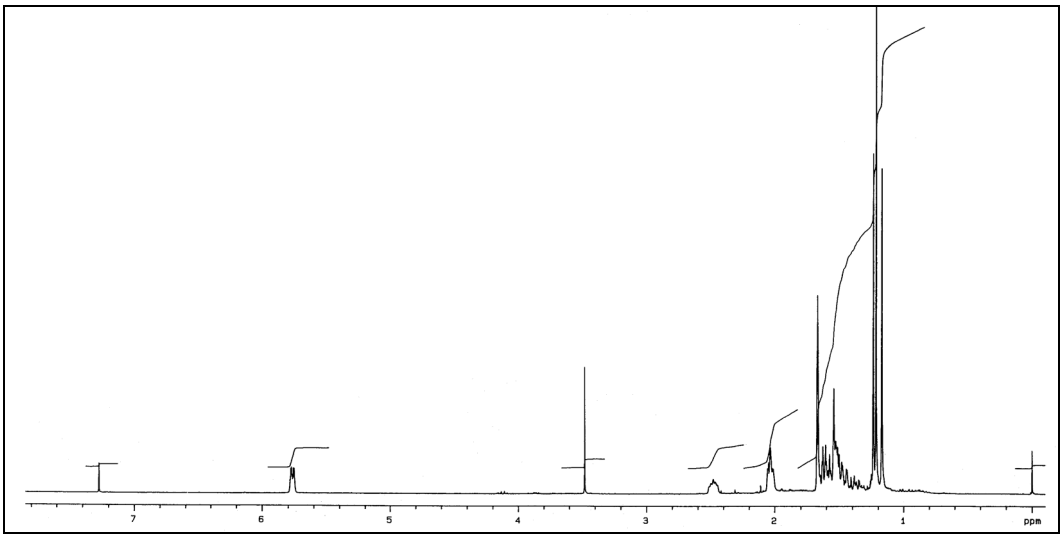


Figure 148: ^{1}H NMR spectrum (300 MHz) of compound **121** in $CDCl_3$

A literature search resulted in trichotomol[209] (**122**), however, comparison of the published data with those of **121** showed differences especially for the atoms C-1 (Δδ = 3.1), C-6 (Δδ = 6.7), C-7 (Δδ =2.9), C-9 (Δδ = 7.6) and C-14 (Δδ = -8.3), indicating that **121** may be a diastereomer of **122**.

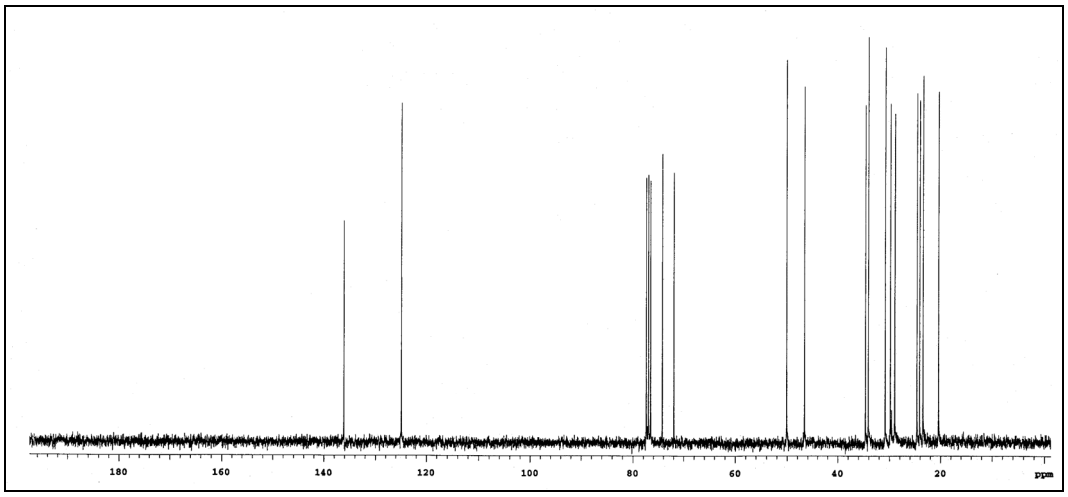
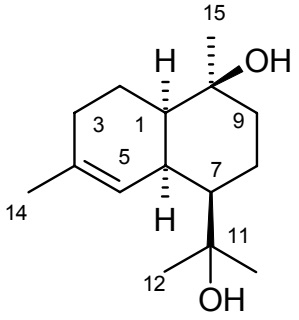
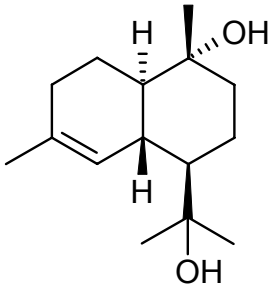


Figure 149: ^{13}C NMR spectrum (300 MHz) of compound **121** in $CDCl_3$

The relative stereochemistry of **121** could not be deduced from the NOESY spectrum due to signal overlapping. The fact that the olefinic proton in **4** appears as a singlet as in **120a**/**120b** but compound **121** gave a doublet as in **119a**/**119b**, shows, however, again *cis*-fused rings. We assume therefore that compound **121** is an amorphane derivative as well and is 10β,11-dihydroxyamorph-4-ene (**121**).



121



122

Table 4: ^{13}C NMR data of compounds **119b**, **121**, **122**, **123**, **124** in $CDCl_3$

C No	**119b**	**121**	**122***	**123**	**124**
1	46.9	46.7	49.8	43.1	44.3
2	37.9	20.4	22.7	35.4	39.7
3	201.7	30.9	30.6	200.0	210.7
4	135.7	136.1	134.3	137.3	50.5
5	153.2	124.9	124.7	151.8	83.5
6	36.9	34.1	40.8	37.2	41.0
7	44.4	50.1	53.0	45.4	45.9
8	20.5	24.1	27.1	19.3	21.4
9	34.6	34.7	42.3	34.1	35.0
10	71.7	72.0	72.1	71.2	71.9
11	29.1	76.6	74.2	27.8	82.1
12	21.7	29.9	24.1	21.3	30.0
13	16.1	24.7	32.1	15.7	24.1
14	28.6	29.0	20.7	28.8	28.7
15	16.0	23.5	24.1	62.2	11.4

* From literature[209]

5.2.3 10β,14-Dihydroxyamorph-4-en-3-one

Compound **123** was isolated as a colourless solid as well; EI HRMS revealed the formula $C_{15}H_{24}O_3$. The ^{13}C NMR spectrum was very similar to that of **119b** exhibiting 15 signals including those of an α,β-unsaturated ketone, in addition to four methines, four methylenes instead of three, and three methyl groups instead of four as in **119b**.

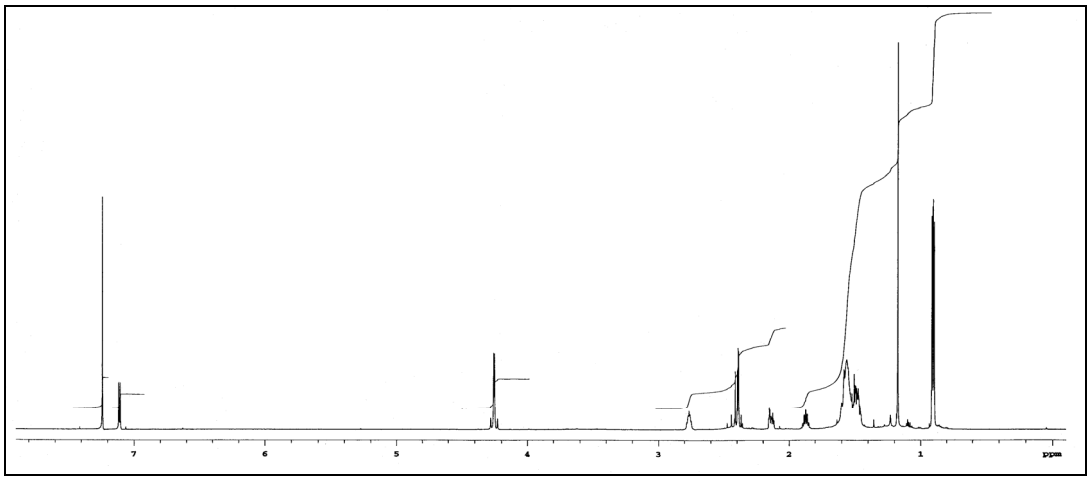
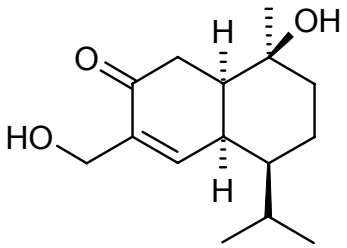


Figure 150: ^{1}H NMR spectrum (300 MHz) of **123** in $CDCl_3$

The major difference was the presence of a methylene group connected to oxygen (δ 62.2) and the absence of one methyl group, which was interpreted as an oxidation of a methyl group in **119b**. The ^{1}H NMR spectrum was similar to that of **119b** as well. The main difference was the substitution of the olefinic methyl signal in **119b** (δ 1.79) by the AB signal of an oxygen-bound methylene group (δ 4.25), which showed an allylic coupling with the double bond proton in the H,H COSY spectrum. This identified compound **123** as 10β-14-dihydroxyamorph-4-en-3-one, which is reported here for the first time.



123

Table 5 : ^{1}H NMR (δ, *J* Hz) data of compounds **119b, 121, 123**, and **124** in $CDCl_3$)

H	**119b*** ($[D_4]$MeOH)	**121**	**123**	**124**
1	2.72 (m)	1.59 (m)	2.13 (m)	2.07 (m)
2	2.50, 2.31 (ABX, 14.7,17.3, 4.7)	2.03 (m)	2.40 (dd, 17.2, 9.6) ; 2.38 (dd, 17.2, 2.5)	2.28, 2.24 (ABX, 12.9, 5.3, J_{AX}< 1)
3	-	2.30	-	-
4	-	-	-	2.43 (quint, 7.3)
5	7.08 (dq, 6.4, 1.3)	5.77 (dq, 6.2, 1.5)	7.11 (br d, 6.3)	3.71 (t, 7.2)
6	2.72 (m)	2.48 (m)	2.77 (m)	2.68 (m)
7	1.56 (m)	1.48 (m)	1.50 (m)	1.99 (m)
8	1.58 (m)	1.60 (m)	1-20-1.60 (m)	1.56 (m)
9	1.40-1.52 (m)	1.54 (m)	1.20-1.60 (m)	1.56, 1.70 (m)
11	1.90 (m)	-	1.88 (sept. 6.7)	-
12	0.92 (d, 6.9)	1.24 (s)	0.92 (d, 6.9)	1.35 (s)
13	0.92 (d, 6.9)	1.17 (s)	0.90 (d, 6.9)	1.09 (s)
14	1.14 (s)	1.22 (s)	1.17 (s)	1.17 (s)
15	1.59 (t, 1.2)	1.67 (s)	4.25, 4.22 (AB,13.2)	1.08 (d, 6.5)

5.2.4 5α,10β,11-Triydroxyamorphan-3-one

Compound **124** was isolated as oil with similar physical properties as of **119b**, **121** and **123**. Also the ^{1}H NMR spectrum of **124** was similar to those of **119b**, **121** and **123**, however, the double bond was obviously absent: The olefinic proton was missing, a ketone carbonyl signal appeared now at δ 210.7 and an oxymethine proton at δ 3.71 gave a triplet. Instead of a methyl signal at δ 1.7, a high-field shifted methyl doublet was located at δ 1.08. The proton spectrum showed three additional methyl singlets; however, signals due to an isopropyl group were missing as in **121**.

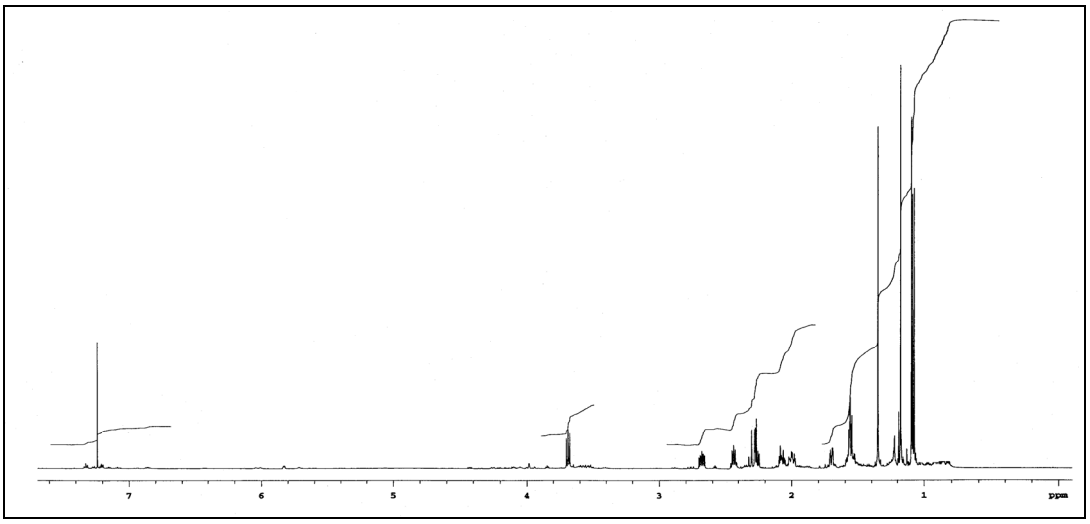


Figure 151: ^{1}H NMR spectrum (300 MHz) of **124** in $CDCl_3$

The ^{13}C NMR spectrum indicated the presence of 15 carbon signals, including signals of four methyls, three methylenes, five methines, and three quaternary C atoms, including the ketone. The major difference here was again the absence of the double bond signals.

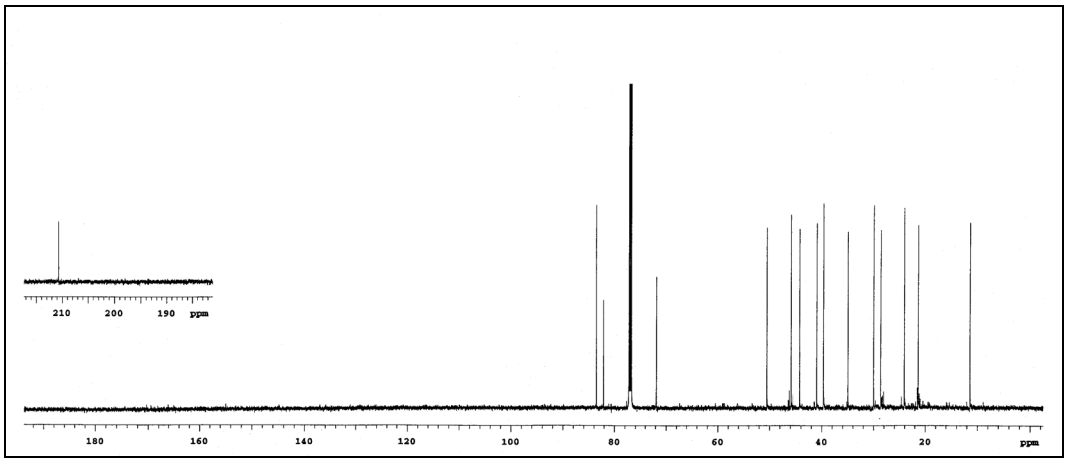
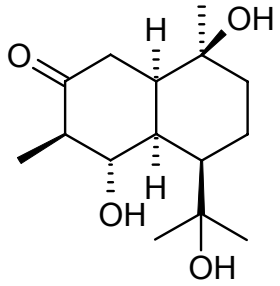


Figure 152: ^{13}C NMR spectrum (300 MHz) of **124** in $CDCl_3$

EI MS indicated no molecular ion, however, an ion at *m/z* 252, which was later found to be due to the loss of water. The relative stereochemistry at position C-4 and C-5 was deduced from the NOE spectrum, which showed a positive NO effect on 14-H_3 and 6-H by irradiation into the 5-H signal. Compound **124** was therefore deduced as 5α,10β,11-triydroxyamorphan-3-one and is also described here for the first time.



124

Compounds **119b**, **121**, **123** and **124** belong to the group of amorphanes, which are *cis*-fused stereoisomers of the cardinene sesquiterpenes. The biosynthetic sequence for the amorphane group starts from an intermediate resulting from a 1,10-cyclisation of farnesyl-pyrophosphate (FPP)[210]. From this intermediate **6**, the cation **7** is formed by a [1,3]hydride shift (fig. 153), and cyclisation of the latter results theoretically in 16 stereoisomeric bicyclic sesquiterpenes. Six series are already known, four were well described, namely bulgaranes, amorphanes, cardinanes, and muurolanes.[211, 212, 213]

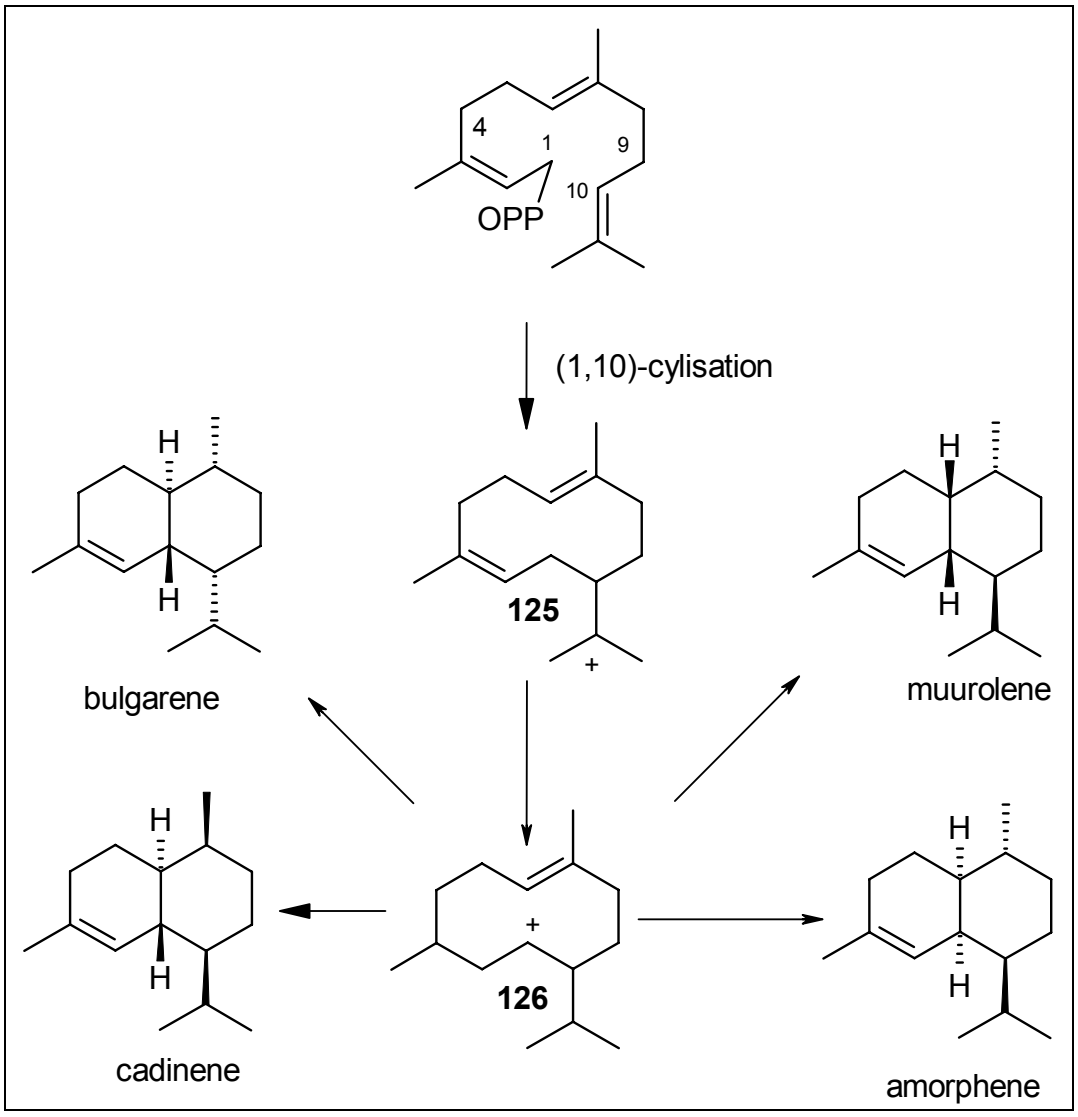


Figure 153: Biosynthesis of sesquiterpene groups

5.3 Marine-derived *Streptomyces* sp. Mei 35

The crude extract of the marine derived Streptomyces sp. Mei 35 showed strong antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Chlorella vulgaris*, *Chlorella sorokiniana and Scenedesmus* subspicatus. The culture on agar exhibited after four days at 28 °C white colonies. These agar plates were used to inoculate 100 of 1-L Erlenmeyer flasks (25 L). After 4 days at 28 °C on a rotary shaker, the beige culture broth was worked up as shown in Figure 154. TLC of the crude extract exhibited yellow and violet spots on spraying with anisaldehyde/sulphuric acid.

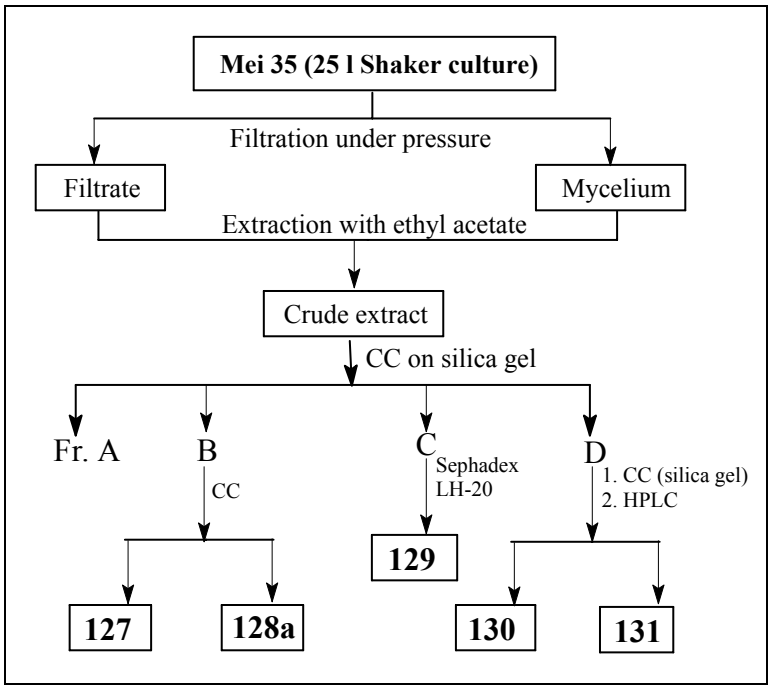
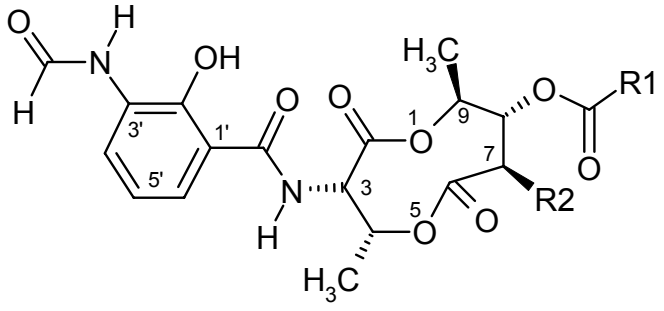


Figure 154: Work-up of the marine *Streptomyces* sp. Mei 35

5.3.1 Antimycin Complex

TLC of fraction B (fig. 154) showed under UV at 366 nm a strong fluorescent spot, which did not give any coloration on spraying with anisaldehyde/sulphuric acid. Column chromatography on silica gel followed by PTLC delivered the compound SFSH3 (**127**). The ^{1}H NMR spectrum of **127** exhibited in the aromatic region three exchangeable protons at δ 12.66 (s), 8.10 (br s) and 7.17 (d). In addition, a doublet at δ 8.52 (^{3}J = 1.7 Hz) and signals for three consecutive aromatic protons at δ 8.56 (d), 7.24 (d) and 6.90 (t) were visible. The aliphatic signals appeared as three complex multiplets in the range of δ 2.60-1.40, 1.40-1.00 and 1.00-080. The complexity of the spectrum and the ratio of the integrals suggested a mixture of similar compounds. The (+)-ESI mass spectrum confirmed this assumption indeed and indicated a mixture of three $[M+Na]^{+}$ ions corresponding to the molecular weights 506, 520, 534 and 548. A search in AntiBase with these data led to the identification of SFSH3 as antimycins A-complex (**127**).



Antimycins (**127**)	R_1	R_2
A_1	$CH(CH_3)CH_2CH_3$	$(CH2)_5CH_3$
A_2	$CH_2CH_2CH_3$	$(CH2)_5CH_3$
A_3	$CH(CH_3)CH_2CH_3$	$CH_2CH_2CH_2CH_3$
A_4	$CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$
A_7	$CH_2CH_2CH_2CH_3$	$(CH2)_2CH(CH_3)_2$
A_8	$CH_2CH(CH_3)_2$	$(CH2)_2CH(CH_3)_2$

The antimycins (**127**) were first isolated from a *Streptomyces* sp. in 1949,[214] the structure determination was completed in 1961[215] and the absolute configuration was established by Kinoshita *et al.*[216] in 1972. This class of compounds is characterized by the presence of a 3-formamidosalicylamide unit, a nine-membered bislactone and two alkyl side chains of varying chain length. Although first identified as antibiotics and used commercially as fungicides,[217] more recently antimycins (**127**) were found to inhibit the electron flow in the mitochondrial respiratory chain between cytochromes b and c_1: They have been used extensively to investigate the energy metabolism in eukaryotic organisms and have been finally reported as ATP-citrate lyase inhibitors.[217]

5.3.2 (4S)-4-Hydroxy-10-methyl-11-oxododec-2-en-1,4-olide

The compound SFSOF2 (**128a**) was obtained as an oil from fraction B. It was not UV-active and showed a violet colour reaction on spraying with anisaldehyde/sulphuric acid, which turned to blue after some minutes. The ^1H NMR spectrum showed in the sp^2 region only two 1H signals at δ 7.48 (dd) and 6.14 (dd). The small coupling constants (5.6 and 1.7 Hz) seemed to indicate a small ring; another 1H signal appeared at δ 5.03 (m) and was assigned to a methine proton bearing an oxygen atom. The aliphatic region delivered a methine quartet at δ 2.50 and a methyl

doublet at δ 1.10, characteristic for the fragment $CHCH_3$. Furthermore, a methyl singlet at δ 2.18 was attributed to an acyl group and a multiplet of ten protons in the range δ 1.70-1.20 seemed to be due to a long methylene chain.

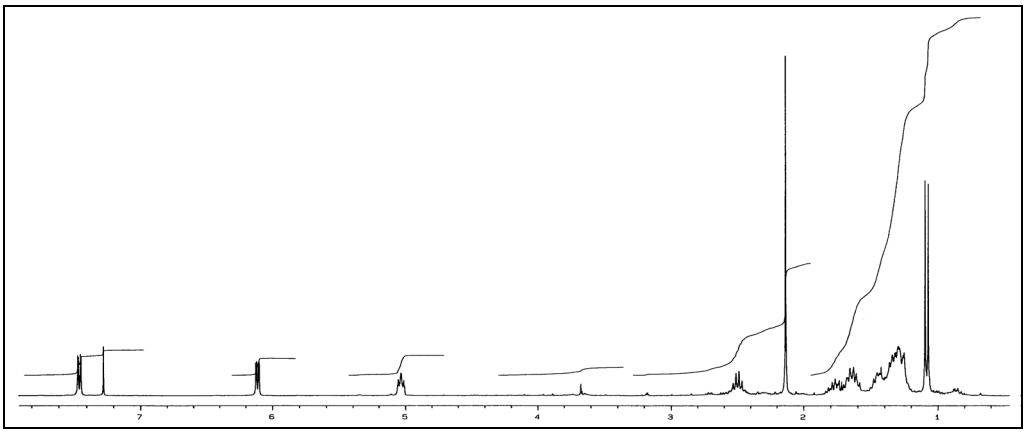
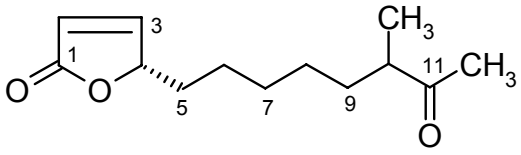


Figure 155: ^{1}H NMR spectrum (300 MHz) of **128a** in $CDCl_3$

The EI MS indicated the molecular peak at *m/z* 224. A search in AntiBase using the substructure and the molecular weight led to the identification of SFSOF2 as (4S)-4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**128a**), which had previously been isolated in our group by Mukku.[218]

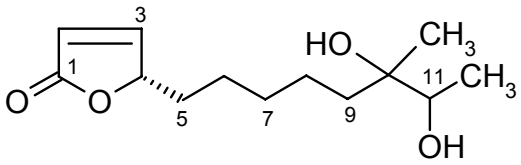


128a

5.3.3 (4S)-4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide

Purification of fraction C on silica gel delivered oily SFSOF3 (**129**). This compound showed on TLC the same characteristics as **128a** on spraying with anisaldehyde/sulphuric acid, and also the ^{1}H NMR spectrum was very similar to that of **128a**. The methyl singlet of the terminal acetyl group was, however, missing. A methine quartet at δ 3.64 and a methyl doublet at δ 1.14 for a $CH_3CH(OH)$-group were observed instead, which revealed that the ketone group in **128a** had been reduced to a secondary alcohol. Additionally, the doublet at δ 1.10 of the methyl group at C-10 of **128a** was replaced by a methyl singlet at δ 1.29. The ^{13}C and APT NMR spectra indicated the presence of five methylene groups, four methines, two methyl groups and

two quaternary carbons. The ketone carbonyl signal at δ 212.6 and the methine signal at δ 40.0 (C-10) in **128a** were replaced by two signals for carbons bearing oxygen at δ 77.4 (C_q) and 74.0 (CH). The (+)-ESI mass spectra showed *quasi*-molecular ions at *m/z* 507 ($[2M+Na]^+$) and 265 ($[M+Na]^+$), which fixed the molecular weight at 242 Dalton. The difference of the molecular weights of Δm = 18 between **128a** and **129** can be interpreted by the substitution of the C-10 methine proton by a hydroxyl group and the reduction of the keto group of **128a** to an alcohol in **129**, which is in accordance with the NMR data. Compound **129** was identified as 4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide, a new natural product.



129

5.3.4 4,10-Dihydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide

TLC of fraction D showed a yellow band and some spots, which revealed similar properties as **128a** and **129** on spraying. The silica gel chromatography followed by preparative HPLC gave an oily compound named SFSH1 (**130**). Its ^{1}H NMR spectrum was very similar to that of **128a** and **129**, the only difference being the replacement of the methyl doublet at δ 1.10 in **128a** by a methyl singlet at δ 1.36 in **130**.

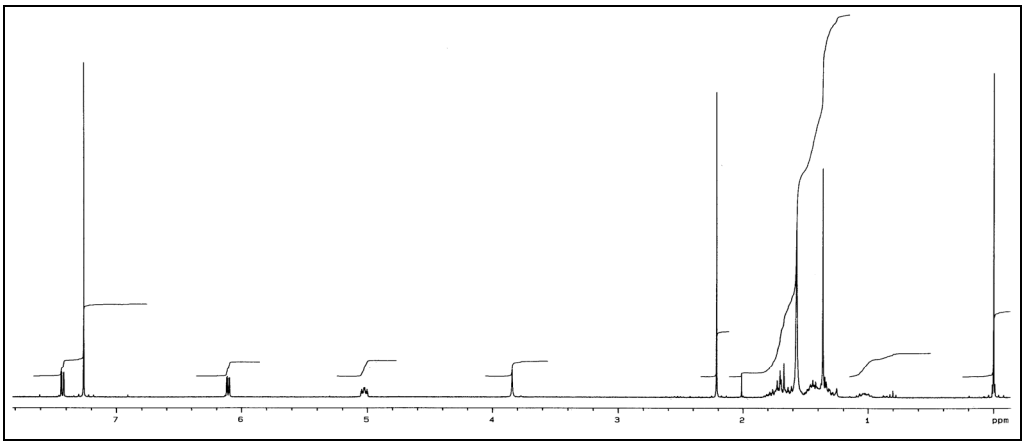
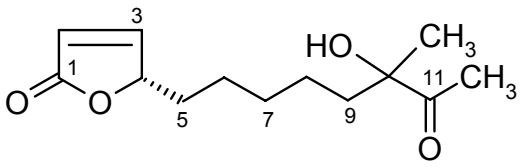


Figure 156: ^{1}H NMR spectrum (300 MHz) of **130** in $CDCl_3$

In the EI mass spectrum, no molecular ion peak was observed, however, the (+)-ESI mass spectrum delivered a molecular weight of *m/z* 240. Obviously the C-10 methine

proton in **128a** was substituted by a hydroxyl group in **130**. This was further confirmed by the ^{13}C and APT spectra, which exhibited a signal of a quaternary carbon bearing an oxygen atom at δ 78.7 in **130** instead of the methine carbon at δ 40.0 in **129**. The structure of **130** was finally assigned as the new 4,10-dihydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide.



130

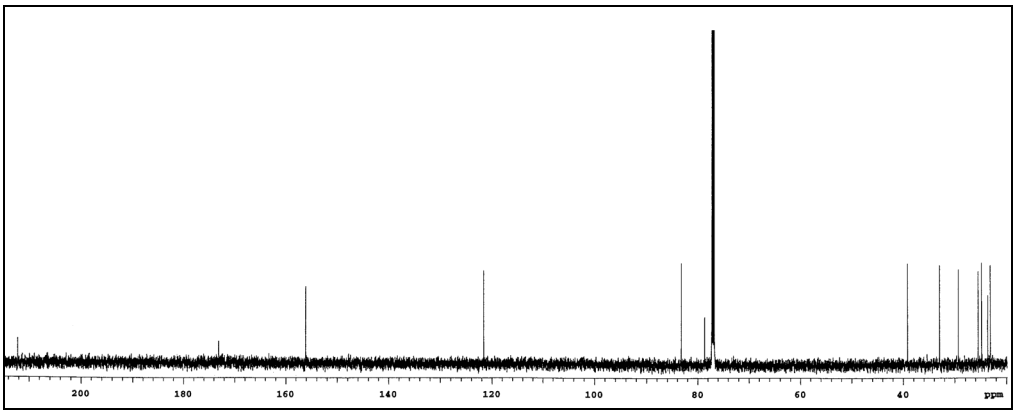


Figure 157: ^{1}H NMR spectrum (300 MHz) of **130** in $CDCl_3$

Butenolides (mainly but-2-enolides) are occurring widespread in fungi,[219] bacteria[220] and higher forms of life, e.g. gorgonians.[221] Their saturated analogues act as signalling substances in bacteria[222] and enhance the spore formation in streptomycetes or induce metabolite production.[223] They are inactive in antibacterial tests.

5.3.5 7-Methylamino-3,4-dimethyl-isoquinoline-5,8-dione

Compound SFSH2 (**131**) obtained as a red solid by preparative HPLC of fraction D, delivered a yellow solution and remained also yellow on TLC and on spraying with anisaldehyde/sulphuric acid. It exhibited also no colour reaction with sodium hydroxide. The molecular weight was deduced from the ESI and EI mass spectra to be *m/z* 216, and EI HRMS delivered the molecular formula $C_{12}H_{12}N_2O_4$. The ^{1}H NMR spectrum showed only three singlets at δ 9.00, 5.82 and 5.72, which disappeared on H/D

exchange, a methyl doublet at δ 2.93 pointing to a CH_3NH- fragment and two singlets at δ 2.76 and 2.70 for aromatic methyl groups.

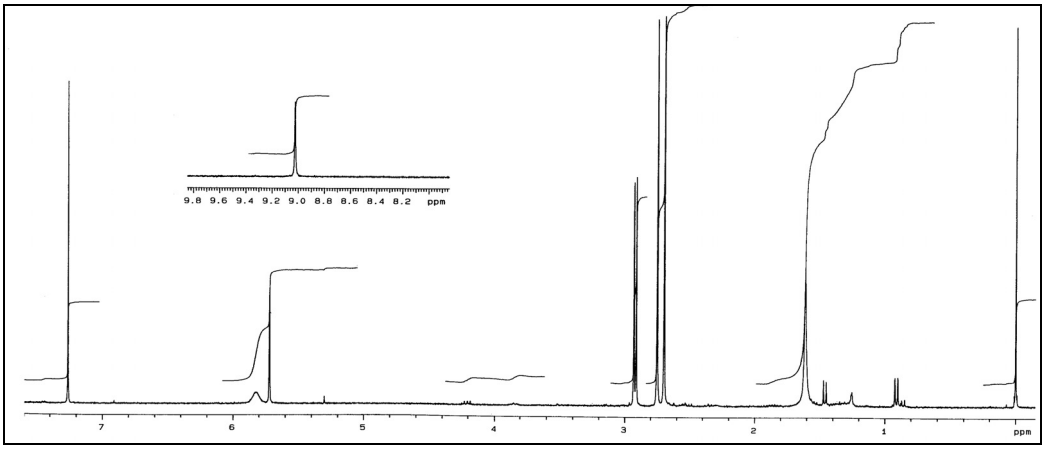


Figure 158: ^{1}H NMR spectrum (300 MHz) of **131** in $CDCl_3$

The ^{13}C and APT NMR spectra indicated two aromatic methine and three methyl carbon signals. Furthermore, signals of seven quaternary carbon atoms were observed, of which two at δ 185.0 and 181.9 represented carbonyl groups.

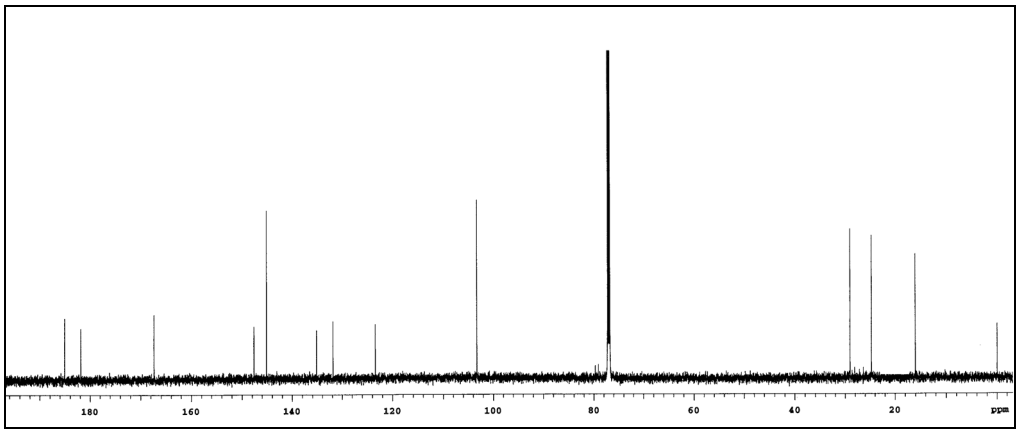
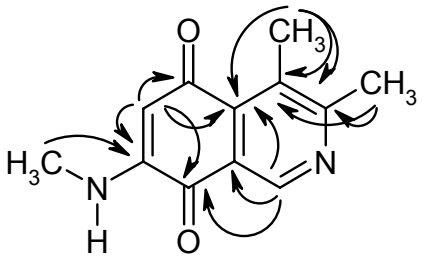


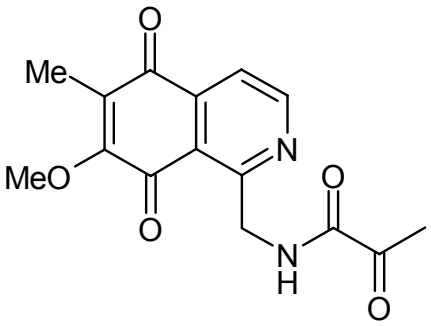
Figure 159: ^{13}C NMR spectrum (300 MHz) of **131** in $CDCl_3$

The search in AntiBase with these data gave 7-methylamino-3,4-dimethyl-isoquinoline-5,8-dione (**131**) as result, which was previously isolated by Speitling from the marine streptomycete B3497[80], however, has not been published so far. Compound **131** belongs to the class of isoquinolinequinones like the cribrostatins,[224,225] renierone[224,226] and *O*-demethyl renierone.[224] The latter three compounds have been isolated from marine sponges and are known to exhibit strong antimicrobial activity[227-229] against *Staphylococcus aureus*, *Bacillus subtilis* and *Can-*

dida albicans and a mild cytotoxicity against a L1210 cell line.[229] From microorganisms (*Streptomyces lavendulae*), only one example [mimocin (**132**)] has been reported so far,[230] however, in the meantime several further derivatives have been isolated in our group by Shaaban.[231] Biologically active isoquinolinquinones are also known as synthetic products: 7-Methylamino-3-methylisoquinoline-5,8-dione[232] e.g. is a 4-demethyl derivative of **131**. The isolation of **131** from a marine streptomycete further supports the idea that bacteria are involved in the biosynthesis of 'sponge metabolites'.



131



132

5.4 Marine-derived *Streptomyces* sp. Mei 23

The marine derived Streptomyces sp. Mei 23 showed in the biological screening antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Mucor miehei*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*. The Streptomycete was grown on agar plates for four days at 28 °C. These plates were used for the inoculation of a 15 L-fermentation in 1 L Erlenmeyer flasks on a rotary shaker, which were cultivated for four days at 95 rpm and 28 °C. The light green culture broth was filtered under pressure and an oily crude extract was obtained and worked up following the scheme shown in Figure 160.

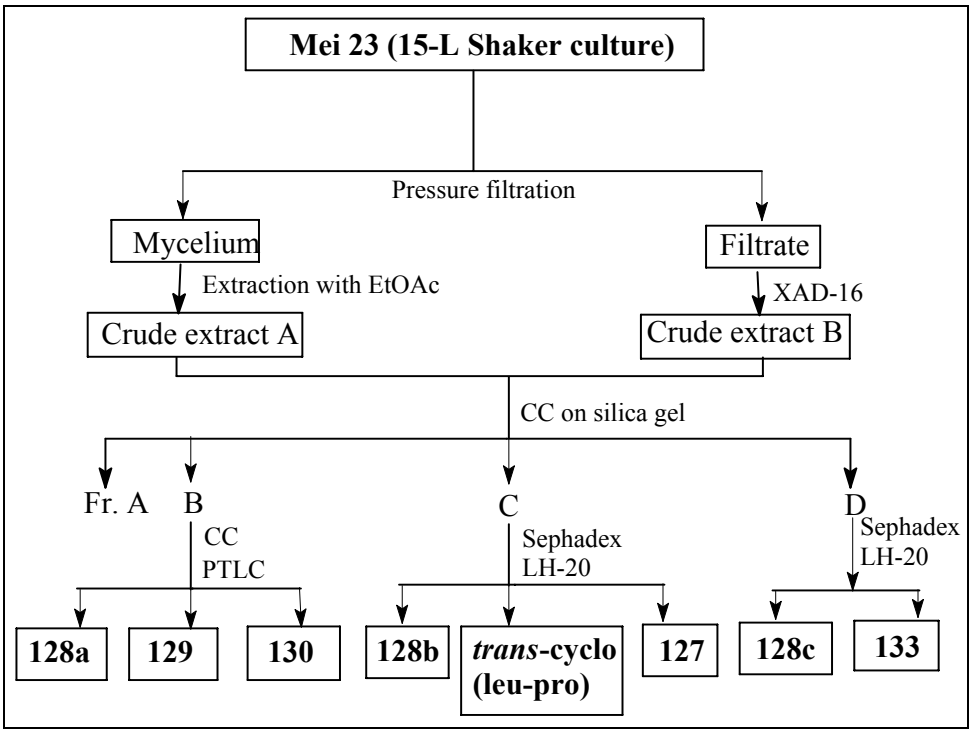


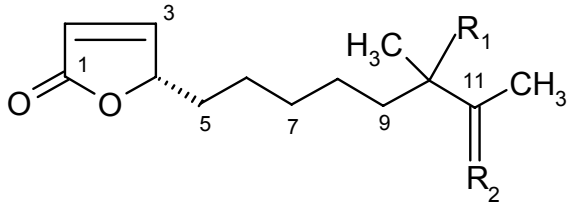
Figure 160: Work-up of the marine-derived strain Mei 23

Due to the same physical and chemical properties and similarities in the spectra (^{1}H NMR, EI MS), the compounds SFUS2, SFU1a and SFUM6 were identified as butenolides **128a, 129,130** respectively, previously isolated from strain Mei 35. In addition, compounds SFUMa and SFUS5a were identified as *trans*-cyclo-(Leucyl-Prolyl) and antimycin A-complex (**127**).

5.4.1 Diastereoisomers

The compound SFUMiX (**128b**) was isolated as an oil, which showed the characteristics of a butenolide on spaying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed two sp^2 protons at δ 7.45 (dd) and 6.12 (dd), two multiplets at δ 5.05 and 3.68 of methine protons attached to oxygen, and confirmed this assumption. The aliphatic region showed a multiplet in the range of δ 1.80-1.20 and two pairs of 3H doublets at δ 1.10 and 0.85. The molecular ion was determined from the ESI MS to be 226. The mass difference of Δm = 2 between **128a** and **130** can result from the reduction of the ketone carbonyl to alcohol. The ^{13}C NMR spectrum (Table 6) was typical for a butenolide, but indicated also pairs of signals at δ 71.6, 40.0, 14.5 for carbons at position 11, 10 and 13. These observations suggested that **128b** is a mix-

ture of diastereoisomeric 4,11-dihydroxy-10-methyl-dodec-2-en-1,4-olides (**128b**), which were also described by Mukku *et al*.[218]



128a: R_1 = H R_2 = O
128b: R_1 = H R_2 = H, OH
128c: R_1= OH R_2 = H, H

Table 6: ^{13}C NMR data ($CDCl_3$, 75.5 MHz) for diastereoisomers **128b**

C-No	**128b**	**128b**	**C-No**	**128b**	**128b**
1	173.2	173.2	8	27.1	26.9
2	121.4	121.4	9	32.3	32.3
3	156.3	156.3	10	39.9	39.6
4	83.4	83.4	11	71.6	71.2
5	33.1	32.3	12	19.4	20.2
6	24.9	24.9	13	14.1	14.5
7	29.5	29.5			

5.4.2 4(S)-4,10-Dihydroxy-10-methyl-dodec-2-en-1,4-olide

Compound SFUS5 (**128c**) from fraction D showed physical, chemical and spectral similarities with **128a-130** and **128b**. In the ^{1}H NMR spectrum, the only difference was the presence of two coupling methyl groups, which formed a triplet and singlet at δ 1.17 and 0.88, respectively, pointing to a C_q-methyl and an ethyl fragment. The methylene signals appeared together with other multiplets in the range of δ 1.50-1.20. The compound **128c,** which was identified as 4(S)-4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**128c**) may have resulted from the reduction of the ketone group in compound **130** to methylene and was also reported by Mukku *et al*.[218] The presence of two position isomers **128b**/**128c** and the diastereoisomers **128b** may indicate an epoxide precursor in their biosynthesis, however, the corresponding precursor has not yet been reported.

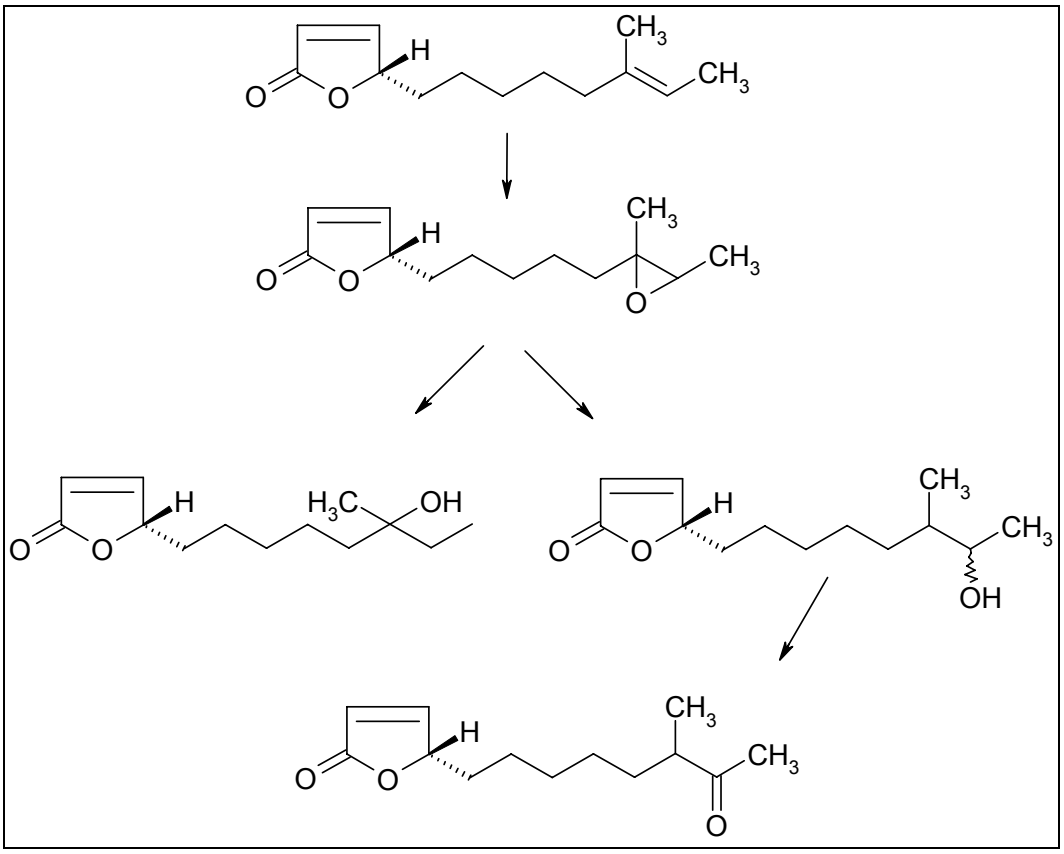
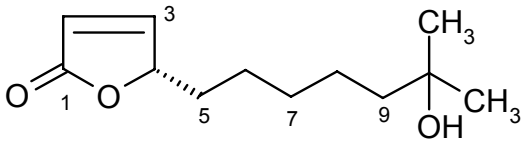


Figure 161: Hypothetic biosynthesis of butenolides from 5-(6-methyl-oct-enyl)-5H-furan-2-one

5.4.3 MNK-003B

The spectral data of compound SFUM2 (**133**) were highly compatible with those obtained from other butenolides and especially of **128a**. Here too, the difference between the chemical shift of the methyl protons at δ 2.10 in **128a** to δ 1.21 in **133** and the 6H singlet can be attributed to a *gem*-dimethyl group. The ESI MS indicated a *pseudo*molecular ion at *m/z* 235. A literature search resulted in the identification of compound **133** as MNK-003B, which was isolated by Ki Woong *et al*.[233] from a marine-derived bacterium.



133

5.5 Marine-derived *Streptomyces* sp. Mei 22

The crude extract of the marine streptomycete Mei 22 exhibited strong activity in the biological screening against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Candida albicans* and *Mucor miehei*. TLC of the crude extract did not present visible spots on daylight but revealed the presence of several black to brown spots on spraying with anisaldehyde/sulphuric acid. For the isolation of these secondary metabolites, the strain was fermented on a 25 L scale using M_2^+ medium with 100% seawater under addition of calcium carbonate. The extraction of the culture broth with ethyl acetate gave after evaporation 2.5 g of an oily crude extract, which was worked up following the scheme given in Figure 162.

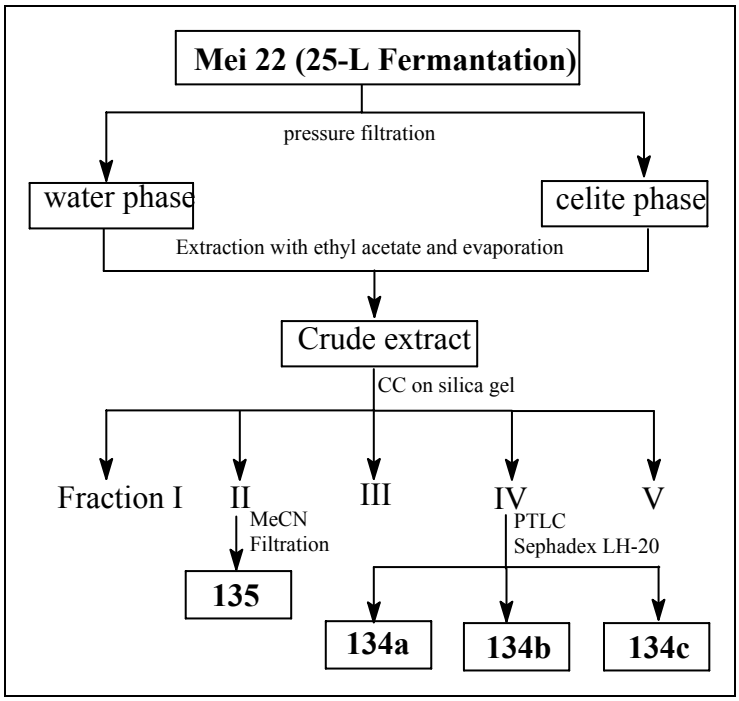
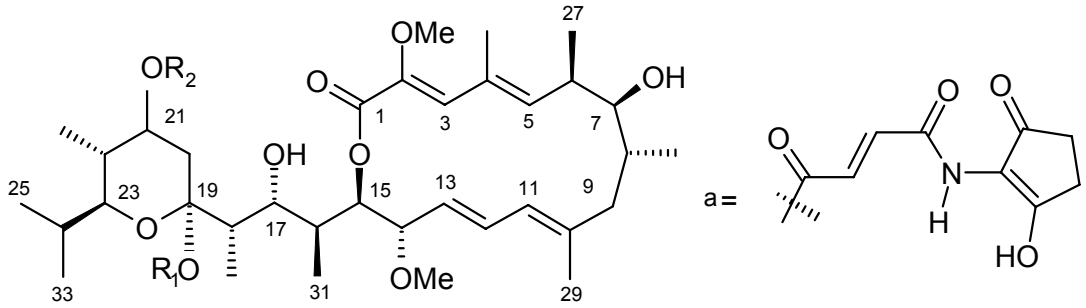


Figure 162: Work-up and isolation scheme of *Streptomyces* sp. Mei 22

5.5.1 Bafilomycin A_1

TLC of fraction IV showed the presence of three brown spots after spraying with anisaldehyde/sulphuric acid. Purification by PTLC followed by Sephadex LH-20 resulted in the isolation of three compounds. Compound MSF4a (**134a**) was obtained as colourless solid. The molecular weight was determined by EI and (+)-ESI mass spectra to be *m/z* 622, in the ^{1}H NMR spectrum six sp^2 signals containing five olefinic methines were observed at δ 6.70 (s), 6.50 (dd), 5.80 (2H, t) and 5.18 (dd), both

doublets of doublets was found to indicate protons in a *trans* configuration. The aliphatic region was more complex than the olefinic one and exhibited a doublet of doublet at δ 4.96, five multiplets in the range of δ 4.10-3.30 integrating each for one proton were attributed to methine groups connected to oxygen. The signals of two methoxy groups appeared at δ 3.63 and 3.22, two 3H singlets at δ 1.98 and 1.93 indicated methyl groups bound to a double bond. Finally, seven methyl doublets appeared in the range of δ 1.08-0.70. With these data, the search in AntiBase led to the identification of MSF4a as bafilomycin A_1 (**134a**), which was confirmed by comparison of the data with the literature[234]. Bafilomycin A_1 is reported as a potent vacuolar Na^+, K^+, H^+-ATPase inhibitor[235], and inhibits cell growth by apoptosis.[236] Due to this activity, a total synthesis of bafilomycin A_1 (**134a**) had been carried out by Roush *et al*.[2355]



134a: R_1 = H R_2 = H
134b: R_1 = H R_2 = a
134c: R_1 = CH_3 R_2 = a

5.5.2 Bafilomycin B_1

From the same fraction IV, a yellow solid was obtained, which showed the same colour reaction on spraying with anisaldehyde/sulphuric acid as bafilomycin A_1 (**134a**). The ^{1}H NMR spectrum of **134b** was also very similar to that of bafilomycin A_1 (**134a**), but some differences appeared mainly in the downfield region with two H/D exchangeable protons at δ 13.4 and 8.66 and two doublets (J = 15.0 Hz) of *trans*-protons at δ 6.90 and 7.20. The only difference in the aliphatic region was the appearance of two methylene multiplets in the range of δ 2.70-2.50. In (+)-ESI MS,

signal of $[M+Na]^+$ and $[M-H]^-$ at *m/z* 838 and 814, respectively, indicated a molecular weight of 815 Dalton.

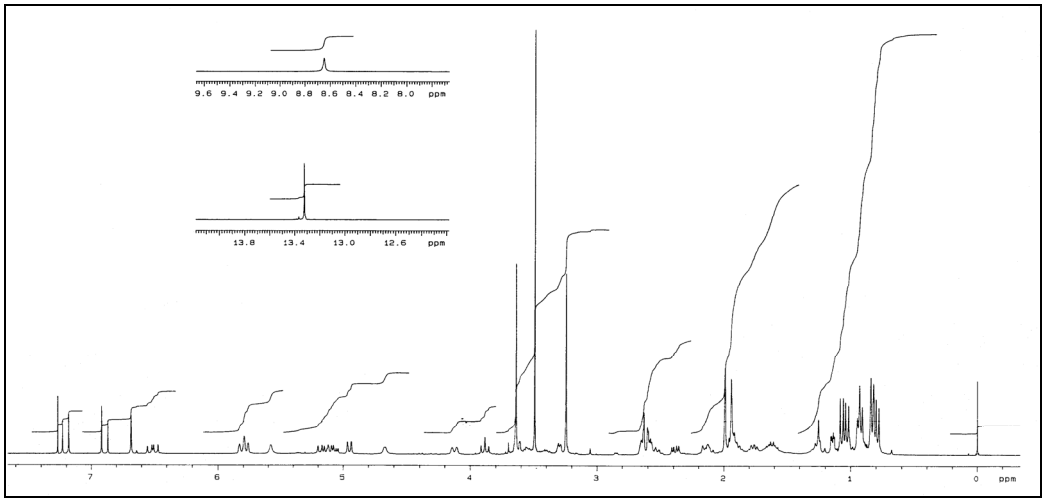


Figure 163: ^{1}H NMR spectrum (300 MHz) of bafilomycin B_1 (**134b**) in $CDCl_3$

The ^{13}C NMR spectrum indicated the presence of 44 signals, three of them could be attributed to carboxyl groups. Comparison of the ^{13}C NMR data of **134b** with those of **134a** from the literature [2344] revealed an additional C_9-fragment. The ESI mass spectrum of **129b** displayed signals at *m/z* 838 $[M+Na]^+$, 1653 $[2M+Na]^+$, 814 $[M-H]^-$ and 1651 $[2M+Na-2H]^-$ in the positive and negative mode, respectively. The odd molecular weight indicated that the additional C_9 fragment contained an odd number of nitrogen atoms. With these data, a literature search gave bafilomycin B_1 (**134b**), which is the N-(3-hydroxy-2-cyclopentenon-2-yl)-fumaryl ester monoamide [3-(Carbamoyl acrylic acid)] of **134a**.[2344] Bafilomycin B_1 (**134b**) is among all natural bafilomycins the most potent nematode inhibitor.[237]

5.5.3 Bafilomycin B_2

The compound **134c** showed the same physical and chemical properties as **134a** and **134b**. The ^{1}H NMR spectrum was very similar to that of **134b**. Whereas **134a** and **134b** had two methoxy groups, the ^{1}H NMR spectrum of **134c** counted for three, with the additional methoxy signal at δ 3.06. The molecular weight of *m/z* 829 was deduced from the (+)-ESI and (-)-ESI mass spectrum. The mass difference of Δm = 14 between **134c** and **134b** confirmed the presence of the additional methoxy group.

A search in AntiBase led easily to the identification of compound **134c** as bafilomycin B_2 (**134c)**.

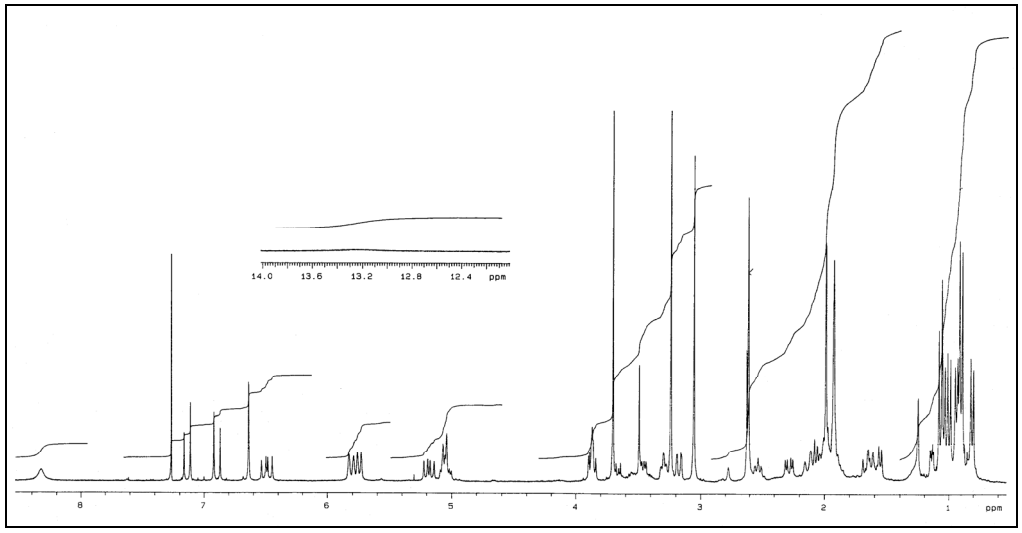


Figure 164: ^{1}H NMR spectrum (300 MHz) of bafilomycin B_2 (**134c**) in $CDCl_3$

The bafilomycins are 16-membered macrolide antibiotics consisting of an α-methoxy or α-methyl α,γ-unsaturated lactone ring. They are members of the pleco-macrolide family (formerly known as the hygrolide family)[238] of macrolide antibiotics that includes the hygrolidins,[239] concanamycins[240] and formamycin.[241] Bafilomycins possess a broad activity spectrum against Gram-positive bacteria, fungi and yeasts; Gram-negative bacteria seem to be insensible.

5.5.4 Deboroaplasmomycin C

Compound MSF2 (**135**) was obtained as middle polar colourless solid, which was fairly soluble in middle polar solvents like dichloromethane, chloroform and ethyl acetate, but hardly soluble in methanol. On spraying with anisaldehyde/sulphuric acid, the compound turned dark grey to black depending on the concentration. The IR spectrum showed a strong ester carbonyl absorption at ν 1724 cm^{-1}. The complex ^{1}H NMR spectrum exhibited signals for 34 protons between δ 5.7 and 0.6, where those at δ 5.24 and 4.89 represented two acidic protons. It contained further signals of two olefinic protons at δ 5.70 and 5.50, seven aliphatic methine groups, six of them bearing oxygen. In addition, four methylene and four methyl groups were observed.

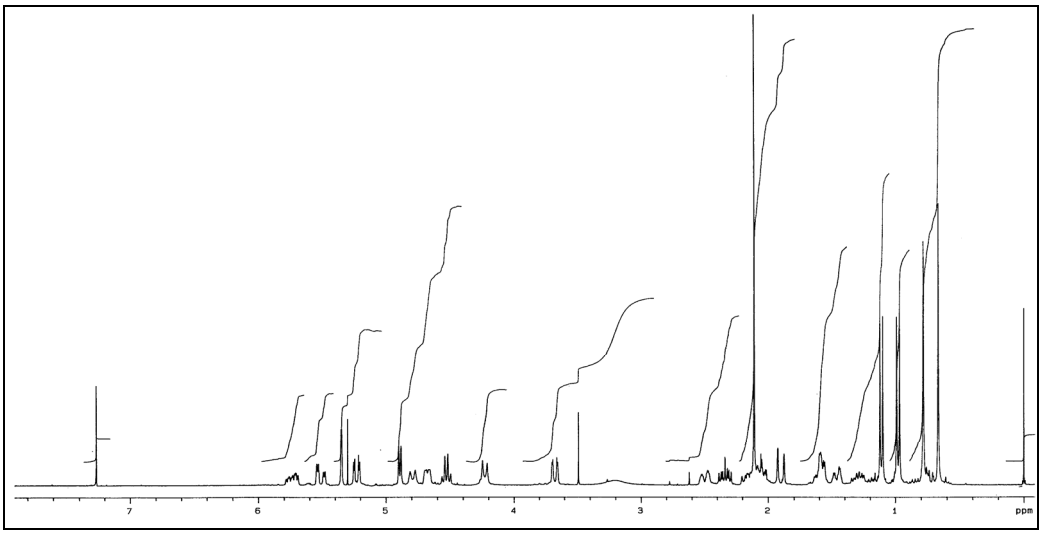


Figure 165: ^{1}H NMR spectrum (300 MHz) of deboraoaplasmomycin C (**135**) in $CDCl_3$

The ^{13}C NMR and ATP spectra exhibited 22 carbon signals. The signals at δ 171.0 and 168.7 were assigned to acid or ester carbonyls, those at δ 133.4 and 126.2 to double bond methine and that at δ 98.7 to an acetal or hemiacetal carbon. Furthermore, there were seven methine carbons, of which six were connected to oxygen; additionally five methylene and five methyl carbon signals were observed.

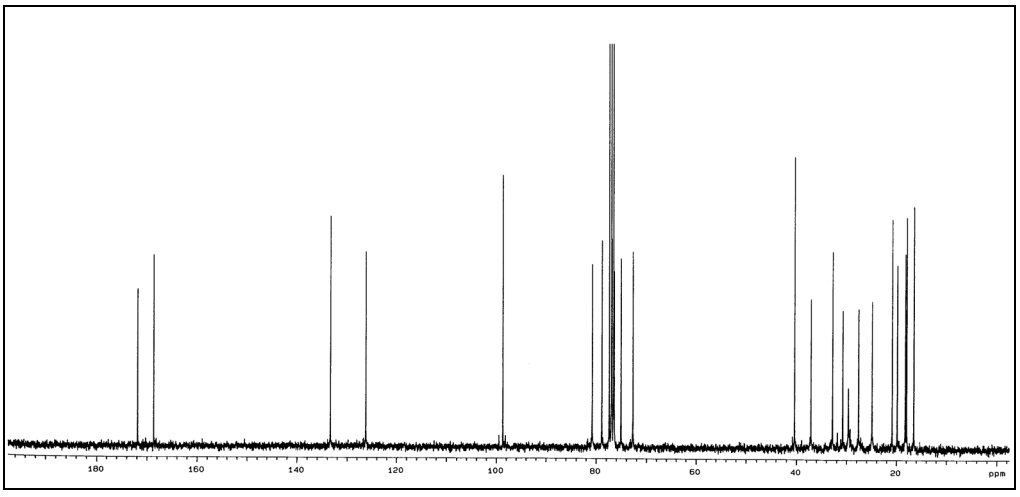
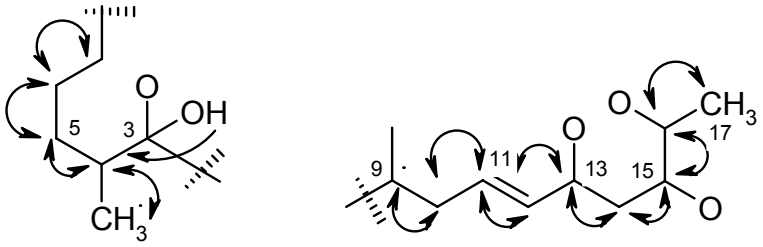


Figure 166: ^{13}C NMR spectrum (75.5 MHz) of deboraoaplasmomycin C (**135**) in $CDCl_3$

With the couplings in the H,H COSY spectrum, two spin systems can be constructed (fig.167).



I II

Figure 167: Fragments I and II of deboroaplasmoycin C (**135**) constructed by H,H COSY (↔) couplings

The spectrum exhibited cross signals between the 2-H at δ 4.19 (d, ^3J = 11.9 Hz) and the OH group at (4.89, d, ^3J = 11.9 Hz) C-2, suggesting a methine group bearing a hydroxyl group without any other protons on the neighbouring carbons. The HMBC experiment permitted the construction of the fragment in Fig. 168 containing all the 22 carbons atoms. The search in AntiBase with this sub-structure delivered deboroaplasmomycin (**135**), which was confirmed by comparison of the data with those of the literature.[242]

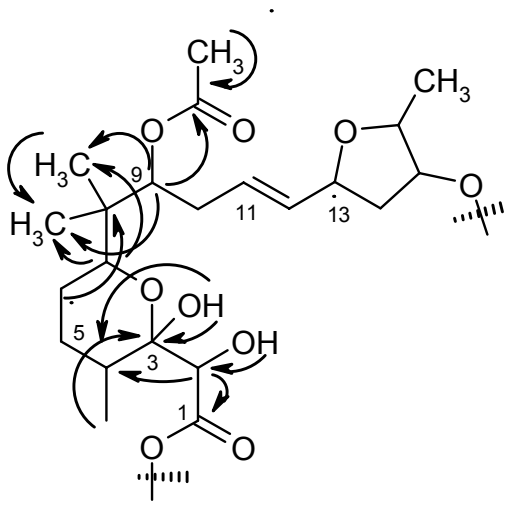
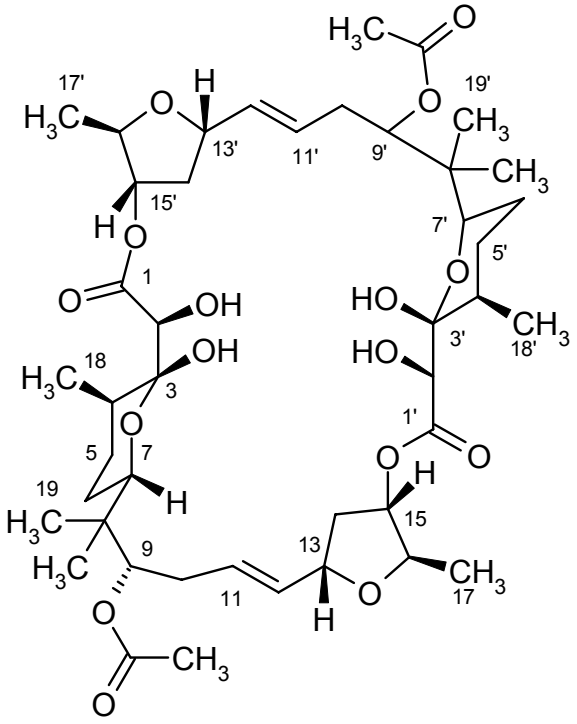
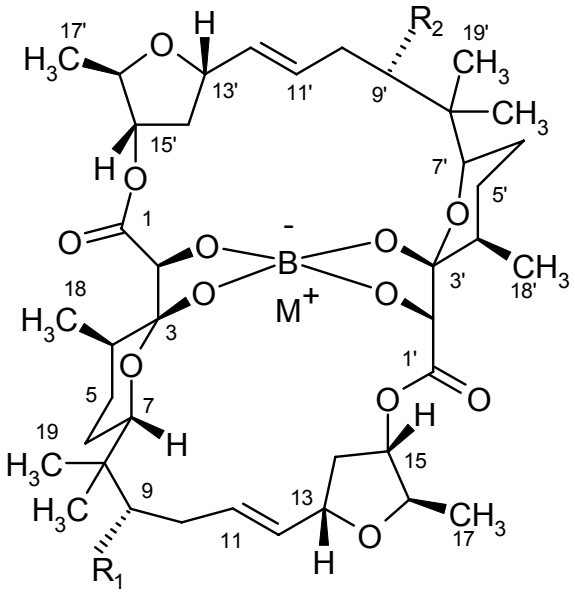


Figure 168: HMBC (→) couplings in deboroaplasmomycin C (**135**)



135

Deboroaplasmomycin C (**135**) was firstly isolated as natural product by W. Fenical *et al.*[243] It belongs to the class of aplasmomycins [A (**136a**), B (**136b**), C (**136c**)], which are further examples among the rare known metabolites containing the element boron. Boromycin[244] was the first known member of these ionophore antibiotics. They exhibited activity against Gram-positive bacteria and also plasmodia.[245]



136a: R_1 =OH R_2 = OH
136b: R_1 = OH R_2 = OAc
136c: R_1 = OAc R_2 = OAc

The unique structure and biological activity of these compounds distinguish these molecules as an unusually interesting target for synthesis.[246, 247] Some experiments were carried out in this thesis in oder to study of the complexation of deboroaplasmomycin C (**135**) with different boron sources but failed mainly due to the poor solubility of deboroaplsmomycin C in water.

5.6 Marine-derived Actinomycete 7617

The crude extract of the marine actinomycete Act 7617 showed in the biological screening a moderate antibacterial activity against *Bacillus subtilis, Escherichia coli*, and *Mucor miehei*. Six days old cultures on agar were used to inoculate a 25 L-fermentation (Erlenmeyer flasks). TLC of the crude extract exhibited three at 254 nm absorbing main bands, which on spraying with anisaldehyde/sulphuric acid became brown. The strain was worked up following the scheme below.

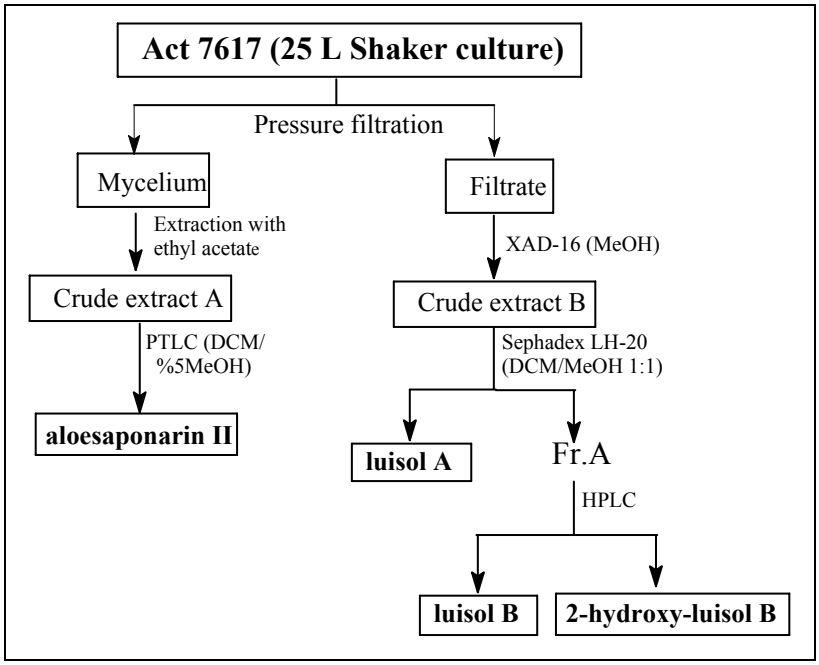


Figure 169: The work-up of the strain Act 7617

5.6.1 Luisol A

The major compound **137** was isolated as colourless amorphous solid and showed a brown colouration on spraying with anisaldehyde/sulphuric acid. In the ^{1}H NMR spectrum, a characteristic ABC pattern composed of a triplet at δ 7.20 and two doublets at δ 6.84 were observed and assigned to aromatic ring protons. Four signals of exchangeable protons at δ 9.60, 5.60, 5.60 and 5.20 were observed and assigned to phenolic and aliphatic hydroxyl groups. Further three methine protons connected to oxygen were at δ 4.60, 4.26 and 4.36, the aliphatic region indicated the presence of a CH_3-CH-fragment characterised by a quartet and a doublet at δ 3.66 and 1.26, re-

spectively, two methylene protons appeared at δ 2.90 (2H) and δ 2.70 (1H), 1.68 (1H).

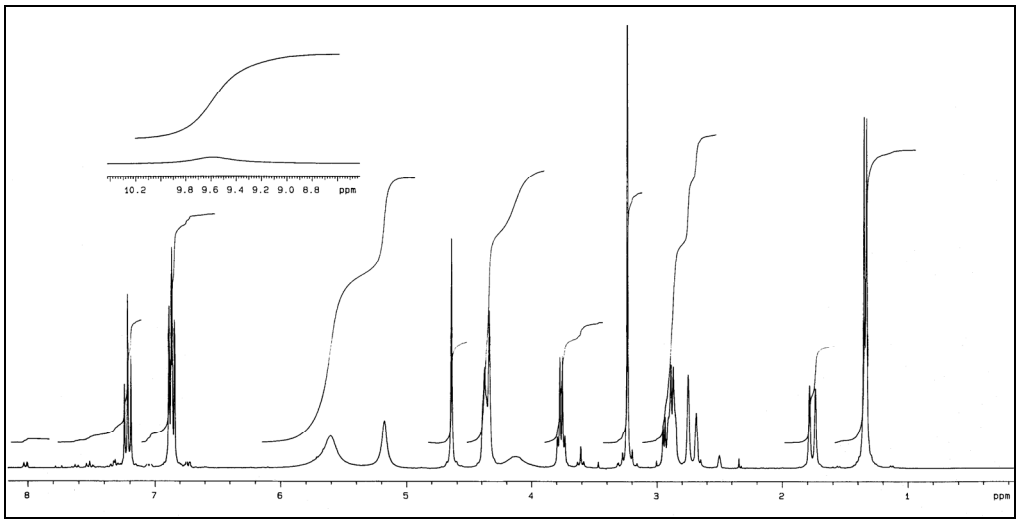


Figure 170 : ^{1}H NMR spectrum (300 MHz) luisol A (**137**) in [D_6]DMSO

The ^{13}C NMR spectrum indicated 16 signals of four quaternary aromatic carbons, three aromatic methines, two quaternary aliphatic and four aliphatic methines, two methylenes and one methine carbon. The ESI mass spectrum indicated a *pseudo*-molecular ion at *m/z* 345 Dalton. The search in AntiBase following the scheme given in Figure 172 delivered luisol A (**137**).

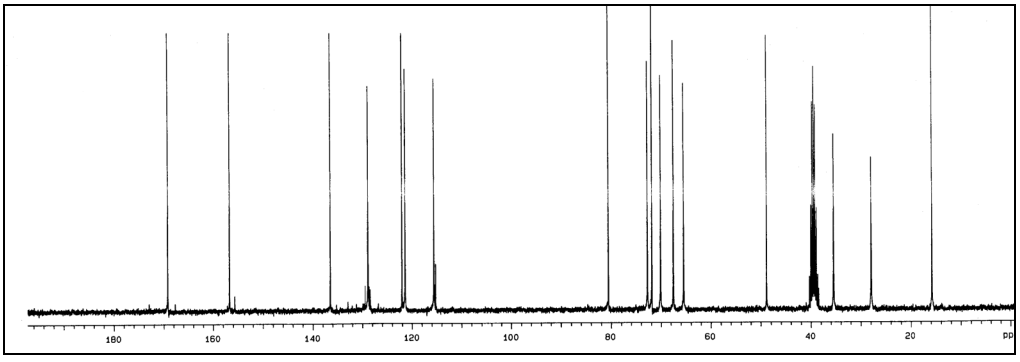


Figure 171: ^{13}C NMR spectrum (300 MHz) of luisol A (**137**) in [D_6]DMSO

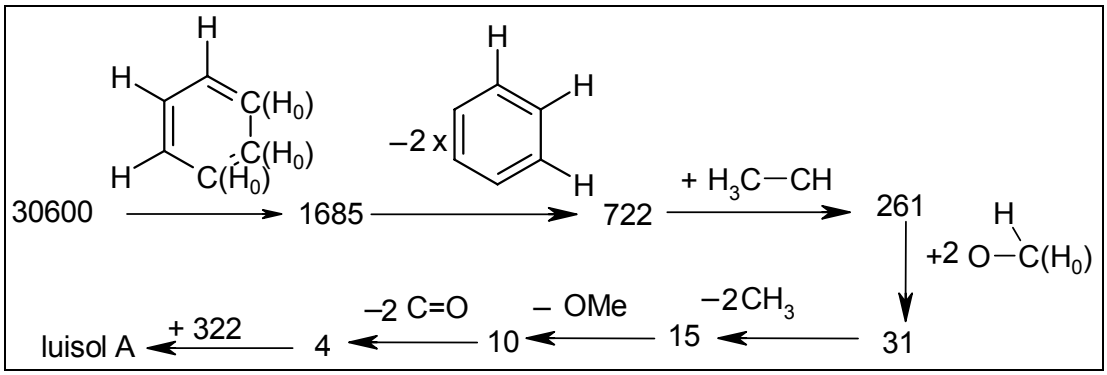
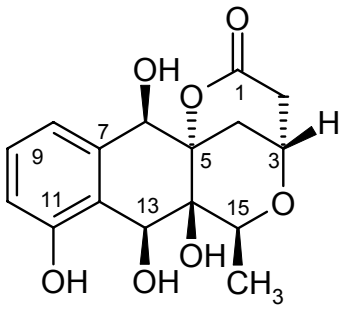


Figure 172: Substructure search of luisol A (**137**) in AntiBase



137

Luisol A (**137**) is a tetraol previously isolated from a marine bacterium by Fenical *et al.*[248]

5.6.2 Luisol B

Compound **138** was obtained as a colourless gum with similar chemical properties as **137**. The ^{1}H NMR spectrum in [D_6]DMSO exhibited in the aromatic region signals due to three consecutive protons. Here too, signals of oxygenated methine protons appeared at δ 5.60 (s) and 4.90 (s). The aliphatic region indicated very few signals, two 1H doublets attributed to a methylene group connected to oxygen at δ 4.18 and 3.86 and finally a methyl singlet at δ 1.50. The molecular weight of 266 Dalton was deduced from the ESI mass spectrum. A similar search as for luisol A (**137**) delivered luisol B (**138**).

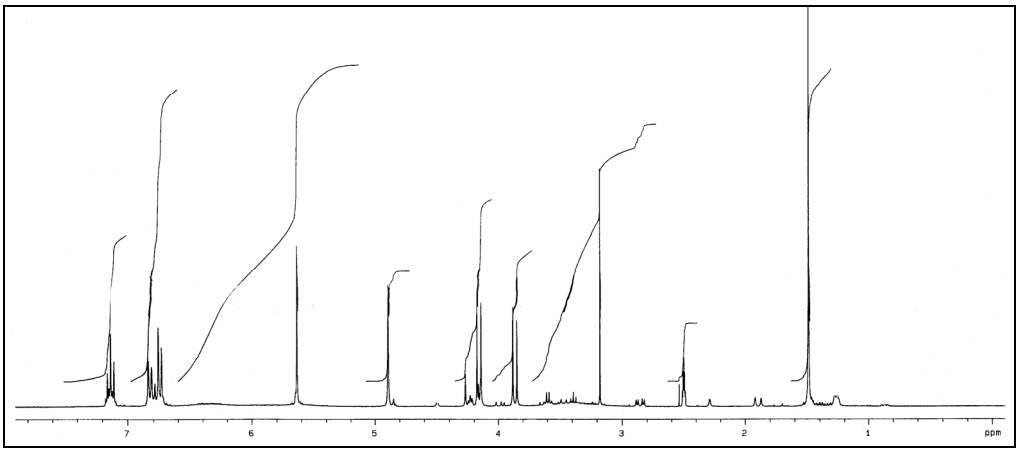
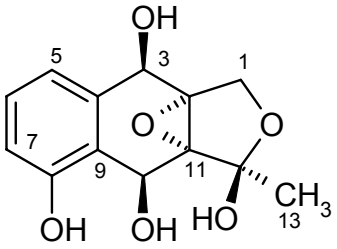


Figure 173: ^{1}H NMR spectrum (300 MHz) of luisol B (**138**) in [D_6]DMSO



138

5.6.3 2-Hydroxyluisol A

Compound **139** was also obtained as colourless gum. The molecular weight of 338 was deduced from the *pseudo* molecular ion at *m/z* 361 $[M+Na]^+$. ESI HRMS delivered *m/z* 361.08940 for the $[M+Na]^+$, corresponding to the molecular formula $C_{16}H_{18}O_8$. The ^{1}H NMR spectrum of **139** was very similar to that of **137** with signals of a 1,2,3-trisubstituted aromatic ring. As in **137**, signals of a CH_3CH-O-fragment, of a methylene group and of four oxygenated methine protons instead of three as in **137** were evident in the aliphatic region.

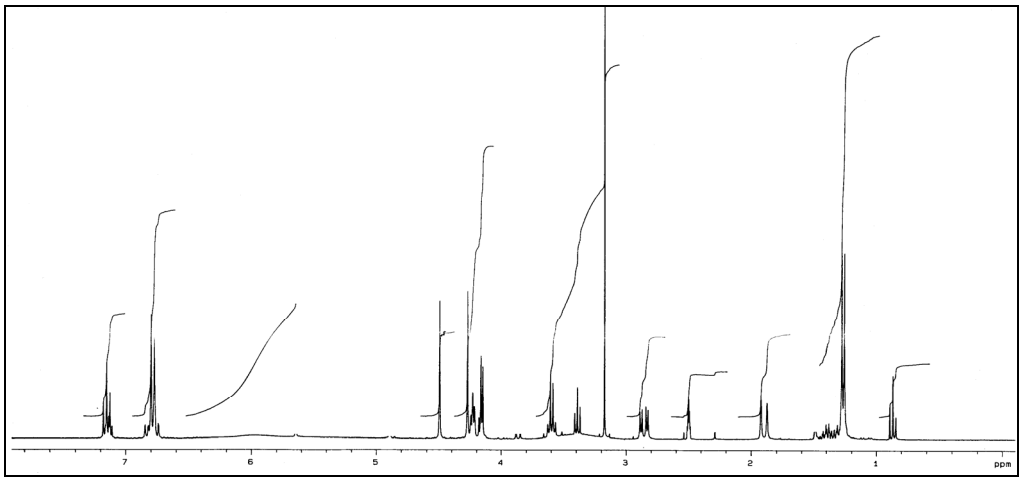


Figure 174: ^{1}H NMR spectrum (300 MHz) of 2-hydroxyluisol B (**139**) in $[D_6]$DMSO

Comparison of the ^{1}H and ^{13}C NMR data (Table 7), the mass difference of Δm = 18 as well as the COSY spectra of **139** and **137** revealed that compound **139** had exactly the same skeleton as **137** but with one proton of the methylene group substituted by a hydroxy group. The relative stereochemistry of **139** at the novel chiral carbon was done by a NOESY spectrum, which indicated a correlations between 13-H/15-H and between 2-H and 3-H, and because **139** is derived from luisol A (**137**), the other stereocenter were supposed to be the same.

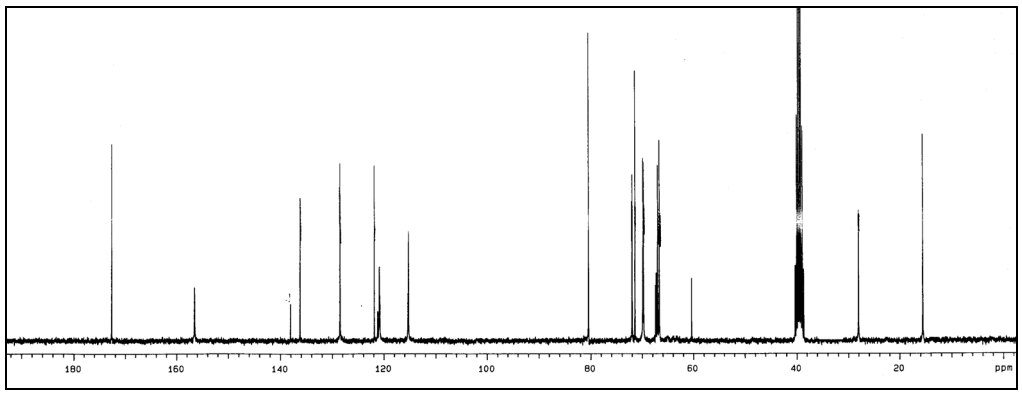
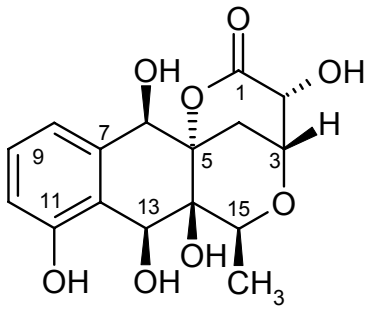


Figure 175: ^{13}C NMR spectrum (300 MHz) of 2-hydroxyluisol B (**139**) in $[D_6]$DMSO

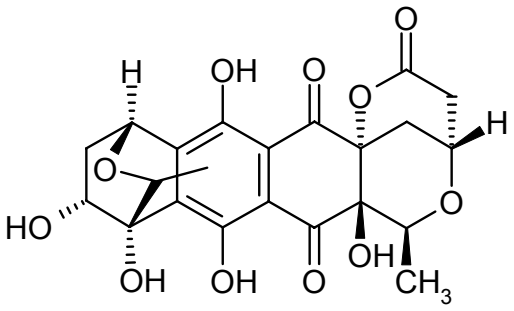


139

Table 7: ^{1}H (300 MHz) and ^{13}C (75.5 MHz) NMR data of luisol A (**137**) and 2-hydroxyluisol A (**139**) in [D_6]DMSO (δ; *J* in Hz)

	luisol A (**137**)		2-hydroxyluisol A (**139**)	
C No.	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	169.2	-	172.7	-
2	35.5	2.71 (d, 19.1) 2.88 (dd, 5, 19)	66.7	4.18 (d, 4.7)
3	65.5	4.38 (m)	69.8	4.23 (t, 4.23)
4α	28.0	1.71 (d, 13.6)	28.1	1.90 (d, 13.7)
4β		2.94 (dd, 5, 13.0)		2.85 (dd, 5, 14.3)
5	80.6	-	80.5	-
6	72.8	4.34 (s)	72.0	4.30 (s)
7	136.5	-	136.3	-
8	121.4	6.85 (d, 7.6)	121.2	6.80 (m)
9	128.9	7.21 (t, 7.8)	128.5	7.15 (t, 7.78)
10	115.6	6.88 (d, 7.6)	115.3	6.76 (m)
11	156.7	-	156.5	-
12	122.1	-	121.9	-
13	67.5	4.63 (s)	67.0	4.50 (s)
14	71.9	-	71.4	-
15	70.1	3.76 (q, 6.1)	69.9	3.60 (q, 6.1)
16	15.9	1.34 (d, 6.1)	15.6	1.27 (d, 6.1)
OH	-	9.60 (br s)	-	-
OH	-	5.60 (br s)	-	-
OH	-	5.15 (br s)	-	-
OH	-	4.15 (br s)	-	-

Luisol A (**137**), B (**138**) and 2-hydroxyluisol A (**139**) are tetrols, formally reduced hydroquinones, which are related to the granaticin group, especially to granaticin C (**140**) and particularly to nanomycin,[249] MM44785[250] and kalafungin;[251] they possess the same hydroxytetrahydropyran ring C and the lactone functionalities. Luisol B (**138**) has the rare epoxy-naphtho[2,3c]furan ring system[248] and is related to anthrinone.[252]



140

6 Plant Metabolites

The persistent use of chemical termiticides is at present of environmental concern and has resulted in the need for plant-derived compounds as alternatives in termite control.[253] Reports published decades ago had revealed that several wood species are naturally resistant to termite infestation, but only a limited number of them had been chemically examined.[254] Following this assumption and in addition to the described work on the isolation and identification of microbial secondary metabolites, we received six pure compounds isolated in Prof. Ganapaty´s group in India from the chloroform extract of the roots of *Diospyros sylvatica*.

6.1 *Diospyros sylvatica* (Ebenaceae)

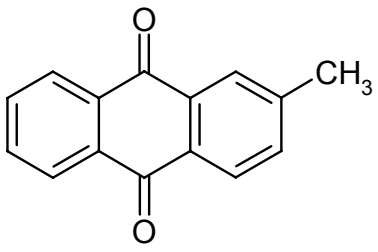
The family of *Ebenaceae* consists of only three genera, of these, the genus *Diospyros* is with 500 species by far the largest.[255] This genus, of which 24 species are native to India, is widespread in the tropics and the warm temperate regions of the world. *Diospyros sylvatica*, also known locally as *gatha* is a moderate-sized tree distributed in the hills of Vizianagram and neighbouring Orissa state.[256]

A number of *Diospyros* sp. is used in herbal medicine for the treatment of whooping cough, leprosy, dysentery, menstrual troubles, abdominal pains and as antibiotic.[257] Chemical examination of a number of species has been done. They reavealed that the stem and leaves of this genus contain triterpenoids, while the roots are well known to contain naphthols and naphthoquinones.[258]

The compounds isolated by Ganapaty were identified using spectroscopic methods as 2-methyl-anthraquinone (**141**), plumbagin (**142**), diosindigo A (**143**), diospyrin (**144**), isodiospyrin (**145**), and microphyllone (**146**). Among these compounds, **141**, **145**, **146** were found to be the major termiticidal components.

6.1.1 2-Methylanthraquinone

The ^{1}H NMR spectrum of the yellow compound **141** contained very few signals and showed in the aliphatic region only the presence of an aromatic bound methyl group by a singlet at δ 2.52. The remaining 2H multiplets at ~δ 8.2 and 7.8 were assigned to an *o*-disubstituted benzene ring, and signals of a 1,2,4-trisubstituted benzene ring gave a typical pattern at δ 8.20 (d, 9.0 Hz), 8.10 (d, 2.0 Hz) and 7.60 (dd). EI MS showed a molecular ion at *m/z* 222 and fragment ions 207 (M-15) for the loss of a methyl radical and 194 (M-28) for a C=O group. The IR spectrum showed absorption peaks at 1680 and 1600 cm^{-1} for carbonyl groups and the aromatic system, respectively. The compound was easily identified as 2-methylanthraquinone by comparison with literature data.[259]

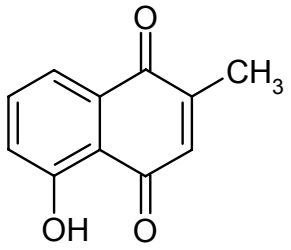


141

6.1.2 Plumbagin

Compound **142** was also obtained as yellow powder with an IR spectrum exhibiting characteristic carbonyl absorptions at 1660 and 1640 cm^{-1}. The ^{1}H NMR spectrum showed a doublet at δ 2.18 (^{4}J = 1.5 Hz) indicating the presence of a C-methyl group

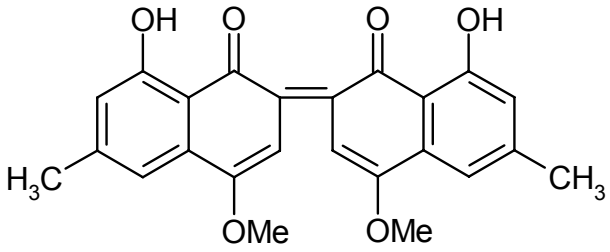
with a small allyl coupling; a quartet at δ 6.80 clearly indicated that the methyl substituent was at C-2 in a naphthoquinone derivative. In addition, signals of a 1,2,3-trisubstituted aromatic system and a chelated hydroxy group at δ 11.98 were present. The EI mass spectrum showed the molecular ion at 188 Dalton and fragment ions at 173 (M-15) and 160 for the loss of C=O. The compound was easily identified as plumbagin (**142**) by comparison with an authentic sample from our collection. Plumbagin was also isolated from *Diospyros greeniwayi*[260], *Diospyros maritime*[261] and other *Ebonaceae*.



142

6.1.3 Diosindigo A

Compound **143** was obtained as blue needles, and found to be sparingly soluble in chloroform, ethanol and other organic solvents. The little amount did not allow NMR-spectroscopic measurements, however, as blue pigments are very rare in plants, only few chromophores had to be taken into account. TLC was done with authentic samples of diosindigos A (**143**) and B from our collection, and the sample clearly identified as **143** by the same R_f values and the origin.[262]

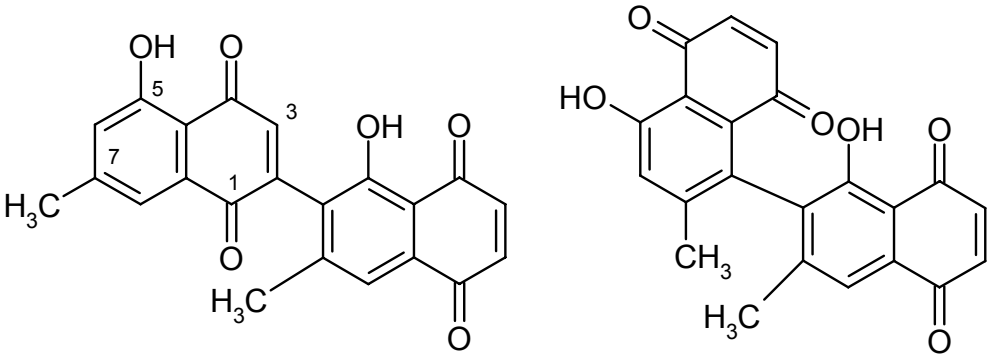


143

6.1.4 Diospyrin and Isodiospyrin

Compound **144** formed orange needles and gave a purple colour with alkali, indicating the presence of a *peri*-hydroxy-quinone. The IR spectrum showed similar absorp-

tions as in **141** and **142** confirming the presence of carbonyl groups. The ^{1}H NMR spectra exhibited in the aliphatic region only two signals of aromatic bound methyl groups. The presence of two chelated hydroxyl protons was supported by signals at δ 12.18 and δ 11.90. Furthermore, three singlets of four protons and two doublets showing *meta* coupling (^{4}J = 1.0 Hz) were present in the aromatic region. The EI MS showed a molecular ion at *m/z* 374 and also fragment ions 359 (M-15) and 346 (M-28). The search in Dictionary of Natural Products delivered two isomeric structures, diospyrin (**144**) and isodiospyrin (**145**).



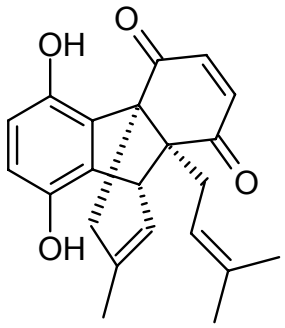
144 **145**

The *meta*-coupled signals and the presence of only three quinonoid protons clearly indicated diospyrin (**144**), which was further confirmed by comparison with the literature data.[263,264] The second compound was easily identified as isodiospyrin (**145**). Both dimers, diospyrin (**144**) and isodiospyrin (**145**) appeared as orange-brown spots in day-light on silica gel; under UV light, diospyrin was orange-red, isodiospyrin dark brown.

Diospyrin **(144)** and isodiospyrin (**145**) are members of a group of more than 90 dimeric naphthoquinones. Such bis-naphthoquinones may arise either by the oxidation of a bisnaphthol formed by radical coupling of two naphthol units (phenol oxidation) or by condensation of a naphthoquinone with a naphthol unit and subsequent oxidation (phenol-quinone addition).[265] Diospyrin (**144**) is a common metabolite of *Diospyros* species[263-267] and was firstly isolated by Kapil *et al*.[268]

6.1.5 Microphyllone

The ^{1}H NMR spectrum of the pale yellow compound **146** indicated 1H doublets at δ 6.47 and 6.34 attributed to a 1,2,3,4-tetrasubstituted benzene, and AB signals at δ 6.73 and 6.43, which can be attributed to quinonoid protons. Two methylene groups appeared as AB and ABX systems. Three methyl groups were connected to double bonds, and at least three protons were appearing as multiplets in the range of δ 4.90-5.85. The ^{13}C NMR spectrum indicated the presence of 22 carbon signals, which were assigned by the APT to two ketone groups, two methylenes, an aliphatic methine, three methyls, six sp^2 methines, and 8 quaternary carbons. The EI mass spectrum indicated the molecular peak at *m/z* 350 Dalton. A search in the Dictionary of Natural Products delivered microphyllone (**146**), a quinone, which was previously isolated from *Ehretia microphylla*.[269]



146

6.2 *Rheum palmatum* (Polygonaceae)

Rheum palmatum is an herbaceous plant belonging to the family of *Polygonaceae*. This family is distributed worldwide in temperate regions except in Africa[270] and commonly comprises herbs, shrubs and rarely trees with about 30 genera and 1000 species. The antiviral activity revealed by the crude extract of the root, which has not been reported for this plant, drew our attention.

6.2.1 Description of *Rheum palmatum*

Rheum palmatum known also as turkey rhubarb, Chinese rhubarb or East Indian rhubarb is one of the 70 species of the genus *Rheum*, which grows in China, Tibet, Russia, Turkey and India. It is a perennial and herbaceous plant of up to 2-3 m height

with large and rounded half palmate leaves. The lobes are acuminate, dull-green and much wrinkled above. Petioles are pale green, marked with short purple lines, round and obscurely channelled above. The flowers are hermaphrodite containing nine stamens inserted into the base of the calyx, and are pollinated by wind.

6.2.2 Medicinal Use and Utilization of the genus *Rheum*

The genus *Rheum* is one of the world's important crude drugs, and particularly rhubarb is one of the most widely used herbs in Chinese prescriptions for a variety of diseases.[271] Decoctions of the root of rhubarb are used as antichloresterolemic, antiseptic, antitumor, aperient, astringent, demulcent, diuretic, laxative, purgative, stomachic and tonic drugs.[272] It is also taken internally in the treatment of chronic constipation, diarrhoea, liver and bladder complaints, haemorrhoids or menstrual problems and skin eruptions due to an accumulation of toxins.[273] The plant is also part of a Nord American formula called Essiac, which is a popular treatment for cancer.[274] The root of rhubarb is not prescribed as a remedy for pregnant and lactating women and for patients with intestinal obstructions. Leaves are poisonous and are used as insecticide.[273] In India and Nepal *R. emodi* is used as stomachic, bitter tonic, cathartic and as a purgative principle.[275] The roots of *R. maximowiczii* are used in central Asia and Uzbekistan as a purgative and for the treatment of stomach disorders.[271] The leaf powder of *R. palaestinum* is used in Syria as a laxative.[276] It is also used because of its anti-leukaemic and antifungal activities. In Europe *Rheum palmtatum* is used as a purgative, as an appetite stimulant, for blood stasis, hypertension, and against mental and renal disorders.[277]

The genus *Rheum* has been extensively studied for its secondary metabolite composition. A variety of phenolic compounds such as: anthraquinones,[275] naphthalenes,[278] stilbenes,[278], flavonoids,[278] procyanidins[277] and tannins[277] have been reported.

6.2.3 Isolation and Identification of compounds

The crude extract of the roots of *R. palmatum* was chromatographed as outlined in Figure 176.

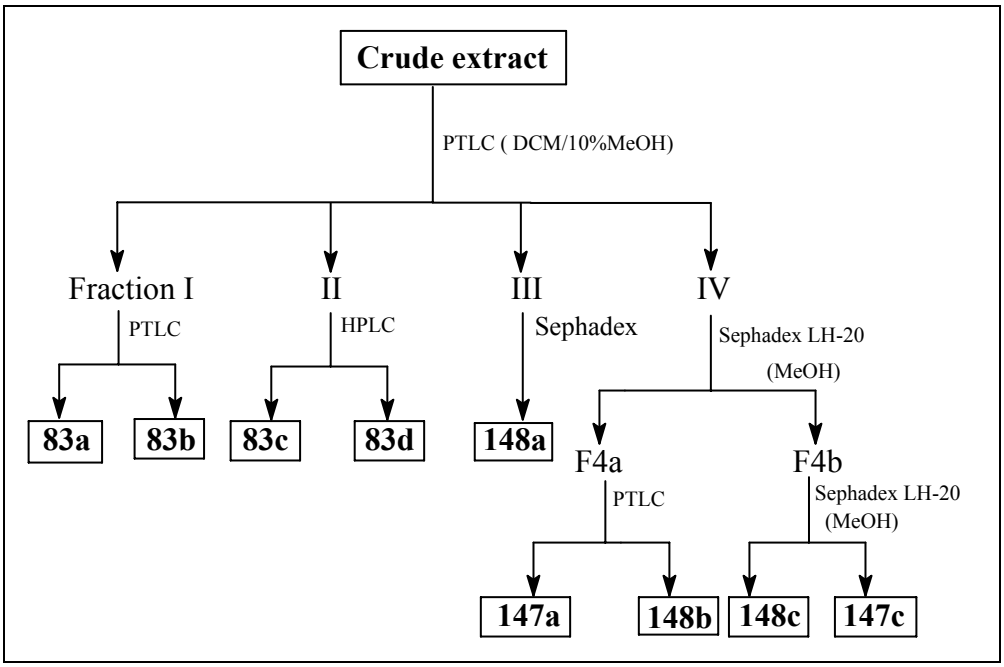
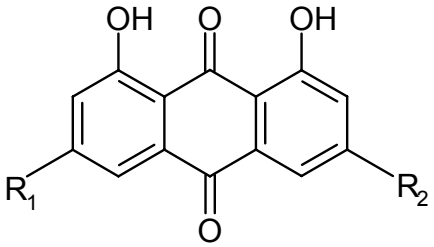


Figure 176: Isolation scheme of *R. palmatum*.

6.2.4 Chrysophanol

Purification of fraction I by PTLC using cyclohexane/ethyl acetate gave pure RP1 (**83a**) as a yellow solid. The yellow spot on TLC remained unchanged on spraying with anisaldehyde/sulphuric acid, but diluted sodium hydroxide solution gave a violet colour reaction characteristic of *peri*-hydroxy-anthraquinones.[279] The ^{1}H NMR spectrum was similar to that of **83a** obtained from the terrestrial *Streptomyces* sp. GW32/698. The EI mass spectrum indicated the molecular weight to be *m/z* 254. The compound RP1 was easily identified as chrysophanol (**83a**).



83a: $R_1 = H$ $R_2 = CH_3$
83b: $R_1 = OCH_3$ $R_2 = CH_3$
83c: $R_1 = H$ $R_2 = CH_2OH$
83d: $R_1 = OH$ $R_2 = CH_3$

6.2.5 Physcion

The compound RP2 (**83b**) was also obtained as yellow solid and gave a characteristic colour reaction of *peri*-hydroxy quinones with diluted alkali solution. The EI mass spectrum indicated the molecular ion at *m/z* 284. The ^{1}H NMR spectrum of **83b** was similar to that of **83a** with two chelated hydroxyl groups at δ 12.32 and 12.12 and an aromatic methyl group at δ 2.43. However, **83b** exhibited only four aromatic protons, two broad singlets at δ 7.62 and 7.08 and two doublets at δ 6.68 and 7.36. In addition, a methyl singlet was observed at δ 3.94, which can be attributed to a methoxy group.

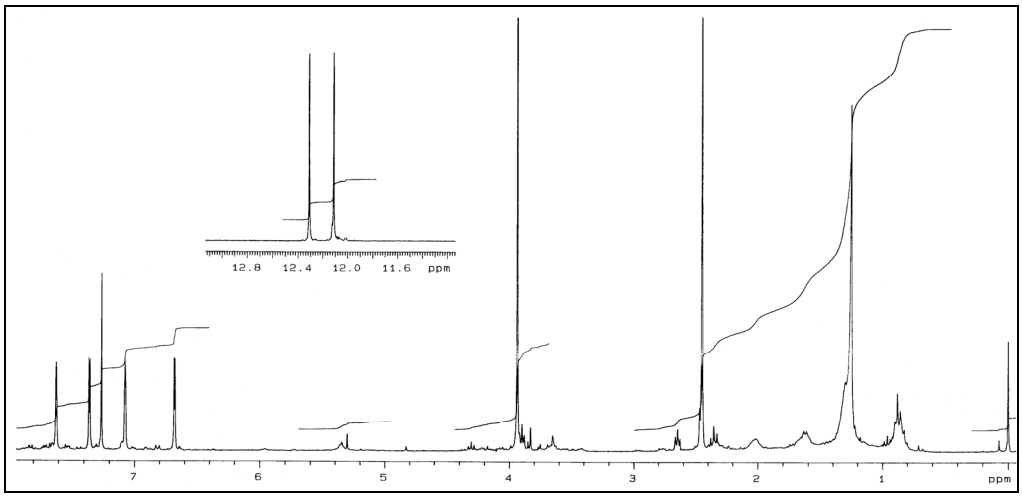


Figure 177: ^{1}H NMR spectrum (300 MHz) of physcion (**83b**) in $CDCl_3$

All the spectroscopic data revealed compound **83b** to be physcion (1,8-dihydroxy-3-methyl-6-methoxyanthraquinone), which has been reported from *Rhamus pallasii*

and other sources; it was confirmed by comparison of the NMR data with the literature.[280]

6.2.6 Aloe-emodin

The orange solid RP5 (**83c**) obtained by purification of fraction II by HPLC depicted similar physical characteristics as chrysophanol (**83a**), and also the ^{1}H NMR spectrum exhibited similarities with that of **83a**. Here also two chelated hydroxyl groups and five aromatic proton signals were observed, the only difference being the methyl signal of **83a**, which was replaced by an oxygenated methylene signal at δ 4.60. With the help of EI MS, the molecular weight *m/z* 270 was determined. The compound was finally identified as aloe-emodin (**83c**) and proved by comparison of the NMR data with the literature.[281]

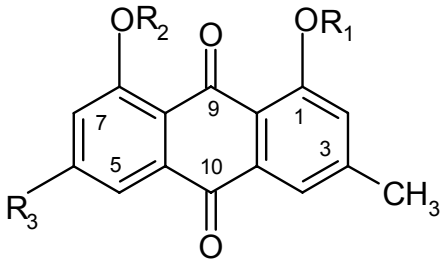
6.2.7 Emodin

The EI mass spectrum of the orange solid RP6 (**83d**) indicated a molecular weight of *m/z* 270, which is identical to that of aloe-emodin (**83c**). The ^{1}H NMR spectrum of **83d** was similar to that of physcion (**83b**), however, the methoxy signal of **83b** was missing. The search in AntiBase with the spectroscopic data led to emodin (**83d**) and the structure was confirmed by comparison of the NMR data with the literature.[282, 283]

6.2.8 Palmatin

The yellow spot of compound Rpa (**147a**) showed strong absorption at 254 nm on TLC and gave a violet colour reation with sodium hydroxide. The aromatic ABC signals, the AB signals at δ 7.72 and 7.56 and an aromatic methyl singlet at δ 2.50 in the ^{1}H NMR spectrum indicated a chrysophanol chromophore as in aloe-emodin (**83c**). However, in **147a** only one chelated OH signal appeared at δ 12.90, which indicated that one of the chelated OH groups in chrysophanol (**83a**) must have been substituted. Signals in the range of δ 5.18-3.20 can be assigned to a sugar moiety, so that Rpa was probably a glycoside. The molecular weight of 416 Dalton was deduced from the ESI and EI mass spectra. The base peak at *m/z* 254 in the EI mass spectrum indicated the [M-sugar] fragment and confirmed the chrysophanol (**83a**) chromophore. The search in Dictionary of Natural Products with the NMR and mass data

gave the 1-*O*-glycoside palmatin (**147a**) and the 8-*O*-glycoside of chrysophanol, chrysophanein (**147b**) as possible structures, which were both reported from *Rheum palmatum*[284]. Comparison of the measured ^{1}H NMR data with the literature values of **147a** and **147b** identified the compound under investigation as palmatin (**147a**). The glycoside **147a** is reported to possess moderate cytotoxicity against HeLa epithelioid and BT-20 human breast carcinoma cells.[284]



147a: R_1 = Glc R_2 = H R_3 =H
147b: R_1 = H R_2 = Glc R_3 = H
147c: R_1 = Glc R_2 = H R_3 = OH

6.2.9 Emodin-1-*O*-ß-D-glucopyranoside

The ESI mass spectrum of the yellow solid RP13 (**147c**) obtained from the fraction 4b indicated the *quasi*-molecular signal at *m/z* 455 $[M+Na]^+$. The aromatic region of the ^{1}H NMR spectrum depicted much similarity to the respective part of emodin (**83d**) and showed an aromatic methyl group as in **83d**. The difference lied, however, in the absence of one of the two chelated OH-signals of **83d** and additional signals between δ 3.80-3.10 and a further doublet of doublet at δ 4.68. The additional signals indicated the presence of a sugar moiety as in **147a**.

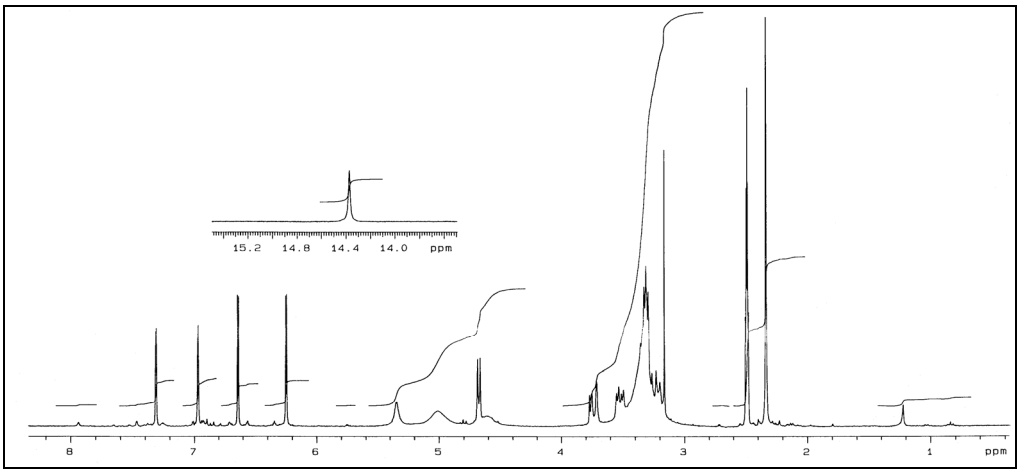


Figure 178: ^{1}H NMR spectrum (300 MHz) of anthraquinone **147c** in [D_6]DMSO

The presence of the base peak in the EI mass spectrum at *m/z* 270 indicated the aglycone to be emodin. Compound **147c** was identified as emodin-1-*O*-ß-D-glucoside previously isolated from *Picramnia teapensis*.[285] It is reported to inhibit the growth of *Leucoagaricus gongilophorus*.

Anthraquinones are common secondary metabolites. They are widely distributed in many families of plants. There are two main pathways leading to anthraquinones in higher plants: the polyketide pathway and the chorismate/*o*-succinylbenzoic acid pathway, the latter is derived from shikimic acid. Anthraquinones are reported to possess antiviral,[286] molluscicidal and antioxidant activities. Physcion (**83b**), aloe-emodin (**83c**) and chrysophanol (**83a**) isolated from *Rheum emodi* rhizomes exhibited antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* (MIC 25-250 µg/ml).[287]

6.2.10 Rhapontigenin

Size exclusion chromatography on Sephadex LH-20 of fraction F5 yielded compound RP7 (**148a**) as colourless solid, which showed a blue fluorescence under 366 nm. The ^{1}H NMR spectrum exhibited signals of an aromatic methoxy group at δ 3.80 and two protons of an isolated *trans*-double bond at δ 6.90 (^{3}J = 16.0 Hz) and 7.00 (^{3}J = 16.0 Hz). It also contained two sets of each three aromatic protons for two isolated benzene rings; the first set of an ABX-signal at δ 6.92 (d, J = 8 Hz), 6.98 (^{3}J = 8, 2 Hz) and 7.09 (d, ^{3}J = 2.0 Hz) can be assigned to an 1,3,4-trisubstituted aromatic

ring, while the other AX_2 signal belongs to an 1,3,5-trisubstituted ring system. Additionally an acidic proton signal was observed at δ 8.20.

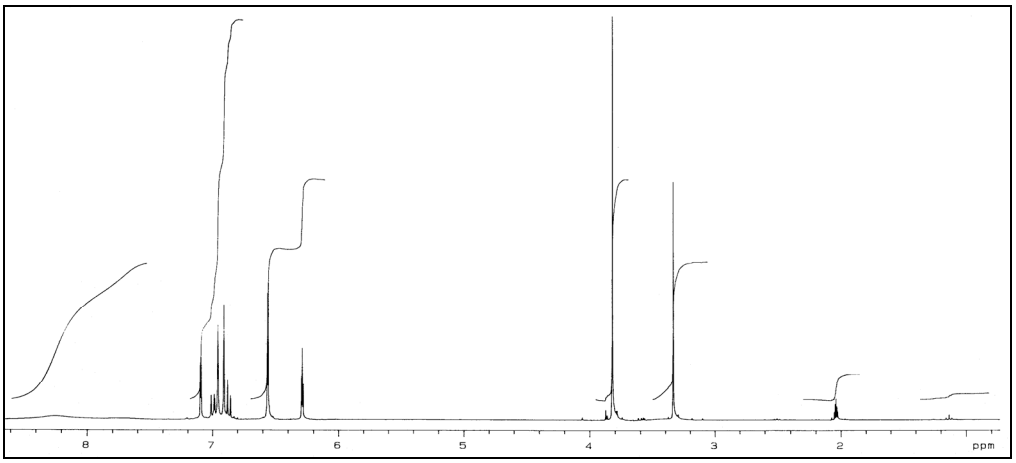


Figure 179: ^{1}H NMR spectrum (300 MHz) of rhapontigenin (**148a**) in [D_6]acetone

EI HRMS of the molecular ion at *m/z* 258 delivered the molecular formula $C_{15}H_{14}O_4$. The ^{13}C and APT NMR spectra consisted of 15 signals representing 14 sp^2 and one sp^3 carbon atoms as demanded by its molecular formula and ^{1}H NMR data.

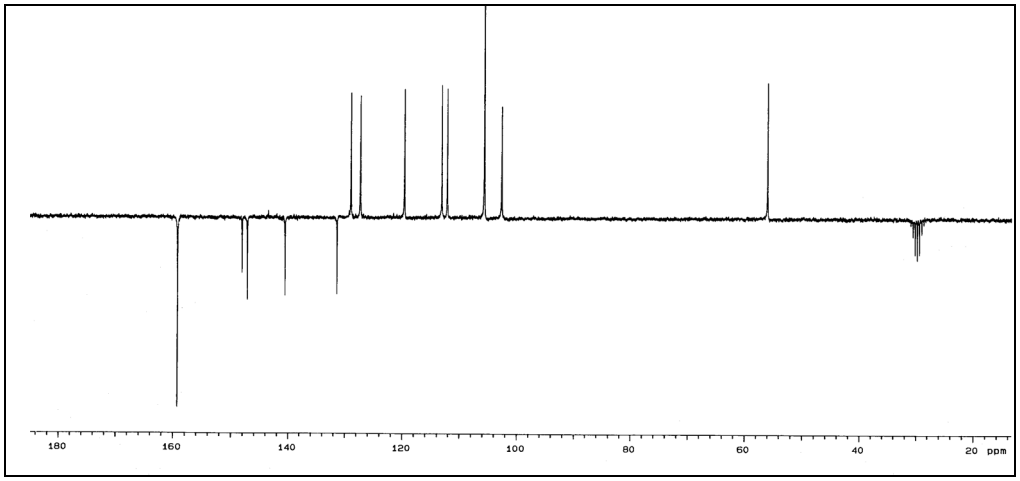
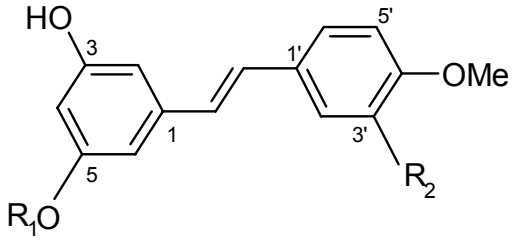


Figure 180: APT NMR spectrum (50.3 MHz) of rhapontigenin (**148a**) in [D_6]acetone

A search in the Dictionary of Natural Products with the NMR-derived substructure and the molecular formula identified compound RP7 as rhapontigenin (**148a**), which had been isolated previously also from rhubarb.[278]



148a: R_1 = H R_2 = OH
148b: R_1 = Glc R_2 = H
148c: R_1 = Glc R_2 = OH

Rhapontigenin (**148a**) and other synthetic *trans*-stilbene derivatives are reported as inhibitors of human cytochrome P450 1B1, they also suppress the growth of human mammary tumour cells and substituted stilbenes may be useful in preventing cancer caused by estrogens and xenobiotics.[288] Stilbenes are also produced by several plants in response to pathogen attacks.[289] In addition to their role as phytoalexins, also antifungal, antibacterial and cytotoxic activities have been reported.[290]

6.2.11 Desoxyrhaponticin

The colourless compound RP10 (**148b**) obtained from fraction F4a showed a molecular peak at *m/z* 404 in the Electron Impact mass spectrum. It depicted similar TLC characteristics like blue fluorescence under 366 nm and black colouration with anisaldehyde/sulphuric acid as rapontigenin (**148a**), which indicated it probably also to contain a stilbene skeleton.

The ^{1}H NMR spectrum resembled that of rhapontigenin (**148a**), however, the ABX system in **148a** was replaced by an A_2B_2-system with signals at δ 6.92 (^3J = 8.0 Hz) and 7.50. The anomeric 1H doublet at δ 4.90 (^3J = 7.0 Hz) and the signals between δ 3.18 - 3.80 displayed the presence of a sugar moiety in **148b**.

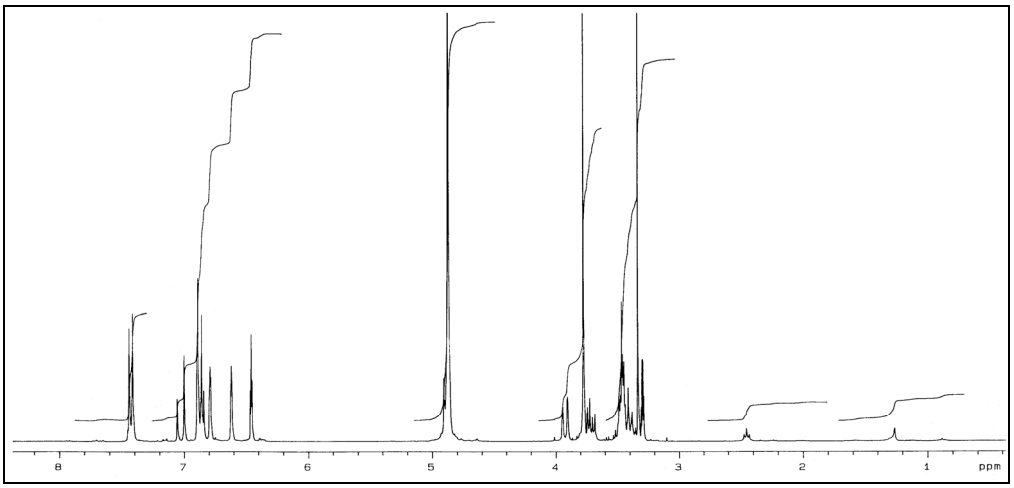


Figure 181: ^{1}H NMR spectrum (300 MHz) of deoxyrhaponticin (**148b**) in $[D_4]$MeOH

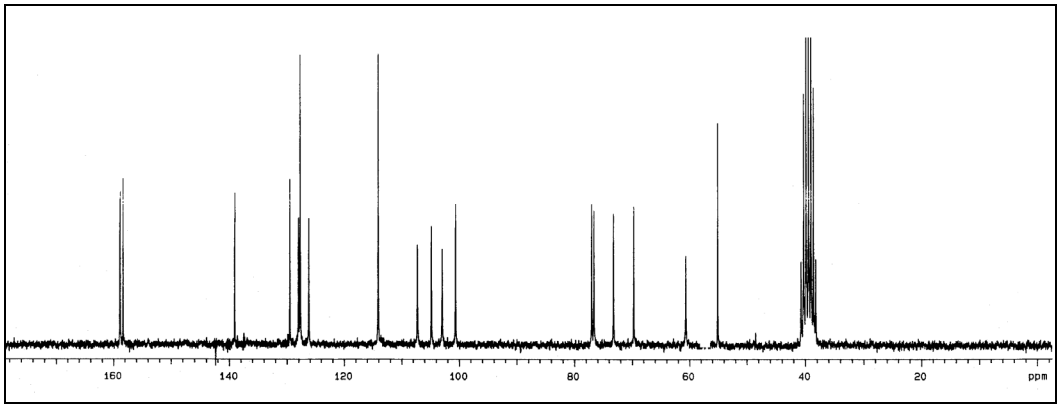


Figure 182: ^{13}C NMR spectrum (50.30 MHz) of deoxyrhaponticin (**148b**) in $[D_4]$MeOH

With the help of the ^{13}C and ^{1}H NMR data, the sugar moiety was identified as glucose with an anomeric carbon signal at δ 100.8. The search in the Dictionary of Natural Products supported by the NMR and MS data led to deoxyrhaponticin (**148b**) as a possible structure, which was confirmed by comparison of the physical and spectral data with the literature.[291]

6.2.12 Rhapontigenin 3-O-ß-D-glucopyranoside (Rhaponticin)

The ^{1}H NMR spectrum of RP11 (**148c**) showed a methoxy group, a *trans*-disubstituted double bond and a 1,3,4- and a 1,3,5-trisubstituted aromatic ring and was related to those of rhapontigenin (**148a**) and desoxyrhaponticin (**148b**). It also

indicated the presence of a sugar moiety as in desoxyrhaponticin (**148b**). The EI mass spectrum showed a molecular signal at *m/z* 420. The difference of the molecular weights by Δm = 16 between **148c** and desoxyrhaponticin (**148b**) indicated that the former could be a hydroxy derivative of the latter. The presence of a sugar moiety was confirmed by the fragment *m/z* 258 [M-sugar] on EI MS. The compound **148c** was identified as rhaponticin previously isolated from *Erythrophleum lasianthum*;[292] rhaponticin shows significant inhibitory effect on platelet aggregation.

6.3 *Canarium schweinfurthii* (Burseraceae)

6.3.1 Generality on genus C*anarium*

The family of *Burseraceae* with 18 genera and 550 species is widespread in the tropical region mainly in Malaysia, South America and Africa. It constitutes shrubs and medium to large sized trees up to 60 m high.[293]

The genus *Canarium* consists of 77 species, where only *Canarium album, Canarium dentica, Canarium indicum, Canarium muelleri, Canarium zentilacum* and *Canarium schweinfurthii*[294] are found in Africa. Two of the African species (*Canarium madagascariencis* and *Canarium schweinfurthii*) constitute big trees of up to 40 - 60 m high. They possess compound leaves with numerous lateral ribs and a massive cylindrical trunk (Ø 1.5 m), which becomes pale grey to brown and scaly with age.[294] *Canarium schweinfurthii* is widely distributed in Africa and mainly in Cameroon, Congo, Gabon, Ivory cost, Nigeria, Uganda and the Democratic Republic of Congo. In Cameroon it is found in Limbe and the Western Province. It is commonly known as Abel in Cameroon, Aiele in Ivory cost and Elemi in Nigeria. It is popular for its edible fruits (Ø 2 - 2.5 cm), which are greenish white with a rather sticky when young and become black when ripening.[295]

6.3.2 Medicinal Use of *Canarium*

Some of the species of the genus *Canarium* are used in traditional medicine. *Canarium album* is used in Chinese traditional medicine to treat poisoning, diarrhoea and dermatitis.[296] In Cameroon resin of *Canarium schweinfurthii* is burned by local people for illumination. Oil from the seeds is used against poisoning and the pow-

dered resin is used in some traditional rites.[299] In Sri Lanka, the gum of *Canarium zeylanicum* is burned by the peasants for light and was commonly used for fumigation.[297]

6.3.3 Isolation and Structure Elucidation

Not many phytochemical studies have been carried out on the genus *Canarium*. Triterpenoids,[302, 298] aromatic compounds, flavonoids[296] and monoterpenoids[297] have been reported as secondary metabolites.

The seeds and trunk of *Canarium schweinfurthii* were colleted from Yaounde (Cameroon). They were separately extracted with CH_2Cl_2/MeOH and then MeOH after drying and grinding. The chromatographic separation of the extract of the seeds on silica gel delivered four compounds (see Figure 183).

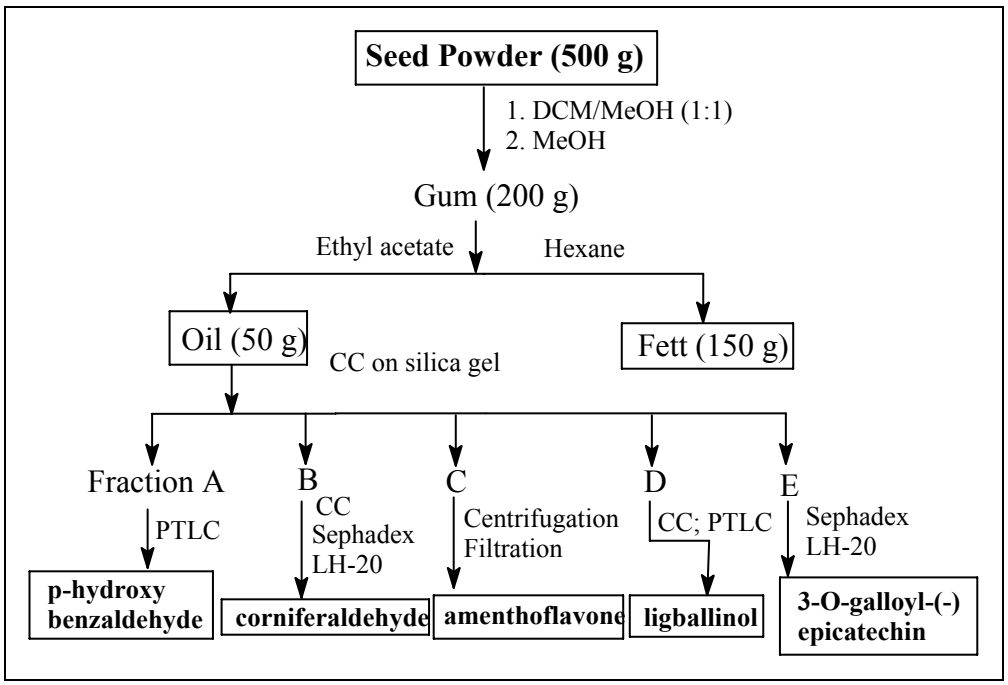


Figure 183: Work-up scheme of the seed powder of *Canarium schweinfurthii*

6.3.4 Amenthoflavone

The yellow powder of **149a** gave a characteristic colour reaction of flavonoids: When 8-9 drops of conc. HCl and magnesium metal were added to **149a**, after 10-12 min at room temperature an orange-red colour of the solution was observed (Shinoda test). The ^{1}H NMR spectrum indicated the presence of 12 aromatic protons. Two

doublets of an A_2B_2 system at δ 7.42 and 6.68 and the set of three proton signals at δ 7.90 (d), 7.80 (dd) and 7.02 (d) can be assigned to a *para*-, and an 1,3,4-trisubstituted aromatic ring. The two 1H signals each with a *meta*-coupling at δ 6.40 and 6.18 belong to a 1,3,4,5-tetrasubstituted benzene ring. Furthermore, a singlet of the intensity 2 at δ 6.50 characteristic for 3-H of flavones, indicated it most probably to be a biflavone. Additionally a 1H singlet was observed at δ 6.30, which can be assigned to 6-H or 8-H of a flavone.

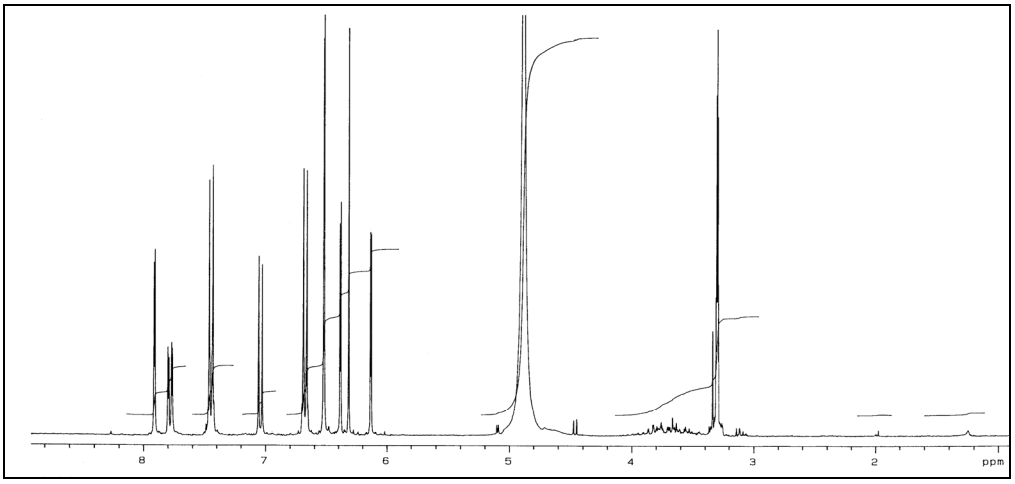


Figure 184: ^{1}H NMR spectrum (300 MHz) of amenthoflavone (**149a**) in $[D_4]$MeOH

The ESI mass spectrum indicated the *pseudo*-molecular peak at *m/z* 561.7 ($[M+Na]^+$), which fixed the molecular weight to 538 Dalton. A search in the Dictionary of Natural Products delivered amenthoflavone (**149a**) and robustaflavone (**149b**), which can explain all the spectroscopic data. The compound was finally identified as amenthoflavone (**149a**) after comparison of the ^{1}H and ^{13}C NMR data with the literature values.[299] Amenthoflavone (**149a**) is reported as a potent anti-inflammatory and anti-ulcer agent.[300]

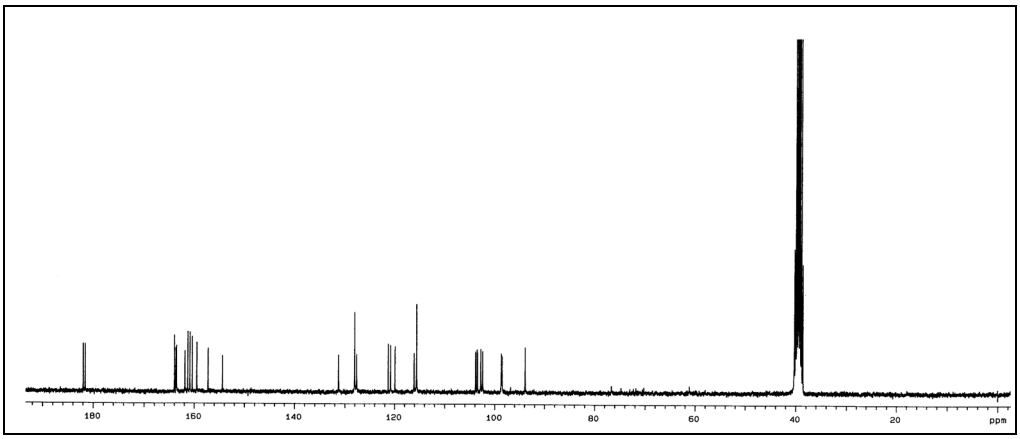
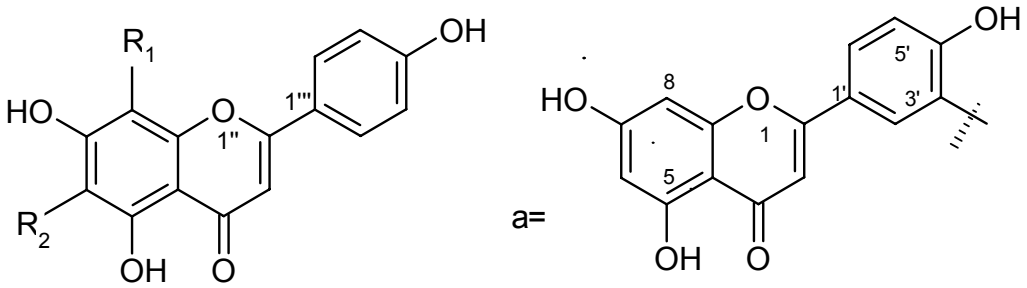


Figure 185: ^{13}C NMR spectrum (75.5 MHz) of amenthoflavone (**149a**) in [D_4]MeOH



149a: R_1 = a R_2 = H
149b: R_1 = H R_2 = a

6.3.5 Ligballinol

Compound **150** was obtained as a colourless solid. The EI mass spectrum indicated the molecular peak at *m/z* 298 and its HRMS gave the molecular formula $C_{18}H_{18}O_4$. In the aromatic region of the ^1H NMR spectrum only two 2H signals appeared as doublets at δ 7.20 and 6.78 representing a *para*-disubstituted benzene ring. The signals in the aliphatic region appeared as four distinct groups, a methine doublet connected to oxygen at δ 4.65, two multiplets at δ 4.20 and 3.10 and a doublet of doublet at δ 3.80. The ^1H NMR spectrum showed signals for only nine protons, which indicates 150 to be a symmetrical dimer in view of its molecular formula.

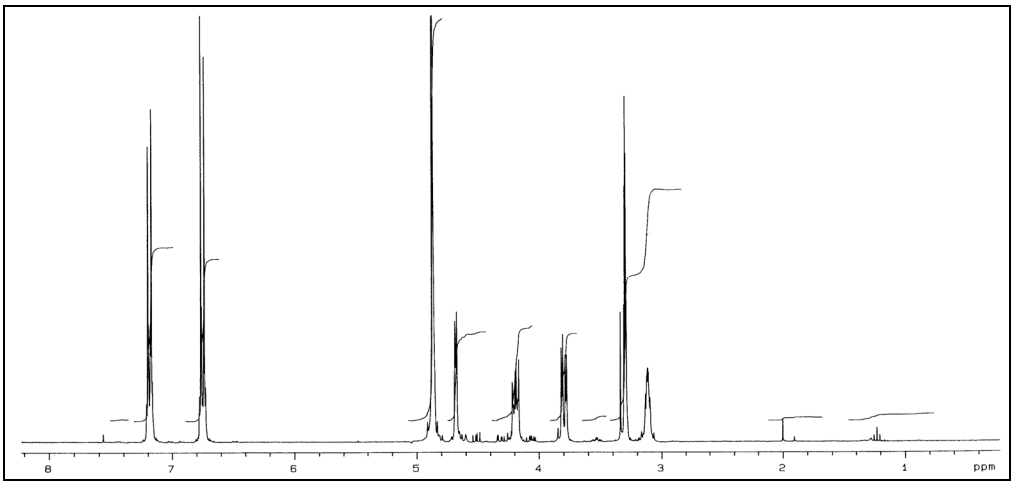
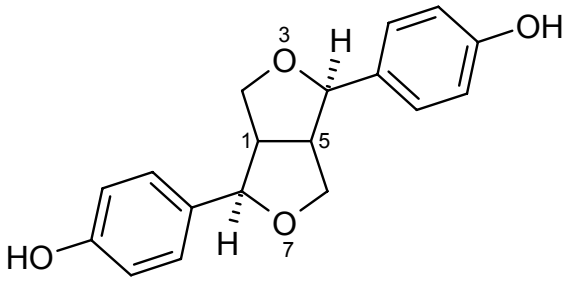


Figure 186: ^{1}H NMR spectrum (300 MHz) of ligballinol (**150**) in [D_4]MeOH

A substructure search was carried out in the Dictionary of Natural Products and compound **150** was identified as ligballinol (**150**).[301] The latter is a bisepoxy lignan and has previously been isolated from *Ecballium elaterium*[301] and was reported as stress metabolite.[302]



150

6.3.6 Coniferaldehyde and *p*-Hydroxybenzaldeyde

Compound **151** was obtained as pale yellow oil from the column chromatography. The ^{1}H NMR spectrum contained a doublet at δ 9.62, which can be assigned to an aldehyde proton. A doublet and a second doublet of doublet of a *trans* double bond at δ 7.39 and 6.59 (J = 15.8, 7.9 Hz) indicated a -CH=CH-CHO moiety. Another significant feature of the ^{1}H spectrum is the presence of a 1,3,4-trisubstituted benzene moiety indicated by the typical signal pattern. An acidic proton signal is seen as a broad singlet at δ 6.18. The aliphatic region exhibited only a methoxy signal at δ 3.94.

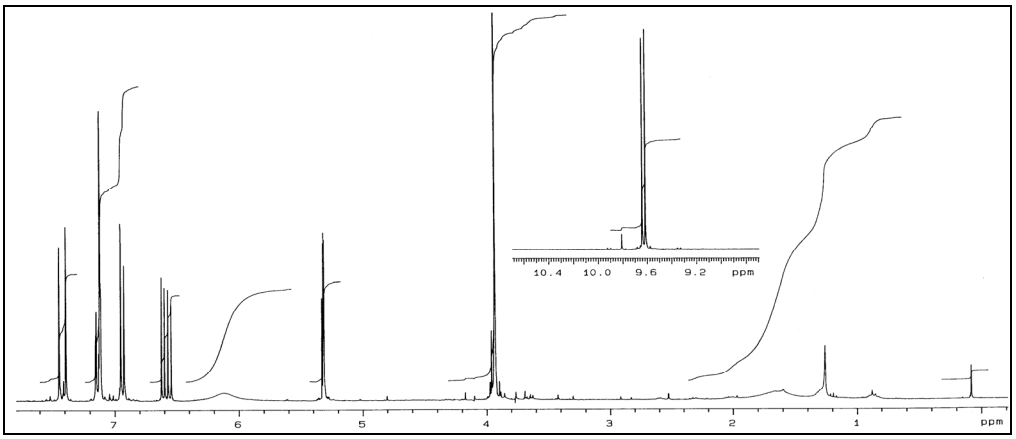
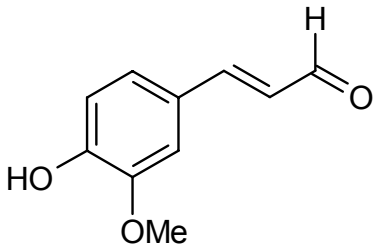


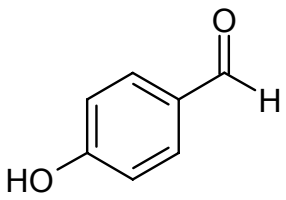
Figure 187: ^{1}H NMR spectrum (300 MHz) of coniferaldehyd (**151**) in CD_2Cl_2

The EI mass spectrum of **151** showed the molecular ion peak at *m/z* 178. Compound **151** was identified as coniferaldehyde previously isolated from roots of *Tamarix nilotica*.[303] Coniferaldehyde (**151**) has been reported as prostaglandin synthase inhibitor and as an antifungal agent.[302]



151

p-Hydroxybenzaldehyde (**152**) was obtained as white solid and its ^{1}H NMR spectrum displayed only three signals at δ 9.82 (1H, s) due to an aldehyde proton and two doublets at δ 7.80 (2H, ^{3}J = 9.0 Hz) and 6.98 (2H, ^{3}J = 8.9 Hz) of a *para*-disubstituted benzene ring. Compound **152** was also reported from *Sorghum bicolor*.[304]



152

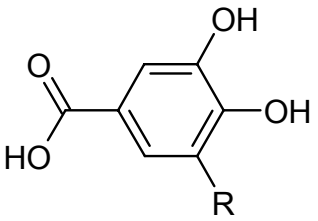
The analysis of the extract of the trunk followed the same scheme as that of the seeds (fig. 1). The chromatography on silica gel of the resin (gum) like crude extract resulted in the isolation of four compounds.

6.3.7 Gallic acid

The brown powder of **153a** was obtained as a major constituent of the trunk extract. Only one signal can be seen at δ 7.02 in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum delivered, however, five carbon signals. The signals at δ 170.4 can be assigned to the carbonyl carbon of a carboxylic acid or amide. The quaternary and methine carbons at δ 146.3 and 110.3, respectively, denote two carbons each. The EI mass spectrum indicated the molecular peak at *m/z* 170, and HRMS delivered the molecular formula $C_7H_6O_5$. The structure was finally derived as gallic acid (3,4,5-trihydroxybenzoic acid, **153a**) and confirmed by comparison of the EI mass fragmentation and the NMR data with the literature. It is reported as a strong natural antioxidant[305] and proven to be one of the anticarcinogenic polyphenols present in green tea.[306] It exhibited a phytotoxic and antifungal activity against *Fusarium semitectum*, *F. fusiformis* and *Alternaria alternata*[307] and it is of great interest in arteriosclerosis prevention.[308]

6.3.8 3,4-Dihydroxybenzoic acid

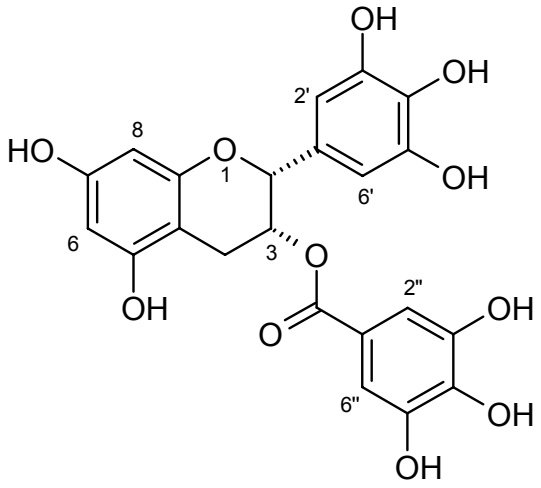
Compound **153b** was obtained as a white solid. The ^{1}H NMR spectrum of **153b** was very simple and showed only two signals of three protons with an unequivocally *o*-coupling in the aromatic region at δ 7.40 (2H) and 6.79 (1H), the *m*- and *o*-coupling overlapped at δ 7.40. The NMR ^{13}C/APT spectrum was similar to that of gallic acid (**153a**) with seven signals, four quaternary carbons including a carbonyl group at δ 170.3 and three aromatic methines. The EI mass spectrum indicated the molecular signal at *m/z* 154, which is 16 mass units (an oxygen atom) less than that of **153a**, i.e. one of the OH groups in **153a** must have been replaced by a proton in **153b** giving 3,4-dihydroxybenzoic acid.



153a: R = OH
153b: R = H

6.3.9 3-O-Galloyl-(-)-epicatechin

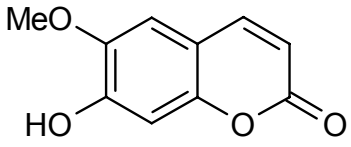
The reddish compound **154** was obtained from fraction D on Sephadex LH-20. The ^{1}H NMR spectrum exhibited in the *sp*^{2} region three 2H singlets at δ 6.87, 6.48 and 5.98. Further signals were seen at δ 5.50 (m), 4.99 (s) and could be assigned to heteroatom bound protons; two doublets of doublets at δ 3.00 and 2.82 indicated a CH_2-CH fragment. The presence of these three singlets resuming six protons could be assigned to three rings having 2 *meta* protons each and showing the same chemical environment. As gallic acid (**153a**) was isolated from the same plant it may contain such features. The molecular weight was deduced from the (+)-ESI mass spectrum to be *m/z* 458. The sub-structure search in DNP (Dictionary of Natural Product) with this information led to 3-O-galloyl-(-)-epicatechin (**154**), which was also reported from green tea leafs[309] and *Myrica rubra*.[310]



154

6.3.10 Scopoletin

The yellow solid **155** showed a strong blue fluorescence under UV at 366 nm. The EI MS indicated the molecular peak at *m/z* 192. The aliphatic region of the ^{1}H NMR spectrum exhibited only a signal of a methoxy group at δ 3.98. The sp^2 region displayed a pair of 1H doublets at δ 7.60 (J = 9.8 Hz) and 6.27 attributed to the *cis*-protons of a double bond, and two 1H singlets at δ 6.92 and 6.85 of an electron rich 1,2,4,5-tetrasubstituted benzene ring. With a search in the Dictionary of Natural Products with the spectroscopic data and by comparison with the literature, it was identified as scopoletin (**155**).[311] This coumarine derivative has been reported to have analgesic properties. Scopoletin caused apoptosis in HL-60 promyelocytic cells[312] and exhibits hypouricemic activities by decreasing uric acid production and as well as a uricosuric mechanism.[313] Scopoletin (**155**) also showed antihypertensive and antihistamine activities.



155

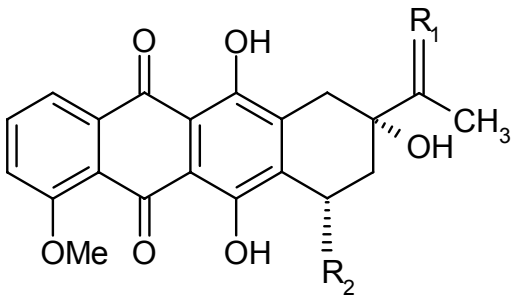
7 Summary

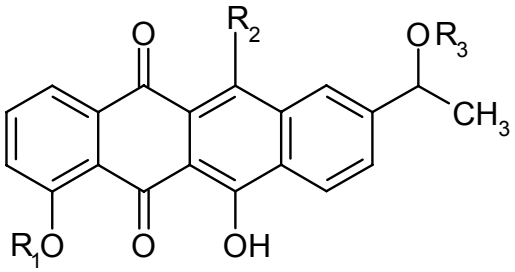
The fascination of natural products from plants with known medicinal properties goes back to ancient times. In the past decades natural products have attracted renewed interest, with bacteria, fungi and especially marine organisms as important sources of biologically active compounds. Today many illnesses are still not curable, and also the increase of resistance urges the development of new medicaments to combat them. During the search for new active secondary metabolites, many known compounds will also be re-isolated making the main task difficult. One way of finding new metabolites may be to carry out research on a direction or test system, which is still unexplored.

In the present work, 15 terrestrial *Streptomycetes* and six marine-derived bacteria strains were selected and based on their chemical and biological screening, fermented under standard conditions. The culture was mixed with Celite and filtrate and mycelium were separated. The mycelium was extracted with ethyl acetate and finally with acetone, while the water phase was extracted with ethyl acetate or passed through XAD-16, the resin washed with distilled water and finally eluted with methanol. A comparative TLC of both extracts was carried out to decide if they could be mixed or had to be worked up separately. The crude extract was separated using various chromatographic methods (silica gel column, Sephadex LH-20, PTLC and HPLC). Isolated compounds were identified using a substructure search including mass and ^{1}H NMR data in databases like AntiBase or the Dictionary of Natural Products (Chapman and Hall). In case of negative search, 2D NMR spectra were recorded for further investigations.

The terrestrial *Streptomyces* sp. GW 37/3236 was found to inhibit in the agar diffusion test the growth of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Streptomyces viridochromogenes*, the fungus *Mucor miehei* and the micro-algae *Chlorella vulgaris,* and *Chlorella sorokiniana*. From a 25 L shaker culture, three new compounds were isolated: 13-O-acetyl-bisanhydro-13-dihydrodaunomycinone (**58**), 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydro-daunomycinone (**59**) and 2-acetylamino-3-hydroxy-benzamide (**60**) together with known compounds: 6,9,11-trihydroxy-4-methoxy-5,12-natphthacenedione (**57**), which is firstly isolated here

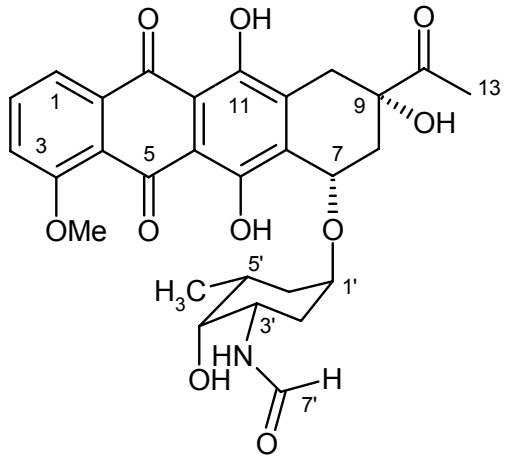
from a natural source, bisanhydro-13-dihydrodaunomycinone (**51**), 7-deoxydaunomycinol (**52**), daunomycinol (**53**), daunomicynone (**54**), baumycin C1 (**55**), and 11-deoxybisanhydro-13-dihydrodaunomycinone (**56**). All compounds were submitted to antibacterial and showed comparable weak activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Streptomyces viridochromogenes*, and no activity against *Candida albicans, Mucor miehei, Chlorella vulgaris, Chlorella sorokiniana* and *Scenedesmus subspicatus*. The new compounds were tested against the human cancer cell lines LXFA 629L and LXFL 529L (lung), MAXF 401NL (breast), MEXF 426NL (melonoma), RXF 944L (kidney) and UXF 1138L (uterus) and had IC_{50} values of > 6 µg/ml.





52: R_1 = OH, H R_2 = H
53: R^1 = OH, H R_2 = OH
54: R_1 = O R_2 = OH

51 $R_1 = CH_3$ R_2 = OH R_3 = H
56 $R_1 = CH_3$ R_2 = H R_3 = H
58 $R_1 = CH_3$ R_2 = OH $R_3 = COCH_3$
59 $R_1 = COCH_3$ R_2 = OH $R_3 = COCH_3$



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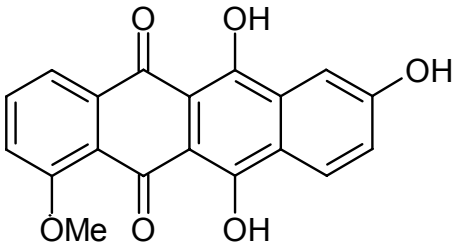

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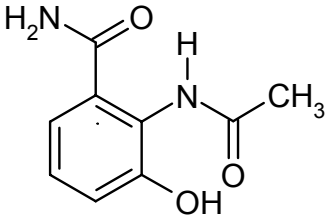
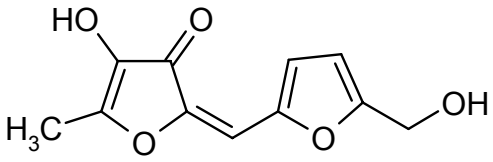


60

The terrestrial isolate GW 21/1313 was found to produce only actinomycin C_2 and D in the standard medium used for our culture. On optimisation of the culture conditions using M_2^+ medium, LB medium, meat extract medium, $CaCl_2$ medium and fish medium, the metabolite spectrum was changed. In $CaCl_2$ medium, the strain produced two new β-carboline derivatives named radhanone A (**80a**) and B (**80d**) and the known metabolite perlolyrin (**78**), a dimer and trimer of 5-hydroxymethylfurfural (**75**) named krishnanone A (**76**) and krishnanone B (**77**), in addition to several simple compounds. 4-Hydroxy-2-(5-hydroxymethyl-furan-2-ylmethylene)-5-methyl-furan-3-one (**73**) is reported here for the first time as a natural product. All β-carbolins were found to be inactive in our agar diffusion tests.



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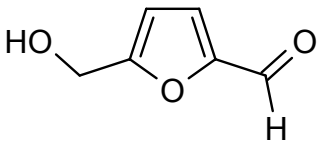

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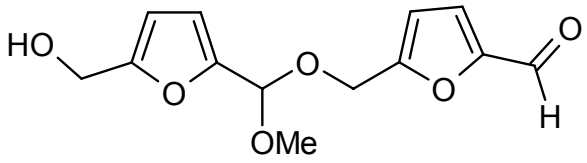

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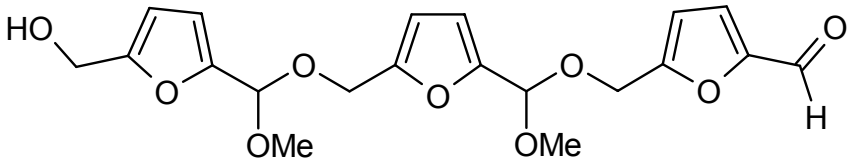

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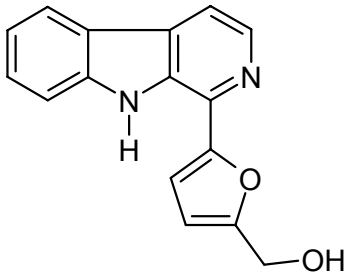
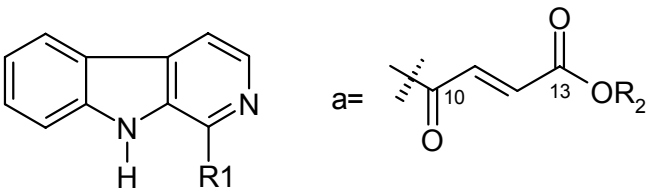
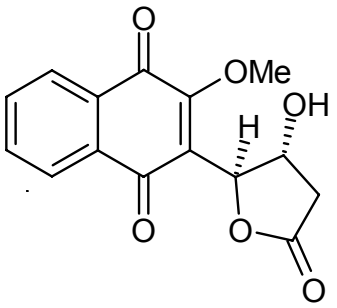


78



80a: R_1 = a R_2 = CH_3
80d: R_1 = a R_2 = H

The terrestrial strain GW 4184 produced a variety of quinones, among them juglomycin A (**62**) and B (**63**), juglorubin (**70**), juglorescein (**71**), the angucyclinone oviedomycin I (**65a**) and its tautomer II (**66a**). The latter and juglomycin J (**64**) are reported here for the first time. They exhibited no antimicrobial activity.



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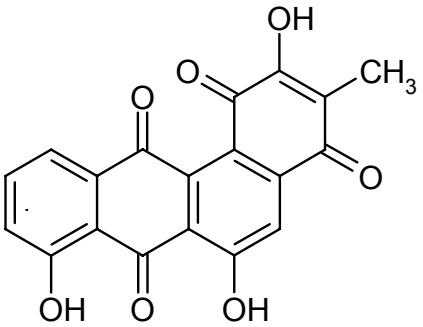
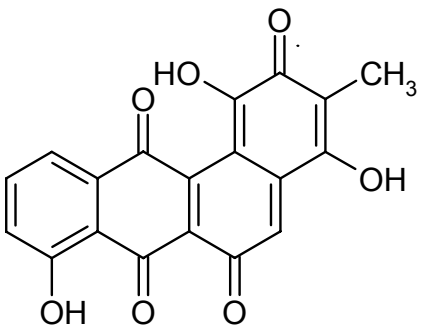
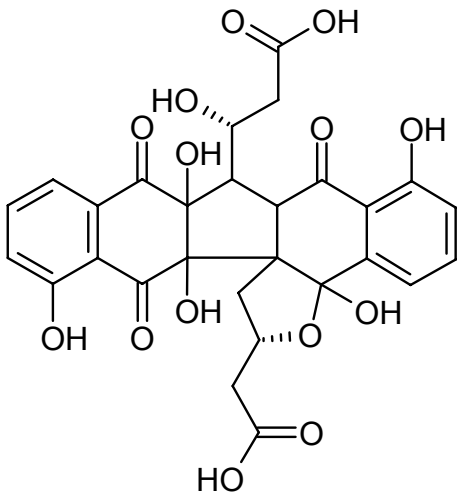


65a

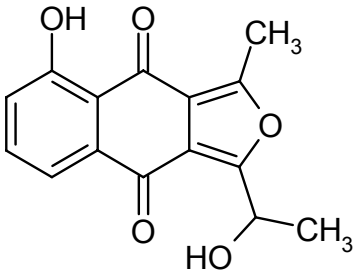


66a

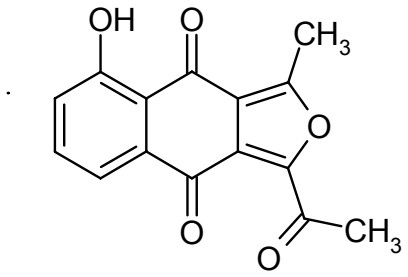


71

Extracts of the strain GW 32/698 showed on TLC an orange fluorescence and several yellow spots and exhibited moderate activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. 16 metabolites were isolated, among them 6 new compounds, bhimamycin A (**88**), B (**89**), C (**93**), D (**94a**) and E (**102**) and bhimanone (**104**). bhimamycin A (**88**) and B (**89**) are the first natural products with the rare naphtho[2,3c]furan-4,9-dione chromophore. In the antibacterial test, only bhimamycin A (**88**), B (**89**) and E (**102**) showed good activity against *Staphylococcus aureus*.



88



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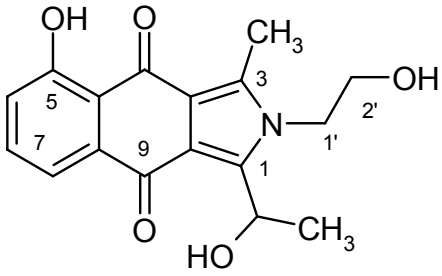

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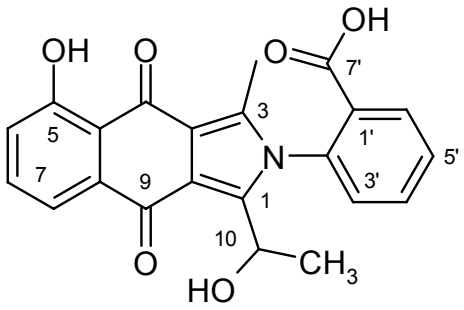
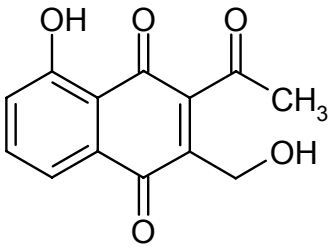
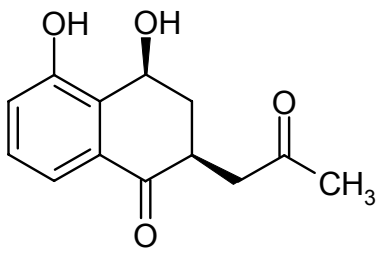


94a



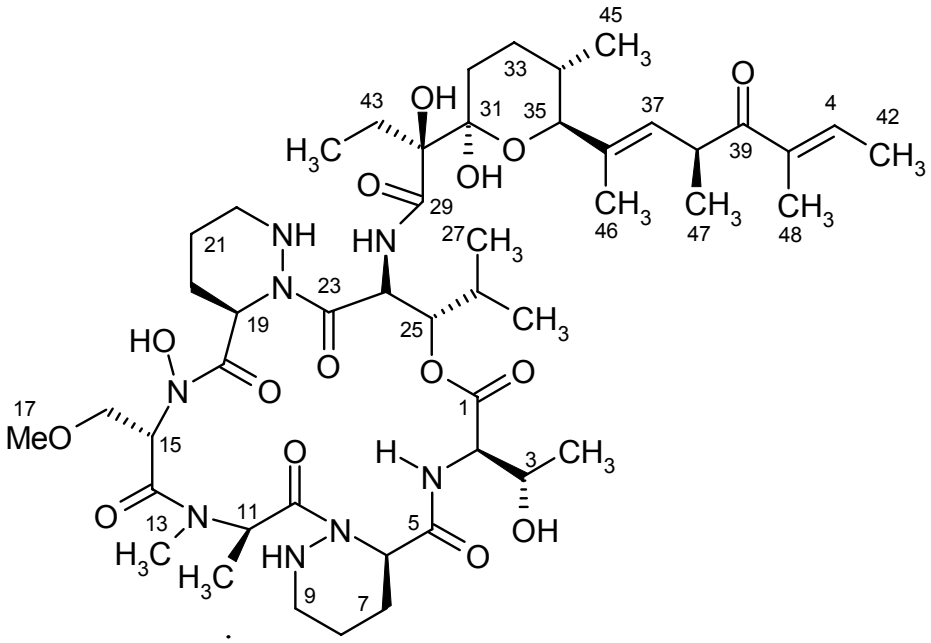
102



104

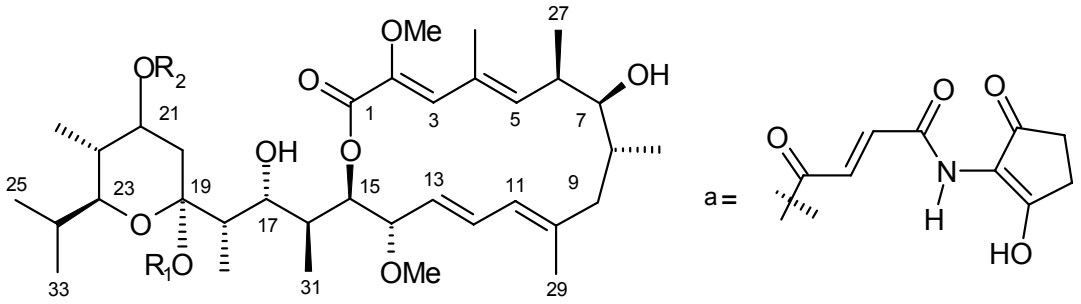
The colourless kettapetin (**82a**) was isolated from the terrestrial *Streptomyces* sp. GW 99/1572 and is described here for the first time. The crude extract also indicated

the presence of known compounds like anthranilic acid, chrysophanol and polyenes, which decomposed during the separation procedure. Kettapeptin shows very good antibacterial activities comparable to bacitracin A. It was also found to be 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. Kettapeptin belongs to the group cyclic hexadepsipeptides of the azinothrizin type, which are characterised by a 19-membered cyclodepsipeptide ring composed of 6 unusual amino acids and an acyl side chain connected through an amide bond. The first member of this class, azinothrizin was reported from *Streptomyces* X-14950.



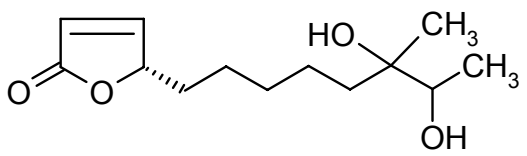
82a

The marine-derived bacteria Mei 22 showed strong activity against *Mucor miehei* and *Staphylococcus aureus*. A 25-L shaker culture using $CaCl_2$ medium and 100% artificial sea water delivered the known compounds bafilomycins A_1 (**134a**), B_1 (**134b**), B_2 (**134c**) and deboroaplasmomycin C (**135**), which all showed activity against the fungus *Mucor miehei*



134a: R_1= H R_2 = H
134b: R_1= H R_2 = a
134c: R_1= CH_3 R_2 = a

Two new butenolides (4S)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (**129**), 4,10-dihydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**130**) and an isoquinoline quinone **131** were isolated from the culture broth of Mei 35 along with the antimycin A-complex (**127**) and (4S)-4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**128a**). Only **131** and antimycin showed strong activity against Gram-positive and Gram-negative bacteria.



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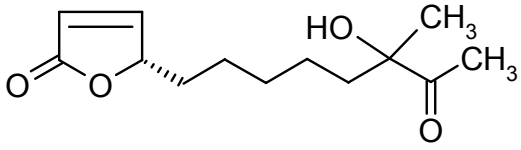

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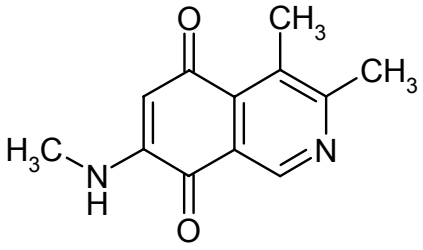
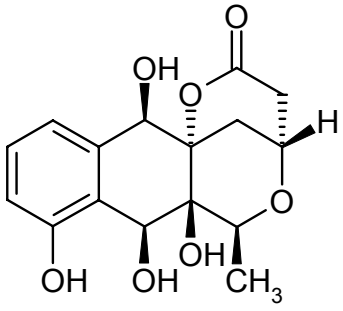


131

The marine actinomycete Act 7617 showed on TLC screening a strong UV absorbing spot at 254 nm, which turned brown to violet with anisaldehyde/sulphuric acid. From the crude extract, the new tetraol 2-hydroxy-luisol A (**139**) and the known luisol A (**137**) und B (**138**) were isolated. They exhibited no antimicrobial activity.



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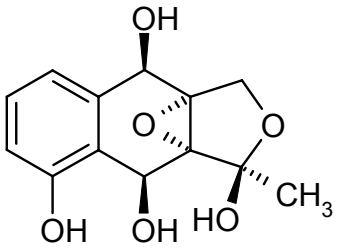

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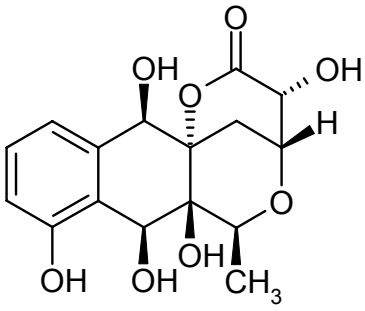
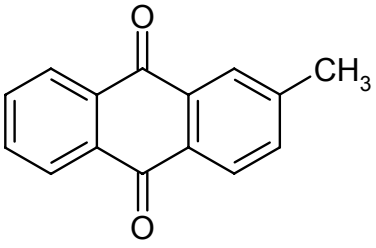


139

The crude extract of the terrestrial *Streptomyces* sp. US80 was obtained from the group of Dr. Mellouli in Tunisia. The strain was collected from Tunisian oasis soil, because *Fusarium oxysporum* sp. *albedinis* (Foa) fungus has caused destruction of a large number of palms in the oases of Algeria and Morocco, but not in Tunisia. This fact could be due to the physico-chemical characteristics of Tunisian oasis soil, and/or to the presence of antagonistic microorganisms, which might inhibit Foa development and dissemination. The separation of the crude extract led to the known metabolites irumamycin (**45**), X-14952B (**46**) and 17-hydroxyventuricidin A (**50**), which exhibited all good antifungal activity against *Mucor miehei*. They were also found to inhibit the growth of the filamentous fungi *Verticillium dahliae* and *Fusarium* sp. as well as *Candida tropicalis* R2CIP203. The highest antifungal activity was obtained with irumamycin (**45**).

Additionally to the bacteria, three plants were analysed: A crude extract from *Rheum palmatum* showed in the primary screening an antiviral activity. The chemical investigation afforded 6 known anthraquinones and 3 stilbenes, which were responsible for the antiviral activity.

In the search for plant-derived compounds as alternative to chemical termiticides, the phytochemical study of *Diospyros sylvatica* in Prof. Ganapaty's group in India gave six pure compounds and their structures were elucidated as 2-methyl-anthraquinone (**141**), plumbagin (**142**), diosindigo A (**143**), diospyrin (**144**), isodiospyrin (**145**), and microphyllone (**146**).



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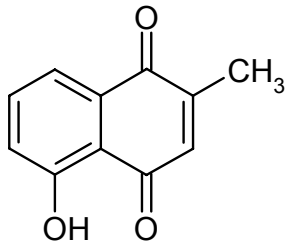

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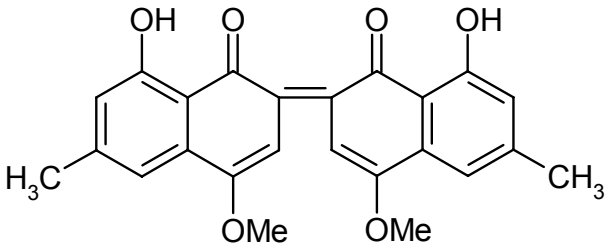

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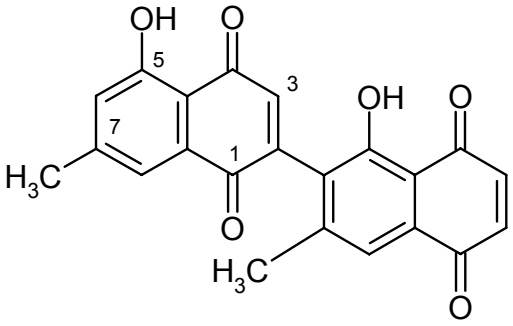

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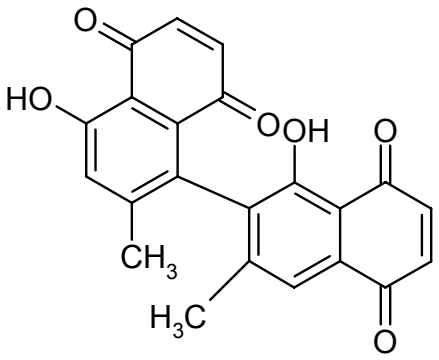

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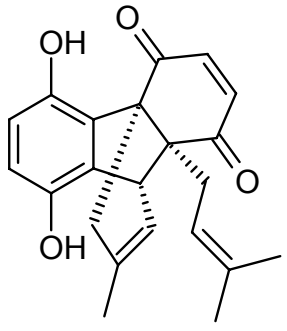

146

All these quinonoid compounds exhibited termiticidal activity.

In this thesis 15 terrestrial *Streptomycetes*, 6 marine-derived bacteria and 2 plants were studied. From the terrestrial strains 62 compounds were isolated, from which 18 compound can be considered as new secondary metabolites from bacteria. The 6 marine strains were found to produce 28 compounds, out of which 11 are reported here for the first time. From the 2 plants, 17 known metabolites were isolated and characterised. The altogether 107 metabolites contained different groups such as peptide, quinones, macrolides, polyene and polymers.

Table 8: Total nummer of isolated compounds from bacteria in this thesis

Strains	**No of Strains**	**No of compounds**	**No of new compounds**
Terrestrial *Streptomycetes*	15	62	17
Marine *Streptomycetes*	6	28	11

From the various activities of the isolated metabolites we can conclude that microorganisms are sources of useful natural product and potent bioactive compounds.

8 Materials and Methods

8.1 General

IR spectra: Perkin-Elmer 1600 Series FT–IR; Perkin-Elmer 297 infrared spectrophotometer; 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 241). – **^{1}H NMR spectra:** Varian Unity 300 (300 MHz), Bruker AMX 300 (300 MHz), Varian Inova 500 (499.8 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet doublet, t = triplet, q = quartet, m = multiplet, br = broad. – **^{13}C NMR spectra:** Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical shifts were measured 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. ESI MS with Quattro Triple Quadruple mass spectrometer Finigan MAT-Incos 50, ESI-MS LCQ (Finnigan). – **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.

8.2 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 for flash chromatography: 30-60 μm (J. T. Baker); Sephadex LH-20 (Pharmacia) was used for size exclusion chromatography.

8.3 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.

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.

8.4 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. (Zwijndrecht). - **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).

8.5 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

8.5.1 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

8.6 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.

8.7 Microbiological and analytical methods

8.7.1 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.

8.7.2 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.

8.7.3 Biological screening

The crude extract was dissolved in $CHCl_3$/10% MeOH (concentration 50 mg/mL), in which the paper disks were dipped, dried under sterile conditions (flow box) and put on an agar plates inoculated with *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Candida albicans* and *Mucor miehei* (Tü 284).

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.

8.7.4 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.

8.7.5 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%.

8.7.6 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.

8.8 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 (+++).

9 Origin of the investigated strains

All Streptomycetes with names starting with the signature "GW" are of terrestrial origin and were obtained from the collection of the "Labor für Bodenkunde" (Dr. Grün-Wollny, Lohra-Kirchvers. Strains Mei are from the Collection of Marine Streptomycetes (Prof. Meiners, Fachhochschule Emden). The origin of a few other strains is mentioned at the corresponding place.

10 Metabolites from selected strains

10.1 Terrestrial *Streptomyces* sp. GW 2/1332

The terrestrial *Streptomyces* sp. GW 2/1332 formed on agar a white aerial mycelium with a red coloration. For screening, the strain was cultivated on a shaker with 95 rpm for three days at 28 °C in four 1-L Erlenmeyer flasks with each 250 ml of M_2 medium. The thus obtained red culture broth was worked up by extracting with ethyl

acetate to deliver 70 mg of a red crude extract. TLC (CH_2Cl_2/10% MeOH) revealed a polar red zone (R_f = 0.65), which turned violet with NaOH. In the biological screening, the extract inhibited the growth of *Streptomyces viridochromogenes* (Tü 57), *Mucor miehei, Chlorella vulgaris and Chlorella sorokiniana*. The results are tabulated below.

Table 9: Biological activity of the crude extract from the strain GW2/1332

Test organisms	Inhibition zone (∅ mm)
Streptomyces viridochromogenes (Tü 57)	13
Mucor miehei	13
Chlorella vulgaris	13
Chlorella sorokiniana	13

10.1.1 Scale up and isolation

For the scale up, the strain GW 2/1332 was cultivated on a 15 L scale under identical conditions as for the screening. The well grown red coloured culture broth was mixed with diatomaceous earth (1 kg) and filtered through a pressure filter. The filtrate and the mycelium were extracted separately with ethyl acetate and the combined solutions were concentrated under vacuum. The dark red residue was chromatographed on silica gel using a CH_2Cl_2/MeOH gradient. Griseorhodin A (**22**, 200 mg) was finally purified by crystallisation from CH_2Cl_2/MeOH and washing with methanol.

Griseorhodin A (22): $C_{25}H_{16}O_{12}$, red powder. – R_f = 0.62 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 13.26 (s, 1H, 5-OH), 11.76 (s br, 1H, 17-OH), 10.76 (s, 1H, 14-OH), 7.26 (s, 1H, 9-H), 6.91 (d, ^3J = 8 Hz, 1H, 6-OH), 6.58 (s, 1H, 10-H), 6.42 (s, 1H, 3-H), 5.30 (d, ^3J = 8 Hz, 1H, 6-H), 4.44 (s br, 1H, 8-H), 4.30 (d, ^3J = 3.1 Hz, 1H, 7-H), 3.90 (s, 3H, 2-OCH_3), 2.23 (s, 3H, 18-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 125.7 MHz): δ = 186.1 (C_q, C-4), 180.3 (C_q, C-1), 165.3 (C_q, C-13), 160.4 (C_q, C-2), 156.3 (C_q, C-5), 153.6 (C_q, C-17), 153.3 (C_q, C-14), 148.8 (C_q, C-16a), 145.9 (C_q, C-14a), 135.7 (C_q, C-9a), 132.0 (C_q, C-13a), 130.2 (C_q, C-5a), 124.0 (C_q, C-8a), 115.8 (CH, C-9), 114.3 (C_q, C-17a), 111.3 (C_q, C-11), 110.2 (CH, C-3),

106.8 (C_q, C-4a), 106.7 (CH, C-10), 103.7 (C_q, C-6a), 75.0 (CH, C-6), 57.1 (OCH_3), 54.7 (CH, C-8), 48.6 (CH, C-7), 18.7 (18-CH_3). – **(+)-ESI MS**: *m/z* (%) = 1038.9 ($[2M+Na]^+$, 50), 531.3 ($[M+Na]^+$, 100). – (-)-ESI MS: *m/z* (%) = 507.6 $[M-H]^-$. – **DCI MS** (NH_3): *m/z* (%) = 526.0 ($[M+ NH_3]^+$, 100), 510 ($[M+ 2H]^+$, 28).

10.2 Terrestrial *Streptomyces* sp. GW 15/1817

The terrestrial *Streptomyces* sp. GW 15/1817 grew on M_2 agar with white aerial mycelium and yellow pigmentation in the agar after three days of incubation at 28 °C. The strain was cultured in 4 of 1 L Erlenmeyer flasks each containing 250 mL M_2 medium on a round shaker with 95 rpm for 72 h at 28 °C, which yielded 120 mg of dark yellow crude extract for the biological and chemical screening.

In the biological screening using agar diffusion method, the crude extract showed antibacterial activities, which are summerized in the following table.

Table 10: Biological activity of the crude extract of the strain GW 15/1817

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	12
Staphylococcus aureus	14
Mucor miehei	21
Chlorella vulgaris	11
Chlorella sorokiniana	12
Scenedesmus subspicatus	10

10.2.1 Pre-Screening

TLC (CH_2Cl_2/ 25% MeOH) of the crude extract showed only one very big UV absorbing yellow spot, which did not change colour with dilute NaOH, but turned brown to black with concentrated sulphuric acid, which is characteristic for polyenes.

10.2.2 Fermentation and Work-up

For scaling up, the strain was cultivated on a 15 L scale at 28 °C for 3 days on a linear shaker. The orange culture broth was mixed with 1 kg diatomaceous earth and

filtered through a pressure filter. The filtrate and mycelium were separately extracted each four times with ethyl acetate (2-L). During the extraction of the mycelial cake, it was treated with ultrasonic radiation for 15 min each time. The yellow organic solutions were combined and the solvent was removed under vacuum resulting in 3 g of dark yellowish brown extract.

10.2.3 Isolation

The whole crude extract (3 g) was triturated with dichloromethane (50 ml) and filtered. The red filtrate showed in addition to oil (fatty acids) a very fast moving red spot (CH_2Cl_2/5% MeOH) of prodigiosin, which turned blue with concentrated sulphuric acid and anisaldehyde/sulphuric acid and yellow with dilute sodium hydroxide, and thus was not followed further. The dichloromethane insoluble yellow powder (1.3 g) exhibited on TLC (CH_2Cl_2/35% MeOH) only one yellow main component. Purification of 100 mg of the powder by PTLC (CH_2Cl_2/35% MeOH) followed by preparative HPLC on RP-18 silica gel (MeCN/50% H_2O) yielded 80 mg of roxacitin (**23**).

Roxaticin (23): $C_{34}H_{54}O_9$, yellow amorphous powder. – R_f = 0.40 (CH_2Cl_2/35% MeOH). – **^{1}H NMR** ([D_4]MeOH, 300.0 MHz): δ = 7.25 (dd, ^3J = 15.8, 11.4 Hz, 1H, 3-H), 6.63 (dd, ^3J = 15.3, 10.0 Hz, 1H, 5-H), 6.50 (dd, ^3J = 14.5, 10.0 Hz, 1H, 7-H), 6.40-6.30 (m, 4H, 8-H, 4-H, 6-H, 9-H), 6.18 (dd, ^3J = 15.0, 10.0 Hz, 1H, 10-H), 5.92 (dd, ^3J = 15.5, 7.0 Hz, 1H, 11-H), 5.83 (d, ^3J = 15.8 Hz, 1H, 2-H), 5.66 (dd, J = 15.5, 4.5 Hz, 1H, 26-H), 5.40 (dd, ^3J = 15.5, 3.0 Hz, 1H, 27-H), 4.80 (dd, ^3J = 7.0, 2,5 Hz, 1H, 29-H), 4.30 (m, 1H), 4.10-3.90 (m, 5H), 3.60 (m, 1H), 2.60 (m, 2H, 12-H, 28-H), 1.94 (m, 1H, Me_2CH), 1.60 (m, 2H), 1.40 (m, 3H), 1.30-1.20 (3 m, 6H), 1.16 (m, 1H), 1.10 (d, ^3J = 7.5 Hz, 3H, 28-Me), 1.08 (d, ^3J = 6.5 Hz, 3H, 12-Me), 0.94, 0.90 (2d, ^3J = 6.4 Hz, 3 H each, Me_2CH). – **^{13}C/APT NMR** (CD_3OD, 75.5 MHz): δ = 169.1 (C_q C-1), 146.9 (CH, C-7), 143.0 (CH, C-5), 139.8 (CH, C-11), 139.3 (CH, C-6), 137.3 (CH, C-3), 133.9 (CH, C-8), 132.6 (CH, C-4), 132.3 (CH, C-9), 131.4 (CH, C-26), 131.2 (CH, C-10), 130.6 (CH, C-27), 121.4 (CH, C-2), 81.8 (CH, C-29), 73.3 (CH, C-25), 72.0 (CH, C-13), 70.0 (CH, C-15), 67.3 (CH, C-17), 66.6 (CH, C-21), 65.5 (CH, C-23), 65.2 (CH, C-19), 48.0 (CH_2, C-24), 47.6 (CH, C-12), 47.5 (CH_2, C-16), 46.0 (CH_2, C-18), 45.8 (CH_2, C-20), 45.1 (CH_2, C-22), 42.8 (CH_2, C-28), 37.9

(CH_2, C-14), 30.5 (CH, C-30), 20.2 (CH_3, C-32), 19.4 (CH_3, C-31), 12.7 (12-CH_3), 11.5 (28-CH_3). **– (-)-ESI MS** *m/z* (%) = 605 ($[M-H]^-$).

10.3 Terrestrial *Streptomyces* sp. GW 3/1122

On agar plate, the strain GW 3/1122 showed a white mycelium and a yellow pigment, which diffused all over the plate. With this plate, a 1 L culture was done with M_2 medium at 28 °C for four days. The intensive yellow or orange culture was extracted with ethyl acetate and the obtained crude extract was used for the biological and chemical screening.

The chemical screening on TLC revealed yellow spots of actinomycins at day light, which turned to orange on spraying with anisaldehyde/sulphuric acid. Other yellow spots did not change with the spray reagent. The extract was found to inhibit the growth of Gram-positive and Gram-negative bacteria, fungi and algae and the results are listed in table 11.

Table 11: Biological activity of the crude extract of the strain GW 3/1122

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	23
Staphylococcus aureus	11
Streptomyces viridochromogenes (Tü 57)	22
Escherichia coli	0
Candida albicans	11
Mucor miehei	11

10.3.1 Fermentation and Isolation.

The strain GW 3/1122 was cultivated on M_2 agar plates for four days at 28 °C and formed white colonies and an orange agar colouration. With the well grown agar culture, a 15 L shaker culture was inoculated and grown at 28 °C for 5 days on a linear shaker. The yellow culture broth was filtered and the mycelial cake was extracted with ethyl acetate. The water phase was given on XAD-16, the resin washed with tap water (20 L) and finally eluted with methanol (5 L). The methanolic phase was

evaporated to dryness and then extracted with ethyl acetate (3 × 1 L). Due to a similar composition of both extracts, they were combined (3 g) and separated by size exclusion chromatography on Sephadex LH-20 (CH_2Cl_2/ 50% MeOH) into two fractions. The first fraction SF839A contained only fatty acids and actinomycins and was not further analysed. The second fraction SF839B was purified by preparative HPLC RP-18 (MeCN/20% H_2O) to yield 26 mg of furanoquinone (**24**) as yellow solid.

Furanoquinone (24): $C_{22}H_{26}O_5$, yellow solid. – R_f = 0.46 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 600.0 MHz): δ = 7.15 (s, 1H, 9-H), 6.52 (*br* s, 1H, 4-OH), 5.06 (m, 1H, 12-H), 4.83 (q, ^3J = 6.6 Hz, 1H, 2-H), 3.99 (s, 3H, 7-OCH_3), 2.04 (s, 3H, 6-CH_3), 1.95, 1.82 (2 m, 4H, 10-H_2 and 11-H_2), 1.62 (s, 3H, 15-H_3), 1.52 (s, 3H, 14-H_3), 1.43 (d, ^3J = 6.6 Hz , 3H, 2-CH_3), 1.23 (s, 3H, 3-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 184.2 (C_q, C-5), 181.3 (C_q, C-8), 161.2 (C_q, C-4), 157.7 (C_q, C-9a), 156.9 (C_q, C-7), 134.0 (C_q, C-6), 133.1 (C_q, C-8a), 131.9 (C_q, C-13), 127.8 (C_q, C-3a), 123.8 (CH, C-12), 109.3 (CH, C-9), 109.1 (C_q, C-4a), 88.0 (CH, C-2), 60.7 (OCH_3), 46.8 (C_q, C-3), 37.7 (CH_2, C-10), 25.6 (CH_3, C-15), 23.6 (CH_2, C-11), 19.6 (3-CH_3), 17.6 (CH_3, C-14), 15.4 (2-CH_3), 9.41 (6-CH_3). – **(+)-ESI MS:** *m/z* (%) = 763.1 ($[2M+Na]^+$, 100), 393.3 ($[M+Na]^+$, 6). – **(-)-ESI MS:** *m/z* = 369.4 ($[M-H]^-$).

10.4 Terrestrial *Streptomyces* sp. GW 24/1229

The terrestrial *Streptomyces* GW 24/1229 was kept for storage as soil culture and in liquid nitrogen. On M_2 agar, the culture showed after incubation for four days at 28 °C a red mycelium on agar. A diffusible pigment stained the agar red.

10.4.1 Primary screening

TLC of the crude extract showed two major red spots with R_f = 0.64 (CH_2Cl_2/10% MeOH), which did not change on spraying with anisaldehyde/sulphuric acid but turned violet with diluted sodium hydroxide.

Table 12: Biological activity of the crude extract of the strain GW 24/1229

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	15
Staphylococcus aureus	18
Streptomyces viridochromogenes (Tü 57)	22
Escherichia coli	18

10.4.2 Fermentation, Work-up and Isolation

The soil culture of the strain GW 24/1229 on three agar plates was used to inoculate 10 L fermentation on a rotational shaker for four days at 28 °C, using M_2 medium. The dark red culture broth resulting was mixed with Celite and filtered under pressure. Extraction of the water phase and mycelium with ethyl acetate and evaporation of the solvent gave 2 g of red crude extract, which was sequentially separated by column chromatography on silica gel and Sephadex LH-20.

The crude extract (2 g) was dissolved in a mixture (CH_2Cl_2/MeOH) and ca. 5 g of silica gel was added and this mixture was dried on the rotatory evaporator and the mixture aerated for 30 min. Chromatography on silica gel using an increasing dichloromethane/methanol gradient afforded three fractions I (200 mg), II (350 mg) and III (400 mg). The red oily fraction I contained fats and prodigiosin. TLC of fraction II showed a major red component with R_f = 0.60 (CH_2Cl_2/10% MeOH). This sparingly soluble fraction was dissolved in 30 ml of CH_2Cl_2/40% MeOH and separated on Sephadex LH-20, whereby β-rubromycin (**28**, 250 mg) was obtained. In the same way, the third fraction delivered γ-rubromycin (**29**, 100 mg), but here a large amount of a violet compound remained on the Sephadex.

β–Rubromycin (30): $C_{27}H_{20}O_{12}$, red powder. – $\boldsymbol{R_f}$ = 0.60 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 12.50 (br s, 1H, OH), 10.64 (br s, 1H, OH), 7.61 (s, 1H, 6-H), 7.22 (s, 1H, 5-H), 7.04 (s, 1H, 6'-H), 3.98 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.40, 3.38 (AB, ^3J = 18.0 Hz, 1H, 3'-$H_{a,b}$), 3.20-2.98 (m, 2H, 4-H_2), 2.55, 2.30 (2 m, 2H, 3-H_2). – **^{13}C/APT NMR** ([D_6]DMSO, 75.5 MHz): δ = 181.1 (C_q, C-9'), 177.9 (C_q, C-4'), 163.4 (C_q, C-9), 159.7 (C_q, 7-

CO_2CH_3), 155.3 (C_q, C-9'a), 154.7 (CH, C-5'), 153.9 (C_q, C-7'), 148.8 (C_q, C-10), 148.4 (C_q, C-8'), 140.4 (C_q, C-10a), 140.3 (C_q, C-7), 131.5 (C_q, C-4a), 127.2 (C_q, C-3'a), 127.0 (C_q, C-5a), 118.6 (CH, C-5), 114.1(C_q, C-8'a), 113.2 (CH, C-6), 111.2 (C_q, C-2), 109.5 (C_q, C-6'), 106.2 (C_q, C-9a), 105.2 (C_q, C-6'), 56.7 (CH_3, 7'-OCH_3), 56.3 (CH_3, 5'-OCH_3), 52.4 (CH_3, 7-CO_2CH_3), 38.7 (CH_2, C-3'), 28.2 (CH_2, C-3), 21.4 (CH_2, C-4). **– (+)-ESI MS:** *m/z* (%) = 1631.8 ($[3M+Na]^+$, 68), 1095.2 ($[2M+Na]^+$, 100), 559.7 ($[M+Na]^+$, 10). **– (-)-ESI-MS:** *m/z* = 535.2 ($[M-H]^-$).

γ–Rubromycin (31): $C_{26}H_{18}O_{12}$, red powder. **–** $\boldsymbol{R_f}$ = 0.63 (CH_2Cl_2/10% MeOH). **–** ^{1}**H NMR** ($[D_6]$DMSO, 300.0 MHz): δ = 13.14 (*br* s, 1H, OH), 11.90 (*br* s, 1H, OH), 10.68 (*br* s, 1H, OH), 7.63 (s, 1H, 6-H), 7.22 (s, 1H, 5-H), 6.37 (s, 1H, 6'-H), 3.85 (s, 6H, 7'-OCH_3, 7-CO_2CH_3), 3.60-3.00 (m, 4H, CH_2), 2.40-2.20 (m, 2H, CH_2). **– EI MS (70 eV):** *m/z* (%) = 522.5 ($[M]^+$, 13), 354 (9), 260 (4), 327 (16), 313 (76), 299 (100), 285 (36), 239 (28), 225 (24), 129 (24), 98 (40), 69 (48), 57 (84), 43 (73). **– (+)-ESI MS:** *m/z* (%) = 1589.1 ($[3M+Na]^+$, 100), 1067.1 ($[2M+Na]^+$, 100), 523.6 ($[M+H]^+$, 2).

10.5 Terrestrial *Streptomyces* sp. GW 22/1326

The *Streptomyces* sp. isolate GW 22/1326 was sub-cultivated in M_2 medium on agar plates at 28 °C for four days. One agar plate was used to inoculate 12 of 1 L Erlenmeyer flasks each containing 250 ml of the medium. The flasks were kept at 28 °C on a rotary shaker (95 rpm) for three days. The resulting broth was filtered and extracted with ethyl acetate to yield 80 mg of oily crude extract.

10.5.1 Pre-screening

The chemical screening of the crude extract showed in addition to the fast moving fats some UV absorbing compounds with (R_f < 0.50, CH_2Cl_2/10% MeOH). For the biological screening results, see table 12. In the pharmacological tests, the crude extract exhibited an anti-interferon-gamma activity.

Table 13: Activity of the crude extract from GW 22/1326 (50 mg/mL)

Test Organisms	Inhibition Zone (∅ mm)
Streptomyces viridochromogenes (Tü 57)	11
Mucor miehei	11
Chlorella sorokiniana	11
Scenedesmus subspicatus	11

10.5.2 Fermentation

The fermentation process was carried as follows: four well-grown agar subcultures of the isolate GW 22/1326 served to inoculate 80 of 1 L Erlenmeyer flasks each containing 250 ml of M_2 medium. The flasks were incubating for 4 days at 28 °C on a linear shaker. The brown mycelium of the growth culture was separated by filtration with the aid of Celite into mycelia cake and filtrate. Both were extracted with ethyl acetate and combined after the analysis of the TLC to give a dark oily crude extract.

The resulting extract was absorbed on silica gel and submitted to column chromatography on silica gel. The elution was performed using CH_2Cl_2 and then a stepwise increasing CH_2Cl_2/MeOH gradient (1 L for each gradient), finally the column was washed with methanol. By TLC monitoring (CH_2Cl_2/5% MeOH), five fractions were obtained and were sent for anti-interferon-gamma activity tests, where fraction V showed activity. Purification of fraction III by PTLC (CH_2Cl_2/7% MeOH) gave 25 mg of the red ε-rhodomycinone (**32**). Chromatography on Sephadex LH-20 (CH_2Cl_2/MeOH 1:1) follows by PTLC (CH_2Cl_2/15% MeOH) of fraction IV delivered 100 mg of dihydrotetrodecamycin (**33**). The active fraction V was also chromatographed on Sephadex LH-20 and then PTLC (CH_2Cl_2/20% MeOH) to result in cosmomycin A (**34**, 6 mg).

ε–Rhodomycinone (**32**): $C_{22}H_{20}O_9$, red powder. – R_f = 0.79 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 13.38 (s, 1H, OH), 12.80 (s, 1H, OH), 11.98 (s, 1H, OH), 7.80 (d, ^3J = 7.5 Hz, 1H, 1-H), 7.65 (t, ^3J = 8.2 Hz, 1H, 2-H), 7.28 (d, ^3J = 8.3 Hz, 1H, 3-H), 5.31 (t, ^3J = 3.0 Hz, 1H, 2-H), 4.23 (s, 1H, 10-H), 3.74 (s, 3H, OCH_3), 2.26 (ABX, ^3J = 15.4, 4.5, 3.0 Hz, 2H, 8-H_2), 1.80 (sextet, ^3J = 7.5 Hz, 1H, 13-H), 1.58 (sextet, ^3J = 7.6 Hz, 1H, 13-H), 1.17 (t, ^3J = 7.3 Hz, 3H, 14-CH_3). –

^{13}C/APT NMR ($CDCl_3$, 50.3 MHz): δ = 190.6 (C_q, C-5), 185.9 (C_q, C-12), 171.3 (C_q, C-15), 162.6 (C_q, C-4), 156.9 (C_q, C-11), 155.8 (C_q, C-6), 137.5 (CH, C-2), 137.1 (C_q, C-10a), 134.9 (C_q, C-6a), 133.2 (C_q, C-12a), 124.8 (CH, C-3), 119.7 (CH, C-1), 115.8 (C_q, C-4a), 111.3 (C_q, C-5a), 111.2 (C_q, C-11a), 71.4 (C_q, C-9), 62.5 (CH, C-7), 52.5 (CH, C-10), 51.5 (CH_3, C-16), 34.3 (CH, C-8), 32.6 (CH_2, C-13), 6.8 (CH_3, C-14). **– (+)-ESI MS:** *m/z* (%) = 879.3 ($[2M+Na]^+$, 100), 451.5 ($[M+Na]^+$, 12). **– (-)-ESI MS:** *m/z* (%) = 877.3 ($[2M+Na-2H]^-$, 100), 427.3 ($[M-H]^-$, 40).

Dihydrotetrodecamycin (**33**) : $C_{18}H_{24}O_6$, colourless solid. **–** R_f = 0.47 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ($[D_6]$DMSO, 300.0 MHz): δ = 5.58 (d, ^3J = 6.4 Hz, 1H, 14-OH), 4.96 (q, ^3J = 6.8 Hz, 1H, 4-H), 4.76 (dd, ^3J = 4.5 Hz, ^4J = 0.9, Hz, 1H, 15-H), 3.93 (d, ^3J = 1.0 Hz, 1H, 13-OH), 3.42 (d, ^3J = 6.2 Hz, 1H, 14-H), 2.70 (qd, ^3J = 7.2 Hz, ^4J = 3.7 Hz, 1H, 16-H), 1.98 (*br* d, ^3J = 13.9 Hz 1H, 12-H), 1.60 (d, ^3J = 12.1 Hz, 1H, 9-H), 1.58-1.30 (m, 5H), 1.38 (d, ^3J = 6.8 Hz, 3H, 5-H_3), 1.19 (m, 2H, 12-H, 11-H), 1.06 (s, 3H, 17-H_3), 0.83 (d, ^3J = 7.5 Hz, 3H, 18-H_3). **– ^{13}C/APT NMR** ($[D_4]$MeOH, 50.3 MHz): δ = 197.6 (C_q, C-6), 183.1 (C_q, C-3), 171.5 (C_q, C-1), 100.9 (C_q, C-2), 94.9 (CH, C-15), 79.9 (CH, C-14), 75.4 (CH, C-4), 69.5 (C_q, C-13), 54.2 (C_q, C-7), 44.3 (CH, C-8), 40.1 (CH_2, C-12), 33.7 (CH, C-16), 27.2 (CH_2, C-10), 24.8 (CH_2, C-9), 22.0 (CH_2, C-11), 17.9 ($2CH_3$, C-5, C-17), 13.5 (CH_3, C-18). **– EI MS (70 eV):** *m/z* (%) = 336 ($[M]^+$, 20), 300 (8), 260 (4), 248 (8), 208 (24), 178 (36), 163 (32), 135 (30), 123 (19), 98 (100), 79 (19), 67 (25), 41 (50).

Cosmomycin A (**34**)**:** $C_{40}H_{53}O_{13}N$, red powder. **–** R_f = 0.54 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 13.90 (*br* s, 1 H, OH), 12.76 (*br* s, 1H, OH), 12.24 (*br* s, 1H, OH), 7.84 (d, ^3J = 8.0 Hz, 1H, 1-H), 7.70 (t, ^3J = 7.8 Hz, 1H, 2-H), 7.30 (d, ^3J = 7.8 Hz, 1H, 3-H), 5.40 (d, ^3J = 2.6 Hz, 1H, 1'-H), 4.98 (*br* s, 1H, 10-H), 4.96 (*br* s, 1H, 1''-H), 4.80 (d, ^3J = 3.0 Hz, 1H, 1'''-H), 4.40 (q, ^3J = 6.0 Hz, 1H, 5''-H), 4.04 (q, ^3J = 6.0 Hz, 1H, 5'''-H), 3.90 (m, 1H, 5'-H), 3.78 (*br* s, 2H, 4'-H, OH), 3.58 (*br* s, 1H, 4'''-H), 3.44 (*br* s, 1H, 4''-H), 2.90 (m, 2H, 7-H_2), 2.22 (*br* s, 6H, $N(CH_3)_2$), 2.10-1.60 (m, 15H), 1.25 (m, 6H, 6'-CH_3, 6'''-CH_3), 1.18 (d, ^3J = 7.2 Hz, 3H, 6''-CH_3), 1.10 (t, ^3J = 7.2 Hz, 3H, 14-CH_3), 1 OH signal is missing. **– (+)-ESI**

MS: *m/z* = 756.4 $[M+H]^+$. **– (-)-ESI MS:** *m/z* (%) = 1531.8 ($[M+Na-2H]^-$, 100), 755.0 ($[M-H]^-$, 90).

10.6 Terrestrial *Streptomyces* sp. GW 28/1818

The terrestrial actinomycete GW 28/2818 came from a soil sample collected in Malta. It grew on M_2 agar with a white aerial mycelium and dark brown agar background on incubating at 28 °C for four days. The diffusion of the yellow pigment into agar was responsible for its dark brown colouration. The extract from a 1 L shaker culture exhibited biological activity against different microorganisms, which is listed in the table below:

Table 14: Biological activity of the crude extract from the strain GW 28/2818

Test Organisms	Inhibition Zone (∅ mm)
Bacillus subtilis	13
Staphylococcus aureus	15
Streptomyces viridochromogenes (Tü 57)	13
Escherichia coli	18
Mucor miehei	12

In the chemical screening on TLC it showed, beside some colourless UV active bands, a yellow zone, which gave an orange fluorescence under UV 366 nm and turned red with diluted sodium hydroxide and became orange on spraying with anisaldehyde/sulphuric acid.

10.6.1 Fermentation, Work-up and Isolation

The strain was cultivated at 28 °C for four days using a linear shaker. 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2 medium were inoculated with well grown agar cultures. The dark brown culture broth was mixed with 1.2 kg diatomaceous earth and filtered through a pressure filter. The filtrate and the residue were extracted separately each four times with ethyl acetate (2 L). The combined organic solutions were evaporated under vacuum to yield 2 g of dark brown extract, which was pre-separated into three fractions by column chromatography on silica gel with a CH_2Cl_2/MeOH-gradient (0-20% MeOH). The first fraction contained the yellow

compound, which could be purified easily by triturating it with CH_2Cl_2 and filtering the insoluble yellow solids off. Further size exclusion chromatography on Sephadex LH-20 (CH_2Cl_2/50% MeOH) delivered 100 mg resistomycin (**35**). Preparative HPLC of the second fraction on RP-18 silica gel (MeCN/90-40% H_2O in 30 min, 100% MeCN for 5 min) delivered N-acetyldopamine (**36**, 6 mg, R_t = 25 min) and 3-indolylethanol (4 mg, R_t = 27 min). Chromatography of the third fraction on Sephadex LH-20 gave only uracil.

Resistomycin (35): $C_{22}H_{16}O_6$, yellow powder. – R_f = 0.66 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 14.54 (s, H/D exchangeable, 1H, OH), 14.34 (s, H/D exchangeable, 1H, OH), 14.02 (s, H/D exchangeable, 1H, OH), 11.98 (br s, 1H, OH), 7.25 (s, 1H, 11-H), 7.05 (s, 1H, 8-H), 6.38 (s, 1H, 4-H), 2.90 (s, 3H, CH_3), 1.58 (s, 6H, $C(Me)_2$). – **(+)-ESI MS:** *m/z* (%) = 775 ($[2M+Na]^+$, 20), 377 ($[M+H]^+$, 6). – **(-)-ESI MS:** *m/z* = 375 $[M-H]^-$.

N-Acetyl dopamine (36): $C_{10}H_{13}NO_3$, colourless solid. – R_f = 0.57 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ([D_6]acetone, 300.0 MHz): δ = 7.85 (*br* s, 2 H, H/D exchangeable, OH), 7.20 (*br* s, 1H, H/D exchangeable, NH), 6.74 (d, ^3J = 8.2 Hz, 1H, 5-H), 6.70 (d, ^4J = 2.0 Hz, 1H, 2-H), 6.50 (dd, ^3J = 8.2 Hz, ^4J = 2.0 Hz, 1H, 6-H), 3.30 (td, ^3J = 5.3, 7.3 Hz, 2 H, 1'-H), 2.60 (t, ^3J = 7.3 Hz, 1H, 2'-H), 1.83 (s, 3H, CH_3). – **^{13}C/APT NMR** ([D_6]acetone, 75.7 MHz): δ = 170.7 ($OCOCH_3$), 145.8 (C_q, C-4), 144.3 (C_q, C-3), 131.7 (C_q, C-1), 120.6 (CH, C-6), 116.5 (CH, C-5), 115.9 (CH, C-2), 41.8 (CH_2 C-1'), 35.7 (CH_2, C-2'), 22.8 (CH_3). **(+)-ESI MS:** *m/z* (%) = 413.4 ($[2M+Na]^+$, 96), 218.5 ($[M+Na]^+$, 100), 196.4 ($[M+H]^+$, 14). – **(-)-ESIMS:** *m/z* (%) = 389.4 ($[2M-H]^-$, 14), 194.4 ($[M-H]^-$, 100). – **EI MS (70 eV):** *m/z* (%) = 195 ($[M]^+$, 8), 136 (100), 123 (24), 77 (8), 60 (8), 43 (16).

3-Indolethanol: $C_{10}H_{11}NO$, colourless solid. – R_f = 0.48 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 8.08 (br s, H/D exchangeable, 1H, NH), 7.62 (d, ^3J = 7.8. Hz, 1H, 4-H), 7.38 (d, ^3J = 8.1. Hz, 1H, 7-H), 7.22 (td, ^3J = 7.1 Hz, ^4J = 1.1 Hz, 1H, 6-H), 7.14 (td, ^3J = 7.1 Hz, ^3J = 1.2 Hz, 1H, 5-H), 7.10 (d, ^3J = 2 Hz, 1H, 2-H), 3.96 (t, ^3J = 6.4 Hz, 2H, 11-H_2), 3.05 (t, ^3J = 6.4 Hz, 2H, 10-H_2).

10.7 Terrestrial *Streptomyces* sp. GW 10/580

The strain GW 10/580 grew with a white thick aerial mycelium on M_2 agar after incubation at 28 °C for three days. With the well grown strain on agar, eight of 1 L Erlenmeyer flasks each with 250 ml of the same medium were inoculated and grown at 28 °C on a linear shaker with 95 rpm, whereby the strain grew with light brown broth. The dark brown extract obtained from the 2 L shaker culture was used for the biological and chemical screening.

10.7.1 Primary screening

The extract was found to inhibit the growth of Gram-positive and Gram-negative bacteria, fungi and algae. The semiquantitative results of antibacterial, antifungal and phytotoxic tests are listed in the following table. The TLC of the crude extract exhibited a light yellow spot and three colourless UV inactive bands, which became first brown and later turned to violet with anisaldehyde/sulphuric acid.

Table 15: Biological activity of the crude extract of the strain GW 10/580

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	30
Staphylococcus aureus	20
Streptomyces viridochromogenes (Tü 57)	30
Escherichia coli	23
Candida albicans	20
Mucor miehei	21
Chlorella vulgaris	20
Chlorella sorokiniana	30
Scenedesmus subspicatus	34

10.7.2 Scale-up of the strain and isolation

For the isolation the strain was cultured on a 25 L scale on a linear shaker with 95 rpm in 1 L Erlenmeyer flasks each containing 250 ml of M_2 medium at 28 °C for four days. The resulting light brown culture broth was filtered through a filter press and both filtrate and mycelium were extracted with ethyl acetate. The combined ethyl acetate extract was evaporated to dryness yielding a dark brown oily material (3 g). Chromatography of the crude extract (3 g) on silica gel using (CH_2Cl_2/MeOH) gradi-

ent resulted in four fractions. The first fraction on TLC showed non-polar fats and fatty acids and was not investigated further. The second fraction containing a yellow compound was purified by PTLC with CH_2Cl_2/5% MeOH to give phenazin-1-carboxylic acid (**38**). Fraction 3 showed two colourless spots, which turned brown and then violet with anisaldehyde/sulphuric acid. Separation by preparative HPLC on RP-18 using a MeCN/H_2O gradient (10:90, 60:40, 100:0, 100:0, 10:90) resulted in feigrisolide B (**39a**) and A (**39b**). PTLC of fraction 4 followed by preparative HPLC using the same gradient gave feigrisolide C (**40**) and (4-hydroxy-phenyl)-acetic acid.

Phenazin-1-carboxylic acid (38): $C_{13}H_8N_2O_2$, yellow solid**,** no colour change with anisaldehyde/sulphuric acid. **– R_f** = 0.78 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 15.60 (s br, 1H, COOH), 9.00 (dd, ^3J = 7.0 Hz, ^4J = 1.0 Hz, 1H, Ar-H), 8.58 (dd, ^3J = 7.0 Hz, ^4J = 1.0 Hz, 1H, Ar-H), 8.20-8.40 (2dd, ^3J = 7.7 Hz, ^4J = 1.2 Hz, 2H, Ar-H), 8.10-7.98 (m, 3H, Ar-H). **– EI MS (70 eV):** *m/z* (%) = 224 ($[M]^+$, 8), 180 (100), 153 (4).

Feigrisolide B (39a): $C_{11}H_{20}O_4$, colourless oil, turns first brown and then violet with anisaldehyde/sulphuric acid. **– R_f** = 0.40 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 6.40, in other spectra at 5.60 (s br, 2H, OH), 4.22 (m, 1H, 6-H), 4.00 (q, ^3J = 8.3 Hz, 1H, 3-H), 3.79 (m, 1H, 8-H), 2.50 (dq, ^3J = 8.3, 7.0 Hz, 1H, 2-H), 1.98-2.03 (m, 2H), 1.80-1.60 (m, 4H), 1.50 (m, 2H), 1.18 (d, ^3J = 7.0 Hz, 3H, 1'-CH_3), 0.92 (t, ^3J = 7.3 Hz, 3H, 10-CH_3). **– ^{13}C/APT NMR** ($CDCl_3$, 125.7 MHz): δ = 178.2 (C_q, C-1), 80.9 (CH, C-3), 77.2 (CH, C-6), 70.3 (CH, C-8), 45.3 (CH, C-2), 40.8 (CH_2, C-8), 30.6 (CH_2, C-5), 29.7 (CH_2, C-9), 28.7 (CH_2, C-4) 13.5 (CH_3, C-1'), 10.0 (CH_3, C-10). **– (+)-ESI MS:** *m/z* (%) = 261.3 ($[M-H+2Na]^+$, 17), 239.3 ($[M+Na]^+$, 5). **– (-)-ESI MS:** *m/z* (%) = 215.4 ($[M-H]^-$, 53). **– EI MS (70 eV):** *m/z* (%) = 216 ($[M]^+$, 4), 198 (20), 187 (20), 169 (100), 143 (84), 125 (64), 113 (56).

Feigrisolide A (39b): $C_{10}H_{18}O_4$, colourless oil, turns first brown and then violet with anisaldehyde/sulphuric acid. **– R_f** = 0.38 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 6.70 (*br* s, 2 H, H/D exchangeable, OH), 4.22 (m, 1H, 6-H), 4.08 (m, 1H, 8-H), 3.98 (q, ^3J = 8.3 Hz, 1H, H-3), 2.50 (dq, ^3J = 8.3, 7.2 Hz, 1H, 2-H), 1.96-2.05 (m, 2 H), 1.76-1.60 (m, 4H), 1.22 (d, ^3J = 6.3 Hz, 3H, 9-H_3), 1.16 (d, ^3J =

7.2 Hz, 3H, 2-CH_3). – **(+)-ESI MS:** *m/z* (%) = 471.5 ($[2M-2H+3Na]^+$, 62), 247.4 ($[M-H+2Na]^+$, 24), 225.3 ($[M+Na]^+$, 9). – **(-)-ESI MS:** *m/z* (%) = 425.8 ($[2M-H+Na]^-$, 26), 201.4 ($[M-H]^-$, 78).

Feigrisolide C (40): $C_{21}H_{36}O_7$, colourless oil, turns first brown and then violet with anisaldehyde/sulphuric acid. – R_f = 0.2 (CH_2Cl_2/5 % MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 6.50 (*s br*, 2H, 2 OH), 5.02 (m, 1H, 12-H), 4.18 (m, 1H, 5-H), 4.00 (m, 3H, 1-H, 8-H, 14-H), 3.78 (m, 1H, 20-H), 2.50 (dq, ^3J = 8.3, 7.0 Hz, 2H, 2-H, 9-H), 2.05-1.90 (m, 4H), 1.40-1.80 (m, 10 H), 1.22 (d, ^3J = 6.3 Hz, 3H, 24-CH_3), 1.10 (d, ^3J = 7.0 Hz, 3H, 23-CH_3), 1.15 (d, ^3J = 7.0 Hz, 3H, 18-CH_3), 0.92 (t, ^3J = 7.4 Hz, 3H, 22-CH_3). – **^{13}C/APT NMR** ([D_6]acetone, 75.7 MHz): δ = 176.0 (C_q, C-10), 174.4 (C_q, C-3), 81.2 (CH, C-1), 81.0 (CH, C-8), 77.4 (CH, C-5), 76.8 (CH, C-14), 70.3 (CH, C-20), 69.4 (CH, C-12), 46.2 (CH, C-9), 45.5 (CH, C-2), 43.5 (CH_2, C-13), 43.3 (CH_2, C-19), 32.0 (CH_2, C-15), 31.8 (CH_2, C-7), 31.3 (CH_2, C-21), 30.1 (CH_2, C-6), 29.8 (CH_2, C-16), 20.8 (CH_3, C-24), 13.6 (CH_3, C-23), 13.4 (CH_3, C-18), 10.3 (CH_3, C-22). – **(+)-ESI MS:** *m/z* (%) = 423.7 ($[M+Na]^+$, 28). – **(-)-ESI MS:** *m/z* (%) = 399.5 ($[M-H]^-$, 46).

(4-Hydroxy-phenyl)-acetic acid: $C_8H_8O_3$, colourless solid. – R_f = 0.35 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ([D_4]MeOH, 300.0 MHz): δ = 7.10 (d, ^3J = 8.5 Hz, 2H, 3-H, 5-H), 6.70 (d, ^3J = 8.5 Hz, 2H, 2-H, 6-H). – **EI MS (70 eV):** *m/z* (%) = 152.1 ($[M]^+$, 26), 138 (8), 121 (12), 107 (100), 77 (19).

10.8 Terrestrial *Streptomyces* sp. GW 9/2335

The terrestrial strain GW 9/2335 grew on M_2 agar with a white aerial mycelium after four days of incubation at 28 °C. The well-grown agar culture was used to inoculate a 1 L shaker culture in M_2 medium, where it grew with light yellow broth after four days at 28 °C with 110 rpm. The culture broth was extracted by the standard method and the resulting extract was used for pre-screening.

10.8.1 Pre-screening

In pharmacological screening, the crude extract inhibited the proliferation of human tumor cells MCF7. In the biological screening using the agar diffusion method, it exhibited activity against Gram-positive and -negative bacteria as well as microalgae. The results of the tests are summarized in the following table. The chemical screening of the extract by TLC depicted, beside others, colourless bands and some UV absorbing compounds (254 nm), which turned first violet and then dark grey with anisaldehyde/sulphuric acid.

Table 16: Biological activity of the crude extract from GW 9/2335 (50 mg/mL)

Test organisms	Inhibition Zone (∅ mm)
Bacillus subtilis	14
Staphylococcus aureus	15
Streptomyces viridochromogenes (Tü 57)	25
Escherichia coli	22
Chlorella vulgaris	14
Chlorella sorokiniana	12
Scenedesmus subspicatus	17

10.8.2 Fermentation, work-up and isolation

For the cultivation, small pieces of the grown agar were used to inoculate a 5 L shaker culture of M_2 medium for five days at 28 °C. The light yellow culture broth was used to inoculate a 25 L fermentation. The pH of the fermentation solution was controlled not to be less than 6.5. To the culture broth, ca. 1 kg of Celite was added and the mixture filtered under reduced pressure. The water phase was extracted with ethyl acetate and the mycelium was extracted successively with 5 L acetone and ethyl acetate. The resulting crude extracts were combined due to their similarity on TLC and yielded 3.4 g of an oily crude extract. The latter was submitted to middle pressure column chromatography on silica gel using a CH_2Cl_2/MeOH-gradient, which afforded four fractions. Separation of fraction II yielded N-acetyltyramine. Fraction IV was again subjected to silica gel chromatography and yielded 3 subfractions. PTLC (CH_2Cl_2/5% MeOH) of subfraction I followed by size exclusion chro-

matography on Sephadex LH-20 (CH_2Cl_2/40% MeOH) gave colourless solid irumamycin (**44**, 18 mg). 5-Deoxyenterocin (**43a**) was separated together with *p*-hydroxybenzoic acid and 2-(*p*-hydroxyphenyl)ethanol by RP-18 preparative HPLC using (MeCN/80% H_2O). The subfraction II showed by TLC a spot having similar physical properties as irumamycin (**44**). The fraction was separated using silica gel with CH_2Cl_2/5% MeOH and gave X-14952B (**45**, 10 mg). The subfraction III was chromatographed on silica gel and finally subjected to preparative HPLC and delivered the orange powdery P-371-A2 (**47**, 4 mg) and slightly yellow KSM-2690 B (**46a,** 10 mg).

5-Deoxyenterocin (43a): $C_{22}H_{20}O_9$, white amorphous solid. – R_f = 0.46 (CH_2Cl_2/8% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.82 (d, ^3J = 7.9 Hz, 2H, 17-H, 17'-H), 7.58 (t, ^3J = 7.5 Hz , 1H, 19-H), 7.44 (t, ^3J = 8.0 Hz, 2H, 18-H, 18'-H), 6.30 (d, ^4J = 2.0 Hz, 1H, 11-H), 5.44 (d, ^4J = 2.0 Hz, 1H, 13-H), 4.84 (br s, 1H, 6-H), 4.30 (br s, 2H, 3-H, 9-H), 3.99 (s, 1H, 15-H), 3.79 (s, 3H, OCH_3), 2.78 (dd, ^3J = 15.0 Hz, ^4J = 4.0 Hz, 1H, 5-Ha), 2.54 (dd, ^3J = 15.0 Hz, ^4J = 4.0 Hz, 1H, 5-He), 2.18 (br d, ^4J = 14.0 Hz, 1H, 7-Ha), 1.80 (br d, ^3J = 14.0 Hz, 1H, 7-He); OH signals were not visible. – **(+)-ESI MS:** *m/z* (%) = 901.1 ($[2M–H+2Na]^+$, 28), 879.1 ($[2M+Na]^+$, 100), 451.6 ($[M+Na]^+$, 40). – **(-)-ESI MS:** *m/z*(%) = 855.1 ($[2M-H]^-$, 26), 427.4 ($[M-H]^-$, 100). – **EI MS (70 eV):** *m/z* (%) = 428.1 (M^+, 76), 323.1 (24), 253.1 (12), 235.1 (100), 193.1 (12), 167.0 (48), 125 (40), 105 (60), 69 (24), 44 (12).

4-Hydroxyphenylethanol: $C_8H_{10}O_2$, white solid. – R_f = 0.54 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.10 (d, ^3J = 8.0 Hz, 2H, 2-H, 6-H), 6.78 (d, ^3J = 8.0 Hz, 2H, 3-H, 5-H), 4.90 (s br, 1H, OH), 3.80 (t, ^3J = 8.0 Hz, 3H, 1'-H_2), 2.80 (t, ^3J = 8.0 Hz, 2 H, 2'-H_2). ― **EI MS (70 eV):** *m/z* (%) = 138.1 ($[M]^+$, 32), 107 (100).

N-Acetyltyramine: $C_{10}H_{13}NO_2$, colourless oil. – **^{1}H NMR** ($[D_4]$MeOH, 300 MHz): δ = 7.00 (d, ^3J = 7.9 Hz, 2H, 2-H, 6-H), 6.70 (d, ^3J = 8.0 Hz, 2H, 3-H, 5-H), 3.30 (m, 2H, 1'-H_2), 2.64 (t, ^3J = 6.7 Hz, 2H, 2'-H_2), 1.87 (s, 3H, CH_3).

Irumamycin (44): $C_{41}H_{65}NO_{12}$, white solid. – R_f = 0.51 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 5.56 (dd, ^3J = 15.5, 8.6 Hz, 1H, 14-H), 5.50 (m, 1H,

5-H), 5.45 (m, 1H, 9-H), 5.24 (dd, ^3J = 15.5, 8.6 Hz, 1H, 15-H), 4.82 (dd, ^3J = 9.3, 1.5 Hz, 1H, 19-H), 4.63 (m, 1H, 3'-H), 4.58 (dd, ^3J = 9.7, 1.7 Hz, 1H, 1'-H), 4.46 (s br, 1H, 7-H), 3.92 (m, 1H, 13-H), 3.40-3.18 (m, 4H, 17-H, 23-H, 4'-H, 5'-H), 2.72 (d, ^3J = 6.8 Hz, 1H), 2.67, 2.58 (AB, ^3J = 17.2 Hz, 2H, 2-CH_2), 2.58-2.20 (m, 3H), 2.18-2.10 (m, 2H), 1.90-1.60 (m, 8H), 1.48 (br s, 3H, 6-CH_3), 1.42 (s, 3H, 24-CH_3), 1.38 (br s, 3H, 8-CH_3), 1.30-1.20 (m, 2H, 11-H_2), 1.28 (d, ^3J = 6.5 Hz, 3H, 5'-CH_3), 1.10 (m, 1H, 21-H), 1.10 (d, ^3J = 6.5 Hz, 3H, 22-CH_3), 1.00 (t, ^3J = 7.2 Hz, 3H, 27-CH_3), 0.95 (m, 1H, 21-H), 0.88 (d, 3H, d, ^3J = 6.4 Hz, 16-CH_3), 0.84 (d, ^3J = 7.5 Hz, 3H, 18-CH_3), 0.80 (d, ^3J = 6.5 Hz, 3H, 20-CH_3); acidic protons were not visible. – **^{13}C/APT NMR** ($CDCl_3$, 125.7 MHz): δ = 210.9 (C_q, C-25), 173.3 (C_q, C-1), 157.6 (C_q, 3'-$OCONH_2$), 134.9 (C_q, C-8), 134.3 (CH, C-14), 134.1 (CH, C-15), 132.9 (C_q, C-6), 129.3 (CH, C-9), 116.8 (CH, C-5), 98.4 (CH, C-1'), 94.0 (C_q, C-3), 82.4 (CH, C-13), 81.6 (CH, C-19), 80.1 (CH, C-7), 77.5 (CH, C-17), 75.2 (CH, C-3'), 75.0 (CH, C-4'), 72.0 (CH, C-5'), 66.3 (CH, C-23), 64.4 (CH, C-24), 43.4 (CH_2, C-2), 42.1 (CH, C-16), 36.9 (CH_2, C-26), 36.7 (CH_2, C-21), 35.9 (CH_2, C-2'), 35.3 (CH_2, C-12), 35.1 (CH_2, C-4), 34.6 (CH, C-18), 31.9 (CH, C-20), 30.5 (CH, C-22), 28.8 (CH_2, C-10), 27.0 (CH_2, C-11), 19.2 (CH_3, 6-CH_3), 17.8 (CH_3, 5'-CH_3), 17.2 (CH_3, 16-CH_3), 17.0 (CH_3, 20-CH_3), 15.9 (CH_3, 22-CH_3), 12.9 (CH_3, 24-CH_3), 10.8 (CH_3, 8-CH_3), 7.3 (CH_3, 27-CH_3), 5.5 (CH_3, 18-CH_3). – **(+)-ESI-MS:** *m/z* = 786.6 $[M+Na]^+$. – **(-)-ESI-MS:** *m/z* = 762.7 ($[M-H]^-$).

X-14952B (45): $C_{42}H_{69}NO_{12}$, white solid. – R_f = 0.49 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 5.57 (dd, ^3J = 15.0, 8.8 Hz, 1H, 14-H), 5.50 (m, 1H, 5-H), 5.45 (m, 1H, 9-H), 5.25 (dd, ^3J = 15.0, 8.8 Hz, 1H, 15-H), 4.85 (dd, ^3J = 8.7, 0.9 Hz, 1H, 19-H), 4.64 (m, 1H, 3'-H), 4.58 (dd, ^3J = 9.6, 1.1 Hz, 1H, 1'-H), 4.46 (s br, 1H, 7-H), 3.96 (m, 1H, 13-H), 3.57 (m, 1H, 23-H), 3.28 (m, 1H, 5'-H), 3.20 (m, 2H, 4'-H, 17-H), 2.67 (m, 1H, 24-H), 2.65, 2.58 (AB, ^3J = 18.0 Hz, 1H, 2-H), 2.50 (q, ^3J = 7.2 Hz, 1H, 26-H), 2.28 (m, 1H, 2'-H), 2.20-2.10 (m, 2H), 2.04-1.98 (m, 2H), 1.80-1.60 (m, 7H), 1.60-1.50 (m, 4H), 1.49 (br s, 3H, 6-CH_3), 1.42 (br s, 3H, 8-CH_3), 1.30 (d, ^3J = 6.5 Hz, 3H, 5'-CH_3), 1.30-1.20 (m, 3H), 1.10 (t, 3H, ^3J = 7.0 Hz, 27-CH_3), 0.98 (d, ^3J = 6.7 Hz, 3H, 16-CH_3), 0.92 (d, ^3J = 7.5 Hz, 3H, 18-CH_3), 0.83 (d, ^3J = 6.6 Hz, 3H, 22-CH_3), 0.82 (br t ^3J = 9.4 Hz, 3H, CH_3), 0.81 (d, ^3J = 6.8 Hz,

3H, 20-CH_3); acidic protons are not visible. – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 217.2 (C_q, C-25), 173.4 (C_q, C-1), 157.5 (C_q, 3'-$OCONH_2$), 134.7 (C_q, C-8), 134.3 (CH, C-14), 134.0 (CH, C-15), 132.8 (C_q-6), 129.3 (CH-9), 116.9 (CH-5), 98.2 (CH-1'), 94.0 (C_q-3), 82.2 (CH-13), 81.9 (CH, C-19), 80.0 (CH, C-7), 77.7 (CH, C-17), 76.7 (CH, C-23), 75.2 (CH, C-3'), 74.9 (CH, C-4'), 71.9 (CH, C-5'), 55.1 (CH, C-24), 43.3 (CH_2, C-2), 42.0 (CH, C-16), 37.9 (CH_2, C-26), 37.0 (CH_2, C-21), 36.8 (CH_2, C-2'), 35.1 (CH_2, C-12), 35.0 (CH_2, C-4), 34.5 (CH, C-18), 33.2 (CH, C-20), 32.5 (CH, C-22), 27.0 (CH_2, C-10), 26.0 (CH_2, C-11), 22.6 (CH_2, 24-*CH_2*CH_3), 19.1 (CH_3, 6-CH_3), 17.8 (CH_3, 5'-CH_3), 17.1 (CH_3, 16-CH_3), 15.8 (CH_3, 20-CH_3), 12.6 (CH_3, 22-CH_3), 11.7 (CH_3, 24-CH_2*CH_3*), 10.7 (CH_3, 8-CH_3), 7.20 (CH_3, 27-CH_3), 5.5 (CH_3, 18-CH_3). – **(+)-ESI-MS:** *m/z* (%) = 1581.9 ($[2M+Na]^+$, 13), 802.7 ($[M+Na]^+$, 100).

KSM-2690 B (46a): $C_{36}H_{51}N_3O_9$, pale yellow solid. – R_f = 0.56 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 8.22 (s, 1H, 13'-H), 7.63 (t, 1H, ^3J = 5.0 Hz, NH), 6.87 (s, 1H, 12'-H), 6.75 (dd, ^3J = 15.0, 12.0 Hz, 1H, 8'-H), 6.40 (d, ^3J = 12.0 Hz, 1H, 5'-H), 6.32 (t, ^3J = 11.0 Hz, 1H, 6'-H), 6.14 (d, ^3J = 15.0 Hz, 1H, 9-H), 6.08 (d, ^3J = 15.0 Hz, 1H, 10-H), 5.94 (t, ^3J = 11.0 Hz, 1H, 7'-H), 5.79 (m, 1H, 9'-H), 5.60 (m, 2H, 8-H, 11-H), 5.43 (d, ^3J = 5.0 Hz, 1H, 3'-OH), 5.26 (s, 1H, 3-OH), 4.98 (q, ^3J = 7.0 Hz 1H, 16-H), 4.80 (d, ^3J = 3.0 Hz, 1H, 7-OH), 4.62 (d, ^3J = 4.0 Hz, 1H, 3'-H), 3.83 (m, 1H, 7-H), 3.70 (t, ^3J = 6.8 Hz, 2H, 12-H), 3.56 (d, ^3J = 7.0 Hz, 2H, 10'-H), 3.38 (t, J = 5.0 Hz, 1H, 4-H), 3.17 (s, 3 H, OCH_3), 2.78 (s, 3H, NCH_3), 2.38 (q, ^3J = 7.0 Hz, 1H, 2-H), 1.98 (dt, ^3J = 15.5, 5.0 Hz, 1H, 5-H), 1.72 (s, 3H, 16'-H_3), 1.71 (s, ^3J = 6.0 Hz, 3H, 16-CH_3), 1.60 (m, 1H, 6-H), 1.18 (m, 1H, 5-H), 1.11 (s, 3H, 14'-H), 1.04 (d, ^3J = 7.0 Hz, 3H, 13-H), 0.98 (s, 3H, 15'-H), 0.85 (s, 3H, 14-H). – **^{13}C/APT NMR** ([D_6]DMSO, 125.7 MHz): δ = 176.0 (C_q, C-1'), 174.3 (C_q, C-1), 170.0 (C_q, C-17), 151.2 (CH, C-13'), 150.5 (C_q C-12'), 139.8 (C_q, C-4'), 134.4 (CH, C-8), 130.2 (CH, C-10), 129.8 (CH, C-11), 129.7 (CH, C-9), 128.9 (CH, C-9'), 128.0 (CH, C-8'), 127.0 (CH, C-7'), 124.4 (CH, C-6'), 123.4 (CH, C-5'), 121.9 (CH,C-12'), 83.5 (C_q, C-15), 82.6 (CH, C-4), 80.7 (C_q, C-3), 77.2 (CH, C-16), 75.1 (CH, C-7), 73.1 (CH, C-3'), 55.9 (OCH_3), 48.5 (CH_2, C-12), 45.7 (C_q, C-2'), 43.5 (CH, C-2), 36.7 (CH, C-6), 32.0 (CH_2, C-5), 28.2 (CH_2, C-10'), 25.9 (CH_3,

NCH_3), 24.7 (CH_3, C-14'), 21.5 (CH_3, 15'-CH_3), 19.8 (CH_3, 16'-CH_3), 16.8 (CH_3, 16-CH_3), 16.1 (CH_3, 14-CH_3), 9.7 (CH_3, 13-CH_3). **– (+)-ESI MS:** *m/z* (%) = 1361.1 ($[2M+Na]^+$, 100), 692.5 ($[M+Na]^+$, 96). **– (-)-ESI MS:** *m/z* = 668.9 ($[M-H]^-$).

P-371-A2 (47): $C_{50}H_{68}N_2O_{22}$, orange powder. **–** R_f = 0.45 (CH_2Cl_2/7% MeOH). **–** ^{1}**H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.46 (s, 1H, 8-OH), 7.85 (d, ^3J = 7.8 Hz, 1H, 10-H), 7.65 (d, ^3J = 7.8 Hz, 1H, 11-H), 6.00 (d, ^3J = 4.8 Hz, 1H, 2-H), 5.81 (d, ^3J = 6.5 Hz, 1H, 5-H), 5.35 (d, ^3J = 4.2, 1H, 4-H), 5.02 (dd, ^3J = 11.8 Hz, ^4J = 6.5 Hz, 1H, 6-H), 4.84 (d, ^3J = 11.1 Hz, 1H, 2'-H), 4.70-4.58 (m, 2H, 1-H_A, 1-H_B), 4.44 (d, ^3J = 9.2 Hz, 1H, 1_C-H), 4.40-4.30 (m, 3 H, 1-H, 5-H_A, 4-H_C), 3.70 (m, 1H, 4'-H), 3.60-3.42 (m, 4H, 6'-H, 4-H_A, 5-H_B, 5-H_C), 3.40 (s br, 1H, 3-H_A), 3.30 (s, 3H, 3_A-OMe), 3.18 (t, ^3J = 8.7 Hz, 1H, 5'-H), 3.13 (d, ^3J = 9.4 Hz, 1H, 4-H_B), 2.46 (dd, ^3J = 11.7 Hz, ^3J = 4.4 Hz, 1H, 3'-H), 2.30 (s, 3H, 5-MeCO), 2.21 (m, 1H, 3-H_C), 2.18 (s, 3H, 4-MeCO), 2.00-1.80 (m, 3H, 2-H_A, 2-H_B, 2-H_C), 1.78 (s, 3H, 3-Me), 1.70 (m, 2H, 2_B-H, 2_C-H), 1.60-1.50 (m, 2H, 3'-H, 3-H_C), 1.42 (d, ^3J = 6.0 Hz, 3H, 7'-H_3), 1.42 (m, 1H, 2_A-H), 1.30 (d, ^3J = 6.0 Hz, 3H, 6_B-H_3), 1.25 (s, 3H, 3_B-Me), 1.24 (d, ^3J = 6.0 Hz, 3H, 6_C-H_3), 1.21 (d, ^3J = 6.7 Hz, 3H, 6_A-H_3). **–** ^{13}**C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 188.3 (C_q, C-7), 187.1 (C_q, C-12), 171.1 (C_q, Me*CO*-5), 170.9 (C_q, Me*CO*-4), 157.8 (C_q, C-8), 155.7 (C_q, NH_2CONH), 145.9 (C_q, C-6a), 140.0 (C_q, C-12a), 139.2 (C_q, C-9), 134.0 (C_q, C-3), 133.0 (CH, C-10), 130.1 (C_q, C-11a), 124.1 (CH, C-2), 119.7 (CH, C-11), 114.0 (C_q, C-7a), 103.2 (CH, C-1C), 99.6 (CH, C-1_B), 99.3 (CH, C-1_A), 89.6 (CH, C-4_B), 83.4 (CH, C-4'), 79.3 (CH, C-1), 77.1 (C_q, C-12b), 76.8 (CH, C-6'), 76.4 (CH, C-3A), 76.1 (CH, C-5'), 75.4 (CH, C-5), 74.14 (CH, C-4a), 74.05 (CH, C-4_A), 73.9 (CH, C-5_A), 72.9 (CH, C-2'), 71.1 (CH, C-5_B), 70.7 (C_q, C-3_B), 69.7 (CH, C-6), 68.1 (CH, C-4), 65.7 (CH, C-5C), 61.9 (CH, C-4C), 57.3 (CH_3, OMe), 44.2 (CH_2, C-2_B), 37.7 (CH_2, C-3'), 30.2 (CH_2, C-2_C), 30.0 (CH_2, C-2_A), 27.8 (CH_2, C-3_C), 22.3 (CH_3, Me-3_B), 21.0 (CH_3, 3-Me), 20.90 (CH_3, 5-MeCO), 20.86 (CH_3, 4-MeCO), 18.3 (CH_3, C-7'), 18.1 (CH_3, C-6_B), 17.7 (CH_3, C-6_C), 16.4 (CH_3, C-6_A). **– (+)-ESI MS:** *m/z* (%) = 1072 ($[M+Na]^+$, 100).

10.9 Terrestrial *Streptomyces* sp US 80

The crude extract of the strain US 80 was fermented in Tunisia on a 20 L scale and the crude extract was sent to our group for further investigations. On TLC, the crude extract showed no UV absorbing zones but several colourless spots, which turned first green and after some time black on spraying with anisaldehyde/sulphuric acid; also some red spots were observed.

Chromatography of 1.87 g extract on Sephadex LH-20 using CH_2Cl_2/MeOH (1:1) afforded four main fractions I-IV. The fraction I was subjected to silica gel chromatography and eluted with CH_2Cl_2/MeOH (increasing gradient), delivering irumamycin (**44**) (7.5 mg). The fraction II was re-chromatographed on silica gel, then Sephadex LH-20 (CH_2Cl_2/50% MeOH) and delivered X-14952B (**45**). The chromatography of the fraction III on Sephadex LH-20 followed by PTLC with CH_2Cl_2/MeOH (95:5) delivered the compound 17-hydroxy-venturicidin A (**50**). The fraction IV was not submitted to further chromatographic methods due to the absence of biological activity.

17-Hydroxy-venturicidin A (**150**): $C_{41}H_{67}NO_{12}$, white amorphous powder. **– R_f** = 0.18 ($CHCl_3$/5% MeOH). **– ^{1}H** and **^{13}C-NMR** see table 1. **– (+)-ESI MS:** *m/z* (%) = 1552 ($[M+Na]^+$, 40), 788 ($[M+Na]^+$, 100). **– (-) – ESI MS:** *m/z* (%) = 764 ($[M-H]^-$). **– (+)-ESI HR MS:** *m/z* = 788.45537 $[M+Na]^+$, 783.50007$[M+NH_4]^+$ (calc. 788.45556 for $C_{41}H_{67}NO_{12}$ Na).

10.10 Terrestrial *Streptomyces* sp. GW 37/3236

The terrestrial S*treptomyces* sp. GW 37/3236 was cultivated on M_2 agar plates for four days at 28 °C and showed a red pigmentation of the agar. The plates were used to inoculate a 1 L culture using M_2 medium during four days at 28 °C. The red culture broth was worked up at standard conditions and the resulting red crude extract was used for biological activity tests (table 17). In the chemical screening, many red bands showing orange fluorescence under UV 366 nm were seen.

Table 17: Biological activity of the crude extract of the strain GW 37/3236

Test organisms	Inhibition zone (∅ mm)
Mucor miehei	13
Chlorella sorokiniana	11
Chlorella vulgaris	12
Streptomyces viridochromogenes (Tü 57)	15
Bacillus subtilis	15
Escherichia coli	17
Staphylococcus aureus	17

10.10.1 Fermentation and Isolation

With a well grown agar culture of the terrestrial *Streptomyces* sp. isolate GW 37/3236, 100 of 1 L Erlenmeyer flasks each containing 250 mL of M_2 medium were inoculated and incubated for 3 days at 28 °C on a linear shaker. The culture broth was mixed with ca. 1 kg diatomaceous earth and filtered through a filter press to separate mycelium and water phase. The mycelial cake and the filtrate were separately extracted each three times with ethyl acetate (ca. 2 L each). Since the chemical composition of both organic phases was similar, they were combined and concentrated under reduced pressure to yield 5 g of a dark red oily crude extract.

The resulting crude extract was subjected to flash column chromatography using a CH_2Cl_2/MeOH gradient and separated into fractions I-V. Further purification of these fractions by PTLC and Sephadex LH-20 yielded six known and four new natural products baumycin C1 (**55**, 3.5 mg), 7-deoxy-13-dihydrodaunomycinone (**52,** 3.4 mg), daunomycinone (**54**, 1.9 mg, R_f = 0.58), 13-dihydrodaunomycinone (**53**, 4.3 mg), 11-deoxybisanhydro-13-dihydrodaunomycinone (**56**, 2.5 mg), bisanhydro-13-dihydrodaunomycinone (**51**, 8.5 mg), 13-O-acetyl-bisanhydro-13-dihydrodaunomycinone (**58**, 6.4 mg), 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (**59**, 0.9 mg), 6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (**57**, 0.9 mg) and 2-acetamido-3-hydroxybenzamide (**60,** 4.4 mg).

Bisanhydro-13-dihydrodaunomycinone (51): $C_{21}H_{16}O_6$, red powder. – R_f = 0.63 (CH_2Cl_2/ 5% MeOH). – **^{1}H NMR** (C_5D_5N, 300.0 MHz): δ = 16.60 (s, 1H, 6-OH), 15.64 (s, 1H, 11-OH), 8.85 (br s, 1H, 10-H), 8.58 (d, ^3J = 7.8 Hz, 1H, 7-H), 8.18 (d, ^3J = 8.0 Hz, 1H, 1-H), 8.07 (d, ^3J = 7.9 Hz, 1H, 8-H), 7.70 (t, ^3J = 8.0 Hz, 1H, 2-H), 7.30 (d, ^3J = 7.8 Hz, 1H, 3-H), 5.37 (q, ^3J = 6.7 Hz, 1H, 13-H), 3.98 (s, 3H, 4-OCH_3), 1.70 (d, ^3J = 6.7 Hz, 3H, 14-H_3). – **^{13}C/APT NMR** (C_5D_5N, 75.5 MHz): δ = 175.7 (C_q, C-5), 173.2 (C_q, C-12), 170.0 (C_q, C-11), 171.3 (C_q, C-6), 161.3 (C_q, C-4), 153.5 (C_q, C-9), 134.9 (C_q, C-12a), 134.4 (CH, C-2), 132.0 (C_q, C-6a), 131.6 (C_q, C-10a), 130.8 (CH, C-8), 126.3 (CH, C-7), 122.7 (CH, C-10), 121.3 (C_q, C-4a), 118.6 (CH, C-1), 116.3 (CH, C-3), 107.0 (C_q, C-11a), 107.4 (C_q, C-5a), 69.1 (CH, C-13), 56.5 (OCH_3), 26.2 (C-14); signals temptatively assigned according to ACD calculations. – **EI MS (70 eV):** *m/z* (%) = 364.2 ($[M]^+$, 100), 346.2 (12), 331(8), 303(8). – **(+)-ESI MS:** *m/z* (%) = 751 ($[2M+Na]^+$, 100), 387 ($[M+Na]^+$, 2). – **(-)-ESI MS:** *m/z* (%) = 749 ($[2M-2H+Na]^-$, 62), 363 ($[M-H]^-$, 100).

7-Deoxydaunomycinol (**52**): $C_{21}H_{20}O_7$, red powder. – R_f = 0.49 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (C_5D_5N, 300.0 MHz): δ = 14.48 (s, 1H, 6-OH), 13.86 (s, 1H, 11-OH), 8.06 (d, ^3J = 7.9 Hz, 1H, 1-H), 7.67 (t, ^3J = 8.1 Hz, 1H, 2-H), 7.36 (d, ^3J = 8.0 Hz, 1H, 3-H), 6.22 (br s, 1H, H/D exchangeable, OH), 5.63 (br s, 1H, H/D exchangeable, OH), 4.18 (q, ^3J = 6.5 Hz, 1H, 13-H), 3.90 (s, 3H, 4-OCH_3), 3.38, 3.10 (AB, ^3J = 18.4 Hz, 2H, 10-H_2), 3.23 (br s, 2H, 7-H_2), 2.40 (m, 1H, 8-H_{eq}), 1.90 (m, 1H, 8-H_{ax}), 1.60 (d, ^3J = 6.5 Hz, 3H, 14-H_3). – **^{13}C/APT NMR** (C_5D_5N, 75.5 MHz): δ = 186.8 (C_q, C-5), 186.4 (C_q, C-12), 161.3 (C_q, C-4), 157.2 (C_q, C-6), 156.6 (C_q, C-11), 139.8 (C_q, C-6a), 139.7 (C_q, C-12a), 136.5 (C_q, C-10a), 121.2 (C_q, C-4a), 119.3 (2CH, C-1, C-2), 118.8 (CH, C-3), 110.5 (C_q, C-11a), 109.8 (C_q, C-5a), 73.7 (CH, C-13), 71.6 (C_q, C-9), 56.4 (CH_3, OCH_3), 32.4 (CH_2, C-10), 28.2 (CH_2, C-7), 21.2 (CH_2, C-8), 17.9 (CH_3, C-14). – **EI MS (70 eV):** *m/z* (%) = 384.2 ($[M]^+$, 100), 339.2 (96), 323 (8).

Daunomycinol (53): $C_{21}H_{20}O_8$, red powder. – R_f = 0.45 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (C_5D_5N, 300.0 MHz): δ = 14.86 (br s, 1H, 6-OH), 13.74 (br s, 1H, 11-OH), 8.07 (d, ^3J = 7.9 Hz, 1H, 1-H), 7.70 (t, ^3J = 8.2 Hz, 1H, 2-H), 7.36 (d, ^3J = 8.4 Hz,

1H, 3-H), 5.84 (t, ^3J = 6.3 Hz, 1H, 7-H), 4.23 (q, ^3J = 6.7 Hz, 1H, 13-H), 3.90 (s, 3H, 4-OCH_3),3.41(br s, 2H, 10-H_2), 2.84, 2.56 (AB, ^3J = 15.0, 6.9, 6.3 Hz, 2H, 8-H_2), 1.62 (d, ^3J = 6.7 Hz, 3H, 14-H_3). **– ^{13}C/APT NMR** (C_5D_5N, 75.5 MHz): δ = 187.3 (C_q, C-5), 187.0 (C_q, C-12), 161.6 (C_q, C-4), 157.4 (C_q, C-6), 156.7 (C_q, C-11), 137.4 (C_q, C-10a), 136.0 (C_q, C-12a), 135.6 (CH, C-2), 135.4 (C_q, C-6a), 121.6 (C_q, C-4a), 119.7 (CH, C-1), 119.6 (CH, C-3), 111.7 (C_q, C-11a), 111.2 (C_q, C-5a), 73.5 (C_q, C-13), 73.4 (C_q, C-9), 64.7 (CH, C-7), 56.7 (OCH_3), 38.6 (CH_2, C-8), 34.0 (CH_2, C-10), 17.8 (CH_3, C-14). **– EI MS (70 eV):** *m/z* (%) = 400.3 ($[M]^+$, 56), 384.3 (16), 364.2 (32), 338.2 (100), 309 (32). **– (+)-ESI MS:** *m/z* (%) = 1222.7 ($[3M+Na]^+$, 24), 823.3 ($[2M+Na]^+$, 100), 423.4 ($[M+Na]^+$, 8). **– (-)-ESI MS:** *m/z* (%) = 821.8 ($[2M-2H+Na]^-$, 100), 399.6 ($[M-H]^-$, 90).

Daunomycinone (54): $C_{21}H_{18}O_8$, red powder. **– R_f** = 0.45 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** (C_5D_5N, 300.0 MHz): δ = 14.68 (br s, 1H, 6-OH), 13.7 (br s, 1H, 11-OH), 8.10 (d, ^3J = 8.0 Hz, 1H, 1-H), 7.72 (t, ^3J = 8.3 Hz, 1H, 2-H), 7.40 (d, ^3J = 8.2 Hz, 1H, 3-H), 5.68 (m, 1H, 7-H) 3.96 (s, 1H, 4-OCH_3), 3.58, 3.37 (AB, ^3J = 18.6 Hz, 2H, 10-H_2), 2.63, 2.30 (ABX, ^3J = 14.1 6.4 Hz, J_{BX} not seen, 1H, 8-H_2), 2.56 (s, 3H, 14-H_3).**– EI MS (70 eV):** *m/z* (%) = 398.3 ($[M]^+$, 64), 382.3 (16), 362.3 (100), 344.2 (44), 337.2 (40), 309.2 (34), 301.2 (18). **– (+)-ESI MS:** *m/z* (%) = 819.4 ($[2M+Na]^+$, 24), 443.5 ($[M-H+2Na]^+$, 20), 421.5 ($[M+Na]^+$,100). **– (-)-ESI MS:** *m/z* = 397.6 ($[M-H]^-$).

Baumycin C1 (55): $C_{28}H_{29}NO_{11}$, red powder. **– R_f** = 0.42 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 13.97 (s, 1H, 6-OH), 13.26 (s, 1H, 11-OH), 8.08 (br s, 1H, 7'-H), 8.03 (d, ^3J = 8.1 Hz, 1H, 1-H), 7.77 (t, ^3J = 8.3 Hz, 1H, 2-H), 7.39 (d, ^3J = 8.0 Hz, 1H, 3-H), 6.16 (d, ^3J = 7.5 Hz, 1H, H/D exchangeable, 3'-NH), 5.50 (br d, ^3J = 6.0 Hz, 1H, 1'-H), 5.22 (br s, 1H, 7-H), 4.45 (br s H/D exchangeable, 1H, OH), 4.25 (m, 2H, 3'-H, 5'-H), 4.05 (s, 3H, 4-OCH_3), 3.63 (br s, 1H, 4'-H), 3.20, 2.90 (AB, ^3J = 18.2 Hz, 2H, 10-H_2), 2.40 (s, 3H, 14-H_3), 2.30, 2.10 (ABX, ^3J = 15.4, 6.8, 6.1 Hz, 1H, 8-H_2), 1.90 (m, 2H, 2'-H), 1.30 (d, ^3J = 6.6 Hz, 3H, 6'-CH_3); one OH not visible. **– ^{13}C/APT NMR** ($CDCl_3$, 125.7 MHz): δ = 212.4 (C_q, C-13), 187.3 (C_q, C-5), 186.9 (C_q, C-12), 161.4 (C_q, C-4), 160.7 (CHO), 156.6 (C_q,C-6), 156.0 (C_q, C-

11), 136.0 (CH, C-2), 135.7 (C_q, C-12a), 134.7 (C_q, C-6a), 134.2 (C_q, C-10a), 121.6 (C_q, C-4a), 120.1 (CH, C-3), 118.6 (CH, C-1), 111.6 (C_q, C-5a), 111.5 (C_q, C-11a), 100.8 (CH, C-1'), 76.9 (C_q, C-9), 70.3 (CH, C-7), 69.6 (CH, C-5'), 67.2 (CH, C-4'), 56.9 (OCH_3), 44.4 (CH, C-3'), 35.3 (CH_2, C-8), 33.6 (CH_2, C-10), 30.2 (CH_2, C-2'), 25.2 (CH_3, C-14), 17.0 (CH_3, C-6'). **– (+)-ESI MS:** *m/z* (%) = 1133.0 ($[2M+Na]^+$, 100), 578.5 ($[M+Na]^+$, 100).

11-Deoxybisanhydro-13-dihydrodaunomycinone (56): $C_{21}H_{16}O_5$, red powder. **–** R_f = 0.60 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 14.87 (s, 1H, 6-OH), 8.48 (d, ^3J = 8.1 Hz, 1H, 7-H), 8.20 (s, 1H, 11-H), 8.06 (d, ^3J = 8.4 Hz, 1H, 8-H), 7.92 (s, 1H, 10-H), 7.77 (t, ^3J = 8.3 Hz, 1H, 2-H), 7.66 (d, ^3J = 8.4 Hz, 1H, 1-H), 7.39 (d, ^3J = 8.2 Hz, 1H, 3-H), 5.10 (q, J = 6.3 Hz, 1H, 13-H), 4.10 (s, 3H, 4-OCH_3), 1.60 (d, J = 6.3 Hz, 3H, 14-H_3). **– ^{13}C/APT NMR** ($CDCl_3$, 125.5 MHz): δ = 188.3 (C_q, C-5), 182.7 (C_q, C-12), 163.3 (C_q, C-6), 161.0 (C_q, C-4), 148.8 (C_q, C-10a), 136.7 (C_q, C-9), 136.1 (C_q, C-12a), 135.6 (CH, C-2), 129.7 (C_q, C-11a), 128.3 (C_q, C-6a), 126.6 (CH, C-8), 126.0 (CH, C-7), 125.1 (C_q, C-4a), 120.8 (CH, C-1), 120.3 (CH, C-10), 118.2 (CH, C-11), 116.3 (CH, C-3), 110.1 (C_q, C-5a), 70.1 (CH, C-13), 56.7 (OCH_3), 25.3 (CH_3, C-14). **– EI MS (70 eV):** *m/z* (%) = 348 ($[M]^+$, 100), 330 (30), 305 (24), 287 (20), 43 (5).

6,9,11-Trihydroxy-4-methoxy-5,12-naphthacenedione (57): $C_{19}H_{12}O_6$, red powder. **–** R_f = 0.62 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 16.34 (s, 1H, 6-OH), 15.20 (br s, 1H, 11-OH), 8.24 (d, ^3J = 8.6 Hz, 1H, 7-H), 8.02 (d, ^3J = 7.8 Hz, 1H, 1-H), 7.85 (d, ^3J = 8.1 Hz, 1H, 2-H), 7.61 (d, ^4J = 1.5 Hz, 1H, 10-H), 7.56 (d, ^3J = 7.9 Hz, 1H, 3-H), 7.30 (dd, ^3J = 8.6 H, ^4J = 1.4 Hz, 1H, 8-H), 3.99 (s, 3H, 4-OMe). **– EI MS (70 eV):** *m/z* (%) = 336 ($[M]^+$, 100), 318 (72), 290 (22), 265 (8). **– (+)-ESI MS:** *m/z* (%) = 655.1 ($[2M+Na]^+$, 100), 359.5 ($[M+Na]^+$, 2).**– (-)-ESI MS:** *m/z* = 335.7 ($[M-H]^-$).

13-O-Acetyl-bisanhydro-13-dihydrodaunomycinone (58): $C_{23}H_{18}O_7$, red powder. **–** R_f = 0.87 (CH_2Cl_2/5% MeOH). **— UV/VIS (MeOH):** λ_{max} (lg ε) = 263 (4.67), 469 (sh) (4.03), 496 (4.17), 532 (4.11) nm. **– IR (KBr):** ν = 3430, 2925, 2856, 1737, 1616, 1578, 1509, 1461, 1440, 1413, 1372, 1269, 1234, 1066, 1045, 1018, 938, 911,

825, 752, 703, 607, 452 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 15.94 (s, 1H, 6-OH), 15.24 (s, 1H, 11-OH), 8.48 (d, ^3J = 8.1 Hz, 1H, 7-H), 8.42 (d, ^4J = 1.5 Hz, 1H, 10-H), 8.12 (dd, ^3J = 7.9, ^4J = 0.7 Hz, 1H, 1-H), 7.77 (dd, ^3J = 8.1 Hz, ^4J = 1.5 Hz, 1H, 8-H), 7.75 (t, ^3J = 7.9 Hz, 1H, 2-H), 7.30 (d, ^3J = 7.9 Hz, 1H, 3-H), 6.05 (q, ^3J = 6.7 Hz, 1H, 13-H), 4.10 (s, 3H, 4-OCH_3), 2.16 (s, 3H, 13-OCOCH_3), 1.64 (d, ^3J = 6.7 Hz, 3H, 14-H_3). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 177.8 (C_q, C-5), 175.5 (C_q, C-12), 170.2 (13-O*CO*CH_3), 168.9 (C_q, C-11), 168.1 (C_q, C-6), 160.8 (C_q, C-4), 146.0 (C_q, C-9), 134.9 (C_q, C-12a), 134.2 (CH, C-2), 131.5 (C_q, C-10a), 131.3 (C_q, C-6a), 130.0 (CH, C-8), 126.3 (CH, C-7), 122.7 (CH, C-10), 121.1 (C_q, C-4a), 118.8 (CH, C-1), 115.8 (CH, C-3), 106.8 (C_q, C-11a), 106.8 (C_q, C-5a), 71.7 (CH, C-13), 56.6 (CH_3, OCH_3), 22.3 (CH_3, C-14), 21.3 (CH_3, 13-OCO*CH_3*). – **EI MS (70 eV):** *m/z* (%) = 406 ($[M]^+$, 100), 390 (18), 363 (4), 347 (16), 328 (20), 121 (6), 91 (8), 43 (12). – **(+)-ESI MS:** *m/z* (%) = 1240.9($[3M+Na]^+$, 12), 835.4 ($[2M+Na]^+$, 40), 429.5 ($[M+Na]^+$, 100). – **(-)-ESI MS**: *m/z* (%) = 833 ($[2M+Na-2H]^-$, 100). – **EI HR MS:** *m/z*: 406.10530 (calcd. 406.10525 for $C_{23}H_{18}O_7$).

4,13-O-Diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (59): $C_{24}H_{18}O_8$, red solid. – R_f = 0.90 (CH_2Cl_2/5% MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε) = 266 (4.05), 460 (sh), 489 (3.45), 523 (3.47), 554 (sh) nm. – **IR (KBr):** ν = 2924, 2853, 1736, 1661, 1636, 1578, 1464, 1414, 1374, 1243, 1212, 1071, 1036, 962, 887, 861, 757, 722, 702 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 15.61 (s, 1H, 6-OH), 15.20 (s, 1H, 11-OH), 8.48 (d, ^3J = 8.3 Hz, 1H, 7-H), 8.44 (d, ^4J = 1.9 Hz, 1H, 10-H), 8.47 (dd, ^3J = 7.9 Hz, ^4J = 1.2 Hz, 1H, 1-H), 7.83 (t, ^3J = 7.9 Hz, 1H, 2-H), 7.79 (dd, ^3J = 8.3 Hz, ^4J = 1.9 Hz, 1H, 8-H), 7.43 (dd, ^3J = 7.9 Hz, ^4J = 1.2 Hz, 1H, 3-H), 6.05 (q, ^3J = 6.7 Hz, 1H, 13-H), 2.48 (s, 3H, 4-OCOCH_3), 2.15 (s, 3H, 13-OCOCH_3), 1.64 (d, ^3J = 6.7 Hz, 3H, 14-H_3). – **EI MS (70 eV):** *m/z* (%) = 434 ($[M]^+$, 24), 392 (100), 350 (8), 332 (38), 199 (36), 90 (8), 57 (8), 43 (14). – **(+)-ESI MS:** *m/z* (%) = 891.3 ($[2M+Na]^+$, 100), 457.5 ($[M+Na]^+$, 30). – **(-)-ESI MS**: *m/z* (%) = 889 ($[2M+Na-2H]^-$, 100), 433 ($[M-H]^-$, 5). – **EI HR MS:** *m/z*: 434.10020 (calcd. 434.10017 for $C_{24}H_{18}O_8$).

2-Acetamido-3-hydroxybenzamide (60): $C_9H_{10}N_2O_3$, colourless solid.– R_f = 0.16 (CH_2Cl_2/5%MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε) = 240 (sh), 295 (3.68) nm. – **IR (KBr):** ν = 3371, 3187, 3065, 1681, 1657, 1603, 1574, 1467, 1433, 1386, 1367, 1320, 1289, 1259, 1189, 1132, 1030, 990, 939, 793, 742, 718, 608, 579, 524, 440 cm^{-1}. – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 6.97 (dd, *J* = 8.3, 1.5 Hz, 1H, 4-H), 7.12 (t, ^3J = 7.9 Hz, 1H, 5-H), 7.05 (dd, ^3J = 7.9 Hz, ^3J = 1.5 Hz, 1H, 6-H), 2.03 (s, 3H, CH_3), 9.60 (br s, 1H, NH)[a], 7.64, 7.41 (2 br s, 2H, NH_2)[a], 10.08 (br s, 1H, OH)[a]; [a] assignment may be exchanged. – **^{13}C/APT NMR** (75.5 MHz, [D_6]DMSO): δ = 170.1 (C_q, C-7), 169.6 (Cq, C-8), 151.2 (C_q, C-3), 131.5 (C_q, C-1), 126.3 (CH, C-5), 123.1 (C_q, C-2), 119.3 (CH, C-6), 119.1 (CH, C-4), 23.1 (CH_3, C-9). – **EI MS (70 eV):** *m/z* (%) = 194 ($[M]^+$, 36), 177 (43), 152 (48), 135 (100), 107 (72), 79 (12), 52 (16), 43 (36). – **(+)-ESI MS:** *m/z* = 217.5 $[M+Na]^+$. – **(-)-ESI MS:** *m/z* = 193 ($[M-H]^-$). – **EI HR MS:** *m/z*: 194.0689 (calcd. 194.0691 for $C_9H_{10}N_2O_3$).

10.11 Terrestrial *Streptomyces* sp. GW 4284

The terrestrial *Streptomyces* sp. GW 4284 was cultivated on agar plates for three days at 28 °C. The isolate grew with dark brown colonies and produced a red pigment all over the plate. A spore suspension of this strain was used to inoculate 4 of 1 L Erlenmeyer flasks each containing 250 ml of M_2 medium. The flasks were shaken for four days with 95 rpm at 28 °C. The culture broth was extracted with ethyl acetate and the resulting crude extract was used for screening.

10.11.1 Pre-screening

The yellow crude extract exhibited a moderate activity only against *Staphylococcus aureus* and *Escherichia coli*. On TLC using CH_2Cl_2/5% MeOH and CH_2Cl_2/10% MeOH indicated the presence of many yellow and red bands, which changed to violet with diluted sodium hydroxide indicating *peri*-hydroxy-quinones.

Table 18: Biological activity of the crude extract of the strain GW 4184

Test organisms	Inhibition zone (∅ mm)
Escherichia coli	11
Staphylococcus aureus	17

10.11.2 Fermentation and Isolation of Secondary metabolites

A loopful each of mature slant culture of GW 4284 was inoculated into 100 of 1 L Erlenmeyer flasks each containing 200-250 ml M_2 medium. The flasks were cultivated on a linear shaker. The strain grew between 28-32 °C first yellow then intensively red within 2-3 days. The red culture broth was harvested and filtered to separate the mycelium, which was extracted with ethyl acetate, while the culture filtrate was passed through a column of XAD-16. The column was washed with 20 L demineralised water and eluted with 20 L methanol. The eluates were concentrated under reduced pressure and finally extraction of the residue was done with ethyl acetate to afford the crude extract B (3.5 g). The mycelium phase was further extracted with acetone and methanol; the organic phases were dried and dissolved in CH_2Cl_2/MeOH (1:1) to give the crude extract A (6 g). Both crude extracts were mixed in the view of the TLC and submitted to a silica gel chromatography giving four fractions I-IV. Further, chromatography of the first fraction on Sephadex LH-20 (CH_2Cl_2/MeOH 1:1) and finally by preparative HPLC on RP 18 gave juglomycin A (**62,** 8.5 mg), B (**63**, 4 mg**)** and J (**64**, 5 mg). Purification of fraction II on RP18 silica gel (41-65 μm, MeOH) gave 10 mg of oviedomycin (**65a**). Preparative HPLC purification of fraction III yielded 4 mg GTRI-02 (**12**), 8 mg of juglorescein (**70**) and 3 mg of oviedomycin (**65a**). The fraction III was triturated with CH_2Cl_2/MeOH (1:1) and filtered off from an undissolved solid. The soluble part was chromatographed on Sephadex LH-20 (CH_2Cl_2/50% MeOH) and the major polar red fraction was further purified by preparative HPLC to afford 18 mg of juglorubin (**10**). The remaining brown solid was not soluble in organic solvents and was not further studied.

Juglomycin A (62): $C_{14}H_{10}O_6$, yellow solid. – R_f = 0.35 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ([D_6]acetone, 300.0 MHz): δ = 11.92 (s br, 1H, 5-OH), 7.78 (dd, *J* = 8.4, 7.5 Hz, 1H, 7-H), 7.60 (dd, ^3J = 7.5 Hz, ^4J = 1.1 Hz, 1H, 8-H), 7.35 (dd, ^3J = 8.4 Hz, ^4J = 1.2 Hz, 1H, 6-H), 6.96 (d, ^4J = 1.2 Hz, 1H, 3-H), 5.71 (dd, ^3J = 3.8 Hz, ^4J = 1.7, Hz, 1H, 4'-H), 4.92 (td, ^3J = 6.8, 2.0 Hz, 3'-H), 4.76 (s br, 1H, 3'-OH), 3.18, 2.50 (ABX, ^3J = 17.2, 7.8 Hz, 3rd coupling not seen, 2H, 2'-H_2). – **^{13}C/APT NMR** ([D_6]acetone, 75.5 MHz): δ = 190.8 (C_q, C-4), 183.8 (C_q, C-1), 175.1 (C_q, C-1'), 162.1 (C_q, C-5), 147.1 (C_q, C-2), 137.6 (CH, C-7), 135.0 (CH, C-3), 133.0 (C_q, C-8a), 125.0 (CH, C-

6), 119.5 (CH, C-8), 115.7 (C_q, C-4a), 81.3 (CH, C-4'), 70.2 (CH, C-3'), 39.4 (CH_2, C-2').

Juglomycin B (63): $C_{14}H_{10}O_6$, yellow solid. – R_f = 0.37 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ([D$_6$]acetone, 300.0 MHz): δ = 11.86 (br s, 1H, 5-OH), 7.79 (dd, ^3J = 8.4, 7.6 Hz, 1H, 7-H), 7.62 (dd, ^3J = 7.6, ^4J = 1.2 Hz, 1H, 8-H), 7.35 (dd, ^3J = 8.4 Hz, ^4J = 1.2 Hz, 1H, 6-H), 6.80 (d, ^4J = 1.2 Hz, 1H, 3-H), 5.50 (dd, ^3J = 3.8 Hz, ^4J = 1.7, Hz, 1H, 4'-H), 5.25 (br s, 1H, 3'-OH), 4.64 (d, ^3J = 5.3 Hz, 1H, 3'-H), 3.00, 2.40 (dd, ^3J = 17.8, 6.0, 1.1 Hz, 2H, 2'-H_2). – **^{13}C/APT NMR** ([D$_6$]acetone, 75.5 MHz): δ = 190.7 (C_q, C-4), 184.1 (C_q, C-1), 175.9 (C_q, C-1'), 162.0 (C_q, C-5), 148.0 (C_q, C-2), 137.7 (CH, C-7), 133.9 (CH, C-3), 133.2 (C_q, C-8a), 125.0 (CH, C-6), 119.7 (CH, C-8), 119.5 (C_q, C-4a), 84.8 (CH, C-4'), 71.9 (CH, C-3'), 39.5 (CH_2, C-2').

Juglomycin J (64): $C_{15}H_{12}O_7$, yellow solid. – R_f = 0.42 (CH_2Cl_2/5% MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε) = 421 (3.51), 283 (3.94), 241 (4.04) nm. – **IR (KBr):** ν = 3420, 2953, 2926, 2854, 1780, 1642, 1606, 1516, 1457, 1367, 1273, 1193, 1158, 1083, 1049, 1002, 944, 907, 882, 837, 757, 710 cm^{-1}. – **CD** (37 µg/ml, MeOH): λ_{ext} ($[\theta]^{22}$) = 322 (678), 284 (-4342), 258 (+1894) nm. – **^{1}H NMR** ([D$_6$]acetone, 300.0 MHz): δ = 11.60 (br s, 1H, 5-OH), 7.78 (dd, ^3J = 7.5 Hz, ^4J = 1.2 Hz, 1H, 7-H), 7.57 (dd, ^3J = 8.1, 7.5 Hz, 1H, 8-H), 7.28 (dd, ^3J = 8.1 Hz, ^4J = 1.2 Hz, 1H, 6-H), 5.61 (d, ^3J = 3.2 Hz, 1H, 4'-H), 4.62 (td, ^3J = 6.8, 3.2 Hz, 1H, 3'-H), 4.19 (s, 3-OCH_3), 3.15 (dd, ^3J = 16.2, 6.8 Hz, 1H, 2'-H_a), 2.45 (dd, ^3J = 16.2, 3.2 Hz, 1H, 2'-H_b). – **^{13}C/APT NMR** ([D$_6$]acetone, 75.5 MHz): δ = 187.1 (C_q, C-4), 184.1 (C_q, C-1), 176.5 (C_q, C-1'), 162.2 (C_q, C-5), 159.5 (C_q, C-3), 138.0 (CH, C-7), 132.8 (C_q, C-2), 130.5 (C_q, C-8a), 124.6 (CH, C-6), 119.4 (CH, C-8), 115.6 (C_q, C-4a), 82.3 (CH, C-4'), 72.1 (CH, C-3'), 62.9 (OCH_3), 39.1 (CH_2, C-2'). – **(+)-ESI MS:** *m/z* (%) = 631 ($[2M+Na]^+$, 100), 327 ($[M+Na]^+$, 20). – **(-)-ESI MS:** *m/z* (%) = 629 ($[2M+Na-2H]^+$, 37), 303 ($[M-H]^+$, 100). – **EI HRMS** *m/z:* 327.04699 (calcd. 327.04807 for $C_{15}H_{12}O_7Na$).

Oviedomycin (**65a**): $C_{19}H_{10}O_7$, yellow solid. – R_f = 0.53 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ([D$_6$]DMSO, 300.0 MHz): δ =12.1 (br s, 1H, OH), 11.60 (br s, 1H, 8-OH), 7.81 (t, ^3J = 8.0 Hz, 1H, 10-H), 7.61 (s, 1H, 5-H), 7.49 (d, ^3J = 8.0 Hz, 1H, 11-H),

7.38 (d, ^3J = 8.1 Hz, 9-H), 1.90 (s, 3H, 3-CH_3). – **(-)-ESI-MS:** *m/z* (%) = 721.2 ($[2M+Na-2H]^-$, 9), 349.3 ($[M-H]^-$, 100).

Methylation of oviedomycin (**65a**): A solution of 8 mg oviedomycin (**65a**) in 5 ml CH_2Cl_2/MeOH was cooled to 0 °C, ca. 0.5 ml 0.4 N etherial diazomethane solution was added and the mixture evaporated after 1 min. TLC of the reaction product exhibited two yellow spots and a red compound, which remained at the bottom. Purification by PTLC (CH_2Cl_2/5% MeOH) delivered two compounds **65b** and **65c**.

2-*O*-Methyl-oviedomycin (**65b**): $C_{20}H_{12}O_7$, yellow solid. – R_f = 0.90 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.51 (s, 1H, 6-OH), 11.63 (s, 1H, 8-OH), 7.83 (s, 1H, 5-H), 7.75 (m, 2H, 10-H, 11-H), 7.32 (dd, ^3J = 8.1 Hz, ^4J = 1.5 Hz, 1H, 9-H), 4.26 (s, 3H, 2-OCH_3), 2.06 (s, 3H, 3-CH_3), – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 192.4 (C_q, C-7), 182.6 (C_q, C-4), 182.0 (C_q, C-12), 180.4 (C_q, C-1), 164.7 (C_q, C-6), 162.4 (C_q, C-8), 161.7 (C_q, C-2), 139.7 (C_q, C-4a), 138.2 (CH, C-10), 137.4 (C_q, C-12a), 135.1 (C_q, C-11a), 127.7 (C_q, C-12b), 127.0 (C_q, C-3), 124.5 (CH, C-9), 120.2 (CH, C-11), 119.9 (CH, C-5), 119.4 (C_q, C-6a), 115.1 (C_q, C-7a), 60.6 (CH_3, 2-OCH_3), 8.9 (CH_3, 3-CH_3). – **EI MS (70 eV):** *m/z* (%) = 364.0 ($[M]^+$, 100), 321.0 (24), 306.0 (16), 293.0 (14), 266 (16), 57 (15), 43 (13).

2,6-Di-*O*-methyl-oviedomycin (**65c**): $C_{21}H_{14}O_7$, yellow solid. – R_f = 0.83 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.42 (s, 1H, 8-OH), 7.86 (s, 1H, 5-H), 7.63 (m, 2H, 10-H, 11-H), 7.30 (dd, ^3J = 8.1, ^4J = 1.8 Hz, 1H, 9-H), 4.26 (s, 3H, 2-OCH_3), 4.19 (s, 3H, 6-OCH_3), 2.06 (s, 3H, 3-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 187.3 (C_q, C-7), 183.4 (C_q, C-4), 183.0 (C_q, C-12), 180.8 (C_q, C-1), 163.1 (C_q, C-6), 162.1 (C_q, C-8), 161.7 (C_q, C-2), 139.9 (C_q, C-4a), 138.5 (C_q, C-12a), 136.6 (CH, C-10), 134.1 (C_q, C-11a), 127.9 (C_q, C-12b), 126.3 (C_q, C-3), 124.6 (C_q, C-6a), 124.4 (CH, C-9), 118.7 (CH, C-11), 116.6 (C_q, C-7a), 112.5 (CH, C-5), 60.6 (CH_3, 2-OCH_3), 57.4 (CH_3, 6-OCH_3), 8.8 (CH_3, 3-CH_3). – **EI MS (70 eV):** *m/z* (%) = 378.1 ($[M]^+$, 100), 363.1 (12), 348.1 (15), 320.1 (10), 280.1 (10).

Oviedomycin II (**66a**): $C_{19}H_{10}O_7$, red solid. **P**urification on PTLC of fraction II containing the yellow oviedomycin **(65a)** gave a red colour band on contact with the plate, the red band was washed from the silica with CH_2Cl_2/50% MeOH and delivered after evaporation of the solvent the pure red solid (**66a**). – R_f = 0.40 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ([D$_6$]DMSO, 300.0 MHz): δ = 7.54 (t, ^3J = 8.0 Hz, 1H, 10-H), 7.21 (d, ^3J = 8.0 Hz, 1H, 11-H), 7.10 (d, ^3J = 8.0 Hz, 1H, 9-H), 6.98 (s, 1H, 5-H), 1.74 (s, 3H, 3-CH$_3$). – **^{13}C/APT NMR** ([D$_6$]DMSO), 75.5 MHz): δ = 187.6 (C$_q$, C-12), 185.8 (C$_q$, C-7), 185.7 (C$_q$, C-6), 177.6 (C$_q$, C-4), 176.6 (C$_q$, C-1), 174.3 (C$_q$, C-2), 161.8 (C$_q$, C-8), 141.9 (C$_q$, C-4a), 141.8 (C$_q$, C-12a), 136.4 (C$_q$, C-11a), 134.3 (CH, C-10), 124.4 (CH, C-5), 122.9 (CH, C-9), 119.6 (C$_q$, C-12b), 119.4 (C$_q$, C-6a),, 116.8 (C$_q$, C-7a), 115.8 (CH, C-11), 113.5 (C$_q$, C-3), 9.6 (CH$_3$, 3-CH$_3$). – **(-)-ESI MS:** *m/z* (%) = 721.0 ([2M+Na-2H]$^-$, 34), 349.3 ([M-H]$^-$, 100).

4-*O*-Methyloviedomycin B (**66b**): An excess of an ethereal diazomethane solution was added to the methanolic solution of oviedomycin II (**66b**, 20 mg) at 0 °C, the mixture stirred for a few seconds and evaporated to dryness. Chromatography on Sephadex LH-20 (CH_2Cl_2/40 % MeOH) delivered a red zone of 4-*O*-methyloviedomycin B (**66b**, 1 mg) and many other coloured by-products. Dark red solid, R_f = 0.88 (CH_2Cl_2/5 % MeOH). – ^{1}H NMR ([D$_6$]DMSO, 300.0 MHz): δ = 7.58 (dd, ^3J = 7.6, 1.1 Hz, 1H, 10-H), 7.38 (dd, ^3J = 7.5, 1.1 Hz, 1H, 11-H), 7.19 (dd, ^3J= 8.3, 1.1 Hz, 1H, 9-H), 6.87 (s, 1H, 5-H), 4.02 (s, 3H, OC*H$_3$*), 1.85 (s, 3H, 3-C*H$_3$*).

Juglorubin (70): $C_{28}H_{17}O_{11}$, red solid. – R_f = 0.26 (CH_2Cl_2/20% MeOH). – **^{1}H NMR** ([D$_4$]MeOH, 300.0 MHz): δ = 8.02 (d, ^3J = 16.8 Hz, 1H, 17-H), 7.44 (dd, ^3J = 7.3, 0.9 Hz, 1H, 13-H), 7.37 (t, ^3J = 8.1 Hz, 1H, 3-H), 7.22 (t, ^3J = 8.3 Hz, 1H, 12-H), 7.00 (dd , ^3J = 8.3, 0.5 Hz, 1H, 11-H), 6.91 (d, ^3J = 8.4 Hz, 1H, 2-H), 6.90 (d, ^3J = 8.1 Hz, 1H, 4-H), 6.09 (d, ^3J = 16.8 Hz, 1H, 18-H), 5.43 (m, 1H, 7-H), 4.03 (dd, ^3J = 12.5, ^4J = 1.1 Hz, 1H, 8-H$_b$), 2.82 (t, ^3J = 13.5 Hz, 1H, 20-Ha), 2.70-2.50 (m, 2H).– **^{13}C/APT NMR** ([D$_6$]DMSO, 75.5 MHz): δ = 196.5 (C$_q$, C-16), 184.3 (C$_q$, C-9), 177.7 (C$_q$, C-14), 173.5 (C$_q$, C-21), 170.7 (C$_q$, C-19), 167.2 (C$_q$, C-5), 161.4 (C$_q$, C-10), 161.4 (C$_q$, C-1), 137.9 (CH, C-17), 137.4 (C$_q$, C-13a), 135.0 (C$_q$, C-15a), 134.0 (C$_q$, C-4a), 132.9 (CH, C-12), 131.2 (C$_q$, C-16a), 130.9 (CH, C-3), 130.2 (C$_q$, C-8b),

129.7 (CH, C-15), 121.1 (CH, C-11), 120.5 (CH, C-2), 119.5 (C_q, C-8a), 118.3 (CH, C-18), 116.4 (CH, C-4), 76.1 (CH, C-7), 43.7 (CH_2, C-20), 31.1 (CH_2, C-8), [118.0 (CH, C-13), 119.4 (C_q, C-9a), 123.9 (C_q, C-14a)][130] **– (+)-ESI MS:** *m/z* = 375.4 [M –H+Na]$^+$. **– (-)-ESI MS:** *m/z* = 529.3 ([M –H]$^-$).

Juglorescein (71): $C_{28}H_{24}O_{14}$, colourless needles from $CHCl_3$/MeOH/C_6H_{12}. **– R_f** = 0.18 (CH_2Cl_2/5% MeOH). **– UV/Vis (MeOH):** λ_{max} (lg ε) = 344 (3.97), 251 (sh, 4.18), 230 (4.56) nm. **– IR (KBr):** ν = 3406, 2925, 1718, 1636, 1456, 1352, 1252, 1168, 1053, 808, 738, 668 cm^{-1}. **–** $[\alpha]_D^{20}$ = -107.4 (c = 0.29, MeOH). **– ^{1}H NMR** (CD_3OD, 300.0 MHz): δ = 7.68 (t, ^3J = 7.8 Hz, 1H, 7-H), 7.54 (dd, ^3J = 7.5 Hz, ^4J = 1.1 Hz, 1H, 8-H), 7.50 (t, ^3J = 8.1 Hz, 1H, 7'-H), 7.41 (dd, ^3J = 7.7 Hz, ^4J = 1.1 Hz, 1H, 8'-H), 7.23 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 1H, 6-H), 6.85 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 1H, 6'-H), 4.82 (m, 1H, 10'-H), 4.58 (m, 1H, 10-H), 3.52 (t, ^3J = 3.8 Hz, 1H, 9-H), 3.29 (below MeOH signal, 1H, 3'-H), 3.16 (dd, ^3J = 13.9, 8.8 Hz, 1H, 9'-H_a), 2.90 (dd, ^3J = 16.9, 3.8 Hz, 1H, 11-H_a), 2.78-2.62 (m, 3H, 11-H_b, 11'-H_2), 2.41 (dd, ^3J = 13.9, 6.0 Hz, 1H, 9'-H_b). **– ^{13}C/APT NMR** ([D_4]MeOH, 75.5 MHz): δ = 206.6 (C_q, C-4'), 204.0 (C_q, C-4), 193.8 (C_q, C-1), 176.0 (C_q, C-12), 174.8 (C_q, C-12'), 163.6 (C_q, C-5), 162.2 (C_q, C-5'), 144.7 (C_q, C-8'a), 138.2 (CH, C-7), 137.6 (CH, C-7'), 136.8 (C_q, C-8a), 124.7 (CH, C-6), 120.2 (CH, C-8), 118.4 (CH, C-8'), 118.3 (C_q, C-4a), 118.0 (CH, C-6'), 115.6 (C_q, C-4'a), 101.8 (C_q, C-1'), 89.4 (C_q, C-3), 88.7 (C_q, C-2), 73.6 (CH, C-10'), 68.0 (CH, C-10), 64.4 (C_q, C-2'), 56.7 (CH, C-3'), 48.3 (CH, C-9), 42.8 (CH_2, C-11), 41.7 (CH_2, C-11'), 40.2 (CH_2, C-9'). **– (+)-ESI MS:** *m/z* (%) = 1191.0 ([2M+Na]$^+$, 100), 607.3 ([M+Na]$^+$, 40). **– (-)-ESI MS:** *m/z* (%) = 1166.8 ([2M-H]$^-$, 32), 605 ([2M+Na-2H]$^-$, 12), 583.1 ([M-H]$^-$, 100). **–** (+)-**ESI HRMS:** *m/z* = 607.10619 [M+Na]$^+$, 585.12384 [M+H]$^+$ (calcd. 607.10583 for $C_{28}H_{24}O_{14}Na$).

GTRI-02 (72): $C_{13}H_{14}O_4$, white solid. **– ^{1}H NMR** ([D_4]MeOH, 300 MHz): δ = 6.60 (s, 1H, 5-H), 4.23 (m, 1H, 3-H), 3.17, 2.84 (ABX, J_{AB} = 16.1 Hz, J_{BX} = 6.2 Hz, J_{AX} = 3.3 Hz, 2H, 4-H_2), 2.90, 2.60 (ABX, J_{AB} = 16.0 Hz, J_{BX} = 3.1 Hz, J_{AX} = 3.0 Hz, 2H, 2-H_2), 2.44 (s, 3H, 7-CH_3), 2.40 (s, 3H, 8-CH_3). **– ^{13}C/APT NMR** ([D_4]MeOH, 75.5 MHz): δ = 208.3 (C_q, 7-CO), 199.3 (C_q, C-1), 158.8 (C_q, C-6), 147.1 (C_q, C-4a),

140.4 (C_q, C-8), 132.3 (C_q, C-7), 124.9 (C_q, C-8a), 114.6 (CH, C-5), 66.8 (CH, C-3), 50.0 (CH_2, C-2), 40.4 (CH_2, C-4), 32.6 (7-CH_3), 19.2 (8-CH_3). **– (-)-ESI MS:** *m/z* (%) = 489.3 ($[2M+Na-2H]^-$, 20), 233.4 ($[M-H]^-$, 100).

10.12 Terrestrial *Streptomyces* sp. GW 21/1313

The isolate GW 21/1313 was cultivated on M_2 agar and incubated for three days at 27 °C. Small pieces of the agar were used to inoculate eight of 1 L Erlenmeyer flasks each filled with 250 mL of $CaCl_2$ medium. The flasks were kept on a rotary shaker with 110 rpm at 35 °C for four days; the intense orange culture broth was filtered and extracted with ethyl acetate.

10.12.1 Pre-screening

TLC of the crude extract with CH_2Cl_2/7% MeOH showed after spraying with anisaldehyde/sulphuric acid various colour reactions (yellow, orange, and dark spots). The biological activity is shown in the Table 19. The crude extract also exhibited a mortality of 100% in the nematode test.[58]

Table 19: Biological activity of the crude extract from GW 21/1313

Test organisms	Inhibition Zone (∅ mm)
Bacillus subtilis	15
Staphylococcus aureus	17
Streptomyces viridochromogenes (Tü 57)	20
Escherichia coli	14
Candida albicans	11
Mucor miehei	22

10.12.2 Fermentation and Isolation.

With a well grown agar culture of GW21/1313, 100 of 1 L Erlenmeyer flasks each containing 250 ml of $CaCl_2$ medium were inoculated and incubated for 4 days at 35 °C on a round shaker (110 rpm). The 25 L culture broth was filtered and extracted with ethyl acetate. Evaporation of the organic solvent gave 1.5 g of a brown oily extract. The crude extract was subjected to size exclusion column chromatography on Sephadex LH-20 using CH_2Cl_2/50% MeOH and gave only two fractions. Fraction 1

contained mainly fats and fatty acids and was not further investigated. Further purification of the second fraction by PTLC and HPLC delivered 4-hydroxy-2-(5-hydroxymethyl-furan-2-ylmethylene)-5-methyl-furan-3-one (**73**, 25 mg), adenine (32 mg) and 2-(*p*-hydroxyphenyl)ethanol (17 mg).

Due to the presence of many minor components, the strain was fermented on a 50 L scale. The culture broth obtained from the 50 L fermentor was extracted and worked up under similar conditions and delivered 12.6 g of a crude extract, which contained about 6.5 g of Niax added during the fermentation to avoid foaming. The extract was filtered over Sephadex LH-20 (CH_2Cl_2/50% MeOH) to get two fractions. The first fraction contained exclusively Niax and was discarded. Fraction 2 was separated by column chromatography on silica gel into subfractions C_1, C_2, C3, C_4 with a stepwise gradient (CH_2Cl_2 to CH_2Cl_2/10% MeOH). Purification of subfraction C_2 on Sephadex LH-20 yielded 2-(indol-3-yl) ethanol (13 mg), of subfraction C_3 by HPLC 5-hydroxymethylfurfural (**75**, 82 mg), krishnanone A (**76**, 32 mg) and B (**77**, 7 mg) and *p*-hydroxybenzoic acid (11 mg). Subfraction C_4 delivered by size exclusion chromatography on Sephadex LH-20 followed by PTLC with CH_2Cl_2/10% MeOH perlolyrin (**78**, 13 mg), 3-(9*H*-β-carbolin-1-yl)-acrylic acid (**80b**, 2 mg) and 3-(9*H*-β-carbolin-1-yl)-acrylic acid methyl ester (**80a**, 4 mg) after purification by HPLC, Sephadex LH-20 and PTLC (CH_2Cl_2/7% MeOH). Uracil (180 mg) and adenine (459 mg) precipitated from subfraction C_5 on adding CH_2Cl_2 to the CH_3OH solution.

4-Hydroxy-2-(5-hydroxymethyl-furan-2-ylmethylene)-5-methyl-furan-3-one (73): $C_{11}H_{10}O_5$, orange solid. – R_f = 0.30 (CH_2Cl_2/10% MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε) = 248 (3.63), 365 nm (4.38). – **^{1}H NMR** ([D$_4$]MeOH, 300.0 MHz): δ = 7.03 (d, ^3J = 3.4 Hz, 1H, 3'-H), 6.63 (s, 1H, 2-CH), 6.52 (d, ^3J = 3.4 Hz, 1H, 4'-H), 4.56 (s, 2H, 5'-*CH*$_2$OH), 2.33 (s, 3H, 5-CH$_3$). – **^{13}C/APT NMR** ([D$_4$]MeOH, 75.5 MHz): δ = 182.8 (C$_q$, C-3), 164.2 (C$_q$, C-5), 159.9 (C$_q$, C-5'), 149.3 (C$_q$, C-2'), 144.0 (C$_q$, C-2), 137.2 (C$_q$, C-4), 119.9 (CH, C-3'), 111.9 (CH, C-4'), 102.3 (CH, 2-CH), 57.5 (CH$_2$, 5'-CH$_2$OH), 12.2 (CH$_3$, 5-CH$_3$). – **(+)-ESI MS** *m/z* (%) = 689 ([3M+Na]$^+$, 22), 467 ([2M+Na]$^+$, 100), 245 ([M+Na]$^+$, 10), 223 ([M+H]$^+$, 12). – **(-)-ESI MS:** *m/z* (%) = 1929 ([8M-8H+7Na]$^-$, 7), 1685 ([7M-7H+6Na]$^-$, 20), 1441 ([6M-6H+5Na]$^-$, 40), 1197 ([5M-5H+4Na]$^-$, 60), 953 ([4M-4H+3Na]$^-$, 80), 709 ([3M-3H+2Na]$^-$,

54), 465 ([2M-2H+Na]$^-$, 27), 221([M-H]$^-$, 100). – **EI MS (70 eV):** *m/z* (%) = 222 ([M]$^+$, 100), 204 ([M-H_2O]$^{+}$, 24), 191 ([M-CH_2OH]$^+$, 18), 176 (8), 151 (16), 133 (55), 121 (36), 105 (28), 79 (8), 43 (CH_3CO^+, 32). – **CI MS (NH_3):** *m/z* (%) = 257 ([M+NH_4+NH_3]$^+$, 4), 240 ([M+NH_4]$^+$, 100), 223 ([M+H]$^+$, 28).

5-Hydroxymethylfurfural (**75**): $C_6H_6O_3$, light yellow oil. – R_f = 0.40 (CH_2Cl_2/8 % MeOH). – **^{1}H NMR** ([D_6]acetone, 300.0 MHz): δ = 9.58 (s, 1-H), 7.37 (d, ^3J = 3.7 Hz, 1H, 2-H), 6.57 (d, ^3J = 3.7 Hz, 1H, 3-H), 4.71 (dd, ^3J = 6.8, 5.3 Hz, 1H, 6-OH), 4.63 (d, ^3J = 6.0 Hz, 2H, 6-H_2). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ 178.1 (C_q, C-1), 162.8 (C_q, C-5), 153.3 (C_q, C-2), 123.8 (CH, C-3), 110.2 (CH, C-4), 57.4 (CH_2, C-6).

Krishnanone A (76): $C_{13}H_{14}O_6$, light yellow oil. – R_f = 0.35 (CH_2Cl_2/10% MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε): 214 (sh, 4.05) 279 (4.01). – $[\alpha]_D^{20}$ – 4.95° (c 1.01, MeOH). – **IR (KBr):** ν = 3419, 2928, 2850, 1680, 1667, 1524, 1383, 1281, 1195, 1104, 1022, 806 cm^{-1}. – **^{1}H NMR** ([D_6]acetone, 300.0 MHz): δ = 9.61 (s, 1H, 2-C*H*O), 7.37 (d, ^3J = 3.4 Hz, 1H, 3-H), 6.65 (d, ^3J = 3.4 Hz, 1H, 4-H), 6.39 (d, ^3J = 3.0 Hz, 1H, 3'-H), 6.25 (d, ^3J = 3.0 Hz, 1H, 4'-H), 5.59 (s, 1H, 2'-CH), 4.64, 4.60 (AB, ^2J = 13.4 Hz, 2H, 5-CH_2), 4.49 (s, 2H, 5'-C*H*$_2$OH), 4.30 (s br, H/D exchangeable, 1H, 5'-CH_2*OH*), 3.35 (s, 3H, OC*H*$_3$). – **^{13}C/APT NMR** ([D_6]acetone, 75.5 MHz): δ =178.3 (CHO), 158.7 (C_q, C-5), 156.7 (C_q, C-5'), 153.8 (C_q, C-2), 150.9 (C_q, C-2'), 123.3 (CH, C-3), 112.3 (CH, C-4), 110.1 (CH, C-3'), 108.1 (CH, C-4'), 97.8 (CH, 2'-CH), 59.6 (CH_2, 5-CH_2), 57.2 (*C*H$_2$OH), 53.5 (OCH_3). – **(+)-ESI MS:** *m/z* (%) = 289 ([M+Na]$^+$, 20).

Krishnanone B (77): $C_{20}H_{22}O_9$, light yellow oil, yellow fluorescent. – R_f = 0.38 (CH_2Cl_2/10 % MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε): 218 (sh, 4.00), 280 (4.50). – $[\alpha]_D^{20}$ – 1.95° (c 1.54, MeOH). – **IR (KBr):** ν (cm^{-1}): 3417, 2927, 2850, 1680, 1668, 1522, 1385, 1280, 1193, 1074, 1023, 810, 778 cm^{-1}. – **^{1}H NMR** ([D_6]acetone, 300.0 MHz): δ = 9.60 (s, 1H, 2-C*H*O), 7.36 (d, ^3J = 3.2 Hz, 1H, 3-H), 6.64 (d, ^3J = 3.2 Hz, 1H, 4-H), 6.43 (d, ^3J = 3.3 Hz, 1H), 6.38 (d, ^3J = 3.2 Hz, 1H), 6.35 (d, ^3J = 3.4 Hz, 1H), 6.24 (d, ^3J = 3.2 Hz, 1H), 5.62 (s, 1H), 5.52 (s, 1H), 4.66, 4.62 (AB, ^2J = 13.3 Hz, 2H, 5-CH_2), 4.54, 4.50 (AB, ^2J = 13.3 Hz, 1H, 5'-CH_2), 4.48 (d, ^3J = 6.0 Hz, 2H,

5"-C*H*$_2$OH), 4.32 (t, ^3J = 6.0 Hz H/D exchangeable, 1H, 5'-CH$_2$*OH*), 3.36 (s, OC*H*$_3$), 3.30 (s, OC*H*$_3$). – **^{13}C/APT NMR** ([D$_4$]MeOH, 75.5 MHz): δ = 178.3 (CO), 158.7 (C$_q$), 156.6 (C$_q$), 153.8 (C$_q$), 152.7 (C$_q$), 151.8 (C$_q$), 151.2 (C$_q$), 123.3 (CH, C-3), 112.3 (CH, C-4), 110.7 (CH), 110.2 (CH), 109.8 (CH), 108.0 (CH), 97.7 (CH), 97.3 (CH), 59.9 (*CH*$_2$OH), 59.6 (CH$_2$), 57.2 (CH$_2$), 53.6 (OCH$_3$), 53.0 (OCH$_3$). – **(+)-ESI MS:** *m/z* (%) = 429.6 ([M+Na]$^+$, 19). – **EI MS:** *m/z* (%) = 406.1 ([M]$^+$, 1), 328 (2), 249 (4), 141 (60), 109 (100), 81 (16).

Perlolyrin (**78**): $C_{16}H_{12}N_2O_2$, yellow solid giving strong yellow fluorescence. – **^{1}H NMR** (D$_6$]DMSO, 300.0 MHz): δ = 11.18 (s, H/D exchangeable, 1H, NH), 8.37 (d, ^3J = 4.9 Hz, 1H, 3-H), 8.23 (d, ^3J = 7.9 Hz, 1H, 5-H), 8.04 (d, ^3J = 4.9 Hz, 1H, 4-H), 7.76 (d, ^3J = 8.3 Hz, 1H, 8-H), 7.59 (ddd, ^3J = 8.1, 7.1 Hz, ^4J = 1.1 Hz, 1H, 7-H), 7.27 (d, ^3J = 7.9, 7.1 Hz, ^4J = 0.8 Hz, 1H, 6-H), 7.21 (d, ^3J = 3.4 Hz, 1H, 12-H), 6.58 (d, ^3J = 3.4 Hz, 1H, 11-H), 5.45 (br s, H/D exchangeable, 1H, OH), 4.68 (s, 2H, 14-H$_2$). – **^{1}H NMR** (D$_6$]acetone, 150.8 MHz)[142]: δ = 9.72 (s, H/D exchangeable, 1H, NH), 8.38 (d, ^3J = 4.9 Hz, 1H, 3-H), 8.24 (d, ^3J = 7.9 Hz, 1H, 5-H), 8.02 (d, ^3J = 4.9 Hz, 1H, 4-H), 7.70 (d, ^3J = 8.3 Hz, 1H, 8-H), 7.56 (ddd, ^3J = 8.1, 7.1 Hz, ^4J = 1.1 Hz, 1H, 7-H), 7.26 (d, ^3J = 7.9, 7.1 Hz, ^4J = 0.8 Hz, 1H, 6-H), 7.22 (d, ^3J = 3.4 Hz, 1H, 12-H), 6.54 (d, ^3J = 3.4 Hz, 1H, 11-H), 4.72 (s, 2H, 14-H$_2$). – **^{13}C/APT NMR** (D$_6$]DMSO, 75.5 MHz): δ = 156.7 (CH, C-10), 152.1 (C$_q$, C-13), 140.9 (C$_q$, C-8a), 138.1 (CH, C-3), 133.1 (C$_q$, C-1), 130.5 (C$_q$, C-9a), 129.4 (C$_q$, C-4a), 128.3 (CH, C-7), 121.5 (CH, C-5), 120.6 (C$_q$, C-4b), 119.6 (CH, C-6), 113.5 (CH, C-4), 112.4 (CH, C-8), 109.6 (CH, C-12), 109.0 (CH, C-11), 56.0 (CH$_2$, C-14). – **^{13}C /APT NMR** (D$_6$]acetone, 150.8 MHz)[142]: δ = 157.4 (CH, C-10), 154.7 (C$_q$, C-13), 142.1 (C$_q$, C-8a), 139.4 (CH, C-3), 134.5 (C$_q$, C-1), 131.9 (C-9a), 130.8 (C$_q$, C-4a), 129.1 (CH, C-7), 122.3 (CH, C-5), 121.9 (C$_q$, C-4b), 120.6 (CH, C-6), 114.2 (CH, C-4), 113.1 (CH, C-8), 110.1 (CH, C-12), 110.0 (CH, C-11), 57.4 (CH$_2$, C-14). – **(+)-ESI MS:** *m/z* (%) = 287 ([M+Na]$^+$, 26), 265 ([M+H]$^+$, 100). – **(-)-ESI MS:** *m/z* (%) = 263 ([M-H]$^-$, 100).

Radhanone A (**80a**): $C_{16}H_{12}N_2O_3$, light yellow oil. – **^{1}H NMR** (D$_6$]DMSO, 300.0 MHz): δ = 12.12 (s, H/D exchangeable, 1H, NH), 8.68 (d, ^3J = 16.2 Hz, 1H, 11-H),

8.59 (d, ^3J = 4.9 Hz, 1H, 3-H), 8.51 (dd, ^3J = 4.9 Hz, 1H, 4-H), 8.32 (d, ^3J = 8.0 Hz, 1H, 5-H), 7.82 (dd, ^3J = 8.3 Hz, ^4J = 0.8 Hz, 1H, 8-H), 7.62 (ddd, ^3J = 8.2, 7.1 Hz, ^4J = 1.1 Hz, 1H, 7-H), 7.33 (dd, ^3J = 8.0, 7.2 Hz, ^4J = 1.2 Hz, 1H, 6-H), 6.93 (d, ^3J = 16.2 Hz, 1H, 12-H), 3.82 (s, 3H, OCH_3). **– ^{13}C/APT NMR** (D_6]DMSO, 150.8 MHz) δ = 189.2 (C_q, C-10), 165.5 (C_q, C-13), 141.9 (C_q, C-8a), 137.8 (CH, C-3), 136.1 (CH, C-11), 135.1 (C_q, C-9a), 135.0 (C_q, C-1), 131.3 (C_q, C-4a), 130.0 (CH, C-12), 129.1 (CH, C-7), 121.9 (CH, C-5), 120.5 (CH, C-6), 120.2 (CH, C-4), 119.9 (C_q, C-4b), 113.1 (CH, C-8), 52.2 (OCH_3). **– (+)-ESI MS:** *m/z* (%) = 303 ($[M+Na]^+$, 4), 281 ($[M+H]^+$, 2).

Radhanone B (**80b**): $C_{15}H_{10}N_2O_3$, yellow solid, yellow fluorescence. **– ^{1}H NMR** ($[D_6]$DMSO, 300.0 MHz): δ = 12.02 (s, H/D exchangeable, 1H, NH), 8.55 (d, ^3J = 4.9 Hz, 1H, 3-H), 8.45 (dd, ^3J = 4.9 Hz, 1H, 4-H), 8.30 (d, ^3J = 7.9 Hz, 1H, 5-H), 8.23 (d, ^3J = 15.8 Hz, 1H, 11-H), 7.81 (dd, ^3J = 8.3 Hz, 1H, 8-H), 7.59 (ddd, ^3J = 8.3, 7.2 Hz, ^4J = 1.1 Hz, 1H, 7-H), 7.30 (dd, ^3J = 7.9, 7.2 Hz, ^4J = 1.1 Hz, 1H, 6-H), 6.95 (d, ^3J = 15.8 Hz, 1H, 12-H). **– (+)-ESI MS** *m/z* (%) = 267 ($[M+H]^+$, 60). **– (-)-ESI MS** *m/z* (%) = 553 ($[2M+Na-H]^-$, 26), 265 ($[2M-H]^-$, 100).

10.13 Terrestrial *Streptomyces* sp. GW 99/1572

The producing isolate GW 99/1572 was collected from a soil sample in Tenerrifa. It was cultured on M_2 agar at 28 °C and showed a brown agar colour, while the aerial mycelium was orange. The agar of one plate was cut into small pieces to inoculate 8 of 1 L Erlenmeyer flasks containing each 250 ml of M_2 medium. Fermentation was carried out on a shaker at 95 rpm for 72 hours. The light orange coloured broth was harvested and both the filtrate and the mycelial cake were extracted with ethyl acetate.

10.13.1 Pre-Screening

TLC of the ethyl acetate showed the presence of some yellow bands, which did not change with 2N sodium hydroxide, but turned to brown with conc. sulphuric acid. The metabolites had a biological activity against *Bacillus subtilis*, *Streptomyces viri-*

dochromogenes, *Escherichia coli*, *Candida albicans*, *Mucor miehei*, and *Scenedesmus subspicatus*.

Table 20: Biological activity of the crude extract of the strain GW 99/1572

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	17
Mucor miehei	13
Streptomyces viridochromogenes (Tü 57)	18
Escherichia coli	20
Candida albicans	11

10.13.2 Fermentation, Extraction and Isolation

With a well grown agar culture of the terrestrial *Streptomyces* sp. isolate GW99/1572, 100 of 1 L Erlenmeyer flasks each containing 250 mL of M_2 medium were inoculated and incubated for 4 days at 28 °C on a linear shaker. The 25 L culture broth was mixed with ca. 1 kg diatomaceous earth and filtered through a filter-press to separate mycelia and water phase. The mycelia cake and the filtrate were separately extracted each three times with ethyl acetate (ca. 2 L each time). After being evaporated in *vacuo* to dryness, the extracts were dissolved in CH_2Cl_2/5% MeOH and a TLC was done. Since the chemical composition of both organic phases was similar, they were combined and concentrated under reduced pressure to yield 4.7 g of a yellow oily crude extract.

The crude extract was then subjected to column chromatography on silica gel (3 × 200 cm, 200 g) with a CH_2Cl_2/MeOH gradient and separated into fraction I and II under TLC control. The first fraction contained a complex mixture of hydroxylated polyene macrolides as shown by ESI MS and comparison of the UV/VIS spectrum with those isolated in our group and was not further analysed. The second fraction exhibited the biological activity observed during the screening of the strain and contained a colourless band on TLC, which turned to light violet with anisaldehyde/sulphuric acid. Purification of the fraction by PTLC (20 × 20 cm, CH_2Cl_2/7% MeOH) followed by final purification on Sephadex LH-20 (CH_2Cl_2/60% MeOH) delivered 110 mg of kettapeptin (**82a**) as a colourless solid; from the same fraction anthranilic acid was also isolated.

Table 21: Antibacterial activities of kettapeptin (**82a)** and bacitracin A in the agar diffusion test at different concentrations (∅ of inhibition zones, mm).

Compounds	Amount (µg/disk)	BS[a]	SV[b]	SA[c]	EC[d]
kettapeptin	5	13	16	20	12
	10	14	16	17	13
	20	16	16	16	13
bacitacin A	5	0	0	0	0
	10	11	0	11	0
	20	14	0	12	11

[a]*Bacillus subtilis,* [b]*Streptomyces viridochromogenes (Tü 57),* [c]*Staphylococcus aureus,* [d]*Escherichia coli*

Crystal data: $C_{48}H_{78}N_8O_{15}$ + 1 CH_3COCH_3, crystal size: 0.2 mm × 0.1 mm × 0.05 mm, space group $P2_12_12_1$, unit cell a = 9.780 Å, b = 20.355 Å, c = 28.635 Å, α = β = γ = 90°, non-hydrogen atomic volume = 18.3 Å^3, ρ_{calc} = 1.226 g/cm^{-3}, 26627 reflections were measured, 3896 unique reflections, resolution = 28.49-0.95 Å, completeness (%) = 97.1, R (int) (%) = 0.0827, I/Sigma = 12.36, ($R_{int} = \Sigma \mid I - \langle I \rangle \mid / \Sigma I$). The refinement converged to $R_1 = \Sigma \mid \mid F_o \mid - \mid F_c \mid \mid / \Sigma \mid F_o \mid$ = 0.0576 for 3142 reflections $F_o > 4$ Sig and 0.0782 for all 3896 data, $wR_2 = [\Sigma\, w(F_o^2 - F_c^2 / \Sigma\, w(F_o^2)^2]^{1/2}$ = 0.1579 for 3896 data and 2/676 parameters, goodness of fit is 1.034.

Kettapeptin (**82a**): $C_{48}H_{78}N_8O_{15}$, colourless solid. **– R_f** = 0.35 (CH_2Cl_2/5% MeOH). **– IR (KBr):** ν = 3422, 2955, 2938, 1744, 1667, 1650, 1636, 1504, 1458, 1394, 1352, 1318, 1260, 1208, 1148, 1118, 1069, 998, 912, 869, 818, 728, 686 cm^{-1}. **–** $[\alpha]^{20}_D$ = +111.2 (c 1.0, $CHCl_3$). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 9.96 (s, 1H, 15-NOH), 8.24 (d, ^3J = 10.7 Hz, 1H, 24-NH), 6.74 (q, ^3J = 6.9 Hz, 1H, 41-H), 6.29 (s, 1H, 31-OH), 6.23 (d, ^3J = 8.5 Hz, 1H, 2-NH), 6.13 (q, ^3J = 7.0 Hz, 1H, 11-H), 5.63 (dd, ^3J = 9.1 Hz, ^4J = 0.7 Hz, 1H, 37-H), 5.42 (dd, ^3J = 10.7, 2.1 Hz, 1H, 25-H), 5.34 (t, ^3J = 7.1 Hz, 1H, 15-H), 5.20 (dd, ^3J = 5.6, 1.5 Hz, 1H, 6-H), 4.92 (m, 2H, 19-H, 24-H), 4.80 (q, ^3J = 6.5 Hz, 1H, 3-H), 4.54 (d, ^3J = 8.6 Hz, 1H, 2-H), 4.44 (dd, ^3J = 12.2, 1.6 Hz, 1H, 22-NH), 4.37 (s, 1H, 3-OH), 4.09 (dq, ^3J = 8.9, 6.9 Hz, 1H, 38-H), 3.90 (d, ^3J = 10.0 Hz, 1H, 35-H), 3.87 (m, 2H, 9-NH, 16-H_A), 3.76 (dd, ^3J = 10.0, 6.7 Hz, 1H, 16-H_B), 3.37 (s, 3H, 17-H), 3.30 (d, ^3J = 13.2 Hz, 1H, 9-H_A), 3.17 (d, ^3J = 12.8 Hz,

1H, 22-H_A), 3.06 (s, 3H, 13-H), 2.98 (m, 1H, 22-H_B), 2.95 (s, 1H, 30-OH), 2.58 (m, 2H, 7-H_A, 9-H_B), 2.26 (d, ^3J = 13.4 Hz, 1H, 20-H), 2.04 (m, 1H, 43-H_A), 1.88 (m, 1H, 20-H_B), 1.86 (m, 3H, 42-H), 1.78 (s, 3H, 48-H), 1.72 (m, 3H, 7-H_B, 32-H), 1.68 (m, 1H, 26-H), 1.66 (m, 1H, 43-H_B), 1.64 (m, 1H, 8-H_A), 1.59 (s, 3H, 46-H), 1.54 (m, 2H, 8-H_b, 21-H_A), 1.52 (m, 2H, 33-H), 1.48 (m, 2H, 21-H_B, 34-H), 1.26 (d, ^3J = 7.0 Hz, 3H, 12-H), 1.14 (q, ^3J = 6.7 Hz, 3H, 47-H), 1.07 (d, ^3J = 6.7 Hz, 1H, 4-H), 0.86 (t, ^3J = 7.6 Hz, 3H, 44-H), 0.83 (d, ^3J = 6.8 Hz, 3H, 28-H), 0.71(d, ^3J = 6.2 Hz, 3H, 45-H). – **^{13}C/APT NMR** ($CDCl_3$, 150.8 MHz): δ = 203.0 (C_q, C-39), 175.3 (C_q, C-29), 173.1 (C_q, C-10), 172.8 (C_q, C-18), 171.0 (C_q, C-14), 170.8 (C_q, C-23), 170.2 (C_q, C-1), 169.5 (C_q, C-5), 137.5 (C_q, C-40), 136.8 (CH, C-41), 132.8 C_q, C-36), 129.3 (CH, C-37), 99.6 (C_q, C-31), 82.2 (CH, C-35), 80.1 (C_q, C-30), 78.6 (CH, C-25), 68.5 (CH, C-16), 64.7 (CH, C-3), 59.2 (CH, C-17), 54.6 (CH, C-24), 52.4 (CH, C-6), 52.2 (CH, C-2), 53.8 (CH, C-15), 51.3 (CH, C-19), 48.0 (CH, C-9), 47.7 (CH, C-11), 45.6 (CH, C-22), 38.2 (CH, C-38), 32.5 (CH, C-34), 29.4 (CH, C-13), 29.2 (CH, C-26), 28.3 (CH, C-32), 27.2 (CH, C-33), 25.9 (CH, C-43), 24.4 (CH, C-7), 24.0 (CH, C-20), 21.3 (CH, C-8), 21.1 (CH, C-21), 19.5 (CH, C-47), 19.3 (CH, C-27), 18.9 (CH, C-4), 17.6 (CH, C-45), 14.9 (CH, C-42), 14.6 (CH, C-28), 13.1 (CH, C-12), 12.0 (CH, C-46), 11.4 (CH, C-48), 8.4 (CH, C-44).– **(+)-ESI MS:** *m/z* (%) = 1029.8 ($[M+Na]^+$, 100), 1051.4 ($[M+2Na-H]^+$, 8). – **(-)-ESI MS:** *m/z* (%) = 1027.7 ($[M+Na-2H]^-$, 20), 1005.8 ($[M-H]^-$, 100). – **ESI HRMS** *m/z* : 1006.56170 (calcd. 1006.558664 for $C_{48}H_{78}N_8O_{15}$).

10.14 Terrestrial *Streptomycetes* sp. GW 32/698

The terrestrial *Streptomyces* sp. GW 32/698 grew on M_2 agar after incubation at 28 °C and four days with a white mycelium and yellow agar colouration. This culture was used for a 1 L fermentation with M_2 medium on a rotary shaker for four days at 28 °C with 95 rpm. The yellow culture broth was extracted with ethyl acetate and gave 40 mg yellow crude extract, which was used for biological and chemical screening.

Table 22: Biological activity of the crude extract from GW 32/698 (50 mg/mL)

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	15
Staphylococcus aureus	15
Escherichia coli	16

The TLC of the crude extract showed many yellow bands on day light and compounds, which under UV 366 nm presented an orange fluorescence and turned orange, black or remained yellow on spraying with anilsaldehyde/sulphuric acid.

10.14.1 Fermentation, Extraction and Isolation

With a well grown agar culture of the terrestrial *Streptomyces* sp. GW32/698, 100 of 1 L Erlenmeyer flasks each containing 250 mL of M_2 medium were inoculated and incubated for 3 days at 28 °C on a linear shaker. The 25 L culture broth was mixed with ca. 1 kg Celite and filtered through a filter press to separate mycelia and water phase. The mycelial cake and the filtrate were separately extracted each three times with ethyl acetate (ca. 2 L each time). Since the chemical composition of both organic phases was similar, they were combined and concentrated under reduced pressure to yield 3 g of a dark yellow oily extract. The crude extract was dissolved in methanol (150 ml), extracted with cyclohexane (150 ml) and both phases were evaporated separately.

The cyclohexane phase was separated by PTLC (20 × 20 cm, 5 plates, hexane/10% ethyl acetate) to yield chrysophanol (**83a**, 3.6 mg, R_f 0.98) as a yellow solid, 2-aminobenzoic acid methyl ester as oil and phenylacetamide. A second orange more polar zone afforded on further purification by CC with CH_2Cl_2 3.5 mg of bhimamycin A (**88**).

The methanol phase was pre-separated by column chromatography on silica gel (3 × 30 cm, 240 g) with a CH_2Cl_2-MeOH gradient into nine fractions. Purification of fraction 1 on a silica gel column (1 × 10 cm, 15 g, CH_2Cl_2) resulted in bhimamycin B (**89**, 4 mg). The HPLC purification of fraction 6 using MeCN /90% H_2O gave aloesaponarin II (**85**, 2 mg, R_f 0.69). On trituration of fraction 8 with CH_2Cl_2/10% MeOH

(20 ml), an orange solid remained undissolved, which was separated by centrifugation to yield 15 mg of 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**84a**, R_f = 0.06). Purification of the soluble part of fraction 8 by HPLC gave adenine (4 mg) and 2'-deoxy-adenosine (3 mg).

Chromatography of fraction 9 on Sephadex LH-20 using CH_2Cl_2/60% MeOH followed by HPLC and PTLC gave another 3 mg of aloesaponarin II (**83a**) as a brown power, bhimamycin C (**93**, 2 mg) and bhimamycin D (**94a**, 5 mg) both as yellow solids.

The crude extract obtained from a second fermentation on a 50-L scale was separated on silica gel (CH_2Cl_2-MeOH gradient) into six fractions. Bhimamycin B (**96**, 50 mg), bhimamycin A (**95**, 70 mg) and 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**91**, 50 mg) were obtained from fractions I, II and VI, respectively, after PTLC (CH_2Cl_2/5% MeOH) and Sephadex LH-20 (CH_2Cl_2/60% MeOH). Fraction IV delivered in a similar way aloesaponarin II (**85**, 3 mg), bhimamycin C (**93**, 7 mg) and D (**94a**, 17 mg). After separation on Sephadex LH-20 (CH_2Cl_2/60% MeOH) followed by PTLC (CH_2Cl_2/10% MeOH), fraction III gave bhimamycin E (**102**, 10 mg). On crystallisation from CH_2Cl_2-MeOH, fraction V yielded 60 mg of bhimanone (**104**) as colourless needles.

Table 23: Antibacterial activities of bhimamycin A (**88**), B (**89**) and E (**102**)

	µg/disc	EC	SA	SV	BS
bhimamcin A	20	11	13	0	0
	40	11	15	0	14
bhimamcin B	20	0	17	0	11
	40	11	20	0	12
bhimamcin E	20	0	15	0	0
	40	0	20	0	0

BS = *Bacillus subtilis*, EC = *Escherichia coli*, SA = *Staphylococcus aureus*, SV = *Streptomyces viridochromogenes*

2-Aminobenzoic acid methyl ester: $C_8H_9NO_2$. **– R_f** = 0.83 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.84 (dd, ^3J = 8.0 Hz, ^4J = 1.4 Hz, 1H, 3-H), 7.26 (m, 1H, 4-H), 6.65 (m, 2H, 3-, 5-H), 3.88 (s, 3H, CH_3). **– EI MS (70 eV):** *m/z* (%) = 151.1 (M^+, 79), 119.0 (100), 92.1 (42), 65.0 (18).

Phenylacetamid: C_8H_9NO. **– R_f** = 0.24 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.40-7.20 (m , 5H, Ar-H), 6.20 (br s, H/D–exchangeable, 1H, NH), 5.58 (br s, H/D–exchangeable, 1H, NH), 3.58 (s, 2H, CH_2). **– EI MS (70 eV):** *m/z* (%) = 135.1 ($[M]^+$, 32), 91.1 (100), 92.1 (100), 65.0 (20), 44.1 (12).

3,8-Dihydroxy-1-methylanthraquinone-2-carboxylic acid (84a): $C_{16}H_{10}O_6$, orange powder. **– R_f** = 0.46 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 13.40 (s, 1H, 8-OH), 7.64 (t, ^3J = 8.5 Hz, 1H, H-6), 7.58 (dd, ^3J = 7.5 Hz, ^4J = 1.4 Hz, 1H, 5-H), 7.28 (dd, ^3J = 8.1 Hz, ^4J = 1.4 Hz, 1H, 7-H), 7.22 (s, 1H, 4-H), 3.10 (s, 3H, 1-CH_3). **– ^{13}C/APT NMR** ([D_6]DMSO, 75.5 MHz): δ = 188.8 (C_q, C-9), 182.9 (C_q, C-10), 172.1 (C_q, 2-COOH), 169.6 (C_q, C-3), 161.4 (C_q, C-8), 149.8 (C_q, C-1), 136.3 (C_q, C-4a), 135.1 (CH, C-6), 132.5 (C_q, C-10a), 124.1 (CH, C-7), 119.0 (C_q, C-2), 117.6 (CH, C-5), 117.5 (CH, C-8a), 115.3 (CH, C-4), 20.3 (CH_3, 1-CH_3). **– EI MS (70 eV):** *m/z* (%) = 298.1 ($[M]^+$, 4), 254.1 (100), 236.1 (20), 44.0 (28).

Aloesaponarin II (85) : $C_{15}H_{10}O_4$, yellow solid. **– R_f** = 0.5 (CH_2Cl_2/5% MeOH). **– ^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 13.06 (br s, 1H, 8-OH), 7.70 (t, ^3J = 8.3 Hz, 1H, 3-H), 7.62 (dd, ^3J = 8.3, 0.9 Hz, 1H, 4-H), 7.45 (d, ^4J = 0.9 Hz, 1H, 5-H), 7.36

(d, ^4J = 0.9 Hz 1H, 7-H), 7.02 (d, ^4J = 0.9 Hz, 1H, 2-H), 2.70 (s, 3H, 1-CH_3). – **EI MS (70 eV):** *m/z* (%) = 254.1 (M^+, 100), 236.1 (22), 152.1 (4), 115.1 (4).

Bhimamycin A (88): $C_{15}H_{12}O_5$, brown solid. – R_f = 0.89 (CH_2Cl_2/8% MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε) = 244 (4.26), 302 (3.45), 384 (3.92) nm. – **IR (KBr):** ν = 3372, 2980, 2926, 2850, 1636, 1604, 1454, 1419, 1350, 1256, 1163, 1109, 1072, 1011, 892, 860, 836, 769, 717 cm^{-1}. – $[\alpha]_D^{20}$ = + 55.6 (c 0.45, $CHCl_3$). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.72 (s, 1H, 5-OH), 7.66 (dd, ^3J = 7.6 Hz, ^4J = 1.2 Hz, 1H, 8-H), 7.57 (dd, ^3J = 8.3, 7.6 Hz, 1H, 7-H), 7.19 (dd, ^3J = 8.3 Hz, ^4J = 1.2 Hz, 1H, 6-H), 5.44 (br s, 1H, 10-OH), 5.10 (q, ^3J = 6.8 Hz, 1H, 10-H), 2.70 (s, 1H, 12-H), 1.61 (d, ^3J = 6.8 Hz, 3H, 11-H). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 186.1 (C_q, C-4), 180.7 (C_q, C-9), 163.9 (C_q, C-1), 163.1 (C_q, C-5), 158.1 (C_q, C-3), 136.1 (CH, C-7), 135.2 (C_q, C-8a), 124.6 (CH, C-6), 119.5 (CH, C-8), 117.6 (C_q, C-4a), 117.1 (C_q, C-3a), 116.9 (C_q, C-9a), 63.9 (CH, C-10), 21.2 (CH_3, C-11), 13.7 (CH_3, C-12). – **EI MS (70 eV):** *m/z* (%) = 272 ($[M]^+$, 80), 257 (96), 230 (100), 200 (8)), 173 (8), 121 (12), 115 (8). – **CI MS (NH_3):** *m/z* (%) = 562 ($[2M+ NH_4]^+$, 4), 290 ($[M+NH_4]^+$, 28), 273 ($[M+H]^+$, 100). – **EI HRMS:** *m/z* = 272.0694 (calcd. 272.06847 for $C_{15}H_{12}O_5$).

Bhimamycin B (89): $C_{15}H_{10}O_5$, brown solid. – R_f = 0.97 (CH_2Cl_2/8% MeOH). – **UV/VIS (MeOH):** λ_{max} (lg ε) = 224 (4.22), 284 (3.68), 3.90 (3.77) (4.03) nm. – **IR (KBr):** ν = 3429, 2922, 2852, 1681, 1636, 1586, 1535, 1456, 1417, 1373, 1346, 1253, 1238, 1223, 1169, 1149, 1103, 1057, 991, 896, 853, 783, 709, 651, 611 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.61 (s, 1H, 5-OH), 7.83 (dd, ^3J = 7.6 Hz, ^4J = 1.2 Hz, 1H, 8-H), 7.68 (t, ^3J = 8.0 Hz, 1H, 7-H), 7.31 (dd, ^3J = 8.3 Hz, ^4J = 1.2 Hz, 1H, 6-H), 2.88 (s, 3H, CH_3), 2.86 (s, 3H, CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 187.7 (C_q, C-10), 186.0 (C_q, C-4), 178.3 (C_q, C-9), 162.9 (C_q, C-5), 161.9 (C_q, C-2), 148.9 (C_q, C-1), 136.7 (CH, C-7), 135.8 (C_q, C-8a), 124.6 (CH, C-6), 123.2 (C_q, C-9a), 120.2 (CH, C-8), 118.6 (C_q, C-3a), 116.9 (C_q, C-4a), 29.4 (CH_3, C-11), 14.4 (CH_3, C-12). – **EI MS (70 eV):** *m/z* (%) = 270 ($[M]^+$, 100), 255 (15), 242 (68), 227 (8), 199 (20), 171(8), 115 (12). – **CI MS (NH_3):** *m/z* (%) = 558 ([2M+

$NH_4]^+$, 20), 288 ([M+$NH_4]^+$, 100), 271 ([M+H]$^+$, 72). **– EI HRMS:** *m/z* = 270.05390 (calcd. 270.05282 for $C_{15}H_{10}O_5$).

Bhimamycin C (93): $C_{17}H_{17}NO_5$, yellow solid. **–** R_f = 0.57 (CH_2Cl_2/8% MeOH). **– UV/VIS (MeOH):** λ_{max} (lg ε) = 380 (3.39) nm. **– IR (KBr):** ν = 3424, 2924, 2853, 1656, 1620, 1458, 1410, 1368, 1262, 1238, 1168, 1080, 1098, 1056, 808, 785, 777, 713 cm^{-1}. **–** $[\alpha]_D^{20}$ = -23.4 (c 0.555, $CHCl_3$). **–** ^{1}**H NMR** ($CDCl_3$, 300.0 MHz): δ = 13.12 (s, 1H, 5-O H), 7.66 (dd, ^3J = 7.6 Hz, ^4J = 1.1 Hz, 1H, 8-H), 7.53 (t, ^3J = 8.0 Hz, 1H, 7-H), 7.16 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 1H, 6-H), 6.67 (br s, 1H, 10-OH), 5.05 (q, ^3J = 6.8 Hz, 1H, 10-H), 4.28 (m, 2H, 1'-H), 3.84 (m, 2H, 2'-H), 2.70 (s, 3H, 12-H), 1.54 (d, ^3J = 6.8 Hz, 3H, 11-H). **–** ^{13}**C/APT NMR** ($CDCl_3$, 75.5 MHz): δ =188.2 (C_q, C-4), 182.4 (C_q, C-9), 163.9 (C_q, C-5), 147.4 (C_q, C-1), 140.6 (C_q, C-3), 137.1(C_q, C-8a), 136.4 (CH, C-7), 124.6 (CH, C-6), 119.9 (CH, C-8), 118.8 (C_q, C-4a), 118.1 (C_q, C-3a), 117.7 (C_q, C-9a), 117.1 (C_q, C-3a), 64.1 (CH, C-10), 61.9 (CH_2, C-2'), 47.9 (CH_2, C-1'), 23.4 (CH_3, C-11), 11.9 (CH_3, C-12). **– EI MS (70 eV):** *m/z* (%) = 315 ([M]$^+$, 100), 272 (96), 270 (36), 252 (24), 228 (12), 141 (4), 121 (4), 45 (4). **– (+)-ESI MS:** *m/z* (%) = 653 ([2M+Na]$^+$, 100), 338 ([M+Na]$^+$, 20). **– (-)-ESI MS:** *m/z* (%) = 314 ([M-H]$^-$). **– EI HRMS**: *m/z* = 315.11070 (calcd. 315.11067 for $C_{17}H_{17}NO_5$).

Bhimamycin D (94a): $C_{22}H_{15}NO_6$, yellow solid. **–** R_f = 0.63 (CH_2Cl_2/8% MeOH). **– UV/VIS (MeOH):** λ_{max} (lg ε) = 248 (4.43), 404 (3.92) nm. **– IR (KBr):** ν = 3394, 2923, 2852, 1737, 1646, 1463, 1463, 1430, 1382, 1266, 1236, 1165, 1120, 1095, 1028, 686 cm^{-1}. **–** $[\alpha]_D^{20}$ = -2.5 (c 0.790, $CHCl_3$). **–** ^{1}**H NMR** (C_6D_6, 300.0 MHz): δ = 13.45 (s, 1H, 5-OH), 7.86 (d, ^3J = 7.2 Hz, 1H, 6'-H), 7.76 (d, ^3J = 6.4 Hz, 1H, 8-H), 7.02 (m, 2H, 6-H, 7-H), 6.90 (t, ^3J = 7.2 Hz, 1H, 4'-H), 6.84 (t, ^3J = 7.2 Hz, 1H, H-5'), 6.56 (d, ^3J = 7.2 Hz, 1H, 3'-H), 2.76 (s, 3H, CH_3), 2.10 (s, 3H, CH_3). **–** ^{13}**C/APT NMR** (C_6D_6, 75.5 MHz): δ = 192.9 (C_q, C-10), 187.5 (C_q, C-4), 179.9 (C_q, C-9), 167.8 (C_q, C-7'), 163.2 (C_q, C-5), 141.4 (C_q, C-3), 137.3 (C_q, C-2'), 136.6 (C_q, C-8a), 135.6 (CH, C-7), 134.2 (C_q, C-1), 133.2 (CH, C-4'), 132.4 (CH, C-6'), 129.5 (CH, C-3'), 129.4 (CH, C-5'), 128.5 (C_q, C-1'), 123.7 (CH, C-6), 122.1 (C_q, C-9a), 119.3 (CH, C-8), 117.6 (C_q, C-4a), 117.2 (C_q, C-3a), 30.7 (CH_3, C-11), 11.8

(CH_3, C-12). **– EI MS (70 eV):** *m/z* (%) = 489 ($[M]^+$, 100), 344 (85), 330 (21), 254 (44), 210 (4), 121 (3), 43 (6). (+)-ESI-MS: *m/z* (%): 1041 ($[2M+Na]^+$, 100), 412 ($[M+Na]^+$, 12). **– (-)-ESI MS:** *m/z* (%) = 388 ($[M-H]^-$, 100).**– EI HRMS:** *m/z* = 389.09090 (calcd. 389.08994 for $C_{22}H_{15}NO_6$).

Synthesis of bhimamycin D (**94a**): Bhimamycin B (**89**, 30 mg) and anthranilic acid (50 mg) were dissolved in ethanol (5 ml) and refluxed for 18 h and then the solvent was evaporated under vacuum. Purification of the reaction mixture by Sepahdex LH-20 (CH_2Cl_2/60% MeOH) and finally on HPLC yielded 1 mg of bhimamycin D (**94a**, 2.3%). The ^{1}H NMR spectrum was identical with that of the natural compound.

Methylation of bhimamycin D (**94a**): To the solution of bhimamycin D (**94a**, 5 mg) in dichloromethane (1 ml), a few drops of etherial diazomethane solution were added at 20 °C and the mixture was evaporated to dryness after shaking for about 5 seconds to yield bhimamycin D methyl ester (**94b**, 5.2 mg, 100%), $C_{23}H_{17}NO_6$, as a yellow solid. **– R_f** = 0.87 (CH_2Cl_2/7% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.98 (br s, 1H, OH), 7.97 (br d, ^3J = 7.2 Hz, 1H, CH), 7.60 (m, 1H, 1CH), 7.49 (m, 3H, 3CH), 7.14 (m, 2H, 2CH), 3.84 (s, 3H, OCH_3), 2.68 (s, 3H, $COCH_3$), 2.35 (s, 3H, Ar-CH_3). **– EI MS (70 eV):** *m/z* (%) = 403 ($[M]^+$, 84), 374 (8), 360 (20), 344 (100), 329 (28), 316 (16), 301 (13), 272 (10), 254 (36), 244 (16), 77 (20), 45 (36).

Bhimamycin E (102): $C_{13}H_{10}O_5$, yellow solid. **– R_f** = 0.54 (CH_2Cl_2/5% MeOH). **– UV/VIS (MeOH):** λ_{max} (lg ε) = 226 (4.35), 280 (4.24), 404 (3.79) nm. **– IR (KBr):** ν = 3409, 3272, 2924, 2854, 1706, 1636, 1600, 1578, 1482, 1458, 1411, 1363, 1300, 1207, 1173, 1159, 1073, 1053, 875, 823, 748, 699, 678, 599, 577 cm^{-1}. **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 11.08 (s, 1H, 5-OH), 7.64 (m, 1H, 7-H, 8-H), 7.38 (s, 1H, 9-OH), 7.21 (m, 1H, 6-H), 3.76 (s, 2H, 9-H), 2.34 (s, 3H, CH_3). **– ^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 203.5 (C_q, C-10), 184.3 (C_q, C-4), 183.1 (C_q, C-1), 161.4 (C_q, C-5), 153.7 (C_q, C-2), 137.8 (CH, C-7), 132.3 (C_q, C-8a), 123.4 (CH, C-6), 119.9 (CH, C-8), 119.9 (C_q, C-3), 113.0 (C_q, C-4a), 38.1(CH_2, C-9), 30.1 (CH_3, C-11). **– EI MS (70 eV):** *m/z* (%) = 246 ($[M]^+$, 16), 204 (100), 176 (17), 147 (8), 121 (12), 102 (6), 43 (64). **– (-)-ESI MS:** *m/z* (%) = 291 ($[M+2Na-H]^+$, 85), 269

([M+Na]$^+$], 100). – **(-)-ESI MS:** *m/z* (%) = 513 ([2M+Na-2H]$^-$, 60), 245 ([M-H]$^-$, 100). – **EI HRMS:** *m/z* = 246.05282 (calcd. 246.05283 for $C_{13}H_{10}O_5$).

Synthesis of bhimamycin F (98) and G (99): Bhimamycin A (**88**, 17 mg) and anthranilic acid (35 mg) were dissolved in ethanol (5 ml) and refluxed for 12 h and then the solvent was evaporated under vacuum. Purification of the reaction mixture over Sepahdex LH-20 (CH_2Cl_2/60% MeOH) yielded 6 mg of bhimamycin F (**98**, 24.5%) and 3 mg of bhimamycin G (**99**, 12.8%).

Bhimamycin F (**98**): $C_{22}H_{17}NO_6$, yellow solid. – R_f = 0.40 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 13.10 (br s, 1H, 5-OH), 7.91 (d, ^3J = 7.2 Hz, 1H, 6'-H), 7.65 (m, 2H, 2 CH), 7.52 (m, 2H, 2-H), 7.28 (d, ^3J = 7.2 Hz, 1H, CH), 7.20 (d, ^3J = 7.2 Hz, 1H, CH), 6.01 (br s, 1H, 10-OH), 4.58 (q, ^3J = 6.5 Hz, 1H, 10-H), 2.23 (s, 3H, 3-CH_3), 1.33 (d, ^3J = 6.5 Hz, 3H, CH_3). – **^{13}C/APT NMR** ([D_6]DMSO, 125.7 MHz): δ = 186.5 (C_q, C-4), 179.6 (C_q, C-9), 169.2 (C_q, C-7'), 162.6 (C_q, C-5), 146.9 (C_q, C-3), 139.9 (C_q, C-2'), 138.5 (C_q, C-8a), 135.7 (C_q, C-1), 135.7 (CH, C-7), 132.5 (C_q, C-1'), 130.4 (CH, C-6'), 129.7 (CH, C-4'), 129.3 (CH, C-3'), 128.8 (CH, C-5'), 123.2 (CH, C-6), 118.5 (CH, C-8), 117.1 (C_q, C-9a), 115.9 (C_q, C-4a), 115.5 (C_q, C-3a), 61.9 (CH, C-10), 21.7 (CH_3), 12.0 (CH_3, C-11). – **(+)-ESI MS:** *m/z* (%) = 805 ([2M+Na]$^+$, 100), 414 ([M+Na]$^+$, 13). – **(-)-ESI MS:** *m/z* (%) = 390 ([M-H]$^-$).

Bhimamycin G (**99**): $C_{22}H_{15}NO_5$, yellow solid. – R_f = 0.42 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 13.21 (s, 1H, 5-OH), 8.25 (dd, ^3J = 7.2 Hz, ^4J = 2.1 Hz, 1H, 3'-H), 7.79 (d, 2H, ^3J = 7.5 Hz, 4'-H, 8-H), 7.69 (t, ^3J = 7.2 Hz, 1H, 7-H), 7.59 (t, ^3J = 7.2 Hz, 1H, 5'-H), 7.41 (d, ^3J = 7.2 Hz, 1H, 6'-H), 7.22 (d, ^3J = 7.2 Hz, 1H, 6-H), 4.35 (q, ^3J = 6.4 Hz, 1H, 10-H), 2.36 (s, 3H, Ar-CH_3), 1.32 (d, ^3J = 6.4 Hz, 3H, 11-H). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 187.2 (C_q, C-4), 181.7 (C_q, C-9), 166.3 (C_q, C-7'), 162.9 (C_q, C-5), 146.5 (C_q, C-3), 138.8 (C_q, C-2'), 135.6 (C_q, C-8a), 135.4 (C_q, C-1), 135.3 (CH, C-7), 134.8 (C_q, C-1'), 133.0 (CH, C-6'), 130.8 (CH, C-4') 130.0 (CH, C-3'), 127.9 (CH, C-5'), 124.1 (CH, C-6), 119.3 (CH, C-8), 117.8 (C_q, C-9a), 117.3 (C_q, C-4a), 117.3 (C_q, C-3a), 63.4 (CH, C-10), 22.8 (CH_3), 12.0 (CH_3, C-11). – **DCI (NH_3):** *m/z* (%) = 374 ([M+H]$^+$).

Bhimanone (104a): $C_{13}H_{14}O_4$, colourless needles. **– R_f** = 0.31 (CH_2Cl_2/5% MeOH). **– UV/VIS (MeOH):** λ_{max} (lg ε) = 220 (4.12), 256 (3.65), 316 (3.28) nm. **– IR (KBr):** ν = 3504, 3165, 2951, 2893, 1702, 1681, 1859, 1472, 1446, 1414, 1363, 1336, 1290, 1241, 1219, 1202, 1168, 1119, 1096, 1054, 1034, 1017, 973, 955, 918, 901, 809, 749, 594, 564, 532, 518, 502 cm^{-1}. **–** $[\alpha]_D^{20}$ = + 65.5 (c 0.55, $CHCl_3$). **– ^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 9.80 (s, 1H, OH), 7.27 (d, ^3J = 8.0 Hz, 1H, 8-H), 7.20 (t, ^3J = 8.0 Hz, 1H, 7-H), 7.05 (d, ^3J = 8.0 Hz, 1H, 6-H), 5.05 (t, ^3J = 3.0 Hz, 1H, 4-H), 3.50 (m, 1 H, 2-H), 2.90 (dd, ^3J = 17.5, 5.9 Hz, 1H, 9-H_A), 2.50 (dd, ^3J = 17.5, 5.3 Hz, 1H, 9-H_B), 2.15 (s, 3H, CH_3), 2.10 (m, 2H, 3-H_2). **– ^{13}C/APT NMR** ([D_6]DMSO, 75.5 MHz): δ = 206.7 (C_q, C-10), 199.0 (C_q, C-1), 155.2 (C_q, C-5), 132.1 (C_q, C-8a), 130.9 (C_q, C-4a), 128.5 (CH, C-7), 120.2 (CH, C-6), 116.5 (CH, C-8), 58.9 (CH, C-4), 43.2 (CH_2, C-9), 37.2 (CH, C-2), 36.5 (CH, C-3), 30.0 (CH_3, C-11). **– EI MS (70 eV):** *m/z* (%) = 234 (M^+, 12), 216 (12), 198 (13), 176 (100), 173 (75), 145 (32), 131 (13), 127 (14), 121 (2), 115 (19), 91 (8), 77 (8), 65 (12), 43 (44). **– (-)-ESI MS:** *m/z* (%) = 491([2M+Na]$^+$, 50), 257 ([M+Na]$^+$, 100). **– (-)-ESI MS:** *m/z* (%) = 233 ([M-H]$^-$, 100). **– EI HRMS:** *m/z* = 234.08930 (calcd. 234.08921 for $C_{13}H_{14}O_4$).

Acetylation of bhimanone (**104**): To the solution of bhimanone (**104**, 6 mg) in pyridine (0.5 ml), acetic acid anhydride (0.5 ml) and a crystal of 4-dimethylaminopyridine (DMPA) were added. The reaction mixture was left at room temperature for 2 h and worked up to yield bhimanone diacetate (**104b**, 8 mg, 98%), $C_{17}H_{18}O_6$, as colorless solid. **– R_f** = 0.66 (CH_2Cl_2/5% MeOH). **– UV/VIS (MeOH):** λ_{max} (lg ε) = 206 (4.42), 244 (4.01), 287 (3.28) nm. **– IR (KBr):** ν = 3426, 3085, 2930, 1770, 1736, 1692, 1654, 1604, 1582, 1466, 1582, 1371, 1315, 1231, 1194, 1122, 1021, 954, 917, 890, 869, 803, 751, 597, 549, 530, 501, 473, 424 cm^{-1}. **– ^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 7.94 (d, ^3J = 8.1 Hz, 1H, 8-H), 7.52 (t, ^3J = 8.1Hz, 1H, 7-H), 7.35 (d, ^3J = 8.1 Hz, 1H, 6-H), 6.32 (t, ^3J = 3.0 Hz, 1H, 4-H), 3.56 (m, 1H, 2-H), 3.07 (dd, ^3J = 17.5, 6.0 Hz, 1H, 9-H_A), 2.58 (dd, ^3J = 17.5, 5.2 Hz, 1H, 9-H_B), 2.31 (s, 3H, 4-$COCH_3$), 2.27 (s, 3 H, 5-$COCH_3$), 2.24 (m, 2H, 3-H_2), 2.09 (s, 3H, 11-CH_3). **– ^{13}C/APT NMR** ([D_6]DMSO, 75.5 MHz): δ = 206.4 (C_q, C-10), 197.3 (C_q, C-1), 170.2 (C_q, 4-$COCH_3$), 169.2 (C_q, 5-$COCH_3$), 148.6 (C_q, C-5), 133.7

(C_q, C-8a), 131.2 (C_q, C-4a), 130.2 (CH, C-7), 128.1 (CH, C-6), 124.9 (CH, C-8), 62.4 (CH, C-4), 43.0 (CH_2, C-9), 37.9 (CH, C-2), 33.5 (CH, C-3), 30.3 (CH_3, C-11), 20.7 (CH_3, 4-$COCH_3$), 21.0 (CH_3, 4-$COCH_3$). **– EI MS (70 eV):** *m/z* (%) = 318 ($[M]^+$, 4), 276 (2), 258 (5), 216 (63), 198 (9), 173 (100), 145 (11), 131 (6), 127 (5), 115 (7), 91 (4).

10.14.2 Terrestrial *Streptomyces* sp. GW 29/1540

The culture of the terrestrial *Streptomyces* GW 29/1540 on M_2^+ agar (better growth compared to standard M_2 medium for terrestrial strains) showed after incubation at 28 °C for four days a white mycelium and the surrounding agar was stained dark grey. The diffusion of this dark pigment gave overall a black colour to the agar.

10.14.3 Primary screening

The culture of the isolate on a 1 L scale at 28 °C and 95 rpm for four days using glucose/ yeast/malt extract and tap water showed after three days a dark grey culture broth. Filtration and extraction of the latter gave an oily crude extract; the dark pigment remained in the water phase. The crude extract was found to be active against *Mucor miehei*, *Streptomyces viridochromogenes*, *Chlorella sorokiniana*, *Chlorella vulgaris,* and *Scenedesmus subspicatus*.

Table 24: Biological activity of the crude extract of the strain GW 29/1540

Test organisms	Inhibition zone (∅ mm)
Mucor miehei	11
Chlorella sorokiniana	35
Streptomyces viridochromogenes (Tü 57)	12
Chlorella vulgaris	30
Scenedesmus subspicatus	30

10.14.4 Fermentation, Work-up and Isolation

The strain GW 29/1540 was fermented in a 25 L scale on a linear shaker for four days at 28 °C. The resulting dark grey culture broth was filtered under pressure and extracted with ethyl acetate. The solvent was evaporated and the dark oily material (3 g) was sequentially separated by silica gel column chromatography, Preparative TLC

and finally reversed phase HPLC. The 3 g crude extract were chromatographed on silica gel using a CH_2Cl_2/MeOH gradient (1 L CH_2Cl_2, 1 L CH_2Cl_2/5% MeOH, 2 L CH_2Cl_2/10% MeOH, 1 L CH_2Cl_2/15% MeOH, 1 L CH_2Cl_2/20% MeOH, 1 L CH_2Cl_2/25% MeOH, 2 L CH_2Cl_2/50% MeOH, 1 L MeOH). Three fractions were selected under TLC control, which showed colourless, UV absorbing spots. On dissolving fraction A in CH_2Cl_2/MeOH, uracil was precipitated and removed by centrifugation. Preparative TLC of the supernatant with CH_2Cl_2/10% MeOH delivered thymin. Purification of fraction B by preparative HPLC using CH_3CN-H_2O (starting with CH_3CN/H_2O 10:90, increasing to CH_3CN/H_2O 60:40 within 30 min, for 33-35 min 100% CH_3CN) delivered *trans*-cyclo(Leu-Pro) (R_t = 12 min) and chloramphenicol (**105**, R_t = 18 min, 10 ml/min). Chromatography of fraction C on Sephadex LH-20 with CH_2Cl_2/40% MeOH resulted in two fractions C_1 and C_2. Preparative HPLC of C_1 with the same gradient as above gave corynecin C (**106c**) as colourless oil and chloramphenicol (**105**). PTLC of fraction C_2 with CH_2Cl_2/5% MeOH delivered staurosporine (**107a**).

Chloramphenicol (**105**) **:** $C_{11}H_{12}Cl_2N_2O_5$, colourless solid. **–** R_f = 0.30 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ([D_4]MeOH, 300.0 MHz): δ = 8.17 (d, ^3J = 9.1 Hz, 2H, 3'-H, 5'-H), 7.63 (dd, ^3J = 9.0 Hz, ^4J = 0.8 Hz, 2H, 2'-H, 6'-H), 6.23 (s, 1H, $CHCl_2$), 5.15 (d, ^3J = 2.6 Hz, 1H, 1-H), 4.13 (m, 1H, 2-H), 3.80 (dd, ^2J = 10.6 Hz, ^3J = 7.2 Hz, 1H, 3-H_a), 3.60 (dd, ^2J = 12.0 Hz, ^3J = 6.0 Hz, 1H, 3-H_b). **– (+)-ESI MS:** *m/z* (%) = 667 ($[2M+Na]^+$, 80), 345.5 ($[M+Na]^+$, 30). **– (-)-ESI MS:** *m/z* (%) = 321.4 ($[M-H]^-$).

Corynecin C (**106c**)**:** $C_{13}H_{18}N_2O_5$, colourless oil. **–** R_f = 0.38 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ([D_4]MeOH, 300.0 MHz): δ = 8.17 (d, ^3J = 8.7 Hz, 2H, 3'-H, 5'-H), 7.62 (dd, ^3J = 8.7 Hz, ^3J = 0.8 Hz, 2H, 2'-H, 6'-H), 5.12 (d, ^3J = 2.6 Hz, 1H, 1-H), 4.15 (m, 1H, 2-H), 3.76 (dd, ^2J = 10.9 Hz, ^3J = 7.6 Hz, 1H, 3-H_a), 3.54 (dd, ^2J = 10.6 Hz, ^3J = 5.7 Hz, 1H, 3-H_b), 2.39 (sextet, ^3J = 6.8 Hz, 1H, $CH(CH_3)_2$), 0.98 (d, ^3J = 6.8 Hz, 3H, CH_3), 0.86 (d, ^3J = 6.8 Hz, 3H, CH_3). **– (+)-ESI MS:** *m/z* (%) = 587 ($[2M+Na]^+$, 100), 305.5 ($[M+Na]^+$, 30). **– (-)-ESI MS:** *m/z* (%) = 281.6 ($[M-H]^-$).

Staurosporine (**107a**)**:** $C_{28}H_{26}N_4O_3$, 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** ($CDCl_3$, 75.7 MHz): δ = 173.8 (C_q, C-5), 139.7 (C_q, C-11a), 136.6 (C_q, C-13a), 132.2 (C_q, C-4c), 130.7 (C_q, C-12a), 127.1 (C_q, C-12b), 126.5 (CH, C-4), 125.0 (CH, C-2), 124.6 (C_q, C-7c), 124.1 (CH, C-10), 123.4 (C_q, C-4a), 120.6 (CH, C-8), 119.9 (CH, C-9), 119.7 (CH, C-3), 118.4 (C_q, C-7a), 115.3 (C_q, C-4b), 115.2 (CH, C-11), 114.0 (C_q, C-7b), 106.9 (CH, C-1), 91.1 (CH, C-2'), 84.1 (CH, C-3'), 80.1 (CH, C-6'), 57.2 (CH_3, OMe), 50.3 (CH, C-4'), 46.0 (CH_2, C-7), 33.3 (CH_3, NMe), 30.2 (CH, H-5'), 30.0 (CH_3, 2'-Me). **– (+)-ESI MS:** *m/z* (%) = 933.3 ($[2M+H]^+$, 100), 467.5 ($[M+H]^+$, 80).

10.15 Marine *Streptomyces* sp. QD518

The strain QD491 was obtained from the Institute of Oceanology in Qingdao, P.R. China and cultivated in a 1 L scale on a rotatory shaker (95 rpm) using meat extract medium at 28 °C for 7 days. The obtained culture broth was extracted with ethyl acetate and the crude extract used for biological and chemical screening. It exhibited activity against *Escherichia coli*, *Streptomyces viridochromogenes*, and *Mucor miehei*. In the chemical screening, the extract showed fluorescent (366 nm) and UV absorbing (254 nm) bands, which turned light green on spraying with anisaldehyde/sulphuric acid, while others turned red.

10.15.1 Fermentation and Isolation:

The marine *Streptomyces* sp. QD518 was cultivated in a 25 L scale on meat extract medium at 28 °C for 7 days on a linear shaker (110 rpm). The culture broth was mixed with ca. 1.5 kg Celite and filtered under pressure. The water phase was extracted with a XAD-16 column (4× 140 cm), the resin washed with distilled water and eluted with methanol, while the mycelium was extracted firstly with ethyl acetate and then acetone. Both crude extract were combined, evaporated to dryness and separated by chromatography on silica gel using a CH_2Cl_2/MeOH gradient to afford four fractions A-D. Further purification of fractions A on Sephadex LH-20

(CH_2Cl_2/50% MeOH) gave only fatty acids. Separation of fraction B on Sephadex LH-20 (CH_2Cl_2/50% MeOH) delivered three sub-fractions B_1, B_2, B_3, which were further purified by PTLC (CH_2Cl_2/3% MeOH). Fraction B_1 delivered the sesquiterpene selina-4(14),7(11)-dien-8,9-diol (**108a,** 4 mg**)** and 5,7-dihydroxy-5,6,7,8-tetrahydro-1*H*-azocin-2-one (**112**, 5 mg), which shows rapid decomposition in NMR tube. Fraction B_2 and B_3 gave 7 known compounds (acetyl-β-carbolin, 2.5 mg, R_f = 0.63 (CH_2Cl_2/7% MeOH), celastramycin B (1.5 mg), vanillic acid (3.5 mg), anthranilic acid, 2 mg, R_f = 0.29 (CH_2Cl_2/7% MeOH), *m*-hydroxybenzyl alcohol, indole-ethanol, 3-indoleacrylamide). Chromatography of fraction C using Sephadex LH-20 (MeOH), PTLC (CH_2Cl_2/5% MeOH) and finally preparative HPLC (H_2O/MeCN gradient) delivered 5 known compounds namely 4-chloro-5-(3´-indolyl)-oxazole (28 mg, R_f = 0.63 (CH_2Cl_2/7% MeOH), 5-(3´-indolyl)-oxazole (2 mg, R_f = 0.43 (CH_2Cl_2/7% MeOH)), 3-(hydroxyacetyl)-indole (5 mg, R_f = 0.34 (CH_2Cl_2/7% MeOH)), indole-3-acetonitril (2 mg, R_f = 0.43 (CH_2Cl_2/7% MeOH)), indole-3-carboxylic acid (R_f = 0.23 (CH_2Cl_2/7% MeOH)), Trituration of fraction D with methanol delivered polyhydroxy butyric acid (PHB) as insoluble material. The soluble part yielded on Sephadex LH-20 (CH_2Cl_2/50% MeOH) the fractions D_1, D_2, D_3. PTLC followed by preparative HPLC of fraction D_1 gave chartreusin (4 mg). Purification of fraction D_2 on Sephadex LH-20 (MeOH) delivered sub-fractions D_{21} and D_{22}. Sub-fraction D_{22} yielded fine needle crystals of stauroporine (**107a,** 10 mg, R_f = 0.37 (CH_2Cl_2/7% MeOH)) in MeCN. The mother liquor was separated by HPLC (MeCN/H_2O 20:80, within 40 min to 100% MeCN, 10 ml/min) and delivered N-Carboxamido-staurosporine (**107b,** R_t = 16.23 min, 2 mg, R_f = 0.31 (CH_2Cl_2/7% MeOH)) and N-carboxamido-staurosporine (**107c,** R_t = 17.44 min, 4 mg).

N-carboxamido-staurosporine (107c): $C_{29}H_{27}N_5O_4$, light yellow solid. – $\boldsymbol{R_f}$ = 0.46 (CH_2Cl_2/7% MeOH). – The UV spectrum is typical for staurosporine derivative and presents many maxima, **UV/VIS (MeOH):** λ_{max} (lg ε) = 372 (3.30), 354 (3.25), 333 (3.43), 318 (3.43), 292 (4.01), 243 (3.67) nm. – **IR (KBr):** ν = 2855, 2929, 2359, 2344, 1633, 1458, 1385, 1316, 1282, 1120, 1018 cm^{-1}. – **^{1}H** and **^{13}C NMR** data see table 3. – **(+)-ESI MS:** *m/z* (%) = 532 ($[M+Na]^+$, 100), 1041 ($[M+2Na]^+$, 90).– (+)-**ESI HRMS:** *m/z* = 510.21382 ($[M+H]^+$, 510.21359 calcd. for $C_{29}H_{28}N_5O_4$).

Selina-4(14),7(11)-dien-8,9-diol (108a): $C_{15}H_{24}O_2$, colourless oil. – R_f = 0.31 (CH_2Cl_2/3% MeOH). – **UV (MeOH):** λ_{max} (lg ε) = 253 (3.51), 204 (4.78) nm. – **IR (KBr):** ν = 3650, 2928, 2361, 2343, 1636, 1437, 1385, 1107 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 4.81 (q, ^4J = 1.7 Hz, 1H, 14-H_A), 4.79 (d, ^3J = 4.0 Hz, 1H, 8-H), 4.60 (q, ^4J = 1.6 Hz, 1H, 14-H_B), 3.24 (d, ^3J = 4.2 Hz, 1H, 9-H), 2.43, 2.14 (ABX, $J_{AB} = J_{trans}$ = 14.3, J_{cis} = 2.9 Hz, 2H, 6-H_2), 2.32 (dm, 2H, 3-H_2), 1.95 (m, 1H, 1-H_A), 1.79 (d, ^5J = 2.1 Hz, 3H, 12-H_3), 1.73 (d, ^3J = 2.1 Hz, 3H, 13-H_3), 1.70-1.50 (m, 3H, 2-H_2, 5-H), 1.13 (ddd, 2 × J = 12.7, 5.4 Hz, 1H, 1-H_B), 0.95 (s, 3H, 15-H_3). – **^{13}C NMR** ($CDCl_3$, 150.7 MHz): δ = 149.6 (Cq, C-4), 130.0 (C_q, C-7*), 129.9 (C_q, C-11*), 106.4 (CH_2 C-14), 80.2 (CH, C-9), 70.3 (CH, C-8), 48.0 (CH, C-5), 40.8 (C_q, C-10), 38.2 (CH_2, C-1), 36.5 (CH_2 C-3), 23.7 (CH_2, C-6), 22.6 (CH_2, C-2), 20.7 (CH_3, C-12), 20.0 (CH_3, C-13), 12.0 (CH_3, C-15). – **EI MS (70 eV)**: *m/z* (%) = 236.2 ($[M]^+$, 100), 221.2 (18), 203.2 (24), 189.2 (35), 175.2 (35), 147.1 (25), 133.1 (30), 124.1 (48), 109.1 (58), 95.1 (40), 81.1 (32), 69.1 (25), 55.1 (26), 41.0 (37). – **EI HRMS:** *m/z* = 236.17760 (236.17761 calcd. for $C_{15}H_{24}O_2$).

5,7-Dihydroxy-5,6,7,8-tetrahydro-1*H*-azocin-2-one (112): $C_7H_{11}NO_3$, colourless oil, R_f = 0.34 (CH_2Cl_2/3% MeOH). – **UV (MeOH)** λ_{max} (log ε) = 233 (sh), 206 (3.80) nm. – **IR (KBr)** ν = 3650, 2926, 2855, 2361, 2343, 1691 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz) δ = 7.26 (dd, ^3J = 5.8 Hz, ^4J = 1.8 Hz, 1H, 4-H), 6.07 (dd, ^3J = 5.8 Hz, ^4J = 1.6 Hz, 1H, 3-H), 4.83 (br t, ^3J = 5.7 Hz, 1H, 7-H), 4.77 (ddd, J = 6.2, 5.6, 0.6 Hz, 1H, 5-H), 4.05 (dd, ^2J = 13.2 Hz, ^3J = 6.0 Hz, 1H, 8-H_A), 3.51 (d, ^3J = 13.2 Hz, 1H, 8-H_B), 2.39 (dd, ^2J = 13.3 Hz, ^3J = 5.6 Hz, 1H, 6-H_A), 1.64 (ddd, ^3J = 13.3 Hz, ^3J = 10.8 Hz, ^3J = 5.4 Hz, 1H, 6-H_B). – **^{13}C/APT NMR** ($CDCl_3$, 150.8 MHz): δ = 175.6 (C_q, C-2), 149.2 (CH, C-4), 128.6 (CH, C-3), 65.6 (CH, C-5), 62.8 (CH, C-7), 53.0 (CH_2, C-8), 40.1 (CH_2, C-6). – **EI MS (70 eV):** *m/z* (%) = 157.0 ($[M]^+$, 86), 141.0 (16), 129.0 (20), 122.1 (34), 99.0 (26), 95.0 (100), 67.0 (78), 41.0 (48).

1,2-Dihydro-pyrrolizin-3-one (114): C_7H_7NO, colourless oil. – R_f = 0.83 ($CHCl_3$/3% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.02 (d, ^3J = 2.9 Hz, 1H, 5-H), 6.43 (t, ^3J = 3.1 Hz, 1H, 6-H), 5.94 (br d, ^3J = 2.8 Hz, 1H, 7-H), 3.00 (s, 4H, 1,2-H_2). – **^{13}C/APT NMR** ($CDCl_3$, 150.8 MHz): δ = 175.1 (C_q, C-3), 139.6 (C_q, C-

8), 119.0 (CH, C-6), 104.5 (CH, C-7), 110.9 (CH, C-5), 34.8 (CH_2, C-2), 19.3 (CH_2, C-1). **– EI MS (70 eV):** *m/z* (%) = 121.0 ($[M]^+$, 22), 111.0 (6), 93 (12), 83 (12), 69 (16), 57 (44), 41 (28).

Trimer (115): $C_{21}H_{21}N_3O_3$, colourless oil. **–** R_f = 0.31 (CH_2Cl_2/3% MeOH). **– UV/VIS (MeOH)** λ_{max} (log ε) 269 (3.70), 230 (4.14), 209 (4.20) nm. **– IR (KBr)** ν = 2912, 1659, 1642, 1502 cm^{-1}. **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz) δ = 6.26 (d, ^3J = 3.1 Hz, 1H, 7"-H), 6.14 (dd, ^3J = 4.1 Hz, ^4J = 0.9 Hz, 1H, 7'-H), 5.84 (dd, ^3J = 3.1 Hz, ^4J = 1.2 Hz, 1H, 6"-H), 5.78 (dd, ^3J = 3.1 Hz, ^4J = 1.5 Hz, 1H, 6'-H), 3.95 (m, 1H, 7-H), 3.63 (m, 2H, 8-H_2), 3.01 (br s, 4H, 3",4"-H_2), 2.93 (br s, 4H, 3',4'-H_2), 2.78 (dd, J = 12.4, 6.58 Hz, 1H, 6_a-H), 2.70 (m, 1H, 3_a-H), 2.5-2.3 (m, 3H, 3_b-, 4-H_2), 2.10 (dd, ^3J = 11.6 Hz, ^4J = 0.6 Hz, 1H, 6_b-H). **– ^{13}C/APT NMR** ($CDCl_3$, 150.8 MHz): δ = 175.4 (C_q, C-2), 172.6 (C_q, C-2'), 172.4 (C_q, C-2"), 141.2 (C_q, C-5"), 139.9 (C_q, C-5'), 130.3 (C_q, C-8"), 128.5 (C_q, C-8'), 116.8 (CH, C-7"), 115.3 (CH, C-7'), 103.6 (CH, C-6'), 103.5 (CH, C-6"), 46.8 (CH_2, C-8), 44.6 (CH_2, C-6), 37.2 (CH, C-7), 35.1 (CH_2, C-3'), 35.0 (CH_2, C-3"), 34.8 (CH_2, C-4), 33.5 (CH_2, C-3), 19.2 (CH_2, C-4"), 19.0 (CH_2, C-4'). **– (+)-ESI MS:** *m/z* (%) = 749.1 ($[2M+Na]^+$, 100), 386.4 ($[M+Na]^+$, 14), 364.3 ($[M+H]^+$, 11). **– EI MS (70 eV):** *m/z* (%) = 363.2 ($[M]^+$, 14), 242.1 (24), 216.1 (61), 203.1 (57), 187.1 (36), 160.0 (25), 147.1 (20), 118.0 (22), 83.9 (30), 70.1 (40), 57.0 (73), 43.0 (100). **– (+)-ESI HRMS:** *m/z* = 386.14763 ($[M+Na]^+$, calcd. 386.14752 for $C_{21}H_{21}N_3O_3Na$).

10.16 Marine *Streptomyces* sp. QD491

The marine *Streptomyces* sp. QD491 was collected at Qingdao coast and was obtained from the group of Prof. Qin (Institute of Oceanology, Qingdao, P.R. China). The crude extract of QD491 obtained from the fermentation of a 1 L culture in SM medium for 6 days at 28 °C exhibited activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and a weak inhibition of *Streptomyces viridochromogenes* (Tü 57) in the biological screening. The chemical screening indicated non-polar zones, which showed no significant UV absorption, but turned violet to blue with anisaldehyde/sulphuric acid.

10.16.1 Fermentation and Isolation

The marine *Streptomyces* sp. QD491 was cultivated in 100 of 1 L Erlenmeyer flasks each containing 250 mL of SM medium at 28 °C, while shaking for 5 days on a linear shaker. The culture broth was mixed with ca 1.5 kg Celite and filtered under pressure. The water phase was chromatographed in XAD-16, the resin washed with distilled water and eluted with methanol, while the mycelium was extracted firstly with ethyl acetate and then acetone. The extracts were combined and separated on silica gel (column 53.5 × 3 cm, CH_2Cl_2/MeOH gradient) yielding four fractions A-D. Fraction A contained fats. Purification of fraction B on Sephadex LH-20 (MeOH) gave 3 sub-fractions B_1-B_3. From B1 and B2, *o*-hydroxyacetanilide, indole-3-carbaldehyde, and 10β-hydroxyamorph-4-en-3-one (**119b**) were obtained after PTLC and HPLC; B_3 contained genistein. Fraction C was purified by chromatography on Sephadex LH-20 (CH_2Cl_2/50% MeOH) and PTLC (CH_2Cl_2/5% MeOH) and delivered prunetin and 10β,11-dihydroxyamorph-4-en (**121**). Fraction D was separated on Sephadex LH-20 (CH_2Cl_2/40% MeOH) into sub-fractions D_1 and D_2. Purification of D_1 by PTLC delivered chalcomycin A. Sub-fraction D_2 was separated on Sephadex LH-20 (MeOH), by PTLC (CH_2Cl_2/7% MeOH) followed by HPLC (20% MeCN/H_2O) to yield 10β,14-dihydroxyamorph-4-en-3-one (**123**) and 5α,10β,11-triydroxyamorphan-3-one (**124**).

10β-Hydroxyamorph-4-en-3-one (**119b**): $C_{15}H_{24}O_2$, colourless solid. – R_f = 0.53 (CH_2Cl_2/5% MeOH). – **IR (KBr):** ν = 3438, 2960, 2930, 2369, 1700 1458, 1387 1257 1194, 1119, 1092, 1045, 908 cm^{-1}. – $[\alpha]^{25}_D$ = -96.0 (*c*, 0.15, $CHCl_3$). – **^{1}H NMR** see table 5, **^{13}C NMR** see table 4. – **EI MS (70 eV):** *m/z* (%) = 236 (12), 218 (14), 193 (29), 175 (40), 165 (12), 147 (14), 135 (17), 121 (10), 109 (69), 95 (16), 91 (25), 79 (34), 69 (42), 55 (45), 43 (100), 41 (94). – **(+)-ESI HRMS** *m/z* = 237.18488 ($[M+H]^+$, calcd. 237.18492 for $C_{15}H_{25}O_2$).

10β,11-Dihydroxyamorph-4-en (**121**)**:** $C_{15}H_{26}O_2$, colourless solid. – R_f = 0.46 (CH_2Cl_2/5% MeOH). – **IR (KBr):** ν = 3406, 2967, 2932, 2361, 2338, 1457, 1376, 1299, 1163, 1021 932, 906 cm^{-1}. – $[\alpha]^{25}_D$ = – 26.4 (*c* 0.14, $CDCl_3$). – **^{1}H NMR** see table 5, **^{13}C NMR** see table 4. – **(+)-ESI MS:** *m/z* (%) = 499 ($[2M+Na]^+$, 28), 261

([M+Na]$^+$, 100). **– DCI MS (NH_3):** *m/z* (%) = 256 ([M+NH_4]$^+$ 43), 238 ([M-H_2O+NH_4]$^+$, 100), 221 ([M-H_2O+H]$^+$, 20), 163 (74). **– (+)-ESI HRMS** *m/z* = 261.18252 ([M+Na]$^+$, calcd 261.18251 for $C_{15}H_{26}O_2Na$).

10β,14-Dihydroxyamorph-4-en-3-one (123): $C_{15}H_{24}O_3$, colourless solid. **–** R_f = 0.39 (CH_2Cl_2/5% MeOH). **– UV/VIS (MeOH)** λ_{max} (log ε) = 237 (3.79). **–** $[\alpha]^{25}_D$ = **–** 70.0 (*c*, 0.07 $CDCl_3$). **– ^{1}H NMR** see table 5, **^{13}C NMR** see table 4. **– EI MS (70 eV):** *m/z* (%) = 252 (3), 209 (4), 191 (25), 176 (10), 173 (8), 163 (4), 134 (3), 121 (5), 108 (20), 79 (9), 77 (10), 71 (14), 69 (24), 55 (16), 43 (100). **– (+)-ESI HRMS:** *m/z* = 253.17988 ([M+H]$^+$, calcd. 253.17983 for $C_{15}H_{25}O_3$).

5α,10β,11-Triydroxyamorphan-3-one (124): $C_{15}H_{24}O_3$, colourless oil. **–** R_f = 0.43 (CH_2Cl_2/5% MeOH). **– IR (KBr):** ν = 3438, 2960, 2930, 2369, 1700, 1458, 1385, 1194, 1119, 1045, 908 cm^{-1}. **–** $[\alpha]^{25}_D$ = **–** 13.1 (*c* 0.13, $CDCl_3$). **– ^{1}H NMR** see table 5, **^{13}C NMR** see table 4. **– EI MS (70 eV):** *m/z* (%) = 252 ([M-H_2O]$^+$, 86), 237 (19), 219 (21), 194 (13), 177 (25), 163 (100), 154 (36), 134 (32), 121 (24), 107 (21), 93 (31), 81 (23), 57 (21), 43 (94), 41 (38). **– DCI MS (NH_3):** *m/z* (%) = 522 ([2M-2H_2O+NH_4]$^+$, 1), 287 (2), 270.2 ([M-H_2O+NH_4]$^+$, 100). **– (+)-ESI HRMS:** *m/z* = 275.16188 ([M+Na-H_2O]$^+$, calcd. 275.16177 for $C_{15}H_{24}O_3Na$).

10.17 Marine-derived *Streptomyces* sp. Mei 35

The marine derived *Streptomyces* sp. isolate Mei35 obtained from the collection of Prof. Meiners (Emden) was pre-cultivated on M_2 100% seawater + $CaCO_3$ agar plates at 28 °C for 3 days and shows white mycelium, they were used to inoculate a 2 L shaker culture on rotary shaker (95 rpm) at 28 °C for 7 days. The greenish culture broth was extracted with ethyl acetate and yielded a yellow oily crude extract.

10.17.1 Primary screening

The antimicrobial test on agar diffusion test shows the results as consigned in the Table 25. The chemical screening shows on TLC a spot, with yellow fluorescence under UV light at 366 nm and many other spots, which were only visible after spraying with anisaldehyde/ sulphuric acid as violet, then blue on staying.

Table 25: Biological activity of the crude extract of the strain Mei 35 (50 mg/mL)

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	12
Chlorella sorokiniana	25
Escherichia coli	11
Chlorella vulgaris	25
Scenedemus subspicatus	22
Candida albicans	11

10.17.2 Fermentation, Extraction and Isolation

With pieces of well grown agar subculture of the strain, 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2 100% seawater + $CaCO_3$ medium were inoculated and cultivated at 28 °C with 95 rpm for 120 hours. The well-grown culture was mixed with ca. 1.5 kg Celite and filtered by pressure filtration. The mycelium and filtrate were separately extracted with ethyl acetate; the organic phases showed a similar composition and were combined and evaporated *i. vac*. to dryness. The oily residue (1.7 g) was chromatographed on silica gel (column 3 × 60 cm, 200 g) using a step-wise CH_2Cl_2/0-10% MeOH gradient to yield fractions A (270 mg), B (125 mg), C (70 mg) and D (80 mg). Purification of fraction B on silica gel with CH_2Cl_2/1% MeOH led to the isolation of the known butenolide **128a** (5 mg) and the antimycin A-complex (**127**) (30 mg). Size exclusion chromatography of fraction C on Sephadex LH-20 (column 3 × 70 cm, CH_2Cl_2/40% MeOH) yielded **129** (4 mg). Purification of fraction D on silica gel column (CH_2Cl_2) followed by preparative HPLC afforded **130** (2 mg) and **131** (2 mg).

Table 26: Biological activity of 7-methylamino-3,4-dimethylisoquinolin-5,8-dione (**131**)

	BS	EC	SA	SV	CA	MM	CV	CS	SS
131	10	10	12	0	0	0	22	17	25

BS = *Bacillus subtilis*, EC = *Escherichia coli*, SA = *Staphylococcus aureus*, SV = *Streptomyces viridochromogenes*, MM = *Mucor miehei*, CV = *Chlorella vulgaris*, CS = *Chlorella sorokiniana*, SS = *Scenedemus subspicatus*, CA = *Candida albicans*

Antimycin A (127) : $C_{27}H_{38}N_2O_9$, oil. **– R_f** = 0.36 (CH_2Cl_2/2% MeOH). **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz,): δ = 12.66 (s, 1H, 2'-OH), 8.56 (d, ^3J = 8.2 Hz, 1H, 4'-H), 8.52 (d, ^3J = 1.2 Hz, 1H, HCO), 8.10 (br s, 1H, 3'-NH), 7.24 (d, ^3J = 8.3 Hz, 1H, 6'-H), 7.17 (d, ^3J = 7.6 Hz, 1H, 3-NH), 6.90 (t, ^3J = 8.2 Hz, 1H, 5'-H), 5.78 (quintet, ^3J = 7.2 Hz, 1H, 4-H), 5.38 (t, ^3J = 7.6 Hz, 1H, 3-H), 5.10 (m, 1H, 8-H), 4.99 (m, 1H, 9-H), 2.50 (m, 1H, 7-H), 2.42 (m, 1H, 2''-H), 1.80-0.80 (m, CH_2 and CH_3 for the residue R). **– (+)-ESI MS:** *m/z* (%) = 571 ($[M+Na]^+$, 60), 557 ($[M+Na]^+$, 100), 543 ($[M+Na]^+$, 56), 529 ($[M+H]^+$, 10). **– (-)-ESI MS:** *m/z* (%) = 547 ($[M-H]^-$, 70), 533 ($[M-H]^-$, 100), 519 ($[M-H]^-$, 44), 505 ($[M-H]^-$, 8).

(4S)-4-Hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (128a): $C_{13}H_{20}O_3$, oil. **– R_f** = 0.37 (CH_2Cl_2/5% MeOH), violet with anisaldehyde/sulphuric acid. **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.44 (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), 5.04 (m, 1H, 4-H), 2.50 (m, 1H, 10-H), 2.12 (s, 3H, 12-CH_3), 1.70 (m, 1H), 1.60 (m, 2H), 1.50-1.20 (m, 10H), 1.10 (d, ^3J = 7.0 Hz, 3H, 13-CH_3). **– EI MS (70 eV):** *m/z* (%) = 224 ($[M]^+$, 12), 182 (12), 153 (52), 135 (16), 122 (20), 97 (36), 72 (36), 43 (100).

(4S)-4,10,11-Trihydroxy-10-methyldodec-2-en-1,4-olide (129): $C_{13}H_{22}O_4$, colourless solid, blue coloration with anisaldehyde/sulphuric acid. **– R_f** = 0.29 (CH_2Cl_2/5% MeOH). **– CD $[\theta]_{210}$ MeOH:** +1753 (*C* 37 µg/mL, MeOH). **– IR (KBr):** ν = 3435, 2928, 2856,1749, 1717, 1699, 1683, 1668, 1662, 1653, 1635, 1615, 1576, 1567, 1558, 1540, 1521, 1506, 1456, 1384, 1173, 1105, 819 cm^{-1}. **– ^{1}H NMR** (300.0 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, ^4J =

1.9 Hz, 1H, 2-H), 5.02 (m, 1H, 4-H), 3.64 (q, ^3J = 6.4 Hz, 1H, 11-H), 1.90-1.30 (m, 10H, 5-H_2, 6- H_2, 7-H_2, 8-H_2, 9-H_2), 1.14 (d, ^3J = 6.5 Hz, 3H, 12-H_3), 1.09 (s, 3H, 10-CH_3). **– ^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 173.3 (C_q, C-1), 156.6 (C_q, C-3), 121.2 (CH, C-2), 83.4 (CH, C-4), 77.4 (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* (%) = 507.0 ($[2M+Na]^+$, 24), 265.3 ($[M+Na]^+$, 100). **– ESI HRMS:** *m/z* = 265.14096 (calcd. 265.14104 for $C_{13}H_{22}O_4Na$).

(4S)-4,10-Dihydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (130): $C_{13}H_{20}O_4$, colourless solid, blue colouration with anisaldehyde/sulphuric acid. **– R_f** = 0.33 (CH_2Cl_2/5% MeOH). **– IR (KBr):** ν = 3458, 2925, 2853, 2344, 2362, 1749, 1716, 1654, , 1598, 1560, 1466, 1419, 1385, 1169, 1112, 820, 720 cm^{-1}. **– CD $[\theta]_{210}$** (35 µg/ml, MeOH): + 2400. **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.43 (dd, ^3J = 5.6 Hz, ^4J = 1.5 Hz, 1H, 3-H), 6.11 (dd, ^3J = 5.6 Hz, ^4J = 1.9 Hz, 1H, 2-H), 5.03 (m, 1H, 4-H), 3.84 (s, 1H, 10-OH), 2.20 (s, 3H, 12-H_3), 1.78-1.30 (m, 10H, 5-H_2, 6-H_2, 7-H_2, 8-H_2, 9-H_2), 1.36 (s, 3H, 10-CH_3). **– ^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 212.2 (C_q, C-11), 173.1 (C_q, C-1), 156.1 (CH, C-3), 121.6 (CH, C-2), 83.2 (CH, C-4), 78.7 (C_q, C-10), 39.2 (CH_2, C-9), 33.0 (CH_2, C-5), 29.3 (CH_2, C-7), 25.5 (CH_2, C-6), 24.8 (CH_2, C-8), 23.1 (CH_3, 10-CH_3), 23.6 (CH_3, C-12).**– (+) ESI MS:** *m/z* (%) = 503.2 ($[2M+Na]^+$, 26), 263.4 ($[M+Na]^+$, 100). **– (+)-ESI HRMS:** *m/z* = 263.125247 (calcd. 263.12539 for $C_{13}H_{20}O_4Na$).

7-Methylamino-4,4-dimethylisoquinolin-5,8-dione (131): $C_{12}H_{12}N_2O_2$, red solid, yellow colouration with anisaldehyde/sulphuric acid. **– R_f** = 0.11 (C_6H_{12}/40% EtO-Ac). **– UV (MeOH):** λ_{max} (lg ε) = 441 (4.26), 380 (3.99), 338 (4.47), 312 (3.10), 273 (3.56), 258 (3.60), 232 (3.83), 227 (3.90). **– IR (KBr):** ν = 3420, 3270, 290, 2850, 1739, 1675, 1638, 1601, 1567, 1543, 1508, 1461, 1421, 1343, 1268, 1181, 1108, 850, 811, 704 cm^{-1}. **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 9.01 (s, 1H, 1-H), 5.82 (br s, 1H, NH), 5.72 (s, 1H, 6-H), 2.93 (d, ^3J = 5.2 Hz, 3H, NCH_3), 2.76 (s, 3H, 4-CH_3), 2.70 (s, 3H, 3-CH_3).**– ^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 185.1 (C_q, C-5), 181.9 (C_q, C-8), 167.4 (C_q, C-3), 147.7 (C_q, C-7), 145.2 (CH, C-1), 135.2 (C_q, C-4a), 131.9 (C_q, C-4), 123.4 (C_q, C-8a), 103.3 (CH, C-6), 29.0 (CH_3, NCH_3), 24.7 (CH_3, 3-CH_3),

16.0 (CH_3, 4-CH_3). **– (+) ESI MS:** *m/z* (%) = 455.3 ($[2M+Na]^+$, 56), 217.3 ($[M+H]^+$, 20). **– (-) ESI MS:** *m/z* (%) = 431.4 ($[2M-H]^-$, 58), 215 ($[M-H]^-$, 100). **– EI MS (70 eV):** *m/z* (%) = 216 ($[M]^+$, 100), 188 (8), 158 (8), 107 (8), 82 (24). **– EI HRMS** *m/z* = 216.0899 (calcd. 216.08987 for $C_{12}H_{12}N_2O_2$).

10.18 Marine-derived *Streptomyces* sp. Mei23

The marine strain Mei23 was obtained from the collection of Prof. Meiners. The strain was cultivated on agar plates for five days at 28 °C and exhibited a white aerial mycelium. The plate was used to inoculate a 2 L shaker culture. The greenish culture broth was extracted with ethyl acetate and the resulting crude extract used for different activity tests.

10.18.1 Pre-screening

The TLC of the crude extract showed no UV absorbing spots under 254 and 366 nm but a rich colour reaction on spraying with anisaldehyde/sulphuric acid from red, violet and yellow was seen. It shows moderate activity in the agar diffusion test.

Table 27: Biological activity of the crude extract from strain Mei23

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	12
Staphylococcus aureus	16
Candida albicans	16
Mucor miehei	13
Chlorella sorokiniana	16
Scenedesmus subspicatus	15

10.18.2 Fermentation and Isolation

The marine derived *Streptomyces* sp. isolate Mei23 obtained from the collection of Prof. Meiners was precultivated on M_2 100% sea water +$CaCO_3$ medium agar plates at 28 °C for 3 days. With pieces of well grown agar subculture of the strain, 15 L rotary shaker were cultivated at 28 °C with 110 rpm for 3 days. The well-grown culture broth was mixed with ca 1.5 kg Celite and filtered by pressure filtration. The

mycelium and filtrate were separately extracted with ethyl acetate and through XAD-16 respectively; the organic phases showed a similar composition and were combined and evaporated *i. vac*. to dryness. The oily residue (2.9 g) was chromatographed on silica gel using a stepwise CH_2Cl_2/MeOH gradient to yield fractions A-D. The column chromatography of fraction B followed by PTLC (CH_2Cl_2/5% MeOH) gave three compounds SFUS2, SFU1a and SFUM6, the purification of the fraction C on Sephadex-LH20 (MeOH) yielded diastereomers **128b**, *trans*-cyclo (Leucyl-Prolyl) and antimycins A-complex (**127**). Purification of fraction D on Sephadex-LH20 (CH_2Cl_2/50% MeOH) gave 4(S)-4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**128c**) and MKN-003B (**133**). All compounds were obtained as pale yellow oils.

4(S)-4,11-Dihydroxy-10-methyldodec-2-en-1,4-olides (128b): $C_{13}H_{22}O_3$, oil, inseparable mixture of diastereomers. – R_f = 0.55 (CH_2Cl_2/5% MeOH), violet with anisaldehyde /sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz,): δ = 7.45 (dd, ^3J = 5.6 Hz, ^4J = 1.5 Hz, 1H, 3-H), 6.12 (dd, ^3J = 5.6 Hz, ^4J = 2.0 Hz, 1H, 2-H), 5.05 (m, 1H, 4-H), 3.70 (m, 1H, 11-H), 1.80-1.20 (m, 11-H), 1.10 (2d, ^3J = 6.4 Hz, each 3 H, 12-CH_3), 0.85 (2d, ^3J = 6.4 Hz, each 3 H, 13-CH_3). – **(+)-ESI MS:** *m/z* (%) = 457.1 ($[2M+Na]^+$, 100), 249.2 ($[M+Na]^+$, 12).

4(S)-4,10-Dihydroxy-10-methyldodec-2-en-1,4-olide (128c): $C_{13}H_{22}O_3$, oil, violet with anisaldehyde/sulphuric acid. – R_f = 0.54 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.44 (dd, ^3J = 5.6 Hz, ^4J = 1.5 Hz, 1H, 3-H), 6.14 (dd, ^3J = 5.6 Hz, ^4J = 1.8 Hz, 1H, 2-H), 5.05 (m, 1H, 4-H), 1.70 (m, 2H, 5-H_2), 1.50-1.20 (m), 1.17 (s, 3H, 13-CH_3), 0.88 (t, ^3J = 7.4 Hz, 3 H, 13-CH_3).

MKN-003B (133): $C_{12}H_{20}O_3$, oil, violet with anisaldehyde/sulphuric acid. – R_f = 0.50 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.45 (dd, ^3J = 5.7 Hz, ^4J = 1.5 Hz, 1H, 3-H), 6.15 (dd, ^3J = 5.7 Hz, ^4J = 2.0 Hz, 1H, 2-H), 5.04 (dddd, ^3J = 7.3, 5.9, 2.0 Hz, ^4J = 1.5 Hz, 1H, 4-H), 1.80-1.60 (m, 2H, 5-H_2), 1.50-1.30 (m, 8H, 6-H_2, 7-H_2, 8-H_2, 9-H_2), 1.21 (s, 6H, 11-H, 12-H). – **(+)-ESI MS:** *m/z* (%) = 447.1 ($[2M+Na]^+$, 52), 235.3 ($[M+Na]^+$, 35).

10.18.3 Marine-derived *Streptomyces* sp. Mei 22

The marine-derived *Streptomyces* sp. Mei 22 showed a white aerial mycelium after 3 days. The 1 L shaker culture kept at 28 °C and 95 rpm for 7 days produced a slightly black culture broth, which was extracted with ethyl acetate and yielded a yellow oily crude extract.

10.18.4 Primary screening

The oily crude extract obtained was used to perform antibacterial activity as well as chemical screening. The chemical screening reveals the presence of some dark grey spots, which were only visible after spraying with anisaldehyde/sulphuric acid.

Table 28: Biological activity of the crude extract from strain Mei 22 (50 mg/mL)

Test Organism	Inhibition Zone (∅ mm)
Mucor miehei	30
Bacillus subtilis	12
Escherichia coli	11
Staphylococcus aureus	20

10.18.5 Fermentation and Isolation of metabolites

The 25 L of strain Mei22 were fermented at 28 °C for 7 days using M_2 100% sea-water + $CaCO_3$ medium on a linear shaker. The obtained brown culture broth was mixed with ca. 1.5 kg Celite and filtrated under pressure. The water phase was extracted with ethyl acetate, while the mycelium was extracted with ethyl acetate, then with 5 L acetone. Both extracts were combined in the view of their TLC and resulted in 2.5 g oily yellowish crude extract.

Chromatography of the crude extract on silica gel using a CH_2Cl_2/MeOH gradient (each 1 L 0 - 20% MeOH), five fractions were obtained. TLC of fraction I indicated only the presence of some fatty acids. TLC of fraction II revealed two not UV absorbing compounds overlapping each other, which became black on spaying with anisaldehyde/sulphuric acid. Evaporation and precipitation with 10 ml of MeCN (or methanol) and filtration gave deboroaplasmomycin C (**135,** 15 mg) as a white powder. Crystallization with CH_2Cl_2/C_6H_{12} gave transparent needles. PTLC of fraction

IV with CH_2Cl_2/10% MeOH) followed by chromatography on Sephadex LH-20 (CH_2Cl_2 /50% MeOH) delivered bafilomycin A_1 (**134a**, 3 mg) as colourless solid, and bafilomycin B_1 (**134b,** 5 mg) and B_2 (**134c**, 4 mg) as yellow solids. All these compounds became reddish to black on spraying with anisaldehyde/sulphuric acid.

Bafilomycin A_1 (134a): $C_{35}H_{58}O_9$, colourless powder. – R_f = 0.57 (CH_2Cl_2/5 % MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 6.70 (br s, 1H, 3-H), 6.50 (dd, ^3J = 15.0, 9.8 Hz, 1H, 12-H), 5.80 (t, ^3J = 9.2 Hz, 2H, 5-H, 11-H), 5.55 (br s, 1H, OH), 5.18 (dd, ^3J = 15.0, 9.2 Hz, 1H, 13-H), 4.96 (dd, ^3J = 8.5, 1.2 Hz, 1H, 15-H), 4.10 (m, 1H, 17-H), 3.90 (m, 1H, 14-H), 3.69 (m, 1H, 21-H), 3.63 (s, 3H, 2-OCH_3), 3.50 (dd, ^3J = 10.0, 2.2 Hz, 1H, 23-H), 3.30 (br d, ^3J = 5.3 Hz, 1H, 7-H), 3.22 (s, 3H, 14-OCH_3), 2.60 (m, 1H, 6-H), 2.30 (m, 1H, 20-H_a), 2.15- 2.00 (m, 2H, 9-H_a, 16-H), 1.98 (s, 3H, 26-H_3), 1.94 (s, 3H, 29-CH_3), 1.93-1.60 (m, 4H, 8-H, 9-H_b, 18-H, 24-H), 1.20 (m, 1H, 22-H), 1.16 (m, 1H, 20-H_b), 1.06 (d, ^3J = 7.0 Hz, 3H, 27-H_3), 1.04 (d, ^3J = 7.3 Hz, 3H, 31-H_3), 0.96 (d, ^3J = 6.3 Hz, 3H, 32-H_3), 0.92 (d, ^3J = 6.5 Hz, 3H, 28-H_3), 0.90 (d, ^3J = 6.7 Hz, 3H, 25-H_3), 0.80 (d, ^3J = 6.7 Hz, 3H, 30-H_3), 0.78 (d, ^3J = 6.7 Hz, 3H, 33-H_3). – **EI MS (70 eV):** *m/z* (%) = 584 (12), 568 (24), 525 (20), 399 (7), 368 (16), 338 (32), 209 (27), 181 (26), 169 (32), 137 (40), 109 (100). – **(+)-ESI MS:** *m/z* (%) = 1267 ($[2M+Na]^+$, 72), 645 ($[M+Na]^+$, 48).

Bafilomycin B_1 (134b): $C_{44}H_{65}NO_{13}$, yellow solid. – R_f = 0.34 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 13.32 (s, 1H, 10'-OH), 8.66 (s, 1H, NH), 7.20 (d, ^3J = 15.6 Hz, 1H, 2'-H), 6.90 (d, ^3J = 15.6 Hz, 1H, 3'-H), 6.70 (s, 1H, 3-H), 6.50 (dd, ^3J = 14.8, 9.6 Hz, 1H, 12-H), 5.82 (d, ^3J = 9.3 Hz, 1H, 5-H), 5.78 (d, ^3J = 9.2 Hz, 1H, 11-H), 5.58 (br s, 1H, 19-OH), 5.18 (dd, ^3J = 15.0, 9.2 Hz, 1H, 13-H), 5.05 (td, ^3J =10.8, 4.9 Hz, 1H, 21-H), 4.98 (br d, ^3J = 8.5, Hz, 1H, 15-H), 4.64 (br s, 1H, 17-OH), 4.15 (br d, ^3J = 8.5 Hz, 1H, 17-H), 3.88 (t, ^3J = 9.3 Hz, 1H, 14-H), 3.64 (s, 3H, 2-OCH_3), 3.60 (m, 1H, 23-H), 3.30 (br d, ^3J = 4.3 Hz, 1H, 7-H), 3.22 (s, 3H, 14-OCH_3), 2.70-2.50 (m, 6H), 2.40 (dd, ^3J = 11.7, 4.9 Hz, 1H, 20-H_a), 2.18- 2.10 (m, 2H, 9-H_a, 16-H), 1.99 (s, 3H, 26-CH_3), 1.94 (s, 3H, 29-CH_3), 1.94-1.56 (m, 4H, 8-H, 9-H_b, 18-H, 24-H), 1.20-1.10 (m, 2H, 20-H_b, 22-H), 1.04 (d, ^3J = 6.9 Hz, 3H, 27-H_3), 1.05 (d, ^3J = 6.8 Hz, 3H, 31-H_3), 0.94 (d, ^3J = 6.3 Hz, 3H, 32-H_3), 0.90 (d, ^3J = 6.5 Hz, 3H, 28-H_3), 0.80 (2d, ^3J = 6.7 Hz, 6H, 25-H_3, 30-H_3), 0.78 (d, ^3J = 6.8 Hz, 3H,

33-CH_3). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 167.3 (C_q, C-1), 164.3 (C_q, C-4'), 164.2 (C_q, C-1'), 150.7 (C_q, C-5'), 142.8 (CH, C-11), 141.6 (C_q, C-2), 143.0 (C_q, C-4), 133.4 (CH, C-3), 133.0 (CH, C-2'), 132.97 (CH, C-3'), 133.6 (C_q, C-10), 132.6 (CH, C-12), 128.2 (CH, C-13), 124.7 (CH, C-5), 98.9 (C_q, C-19), 82.5 (CH, C-14), 81.3 (CH, C-7), 76.8 (CH, C-15), 76.58 (CH, C-23), 75.5 (CH, C-21), 70.6 (CH, C-17), 60.1 (CH_3, C2-OCH_3), 56.2 (CH_3, C14-OCH_3), 41.7 (CH, C-18), 42.0 (CH_2, C-9), 40.9 (CH_2, C-20), 40.0 (CH, C-22), 39.9 (CH, C-8), 38.8 (CH, C-16), 37.2 (CH, C-6), 29.7 (CH, C-24), 21.7 (CH_3, C-32), 21.1 (CH_3, C-28), 20.5 (CH_3, C-29), 14.0 (CH_3, C-26), 17.2 (CH_3, C-27), 13.5 (CH_3, C-33), 12.7 (CH_3, C-25), 9.8 (CH_3, C-30), 7.1 (CH_3, C-31). – **EI MS (70 eV):** *m/z* (%) = 568 (16), 525 (64), 211 (56) 149 (20), 113 (100), 99 (65). – **(+)-ESI MS:** *m/z* (%) = 1653.5 ($[2M+Na]^+$, 90), 838.9 ($[M+Na]^+$, 100). – **(-)-ESI MS:** *m/z* (%) = 1651.8 ($[2M+Na-2H]^-$, 60), 814.7 ($[M-H]^-$, 100).

Bafilomycin B_2 (134c): $C_{45}H_{67}O_{13}N$, yellow solid. – R_f = 0.42 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ 13.20 (br s, 1H, 10'-OH), 8.34 (br s, 1H, NH), 7.17 (d, ^3J = 15.6 Hz, 1H, 2'-H), 6.90 (d, ^3J = 15.6 Hz, 1H, 3'-H), 6.62 (s, 1H, 3-H), 6.50 (dd, ^3J = 14.8, 9.7 Hz, 1H, 12-H), 5.80 (d, ^3J = 9.3 Hz, 1H, 5-H), 5.78 (d, ^3J = 9.8 Hz, 1H, 11-H), 5.20 (dd, ^3J = 14.8, 9.1 Hz, 1H, 13-H), 5.10-5.00 (m, 2H, 15-H, 21-H), 3.86 (m, 2H, 14-H, 17-H), 3.70 (s, 3H, 2-OCH_3), 3.50 (m, 1H, 23-H), 3.30 (br t, ^3J = 5.5 Hz, 1H, 7-H), 3.24 (s, 3H, 14-OCH_3), 3.05 (s, 3H, 19-OCH_3), 2.70-2.50 (m, 6H), 2.40 (dd, ^3J = 11.5, 5.8, 1H, 20-H_a), 2.18- 2.10 (m, 2H, 9-H_a, 16-H), 1.99 (s, 3H, 26-CH_3), 1.94 (s, 3H, 29-H_3), 1.70-1.50 (m, 4H, 8-H, 9-H_b, 18-H, 24-H), 1.18 (dd, ^3J = 11.0, 2.0 Hz, 1H, 20-H_b), 1.10 (m, 2H, 22-H), 1.04 (d, ^3J = 7.3 Hz, 3H, 27-H_3), 1.02 (d, ^3J = 7.3 Hz, 3H, 31-H_3), 0.96 (d, ^3J = 6.4 Hz, 3H, 32-H_3), 0.94 (d, ^3J = 6.7 Hz, 3H, 28-H_3), 0.92 (d, ^3J = 6.7 Hz, 3H, 25-H_3), 0.90 (d, ^3J = 6.6 Hz, 3H, 30-H_3), 0.79 (d, ^3J = 6.4 Hz, 3H, 33-H_3). – **EI MS (70 eV):** *m/z* (%) = 568 (16), 525 (32), 334 (15) 209 (15), 195 (26), 137 (32), 125 (38), 109 (100), 93 (42), 81 (40). – **(+)-ESI MS:** *m/z* (%) = 1681.6 ($[2M+Na]^+$, 100), 852 ($[M+Na]^+$, 40). – **(-)-ESI MS:** *m/z* (%) = 1679.8 ($[2M+Na-2H]^-$, 40), 828.8 ($[M-H]^-$, 100).

Deboroaplasmomycin C (135): $C_{44}H_{68}O_{16}$, white needles from dichloromethane/cyclohexane. – R_f = 0.57 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0

MHz) : δ = 5.70 (dddd, ^3J = 15.5, 7.5, 4.7, 1.4 Hz, 1H, 11-H), 5.50 (dd, ^3J = 15.5, 4.0 Hz, 1H, 12-H), 5.24 (d, ^3J = 1.8 Hz, 1H, 3-OH), 5.18 (dd, ^3J = 12.0, 2.7 Hz, 1H, 9-H), 4.89 (d, ^3J = 11.9 Hz, 1H, 2-OH), 4.85 (d, ^3J = 5.5 Hz, 1H, 15-H), 4.62 (dd, ^3J = 8.3, 3.4 Hz, 1H, 13-H), 4.53 (q, ^3J = 6.6, Hz, 1H, 16-H), 4.23 (d, ^3J = 11.9 Hz, 1H, 2-H), 3.65 (dd, ^3J = 11.7, 2.0 Hz, 1H, 7-H), 2.47 (m, 1H, 10-H), 2.33 (ddd, ^2J = 14.4 Hz, ^3J = 8.9, 5.4 Hz, 1H, 14-H_A), 2.10 (m, 1H, 10-H_A), 2.10 (s, 3H, 22-H_3), 2.03 (m, 1H, 4-H), 1.86 (d, ^2J = 14.4 Hz, 1H, 14-H_B), 1.55 (m, 2H, 5-H_2), 1.41 (m, 1H, 6-H), 1.23 (qd, ^3J = 14.4, 5.4 Hz, 1H, 6-H), 1.06 (d, ^3J = 6.6 Hz, 3H, 17-H_3), 0.93 (d, ^3J = 6.7 Hz, 3H, 18-H_3), 0.78 (s, 3H, 19-H_3), 0.64 (s, 3H, 20-H_3). – **^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 171.9 (C_q, C-21), 168.7 (C_q, C-1), 133.4 (CH, C-12), 126.2 (CH, C-11), 98.8 (C_q, C-3), 80.8 (CH, C-15), 78.9 (CH, C-16), 76.9 (CH, C-9), 76.4 (CH, C-13), 75.1 (CH, C-2), 72.8 (CH, C-7), 40.4 (C_q, C-8), 37.1 (CH_2, C-9), 32.8 (CH_2, C-4), 30.8 (CH_2, C-10), 27.6 (CH_2, C-5), 25.0 (CH_2, C-6), 20.9 (CH_3, C-22), 19.9 (CH_3, C-17), 18.2 (CH_3, C-19), 17.9 (CH_3, C-20), 16.5 (CH_3, C-18); signals for only one half of the molecule were compted. – **(+)-ESI MS:** *m/z* (%) = 875.6 ($[M+Na]^+$, 100). – **(-)**-ESI MS: *m/z* (%) = 851.5 ($[M-H]^-$, 100). – **DCI (NH_3):** *m/z* (%) = 870 ($[M+NH_4]^+$, 4), 444 (100).

10.19 Marine-derived Actinomycete Act 7617

The actinomycete strain Act 7617 from the Alfred-Wegener-Institut showed an orange colouration on agar at 28 °C after four days. One grown plate was used to inoculate a 1 L shaker culture with M_2^+ medium. The culture broth harvested after four days was extracted three times with ethyl acetate and the obtained dark oily crude extract was used for antibacterial and chemical screening.

10.20 Pre-Screening

In the biological screening, the crude extract showed moderate antibacterial activity as consigned in table 29. TLC of the crude extract showed three big violet spots after spraying with anisaldehyde/sulphuric acid.

Table 29: Biological activity of the crude extract of the strain Act 7617

Test organisms	Inhibition zone (∅ mm)
Bacillus subtilis	12
Staphylococcus aureus	20
Mucor miehei	12
Escherichia coli	13

10.20.1 Fermentation and Isolation of metabolites

The strain Act 7617 was cultivated on a 25 L scale as linear shaking culture using M_2^+ at 28 °C during four days. The brown culture broth was filtered under pressure and extracted. While the water phase was subjected to XAD-16 followed by washing with tap water and finally extracted with methanol to yield an oily crude extract B, the mycelium part was extracted with ethyl acetate and finally with acetone to yield a light yellow extract A. TLC of both crude extracts showed no similarities and work-up was done separately. The crude extract A was washed with cyclohexane and presented only one fast moving spot, which was separated by PTLC (CH_2Cl_2/5% MeOH) and gave aloesaponarin II. Crude extract B was submitted to Sephadex LH-20 (CH_2Cl_2/50% MeOH), resulting in luisol A (**137**) and a mixture of two spots (fraction A). The latter was further separated using preparative HPLC with MeCN/80% H_2O and yielded luisol B (**138**) and 2-hydroxyluisol A (**139**).

Luisol A (137): $C_{16}H_{18}O_7$, colourless gum. – R_f = 0.58 (CH_2Cl_2/15% MeOH). – **^{1}H** and **^{13}C NMR** see table 7. – **(+)-ESI MS:** *m/z* (%) = 667 ($[2M+Na]^+$, 100), 345 ($[M+Na]^+$, 6). – **(-)-ESI MS :** *m/z* (%) = 643 ($[2M-H]^-$, 92), 321 ($[M-H]^-$, 100).

Luisol B (**138**): $C_{13}H_{14}O_6$, oil. – R_f = 0.65 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($[D_6]$DMSO, 300.0 MHz): δ = 7.13 (t, ^3J = 7.7 Hz, 1H, 6-H), 6.83 (d, ^3J = 7.4 Hz, 1H, 5-H), 6.77 (dd, ^3J = 7.9 Hz, ^4J = 1.1 Hz, 1H, 7-H), 6.38 (br s, 1H, OH), 5.63 (s, 1H, 10-H), 4.89 (s, 1H, 3-H), 4.16 (d, ^3J = 9.9 Hz, 1H, 1-H_A), 3.87 (d, ^3J = 9.9 Hz, 1H, 1-H_B), 1.49 (s, 3H, 13-H_3). – **(+)-ESI MS :** *m/z* (%) = 554.8 ($[2M+Na]^+$, 90), 289.1 ($[M+Na]^+$, 40). – **(-)-ESI MS:** *m/z* = 265.1 ($[M-H]^-$).

2-Hydroxyluisol A (139): $C_{16}H_{18}O_8$, colourless gum. – R_f = 0.42 (CH_2Cl_2/15% MeOH). – **^{1}H** and **^{13}C NMR** see table 7. **UV/VIS (MeOH):** λ_{max} (lg ε) = 219 (sh), 280 (2.80) nm. – **IR (KBr):** ν = 3355, 2258, 1745, 1647, 1594, 1466, 13854, 1278, 1154, 1105, 995, 953, 826, 765, 733 cm^{-1}. – **(+)-ESI MS:** *m/z* (%) = 699 ([2M+Na]$^+$, 100), 361 ([M+Na]$^+$, 2). – **(-)-ESI MS :** *m/z* = 337 ([M-H]$^-$).

11 Plant Metabolites

11.1 Quinones from *Diospyros sylvata*

2-Methylanthraquinone (**141**): $C_{15}H_{10}O_2$, yellow needles. – R_f = 0.70 (C_6H_6/50% $CHCl_3$). **–IR (KBr):** ν = 1680, 1600, 1335 cm-^{1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 8.14-8.24 (m, 2H, 5-H and 8-H), 8.20 (d, ^3J = 9.0 Hz, 1H, 4-H), 8.10 (d, ^3J = 2.0 Hz, 1H, 1-H), 7.80-7.76 (m, 2 H, 6-H and 7-H), 7.60 (dd, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 1H, 3-H), 2.52 (s, 3 H, 2-CH_3). **— EI MS (70 eV):** *m/z* (%) = 222 ([M]$^+$, 100), 207 (20), 194 (60).

Plumbagin (**142**): $C_{11}H_8O_3$, red needles. R_f = 0.53 (C_6H_{12}/50% $CDCl_3$). **– IR (KBr):** ν = 1660, 1640, 1385 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 11.98 (s, 1 H, 5-OH), 7.62 (dd, ^3J = 8.0 Hz, ^4J = 1.5 Hz, 1H, 8-H), 7.59 (t, ^3J = 8.0 Hz, 1H, 7-H), 7.25 (dd, ^3J = 8.0 Hz, ^4J = 1.5 Hz, 1H, 6-H), 6.82 (q, ^4J = 1.5 Hz, 1H, 3-H), 2.18 (d, ^4J = 1.5 Hz, 3H, 2-CH_3). **– EI MS (70 eV):** *m/z* (%) = 188 ([M]$^+$, 100), 173 (20.5), 160 (17), 145 (3), 120 (14), 92 (13), 77 (5).

Diospyrin (**144**): $C_{22}H_{14}O_6$, orange red prisms. – R_f = 0.27 (C_6H_6). **– IR (KBr):** ν = 1660, 1640, 1360 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz,): δ = 12.15 (s, 1H, 5'-OH), 11.90 (s, 1H, 5-OH), 7.58 (s, 1H, 8'-H), 7.50 (br s, 1H, 8-H), 7.15 (s, 1H, 6-H), 6.98 (s, 2H, 2'-H, 3'-H), 6.93 (s, 1H, 3-H), 2.45 (s, 3H, 7-CH_3), 2.30 (s, 3H, 7'-CH_3). – **EI MS (70 eV):** *m/z* (%) = 374 ([M]$^+$, 100), 359 (32), 346 (8), 329 (14), 318 (7), 165 (8), 106 (12), 104 (8).

Isodiospyrin (**145**): $C_{22}H_{14}O_6$, orange red needles. – R_f = 0.18 (C_6H_6). **– IR (KBr):** ν = 1662, 1640, 1371 cm^{-1}. – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.42 (s, 1H, 5'-OH), 12.05 (s, 1H, 5-OH), 7.60 (s, 1H, 8'-H), 7.30 (s, 1H, 6-H), 6.98 (s, 2H, 2'-, 3'-H),

6.89, 6.77 (AB, ^3J = 10.0 Hz, 1H, 2-, 3-H), 6.77 (d, ^3J = 10.0 Hz, 1H, 3-H), 2.03 (s, 3H, 7-CH_3), 2.01 (s, 3H, 7'-CH_3). **– EI MS (70 eV):** *m/z* (%) = 374 ($[M]^+$, 100), 359 (60), 346 (7), 329 (7.5), 318 (2), 187 (7.5).

Microphyllone (**146**): $C_{22}H_{22}O_4$, R_f = 0.54 ($CHCl_3$/1% MeOH). **– IR (KBr):** ν = 1670, 1600, 3375 cm^{-1}. **– ^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 6.75 (d, ^3J = 10.5 Hz, 1H, 5'-H), 6.50 (d, ^3J = 8.5 Hz, 1H, 6-H), 6.45 (d, ^3J = 10.5 Hz, 1H, 6'-H), 6.38 (d, ^3J = 8.5 Hz, 1H, 5-H), 5.80 (br d, ^3J = 7.0 Hz, 1H, 8-H), 4.90 (m, 3H, 8'-H, 2OH), 3.87 (d, ^3J = 6.5 Hz, 1H, 7-H), 2.70, 2.28 (d, ^2J = 18.8 Hz, 2H, 10-H_2), 2.47, 2.30 (AB, ^2J = 14.8, 7.8 Hz, 2H, 7'-H_2), 1.64 (s, 3H, 11'-CH_3), 1.62 (s, 3H, 11-CH_3), 1.47 (s, 3H, 10'-CH_3). **– ^{13}C/APT NMR** ($CDCl_3$, 75.5 MHz): δ = 202.7 (C_q, C-1'), 201.6 (C_q, C-4'), 145.0 (C_q, C-4), 143.1 (C_q, C-1), 139.7 (C_q, C-6'), 139.2 (CH, C-5'), 137.7 (C_q, C-2), 135.3 (C_q, C-9'), 133.3 (C_q, C-9), 130.3 (C_q, C-3), 121.8 (CH, C-8), 118.2 (CH, C-8'),117.0 (CH, C-6), 115.3 (CH, C-5), 68.4 (C_q, C-2'), 59.7 (C_q, C-3'), 41.1 (CH, C-7), 32.6 (CH_2, C-10), 28.7 (CH_2, C-7'), 25.8 (CH_3, C-11'), 22.6 (CH_3, C-11), 17.8 (CH_3, C-10'),. **– EI MS (70 eV):** *m/z* (%) = 350 ($[M]^+$, 100), 335 (3), 267 (5), 228 (25), 123 (10), 69 (10).

11.2 *Rheum palmatum*

The obtained 2.4 g crude extract from our collaborator was dissolved in CH_2Cl_2/5% MeOH and chromatographed on three PTLC plates (40 × 20 cm) and developed with CH_2Cl_2/10% MeOH. Four fractions I to IV resulted from this separation. Purification of fraction I using C_6H_{12}/10% EtOAc delivered chrysophanol (**83a**) and physcion (**83b)**. TLC of the fraction II revealed two close spots which could not be separate using PTLC. Preparative HPLC using a gradient (MeCN/H_2O = 20:80, after 30 min MeCN/H_2O = 60:40, 35 min MeCN/H_2O = 100:0) gave emodin (**83d)** and aloe-emodin (**83c)**. Fraction III was purified on Sephadex LH-20 using MeOH as eluent and delivered rhapontigenin (**148a**). Separation of fraction IV on Sephadex LH-20 delivered two sub-fractions F4a and F4b, which were separated using PTLC (CH_2Cl_2/10% MeOH) and Sephadex LH-20 MeOH and delivered palmatin (**147a)**, emodin 1-ß-O-glucopyranoside (**147c)**, deoxyrhaponticin (**148b**). Rhapontigenin-3-O-ß-D-glucopyranoside (**148c**).

Chrysophanol (**83a**) : $C_{15}H_{10}O_4$, yellow solid. – R_f = 0.63 (CH_2Cl_2). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.09 (s, 1H, OH), 11.98 (s, 1H, OH), 7.80 (dd, ^3J = 8.0 Hz, ^4J = 1.2 Hz, 1H, 5-H), 7.64 (t, ^3J = 8.3 Hz, 1H, 6-H), 7.62 (d, ^4J = 0.5 Hz, 1H, 4-H), 7.26 (dd, ^3J = 8.2 Hz, ^4J = 1.2 Hz, 1H, 7-H), 7.05 (d, ^4J = 0.5 Hz, 1H, 2-H), 2.44 (s, 3H, 3-CH_3).

Physcion (83b): $C_{16}H_{12}O_5$, yellow solid. – R_f = 0.60 (CH_2Cl_2). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 12.32 (s, 1H, OH), 12.12 (s, 1H, OH), 7.62 (br s, 1H, 4-H), 7.36 (d, ^4J = 2.5 Hz, 1H, 5-H), 7.08 (br s, 1H, 2-H), 6.68 (d, ^4J = 2.5, 1H, 7-H), 3.94 (s, 3 H, OMe), 2.44 (s, 3H, Me).

Aloe-emodin (83c) : $C_{15}H_{10}O_5$, yellow powder. – R_f = 0.63 (CH_2Cl_2/6% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 11.92 (s, 2H, OH), 7.80 (t, ^3J = 8.0 Hz, 1H, 6-H), 7.72 (d, ^3J = 8.0 Hz, 1H, 5-H), 7.70 (br s, 1H, 4-H), 7.38 (d, ^3J = 7.9 Hz, 1H, 7-H), 7.26 (br s, 1H, 2-H), 5.58 (br s, 1H, OH), 4.60 (s, 2H, CH_2).

Emodin (83d): $C_{15}H_{10}O_5$, yellow powder. – R_f = 0.74 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 12.14 (br s, 2H, 2OH), 7.50 (d, ^4J = 0.9 Hz, 1H, 5-H), 7.18 (br s, 1H, 4-H), 7.04 (d, ^4J = 2.0 Hz, 1H, 7-H), 6.46 (d, ^4J = 2.2 Hz, 1H, 2-H), 2.40 (s, 3H, 6-CH_3).

Palmatin (147a): $C_{21}H_{20}O_9$, yellow powder. – R_f = 0.22 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 12.90 (s, 1H, OH), 7.76 (t, ^3J = 9.4 Hz, 1H, 6-H), 7.72 (br s, 1H, 4-H), 7.64 (dd, ^3J = 9.0 Hz, ^4J = 0.6 Hz, 1H, 5-H), 7.56 (br s, 1H, 2-H), 7.37 (dd, ^3J = 9.4 Hz, ^4J = 0.5 Hz, 1H, 7-H), 5.18 (d, ^3J = 6.8 Hz, 1H, 1'-H), 5.58 (br s, 1H), 5.05 (br s, 3H), 3.75-3.20 (Glc), 2.50 (s, 3H, CH_3). – **EI MS (70 eV):** *m/z* (%) = 254 [M-sugar]$^+$, 100), 226.1 (12), 197.1 (8), 152.1(8). – **(+)-ESI MS:** *m/z* (%) = 855.1 ([2M+Na]$^+$, 100), 439.5 ([M+Na]$^+$, 36). – **(-)- ESI MS:** *m/z* (%) = 831.7 ([2M-H]$^-$, 2), 253.6 ([M-sugar-H]$^-$, 100).

Emodin 1-ß-O-glucopyranoside (147c): $C_{21}H_{20}O_{10}$, yellow solid. – R_f = 0.47 (CH_2Cl_2/15% MeOH). – **^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 14.38 (s, 1H, OH), 7.30 (d, ^4J =1.1 Hz, 1H, H-5), 6.98 (br s, 1H, 4-H), 6.63 (d, ^4J = 2.3 Hz, 1H, 7-H), 6.23 (d, ^4J = 2.3 Hz, 1H, 2-H), 5.37 (br s, 1H, OH), 5.00 (br s, 1H, OH), 4.68 (d, ^3J =

6.8 Hz, anomeric H), 4.60 (br s, 1H, OH), 3.80-3.20 (Glc), 2.36 (s, 3H, CH_3). **– EI MS (70 eV):** *m/z* (%) = 270.0 ([M-sugar]$^+$, 100), 242.0 (12), 197.1 (8), 213.1 (12), 185 (3), 139 (4), 84 (10), 44 (24). **– (+)-ESI MS:** *m/z* (%) = 887.1 ([2M+Na]$^+$, 100), 455.3 ([M+Na]$^+$, 74). **– (-)- ESI MS:** *m/z* = 431.6 ([2M-H]$^-$).

Rhapontigenin (148a): $C_{15}H_{14}O_4$, colourless solid, blue fluorescence under 366 nm. **– R_f** = 0.56 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ([D_6]acetone, 300.0 MHz): δ = 8.30 (br s, 2H, 2OH), 7.10 (d, ^4J = 2.3 Hz, 1H, 2'-H), 7.00 (d, ^3J = 16.7 Hz, 1H, H_α), 6.98 (d, ^3J = 8.1 Hz, 1H, 6'-H), 6.88 (d, ^3J = 16.0 Hz, 1H, H_β), 6.86 (d, ^3J = 7.2 Hz, 1H, 5'-H), 6.56 (d, ^3J = 2.3 Hz, 2H, H-2, 6-H), 6.29 (t, ^4J = 2.3 Hz, 1H, 4-H), 3.80 (s, 3H, OCH_3). **– ^{13}C/APT NMR** ([D_6]acetone, 50.3 MHz): δ = 159.3 ($2C_q$, C-3, C-5), 148.1 (C_q, C-4'), 147.2 (C_q, C-3'), 140.6 (C_q, C-1), 131.4 (C_q, C-1'), 129.0 (CH, C_α), 127.4 (CH, C_β), 119.7 (CH, C-6'), 113.2 (CH, C-2'), 112.2 (CH, C-5'), 105.7 (2CH, C-2, C-6), 102.7 (CH, C-4), 56.1 (CH_3, OMe). **– EI MS (70 eV):** *m/z* (%) = 258.1 ([M]$^+$, 100), 225.0 (8), 197.0 (40). **– (+)-ESI MS:** *m/z* (%) = 539.0 ([2M+Na]$^+$, 100), 281.2 ([M+Na]$^+$, 20). **– (-)-ESI MS:** *m/z* (%) = 515.0 ([2M-H]$^-$, 20), 257.2 ([M-H]$^-$, 100).

Desoxyrhaponticin (148b): $C_{21}H_{24}O_8$, colourless solid, showed, exhibited a blue fluorescence under 366 nm. **– R_f** = 0.50 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 7.51 (d, ^3J = 9.0 Hz, 2H, 2'-H, 6'-H), 7.06 (d, ^3J = 16.2 Hz, 1H, α-H), 6.95 (d, ^3J = 8.7 Hz, 2H, 3', 5'-H), 6.90 (d, ^3J = 16.6 Hz, 1H, ß-H), 6.78 (br s, 1H, 2-H), 6.60 (br s, 1H, 6-H-), 6.38 (br s, 1H, 4-H), 5.20 (br s, 2H, 2OH), 4.80 (d, ^3J = 7.5 Hz, 1H), 3.80 (s, 3H, OCH_3), 3.70-3.10 (m, 6H, sugar-H). **– EI MS (70 eV):** *m/z* (%) = 404.1 ([M]$^+$, 8), 270.0 (15), 242.1 ([M-sugar]$^+$, 100), 181.0 (12), 60.0 (10). **– (+)-ESI MS:** *m/z* (%) = 427.4 ([M+Na]$^+$, 22), 831.1([2M+Na]$^+$, 100). **– (-)- ESI-MS:** *m/z* (%) = 807.5 ([2M-H]$^-$, 32), 403.4 ([M-H]$^-$, 30).

Rhapontigenin-3-O-ß-D-glucopyranoside (148c): $C_{21}H_{24}O_9$, white solid, give a blue fluorescence under 366 nm. **– R_f** = 0.33 (CH_2Cl_2/10% MeOH). **– ^{1}H NMR** ([D_6]DMSO, 300.0 MHz): δ = 7.00 (d, ^3J =16.0 Hz, 1H, H_α), 6.80 (d, ^3J =16.1 Hz, 1H, H_β), 6.99-6.84 (m, 3H, 2', 5', 6'-H), 6.73 (br s, 1H, 6-H), 6.58 (br s, 1 H, 2-H),

6.37 (br s, 1H, 4-H), 4.80 (d, ^3J = 8.0 Hz, 1H, anomeric H), 3.78 (s, 3H, OCH_3), 3.00-3.70 (m, 6H, sugar-H). **– (+)-ESI MS:** *m/z* (%) = 863.1 ($[2M+Na]^+$, 100), 443.3 ($[M+Na]^+$, 6). **– (-)-ESI MS:** *m/z* (%) = 1259.1 ($[3M-H]^-$, 42), 839.4 ($[2M-H]^-$, 100), 419.4 ($[M-H]^-$, 40). **– EI MS (70 eV):** *m/z*(%) = 420.1 ($[M]^+$, 2), 258.1 (100), 225.0 (15), 197.1 (40), 84.0 (22), 66.0 (22).

11.3 *Canarium schweinfurthii*

The seeds and trunk of *Canarium schweinfurthii* was purchased from a vendor in Yaoundé, Central province of Cameroon. The identity of the plant was established by Mr. P. Minzili from the National Herbarium at Yaoundé where the voucher specimen (No 16929) is kept.

600 g of seeds and 200 g of trunk were dried, powdered and work-up in the similar manner. The resulting powder from the seed was extracted with CH_2Cl_2-50% MeOH for 24 h, followed by MeOH for 12 h. Both extracts were mixed due to the similarity of their constituents on TLC, evaporated to dryness and gave a brown gum.

The brown gum (200 g) obtained from the seed powder (500 g) was defatted with hexane and dissolved in ethyl acetate. This solution was separated by silica gel column chromatography using a CH_2Cl_2/MeOH gradient (each 1 L) under TLC control. Five fractions A-E were obtained. Fraction A obtained from 100% CH_2Cl_2 was further purified by PTLC using hexane/10% EtOAc and delivered *p*-hyroxybenzaldehyde (**152**) as oil. Fraction B showed on TLC a spot, which turn pink on spraying with anisaldehyde/sulphuric acid. Chromatography on Sephadex LH-20 (CH_2Cl_2/40% MeOH) delivered coniferaldehyde **(151,** 4.4 mg) as pale yellow compound. From fraction C, a yellow powder of amenthoflavone **(149a)** had been separated, which was collected by centrifugation (9 mg). TLC of fraction of D showed under UV 254 nm a strong absorption but delivered no colour reaction on spraying with anisaldehyde/sulphuric acid. Column chromatography (CH_2Cl_2/MeOH, gradient) followed by PTLC gave 5 mg ligballinol (**150**). 3-O-galloyl-(-)-epicatechin (**154,** 30 mg) was obtained from fraction E but also from the trunk extract, gallic acid (**153a,** 20 mg), 3,4-dihydroxybenzoic acid (**153b,** 15 mg) and scopoletin (**155,** 6 mg) were obtained by chromatography of the trunk extract.

Amenthoflavone (149a): $C_{30}H_{18}O_{10}$, yellow powder. – R_f = 0.45 (CH_2Cl_2/15% MeOH). – **^{1}H NMR** ([D_4]MeOH, 300.0 MHz): δ = 7.90 (d, ^4J = 2.1 Hz, 1H, 2'-H), 7.78 (dd, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 1H, 6'-H), 7.42 (d, ^3J = 8.2 Hz, 2H, 2''', 6'''-H), 7.02 (d, ^3J = 9.0 Hz, 1H, 5'-H), 6.68 (d, ^3J = 8.2 Hz, 2H, 3''', 5'''-H), 6.50 (s, 2H, 3, 3''-H), 6.40 (d, ^4J = 2.2 Hz, 1H, 8-H), 6.30 (s, 1H, 6''-H), 6.13 (d, ^4J = 2.2 Hz, 1H, 6-H). – **^{13}C/APT NMR** ([D_6]DMSO, 75.5 MHz): δ = 182.0 (C_q, C-4), 181.6 (C_q, C-4''), 164.0 (C_q, C-2), 163.7 (C_q, C-7), 163.6 (C_q, C-2''), 161.9 (C_q, C-5), 161.4 (C_q, C-7), 160.9 (C_q, C-5), 160.4 (C_q, C-4''), 159.5 (C_q, C-4'), 157.3 (C_q, C-9), 154.4 (C_q, C-9''), 131.3 (CH, C-6'), 128.1 (2CH, C-6''', C-2'''), 127.7 (CH, C-2'), 121.3 (CH, C-3'), 120.8 (C_q, C-1'), 120.0 (C_q, C-1'''), 116.1 (CH, C-5'), 115.7 (CH, C-5''', C-3'''), 103.9 (C_q, C-8''), 103.6 (C_q,, C-10), 103.5 (C_q, C-10'') 102.9 (CH, C-3), 102.5 (CH, C-3''), 98.8 (CH, C-6), 98.6 (CH, C-6''), 93.9 (CH, C-8). – **EI MS (70 eV):** *m/z* (%) = 538.2 ([M]$^+$, 64), 520.2 (32), 354 (8), 309.2 (12), 229.2 (16), 121.1 (20), 91.1 (24).

Ligballinol (150): $C_{18}H_{18}O_4$, colourless solid. – R_f = 0.55 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ([D_4]MeOH, 300.0 MHz): δ = 7.20 (d, ^3J = 9.0 Hz, 2H, 2'-H, 6'-H), 6.78 (d, ^3J = 9.0 Hz, 2H, 3'-H, 5'-H), 4.65 (d, ^3J = 4.0 Hz, 2H, 2-H), 4.20 (m, 1H, 4-H_e), 3.80 (dd, ^3J = 12.0, 4.0 Hz, 1H, 4-H_a), 3.05 (m, 1H, 2-H); signals of only one part of the symetrical molecule is given. – **EI MS (70 eV):** *m/z* (%) = 298.1 ([M]$^+$, 48), 280.1 (2), 267.1 (16), 179.1 (8), 175.1 (32), 147.1 (16), 133.0 (70), 121.0 (100), 107.0 (58), 94.0 (16). – **DCI (NH_3):** *m/z* (%) = 316.2 ([M+NH_4]$^+$, 92), 333.2 ([M+NH_3+NH_4]$^+$, 8).

Coniferaldehyde (151): $C_{10}H_{10}O_3$, pale yellow oil. – R_f = 0.90 (C_6H_{12}/20% EtOAc). – **^{1}H NMR** (CD_2Cl_2, 300.0 MHz): δ = 9.62 (d, ^3J = 7.9 Hz, 1H, CHO), 7.42 (d, ^3J = 15.8 Hz, 1H β-H), 7.14 (d, J^3= 2.0 Hz, 1H, 2-H), 7.12 (dd, ^3J = 7.0 Hz, ^4J = 2.0 Hz, 1H, 6-H), 6.94 (d, ^3J = 8.0 Hz, 1H, 5-H), 6.60 (dd, ^3J = 7.9, 15.8 Hz, 1H, α–H), 6.18 (s, 1H, 4-OH), 3.94 (s, 3H, OMe). – **EI MS (70 eV):** *m/z* (%) = 178.1 ([M]$^+$, 100), 177.1 (18), 147.1 (24), 135.1 (28), 175.1 (32), 124.1 (12), 107.1 (24), 89.0 (8), 77.0 (20), 51.0 (8).

***p*-Hydroxybenzaldehyde (152):** $C_7H_6O_2$, colourless solid. – R_f = 0.62 (Hexan/10% EtOAc). – **^{1}H NMR** (CD_2Cl_2, 300.0 MHz): δ = 9.82 (s, 1H, CHO), 7.81 (d, ^3J = 8.7 Hz, 2H, 2-H, 6-H), 6.98 (d, ^3J = 8.7 Hz, 2H, 3-H, 5-H).

Gallic acid (153a): $C_7H_6O_5$, brown powder. – R_f = 0.42 (CH_2Cl_2/20% MeOH). – **^{1}H NMR** (CD_3OD, 300.0 MHz): δ = 7.00 (s, 2H, 2, 6-H). – **^{13}C/APT NMR** (CD_3OD, 75.5 MHz) : δ = 170.4 (C_q, CO), 146.3 (C_q, C-3, C-5), 139.5 (C_q, C-4), 122.0 (C_q, C-1), 110.3 (CH, C-2, C-6). **– EI MS (70 eV):** *m/z* (%) = 170.0 ($[M]^+$, 100), 153.1 (72), 125 (12), 135.0 (12), 113.0 (12), 79.0 (8).

3,4-Dihydroxybenzoic acid (153b): $C_7H_6O_4$, white solid. – R_f = 0.45 (CH_2Cl_2/20% MeOH). **^{1}H NMR** ([D$_4$]MeOH, 300.0 MHz): δ = 7.40 (m, 2H, 2-H, 6-H), 6.79 (dd, ^3J = 8.5, ^4J = 1.1 Hz, 1H, 5-H). – **^{13}C/APT NMR** ([D$_4$]MeOH, 75.5 MHz): δ = 170.3 (C_q, COOH), 151.5 (C_q, C-3), 146.0 (C_q, C-4), 123.9 (CH, C-6), 123.2 (C_q, C-1), 117.7 (CH, C-2), 115.8 (CH, C-5). **DCI (NH_3):** *m/z* (%) = 189.1.2 ($[M+NH_4+NH_3]^+$, 5). – **(-)-ESI MS:** *m/z* (%) = 307.0 ($[2M-H]^-$, 54), 153.2 ($[M-H]^-$, 100). – **EI MS (70 eV):** *m/z* (%) = 154.0 ($[M]^+$, 100), 137.0 (80), 164.1(30), 109.0 (12).

3-O-Galloyl-(-)-epicatechin (154): $C_{22}H_{18}O_{11}$, reddish solid: – R_f = 0.35 (CH_2Cl_2/20% MeOH). – **^{1}H NMR** ([D$_4$]MeOH, 300.0 MHz): δ = 6.87 (s, 2H, 2’’-H, 6’’-H), 6.48 (s, 2H, 2’-H, 6’-H), 5.98 (s, 2H, 6-H, 8-H), 5.50 (m, 3-H), 4.99 (br s, 1H, 2-H), 2.98, 2.82 (ABX, J_{AB} = 17.8 Hz, J_{BX} = 4.1 Hz, J_{AX} = 2,3Hz, 2H, 4-H$_2$).– **(+)-ESI MS:** *m/z* (%) = 939.9 ($[2M+Na]^+$, 100), 481.2 ($[M+Na]^+$, 16). – **(-)-ESI MS:** *m/z* (%) = 915.9 ($[2M-H]^-$, 100), 457.0 ($[M-H]^-$, 84).

Scopoletin (155): $C_{10}H_8O_4$, yellow needles. – R_f = 0.53 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300.0 MHz): δ = 7.60 (d, ^3J = 9.8 Hz, 1H, 4-H), 6.92 (s, 1H, 5-H), 6.85 (s, 1H, 8-H), 6.27 (d, ^3J = 9.8 Hz, 1H, 3-H), 6.13 (br s, 1H, OH), 3.98 (s, 3H, OMe). – **EI MS (70 eV):** *m/z* (%) = 192.2 ($[M]^+$, 100), 177.1 (56), 164.1 (30), 149.1 (52), 121.1 (20), 79.0 (16), 69.0 (32), 51.0 (14).

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Lebenslauf

Am 06.06.1973 wurde ich als zweites Kind der Eheleute Moyopo Clemence und Tchuente Joseph in Jaunde, Kamerun geboren.

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1993 immatrikulierte mich an der Universität Jaunde I, Fakultät der Wissenschaft, für das Studienfach Naturwissenschaft und bestand 1997 die Vordiplomprüfung (B.Sc.) für die Fachkombination Physikalische Chemie/Chemie. Von September 1997 bis 1998, machte ich mein Magister an der Universität Jaunde I, Kamerun, Fach Organische Chemie, Abteilung Naturstoffchemie unter der Leitung von Prof. B. T. Ngadjui mit dem Titel: „Contribution on the Phytochemical study of one Cameronian medicinal plant: *Dorstenia mannii* (Moraceae)“.
Von September 1999 bis 2001 machte ich eine Aufnahmeprüfung für Promotion und Arbeite ich als Assistent in Fach Organische Chemie an der Universität Jaunde I, Kamerun.

Von Juni 2001 bis September 2001 kam ich in Deutschland mit einem DAAD Stipendium nach Deutschland und lernte ich für 4 Monate Deutsch in Speak and Write, Marburg.

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Ich besitze die Kamerunische Staatsbürgerschaft.

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18. **Serge Fotso**, Shao jie Wu, Son Qin, Fuchao Li and Harmut Laatsch, New Amorphanes from a Marine *Streptomyces* sp. accepted in *J. Nat. Prod.*

19. Shao Jie Wu, **Serge Fotso**, Fuchao Li, Song Qin, Gerhard Kelter, Heinz H. Fiebig, and Hartmut Laatsch, Carboxamido-staurosporine and Selina-4(14),7(11)-diene-8,9-diol, new Metabolites from a Marine *Streptomyces* sp. submitted to *Journal of Antibiotics*