CLARISSE BLANDINE FOTSO FONDJA YAO

Aqabamycins, Rare Nitro Maleimides and other Novel Metabolites from Microorganisms; Generation and Application of an HPLC-UV-ESI MS/MS Database

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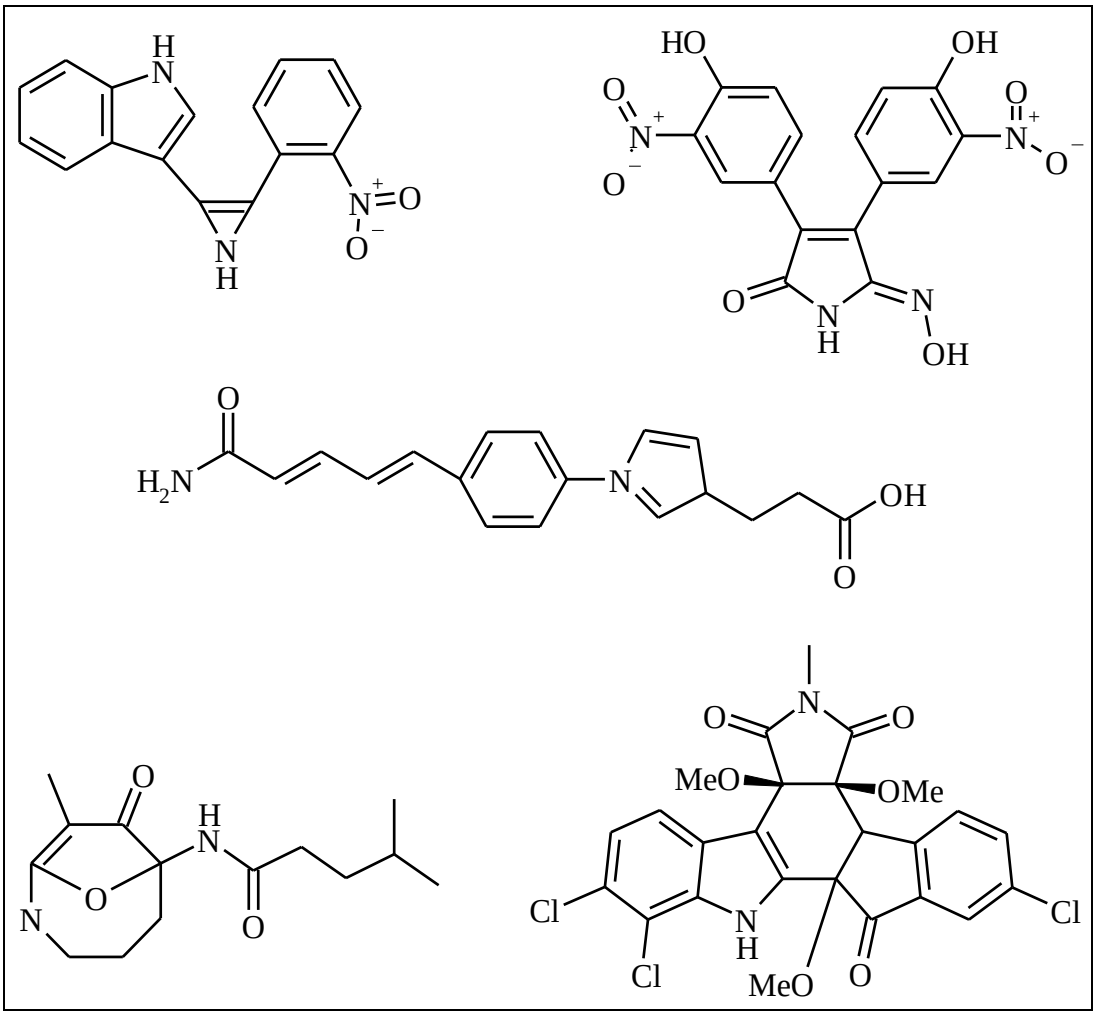

Göttingen 2007

Aqabamycins, Rare Nitro Maleimides and other Novel Metabolites from Microorganisms; Generation and Application of an HPLC-UV-ESI MS/MS Database

Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultäten
der Georg-August-Universität Göttingen

vorgelegt von
CLarisse Blandine Fotso Fondja Yao
aus
Yaoundé (Kamerun)

Göttingen 2007

D7

Referent: Prof. Dr. H. Laatsch

Korreferent: Prof. Dr. A. Zeeck

Tag der mündlichen Prüfung: 22-23 Januar 2008

Die vorliegende Arbeit wurde in der Zeit von Oktober 2004 bis Dezember 2007 am Institut für Organische und Biomolekulare Chemie der Georg-August-Universität Göttingen unter der Anleitung von Prof. Dr. H. Laatsch angefertigt.

Mein herzlicher Dank gilt Herrn Prof. Dr. H. Laatsch für die Bereitstellung des interessanten Themas und die Möglichkeit, diese Arbeit in seinem Arbeitskreis anzufertigen.

Ich widme diese Arbeit meinem Bruder Noussa Yao Joseph, dessen Namen meine Tochter trägt. (Arielle Sorelle Noussa Fotso).

Aqabamycins, Rare Nitro Maleimides and other Novel Metabolites from Microorganisms; Generation and Application of an HPLC-UV-ESI MS/MS Database

Dissertation

For the acquisition of the degree Philosophical Doctorate (PhD)

Division of Mathematics and Natural Sciences

Georg-August-University Göttingen

Submitted by

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from

Yaoundé (Kamerun)

Göttingen 2007

D7

Supervisor:	Prof. Dr. H. Laatsch
Co supervisor:	Prof. Dr. A. Zeeck
Date of the oral exam:	22-23 January 2008

The presented work was carried out from October 2004 until December 2007 at the institute of Organic and Biomolecular Chemistry, Georg-August University Göttingen, under the supervision of Prof. Dr. H. Laatsch.

My heartiest thanks go to Prof. Dr. H. Laatsch for providing the interesting subject and the opportunity to carry out this work in his team.

I dedicate this work to my brother Noussa Yao Joseph whose name my daughter carries (Arielle Sorelle Noussa Fotso).

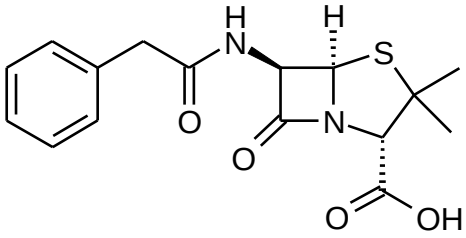
1 Introduction

The WHO (World Health Organisation) estimated that nearly 80% of the world's population uses traditional medicine (mainly medicinal plants) for primary medical treatment, [1] and a major part of the health system is based on the use of natural products. Natural products chemistry is the study of nature for the search of bioactive compounds: It is derived from the use of natural resources by humans for medicinal purposes. This study involves the isolation and structure elucidation of pure compounds, their formation, use and role in organisms. Primarily, the search for new bioactive drugs was focused only on plants as the potential source, and this was based on the knowledge that especially plants are very useful in traditional medicine. This agreed with the citation of Christophe Waith: "Plant species is the very last gift of mother nature in the cause of human health". [2]

The investigation of medicinal plants has suffered in some parts of the world by the limitation of plant material due to environmental protection. Nevertheless new secondary metabolites with large spectra of activity continue to be isolated and reported from plants. The increase in the incidence of multi-drug-resistance bacteria and the recrudescence of new diseases led to urgent investigation of new classes of more potent antibiotics. This need obligated researchers to look for alternative sources such as terrestrial and marine microorganisms and fungi, which have a special adaptability as well as a survival in extreme conditions. This enables them to produce substances with unique and special effectiveness. It also follows the observation that antibiotics from microorganisms are usually more active than antimicrobial compounds from plants.

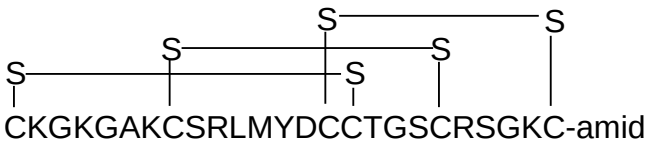
1.1 Marine and terrestrial microorganisms as new sources for drug discovery

The existence of microorganisms was hypothesized during the late middle ages, but they were not observed until the 17th century and later proved by the observation of Louis Pasteur in 1857, who said "I am of the opinion that alcoholic fermentation never occurs without simultaneous organization, development and multiplication of cells….".[3] In 1929, Alexander Fleming succeeded with a breakthrough in the world of microorganism by the accidental discovery of penicillin (**1**) from *Penicillium notatum.*[4]



1

The use of natural products has a long tradition but was mostly focussed on terrestrial sources. Although in ancient times the Phoenicians already employed a chemical secretion from marine molluscs to produce the Tyrian purple for woollen cloth, [5] and seaweeds have long been used to fertilize the soil, [5] oceans remained to be an incredibly hostile environment for humans. Owing to the diversity of flora and fauna of the oceans, that covers a huge surface of the globe (about 70 %) [6] and because of their symbiotic lifestyle,[7] it was postulated that the production of active compounds should far exceed these from plants.[8] The discovery of compound such as the highly active prostaglandin derivative (prostaglandin E2) in gorgonians (*Pseodopteropgorgia elisabethae*) 1968, the anti-viral drug Acyclovir® from sponges (*Cryptotheca crypta*), the pain-killer Prialt® (**2**) from cone snails (*Conus magnus*) [9] and so on, stimulated the research on bioactive secondary metabolites from marine organisms.[10]



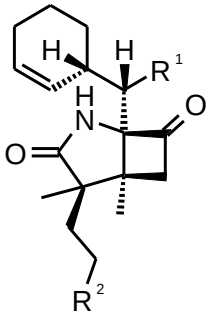
2

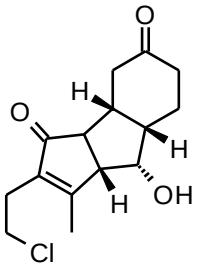
Less than 1 % of bacterial and 5 % of fungal species are currently known, and the potential of novel microbial sources, particularly those found in extreme environments, seems infinite.[11] Most marine bacterial metabolites have been isolated from species of the genus *Streptomyces* and *Alteromonas /Pseudoalteromonas* [12], but other genera remain nearly untouched.

Table 1: Number M of published metabolites from marine bacteria according to their taxonomic origin since 1966 [12]

Genus	M	Genus	M	Genus	M
Streptomyces	214	*Janibacter*	9	*Brevibacterium*	2
Unidentified bacteria	65	*Microbacterium*	9	*Chrysobacter*	2
Alteromonas	47	*Actinomadura*	8	*Enterobacter*	2
Bacillus	37	*Marinobacter*	7	*Pelagiobacter*	2
Vibrio	29	*Salinospora*	7	*Blastobacter*	1
Pseudomonas	28	*Flavobacterium*	6	*Chainia*	1
Actinomyces	25	*Micrococcus*	6	*Cyclobacterium*	1
Pseudoalteromonas	25	*Halomonas*	5	*Deleya*	1
Cytophaga	19	*Ruegeria*	4	*Enterococcus*	1
Micromonospora	19	*Halobacillus*	3	*Erythrobacter*	1
Myxobacteria	17	*Nocardiopsis*	3	*Flexibacter*	1
Chromobacterium	15	*Oceanibulbus*	3	*Maduramyces*	1
Agrobacterium	14	*Alcaligenes*	2	*Photobacterium*	1

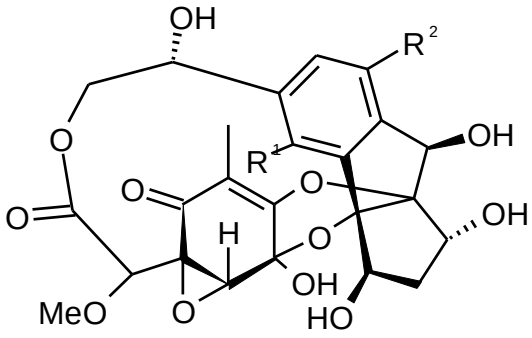
Every year the number of novel compounds isolated from marine microorganisms and phytoplankton, marine algae, sponges, coelenterates, bryozoans, molluscs, tunicates and echinoderms is increasing rapidly [13] and to date, more than 15000 marine natural products have been reported [14] comprising all chemical classes and have established themselves as a diverse group of biologically important compounds. Even deep ocean sediments are a valuable source of new actinomycetes that are unique to the marine environment. The first truly and most exciting marine actinomycete genus named *Salinospora (Salinispora)* has been described by Fenical 2002.[15] *Salinospora* strains produce biologically active secondary metabolites such as the very potent cytotoxin and very potent proteasome inhibitor (IC_{50} = 1.3 nM) salinosporamide A (**3a**).[16] Related metabolites such as salinosporamides B (**3b**) and C (**4**) and the unprecedented chlorinated macrolides sporolides A (**5a**) and B (**5b**) were also isolated from the same source.[17]





3a: $R^1 = CH_3$, $R^2 = Cl$ **4**

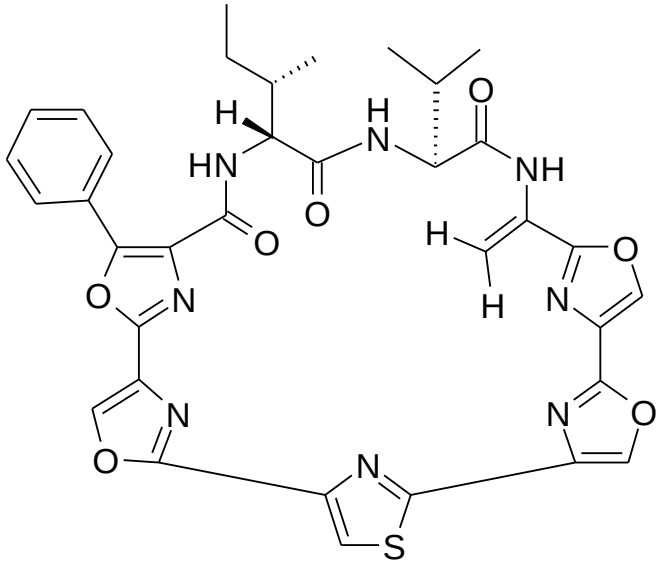
3b: $R^1 = OH$, $R^2 = H$



5a: $R^1 = Cl$, $R^2 = H$

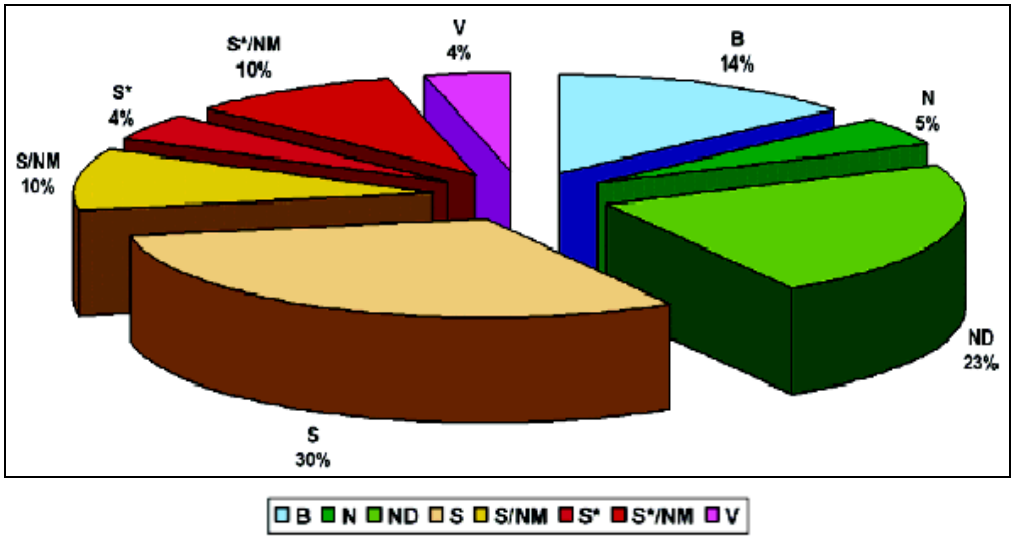
5b: $R^1 = H$, $R^2 = Cl$

A search in the literature [18] revealed that from 1981 to 2006 a large number of new compounds has been isolated from natural sources (28%), or were derived from a natural product e.g. by *semis*ynthetic modification (23%).



6

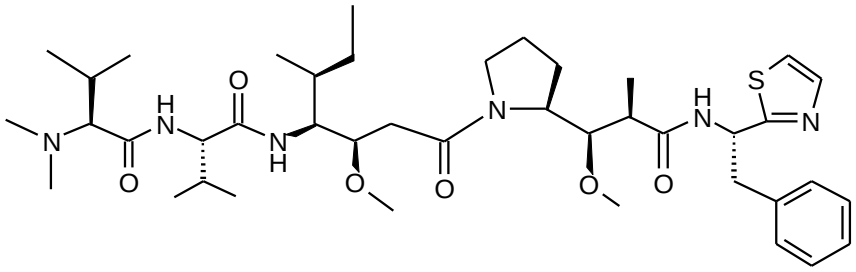
The newly reported metabolites possessed interesting biological activities such as antitumor, antibacterial, and antifungal properties. An example is mechercharmycin A (**6**), which is active against A-549 lung and Jurkat human leukaemia cell lines, and was also reported as a cytotoxic compound in a patent published in 2005.[19]



N	Natural product
ND	ND" Derived from a natural product and is usually a semisynthetic modification.
V	Vaccine
B	Biological; usually a large (>45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means in a surrogate host.
S	Totally synthetic drug, often found by random screening/modification of an existing agent.
S+	Made by total synthesis, but the pharmacophore came from a natural product.
NM	Natural product mimic

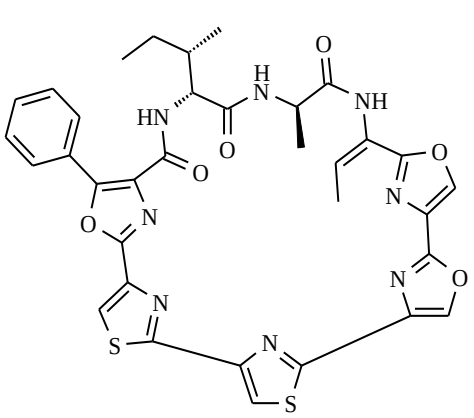
Figure 1: New chemical entities from natural sources, 01/1981- 06/2006[18]

The extremely potent anticancer drug dolastatin 10 (**7**) was isolated in 1970 from the extract of the sea hare *Dolabella auricularia* [20] and its structure was elucidated first 1985 after its isolation from field collections of the marine cyanobacterium *Symploca.* [21]



7

The novel cyclic peptide urukthapelstatin A (**8**) was isolated from the marine-derived thermoactinomycete *Mechercharimyces asporophorigenens* YM11-542.[22] The anticancer urukthapelstatin A (**8**) is a thiopeptide antibiotic and is structurally related to mechercharstatin (or mechercharmycin) [23] (**6**) and YM-216391 (**9**). [24]



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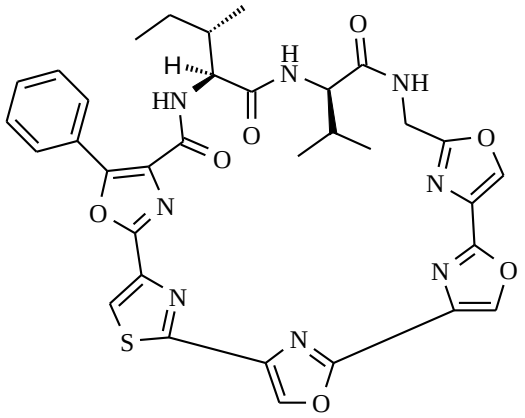
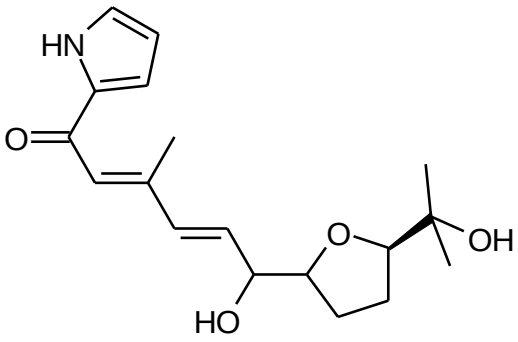
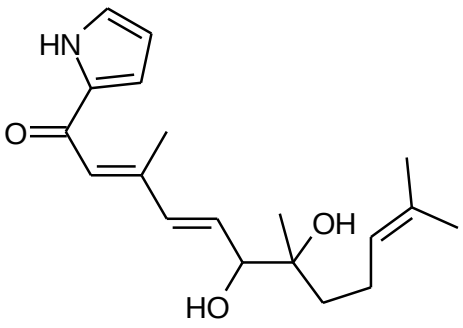


9

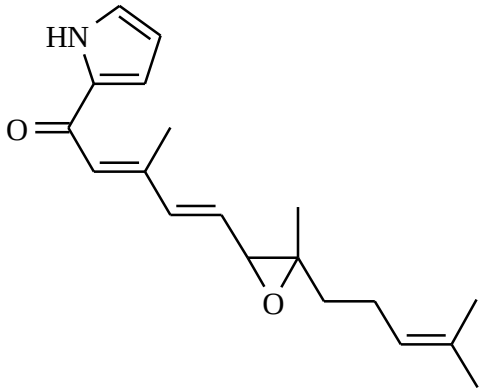
Three new pyrrolosesquiterpenes, glaciapyrroles A–C (**10a-c**) were isolated from a culture of *Streptomyces* sp. obtained from Alaskan marine sediment.[25] Glaciapyrrole A (**10a**) exhibited an IC_{50} of 180 µM toward the colorectal adenocarcinoma HT-29 and melanoma B16–F10 human cancer cell lines.



10a

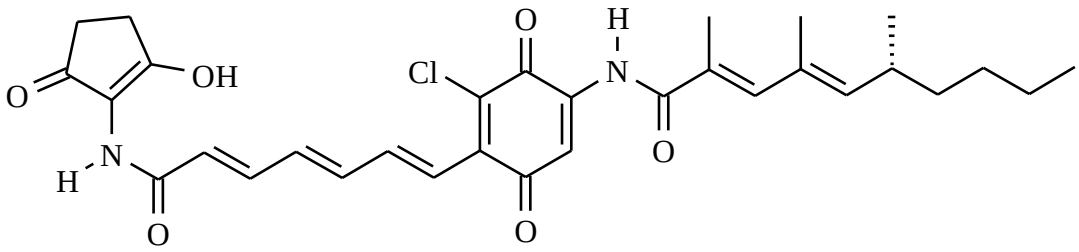


10b

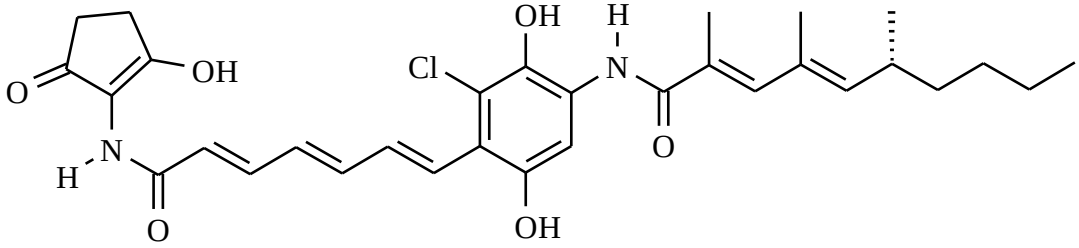


10c

The chlorine containing manumycin derivatives, chinikomycins A (**11a**) and B (**11b**) were isolated from a *Streptomyces* species isolate M045; they displayed anti-tumour activity against a number of human cancer cell lines. [26]

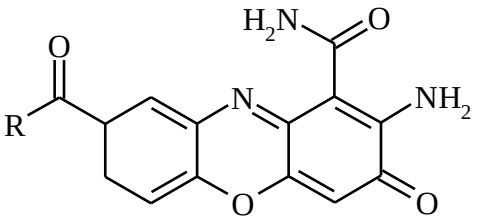


11a



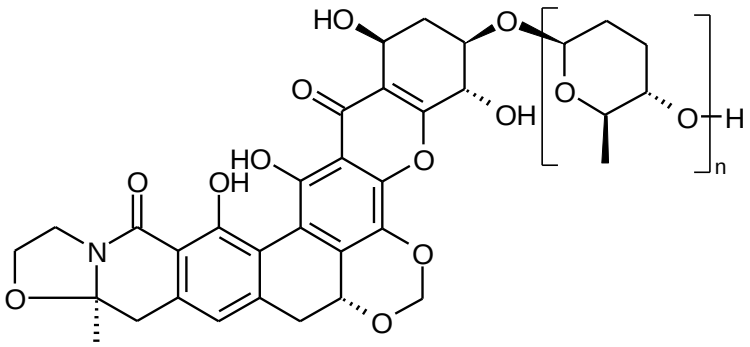
11b

Elloxazinones A (**12a**) and B (**12b**) were isolated from the soil bacterium *Streptomyces griseus* Acta 2871, they showed antitumor activity, a moderate inhibition of the proliferation of human cells from gastric adenocarcinoma *in vitro* but a strong inhibition of hepatocellular carcinoma cells, whereas elloxazinone B (**12b**) strongly inhibited the proliferation of human breast carcinoma cells.[27]

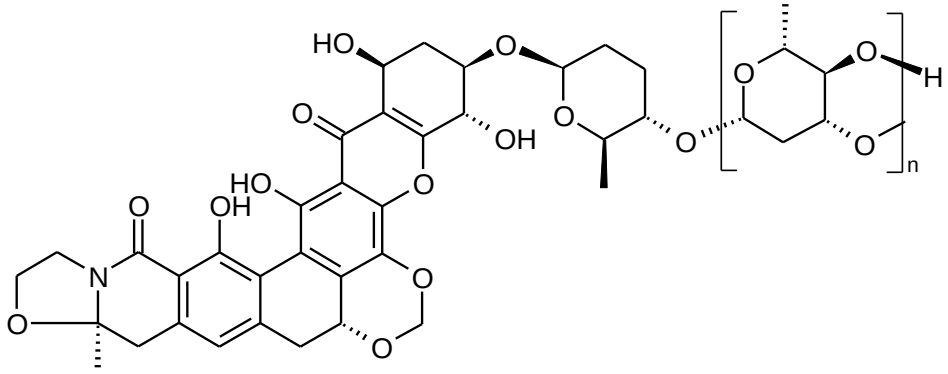


12a: R = OH
12b: R = OCH_3

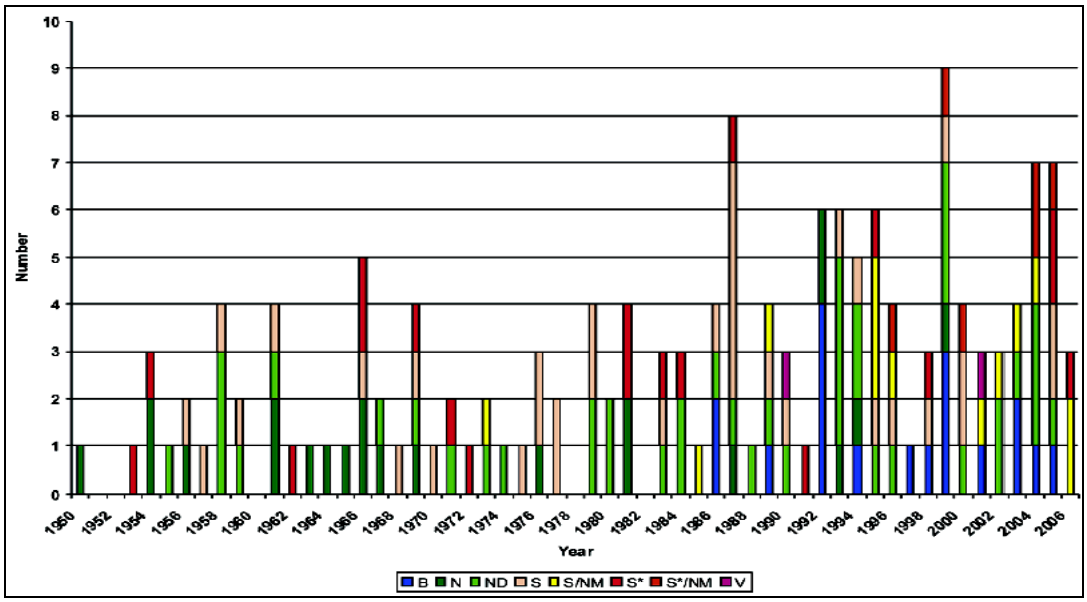
Kigamicins A-E (**13a-b, 14a-c**) were discovered in *Amycolatopsis* sp. ML630-mF1 and showed selective toxicity against PANC-1 cells under nutrient starvation.[28] Kigamicins showed antimicrobial activity against Gram-positive bacteria including methicillin resistant *Staphylococcus aureus* (MRSA). Only kigamicin D (**14b**) inhibited the growth of various mouse tumour cell lines at IC_{50} of about 1 μg/ml. [28]



13a: n =1
13b: n = 2



14a: n = 1
14b: n = 2
14c: n = 3



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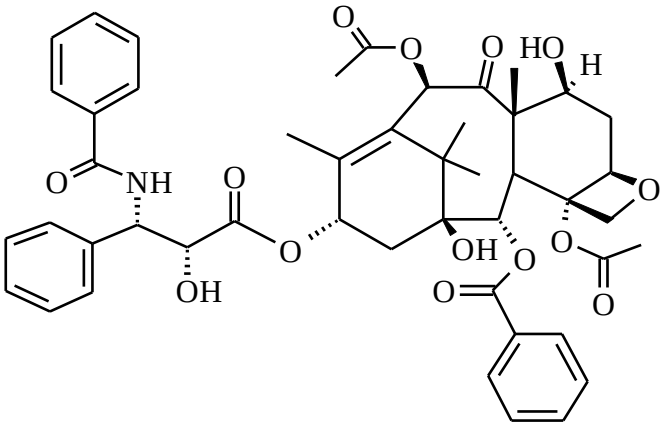
S+	Made by total synthesis, but the pharmacophore came from a natural product.
NM	Natural product mimic

Figure 2: Approved anticancer agents, organized by source/year [18]

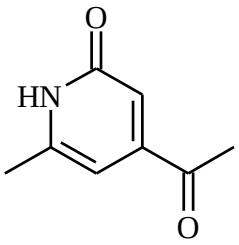
Microorganisms are today the origin of the best-marketed drugs, [29] despite the recognition that the research in anticancer natural products has declined significantly during the last few years. [18]

Taxol (**15**) which was first isolated 1971 from plants [30] and later also found in fungi, [31] was approved for breast and ovarian cancer and acts by blocking depolymerisation of microtubules and promotes tubulin polymerisation. [32] Aside from its anticancer activity it showed also antifungal activity against *Oomycetes*. [33]



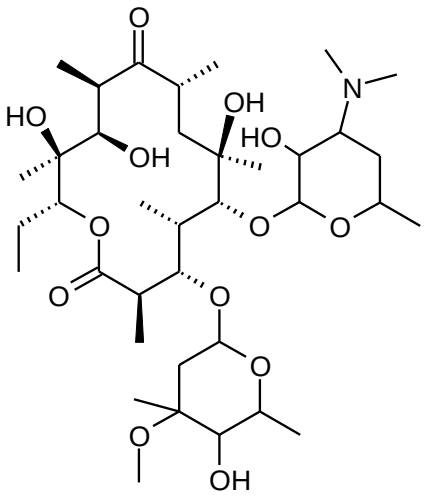
15

Streptokordin A (**16**), a cytotoxic methylpyridine, was isolated from *Streptomyces* sp. KORDI-3238. It displays modest cytotoxicity towards a human leukaemia cell line (K-562) and has a broad-spectrum antibiotic activity. [34]



16

Streptomycetes are not only well known to produce anticancer products, certain species are also valuable because they produce most of our commercial antibiotics like erythromycin (**17**), which was isolated 1949 from *Streptomyces erythreus* and first introduced in the market in 1952 under the brand name Ilosone™. It has a broad-spectrum antimicrobial activity similar to or slightly better than that of penicillin (**1**), [35] the first ß-lactam antibiotic. Another example is the natural product derived from *Streptomyces roseosporus*, daptomycin (Cubicin™) [36] (**18**). This was the first lipopeptide agent to be released onto the market and has a spectrum of activity which is limited to Gram-positive organisms, including a number of highly resistant species (MRSA, VISA, VRSA, VRE) [37] and it appears to be more bactericidal than vancomycin.



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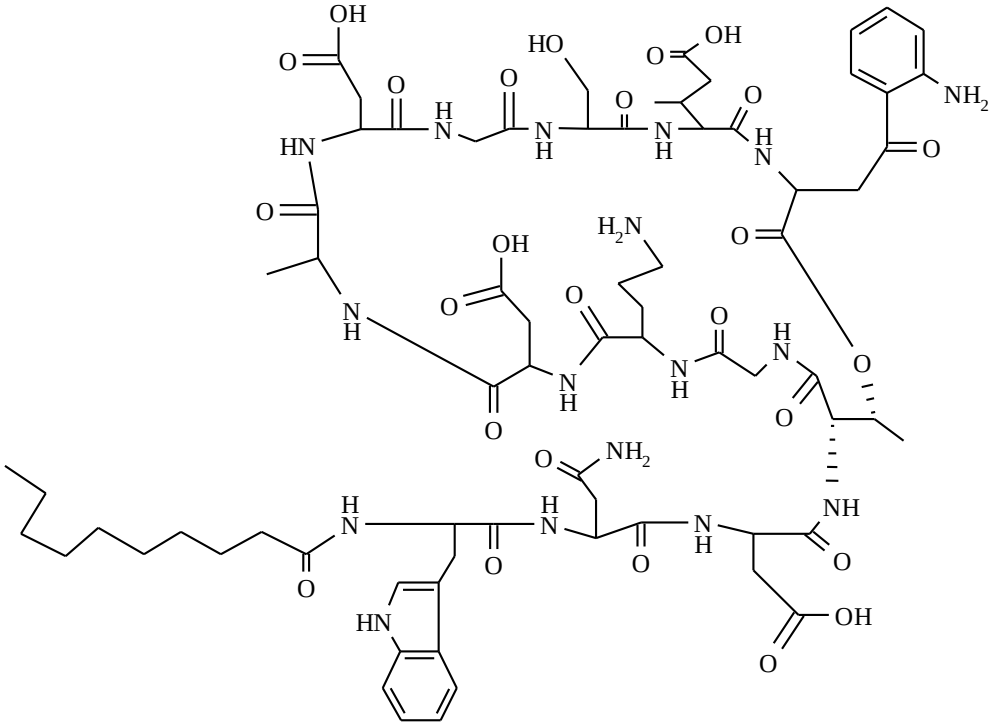
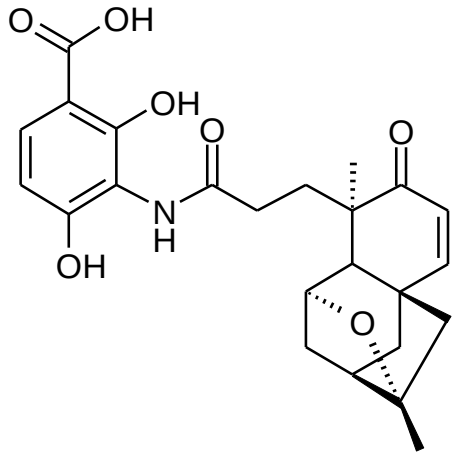


18

Until now only very view classes of antibiotics (mainly ß-lactams, glucopeptides und tetracyclines) have been marketed. [38] Moreover, there is an alarming scarcity of new antibiotic classes in the pipeline of the pharmaceutical industry. Nevertheless, the microbial natural products remain the most promising source of novel antibiotics, although new approaches are required to improve the efficiency of the discovery process. [38] The impact of microbial biodiversity, the influence of growth conditions on the production of secondary metabolites, [39] the choice of the best screening step

and the challenges faced during the isolation and identification of the active compounds must be taken into account before investigation. Furthermore, difficulties can be approximately solved by the application of biotechnology, medicinal chemistry, combinatorial chemistry, biosynthesis or feeding experiments. By taking this view, the combination of target-based whole-cell using antisense differential sensitivity assays [40] and biochemical assays of the extract from a strain *Streptomyces platensis* [41] facilitated the identification of a potent and selective molecule called platensimycin (**19**) from a soil sample collected in South Africa.



19

Platensimycin (**19**) possessed a strong, broad spectrum of Gram positive antibacterial activity, no cross-resistance to other key antibiotic-resistant strains, including methicillin-resistant *Staphylococcus aureus*, vancomycin-intermediate *Staphylococcus aureus* and vancomycin-resistant enterococci and showed a higher potent inhibition for FabF/B condensing enzymes.[41] Platensimycin had been discovered independently in our group earlier, but was described only in a thesis.[42]

After optimisation of the cultivation and growth conditions, which vary depending on microbes, further screening methods were discovered like high-throughput genome scanning which allowed the discovery of clusters of genes that encode bioactive compounds, [43] combinatorial biosynthesis like modification of the PKS cluster through introduction of different loading domains that specified a side chain, [44] complete the natural product chemistry. The application of these discovery methods revolutionized natural product discovery so that it is today no longer impossible to generate derivatives for a bioactive natural product by biological methods.

2 The Aim of the Work

The improvement in natural products chemistry is the basis of new medical applications. This aim can only be reached after several steps including isolation and structure identification, which are preconditions in natural product chemistry. To contribute further results in this field, the present work is divided into two parts:

- The isolation and structure elucidation of new and preferably biologically active secondary metabolites from marine and terrestrial bacteria. To enable this, several steps needed to be completed such as biological assays (mainly based on agar diffusion assays for antibacterial, antifungal and antialgal activity in addition to the toxicity test based on brine shrimps) and the chemical screening, which mainly uses TLC staining with different reagents like anisaldehyde, Ehrlich's reagent or HPLC in combination with hyphenated techniques in order to estimate qualitatively and semi-quantitatively the metabolic pattern.
- The completion of our HPLC-UV-ESI MS/MS database with all compounds previously isolated in our group, so that the dereplication of known as well as new metabolites from crude extracts of bacteria can be sped up in the future.

Within the chemical screening, the use of HPLC-UV/VIS MS/MS data results in and delivers supplementary information that supersedes the results from TLC investigations. Further use of HPLC-UV/VIS-ESI-MS/MS data for the quick identification of new and/or already known compounds saves time, money and efforts during the isolation process (Figure 3). This screening method is not only suited for the rapid dereplication of secondary metabolites, but also for feeding experiments and media optimisation.

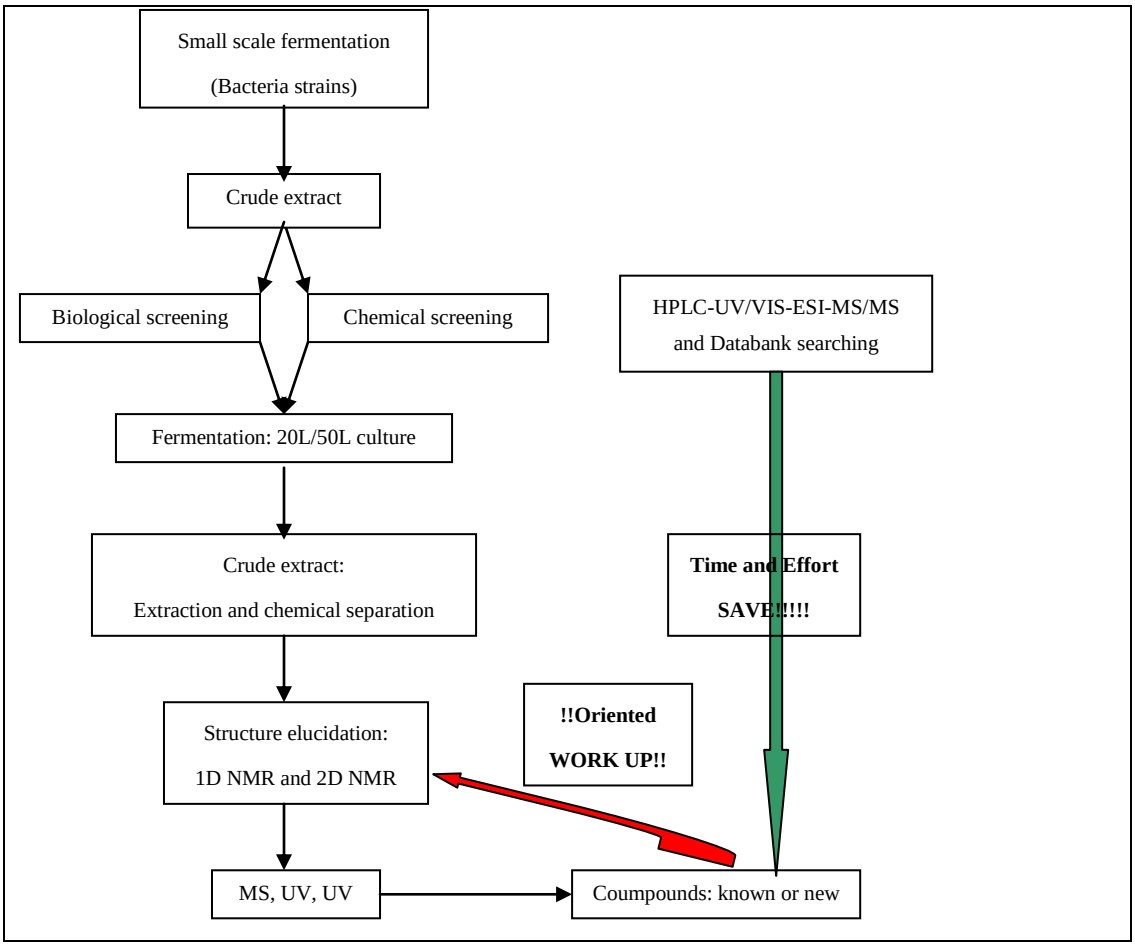


Figure 3: Simplified overview of the data-based dereplication process

3 HPLC-UV-ESI MS/MS-Database

3.1 General

Natural product chemistry is a multiple step process and time consuming, therefore it is very important to find a way to reduce the efforts during this task. In the recent years new techniques have been developed in the natural chemistry such as HPLC MS/MS, which has the capacity to identify compounds from a small sample of complex mixtures. The use of the HPLC MS/MS has increased, so that it is accessible now at many research facilities. In this technique two mass spectrometers are connected in series by a chamber (collision cell) where the molecules are irradiated with a particle beam. Each ion produced in the ion source can then be mass selected and fragmented by CID (Collision-Induced Dissociation) and the fragmentation can be repeated in the same way again (MS/MS^n). Several ion sources can be used: ESI (Electrospray Ionisation) which produces ions from molecules in solution, APCI (Atmospheric Pressure Chemical Ionisation) which uses an electric field with a corona discharge, FAB (Fast Atom Bombardment), LSI (Liquid Secondary Ion) and MALDI (Matrix Assisted Laser Desorption Ionisation). The principles of these sources are based on the secondary emission and are normally used to analyse compounds, which have a high molecular weigh, especially polymers and peptides. The HPLC-MS/MS combination provides a fast method to separate and determine the molecular mass of a compound in a mixture, where each compound provides by fragmentation a characteristic “fingerprint” pattern.

The most commonly used analysers are the triple-quadrupole mass filter, the magnetic sector and the electric sector field.

Analysis of targeted compounds in a mixture can be performed with extreme rapidity by MS/MS because of the essentially simultaneous access to the mixture components. [45] MS/MS is particularly useful for the rapid detection of specific components in a complex mixture with minimal sample preparation. The potential of the MS/MS technique for structural elucidation has been explored [46] and the best method of ionisation depends upon the application and type of sample. For the elucidation of the structure of pure compounds, ionisation by electron impact or electrospray ionisation can be the method of choice. ESI provides efficient ionisation for

very different types of molecules including polar, labile, and high molecular mass drugs and metabolites.

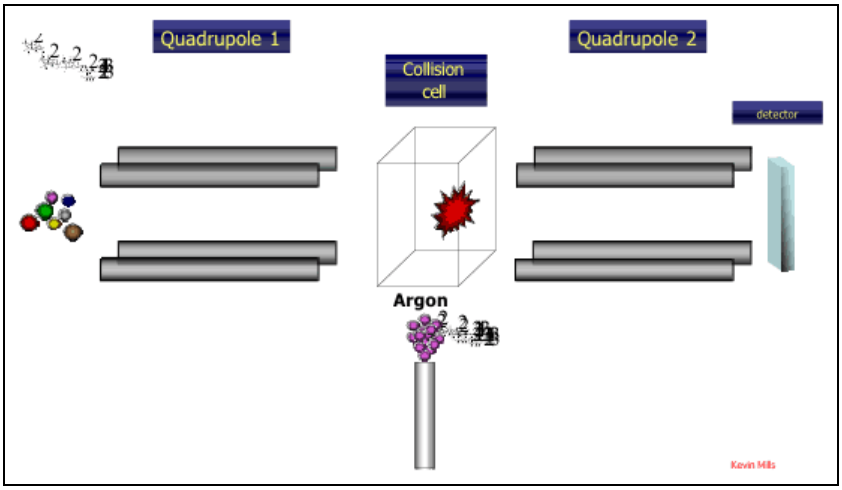


Figure 4: Schematic representation of the Tandem MS/MS [47]

3.2 Liquid chromatography (LC)

In the natural product discovery another goal is the successful separation and purification of complex mixture. This is widely done through HPLC, due the high sensitivity and selectivity, reproducibility and accessibility of this technique. The multiple competences of HPLC distinguish it from other analytical techniques; a chromatogram is defined and simple. Each peak and retention time is characteristic of a component; each chromatogram is diagnostic of an event or experiment associated e.g. with a drug development or a fermentation process. So-called hyphenated techniques, i.e. the coupling of chromatographic principles with spectroscopic investigation methods, like LC NMR, LC-CD, LC UV/VIS MS, [48] GC MS as well as LC-ESI MS [49] enables the creation of multidimensional substance databases and contributes therewith considerably to a simplification of the dereplication process. The selection of an LC method depends on the complexity of the sample matrix and also on the specificity of the mass spectrometric detection method. [50] In addition to appropriate sample preparation, good chromatographic performance is often required for sufficient specificity of the LC/MS analysis. In order to improve and/or to accelerate the identification of compounds, most of the methods can be combined and therefore the respective disadvantages cancelled. The combination of the three on-

line coupling methods HPLC MS/MS, HPLC NMR, and HPLC CD enables the determination of the absolute stereostructures of compounds without the necessity of isolation and purification and was for the first time applied in phytochemical analysis [51].

3.3 Development of an HPLC-UV-ESI MS/MS database

High-performance liquid chromatography (HPLC) coupled to electrospray ionisation tandem mass spectrometry (ESI MS/MS) plays an increasing role in natural product analysis, since it permits the fast screening of crude biological extracts for detailed information about metabolic profiles, with a minimum amount of material.[52,53] Electrospray ionisation (ESI) is the most popular technique for this purpose, as it is considered as soft ionisation technique which usually leads to only protonated or deprotonated molecules. HPLC ESI MS represents the combination of three powerful instruments: the HPLC and two mass spectrometers. Retention time information is obtained from the HPLC, and the mass spectrometer provides molecular mass information. If a UV detector is integrated between both instruments (Figure 5), it generates a LC/UV chromatogram, and based on UV responses, compound purity and information on the chromophore can be assessed as well. It is obvious that application of LC UV/VIS MS/MS in the chemical screening would deliver valuable supplementary information.

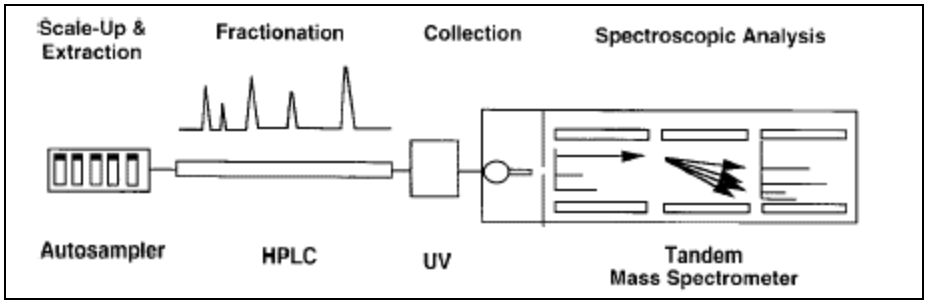


Figure 5: Schematic representation of a tandem LC/MS/MS instrument.

The use of LC/MS and LC/MS/MS techniques can rapidly provide structural information of unknown trace components found in production batches. Another application of the LC/MS and LC/MS/MS technique is the identification of metabolites including reactive species in drug metabolism studies due to their sensitivity and flexibility.[54] Tiller *et al*. have demonstrated an analytical strategy with on-line

LC/UV/MS and LC/MS/MS to rapidly obtain structural information for leaches from a drug-delivery device.[55]

3.3.1 Sample preparation

Sample preparation is still a topic of high importance when an LC/MS/MS method is developed to assay biological material. In addition, the method's performance should remain reasonably consistent over time. The results should be relatively free of systematic errors; any relative error should be characterized and consistent and meet acceptability guidelines for the method. Therefore, sample preparation is used to ensure that a method maintains certain basic elements of ruggedness and consistency that are expected in any assay. Generally a concentration of 1 to 5000 ng / mL is needed. The solvent of preference is methanol.

3.3.2 Chromatographic and Mass Spectrometry Conditions

For the chromatographic separation, two HPLC columns with different adsorbents and sizes from two different manufacturers were compared (RP-C12- column, 150 mm, Phenomenex: RP-C18 silica gel column, 125 mm, 2μm, Macherey & Nagel). Despite of their similar retention behaviour, RP-C12 was preferred, as it possessed a slightly higher number (25% more) of free silanol coverage, which resulted in better separation characteristics, especially for basic and tailing compounds. Because of all these advantages it was selected for the creation of the spectra library (HPLC-UV-ESI-MS/MS).

As a mobile phase, a binary methanol/water gradient with 0.05% formic acid (to increase the sharpness and quality of the peaks) was used. At t = 0, a water/methanol 9:1 mixture was flushed through the column and increased to 100% methanol within 20 min. At this concentration, the gradient was maintained for 10 min and set down subsequently during 2 min to 10% methanol again and held there for the final 8 min of the run (Figure 6). All separation steps were performed at room temperature; the optimal flown rate was 300 µl / min and a volume of 5 µl was injected on to the analytical column, which was connected directly with the UV/VIS DAD detector operating in a wavelength range of 200 - 800 nm.

Electrospray ionisation (ESI) was applied in positive and in negative mode with an electrospray voltage of 4.50 kV and the dwell time was 50 ms in the full scan and

200 ms in SIM (selected ion monitoring) scan with a 5 ms resp. 3 ms pause between scans. The capillary temperature was set at 220 °C. The source was operated in both ions mode using nitrogen sheath gas at 80 psi. The scan range was from 100 to 2000 amu. Due to fluctuations in mass assignment, a single ion is allowed to have a width of ± 0.5 *m*/*z*. Ions also have a width along the time axis corresponding to chromatographic peak width. In most cases the *quasi*monomer ion is given as $[M+H]^+$, $[M+Na]^+$, $[M-H]^-$ as well as the dimer ions $[2M+H]^+$, $[2M+Na]^+$ and $[2M-H]^-$, $[2M+Na-2H]^-$ etc.

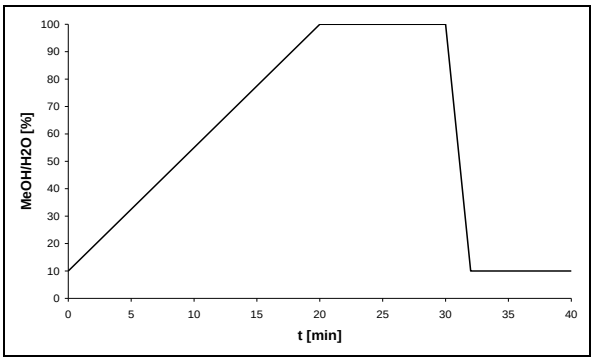


Figure 6: Applied methanol/water gradient for ESI MS/MS measurements.

Sometime the signal intensity of the dimer ions was too high compared to those of the monomeric ions. To avoid this, several trials were performed relating to the ion source collision-induced dissociation (ISCID), which can allow the dimer to decompose back into the monomer, and at the end a collision energy around ISCID: 10V was favoured because of the stability of different molecules.[56]

3.3.3 ESI MS/MS-Method

The utilisation of HPLC with collision induced dissociation or normalized collision energy (CID) has opened further dimensions in the field of mixture analysis. CID refers to the process by which the translational energy of an ion accelerated towards a neutral target species is partitioned into internal energy, resulting in the decomposition of the incident ion into fragment ions. This process may be induced inside the ESI source or within the mass analyser region of the instrument to obtain MS/MS or MS^n spectra. Since the fragmentation achieved using these methods will be representative of analysed structure, these techniques can serve as effective tools for the direct on-line elucidation of the structure and was applied here to decrease the

intensity of the dimer ion, with an increase of the monomeric ion. The presence of the dimer ion observed frequently in the mass spectrum led to difficult interpretation of the fragmentation pattern. The first step was therefore a full-scan mass spectrum to acquire data on positive ions resp. negative ions within the scan range. The second scan event was an MS/MS experiment performed by using a data dependent scan on protonated molecules ($[M+H]^+$ resp. $[M-H]^-$) ions of compounds at a collision energy of 35% of the instrument's maximum. The use of CID in combination with HPLC and ESI is used for example to enable the detection of sialyl Lewis antigen on high pmol level of OMD-GP1.[57] The analysis strategy makes use of "data-dependent" analysis, wherein the mass spectrometer first obtains molecular ions using full-scan techniques, and makes real-time decisions about MS/MS product-ion spectra that must be obtained.[58] In this way, molecular mass, retention time, UV spectrum and substructural information are obtained during a single HPLC run.

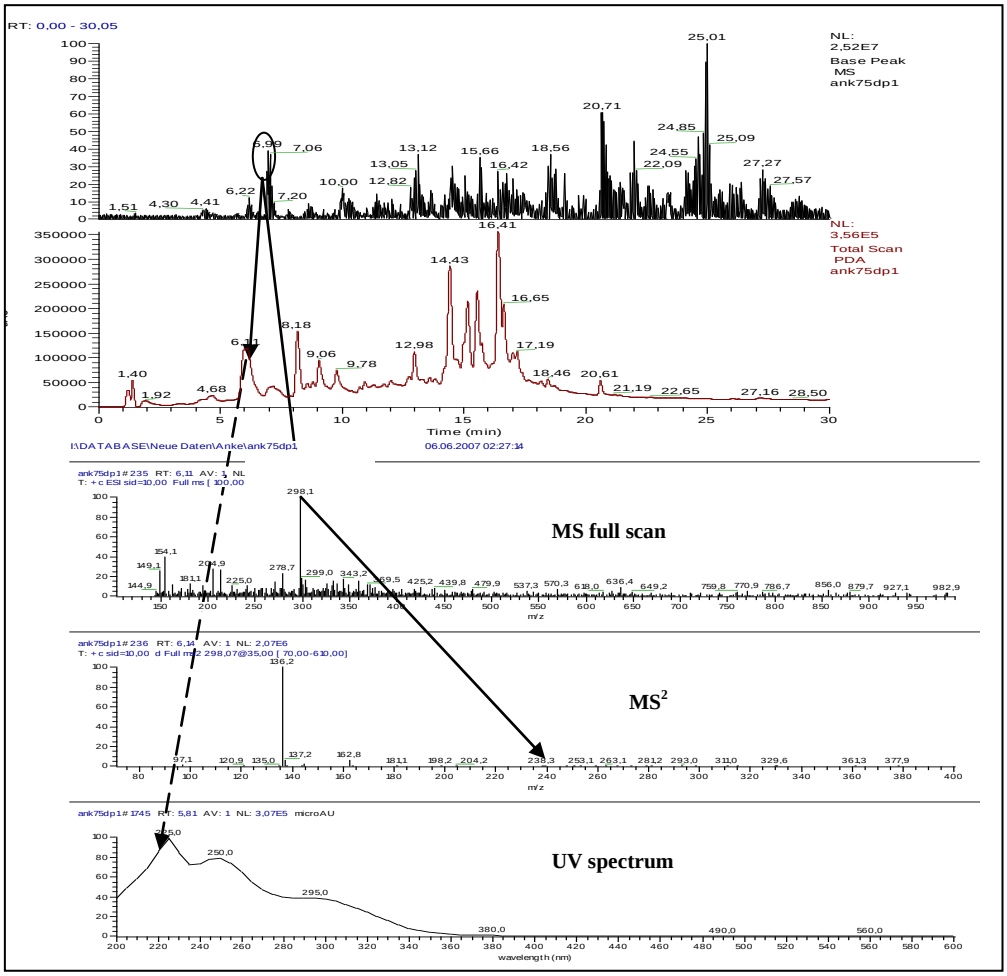


Figure 7: View of a HPLC run in dependent scan mode

3.3.4 Application of the ACD Database

Time and costs are very important in drug discovery. Optimising a dereplication method is always and remains an advantage. Structure databases are generated and provide a quick reference to proposed structure, retention time, molecular mass, and UV properties. The databases provide a comprehensive approach to organizing structure information and the basis for comparison. In this way, LC/MS methods are used during the later stages of drug development to rapidly generate information in support of preclinical development and to provide valuable information in support of registration activities.[59]

The ACD/MS Manager program, which was develop from ACD\Labs, offers different possibilities such as the development of personal databases depending on special requirements. The software can process in its entry-level configuration normal mass spectra (MS), tandem mass spectra (MS/MS, MS/MS/MS, MS^n) or hyphenated data sets (LC/MS, LC/MS/MS, LC/DAD, CE/MS and GC/MS).

HPLC-UV-ESI MS/MS database is a rapid identification method for handling large numbers of crude extracts. Although a modest amount of time and resources is required to implement this strategy, this database has two important benefits:

- Firstly, it provides a user-friendly format to search data. This feature is essential for the rapid identification of known compounds and requires only retention time, UV and molecular mass information *via* LC/MS analysis.
- A second benefit is the efficient extraction of information. The database may be "mined" to detect spectra that may not be noticed otherwise.

In our research group we possess around 1000 compounds, which were isolated in the past from microorganism, especially from bacteria. To build up the database, both crude extracts and pure substances were measured. To further improve the search, the following data were registered:

- The monomeric and dimeric molecular ions as well as the corresponding MS/MS fragmentations.
- The corresponding UV spectra.

The measurement conditions were already mentioned above. To date, 601 pure substances and some crude extracts were recorded in the database.

3.3.5 Search in the database

In the following chapters, the dereplication process by HPLC MS/MS is described step by step. Firstly, the mass spectrum should be imported from Xcalibur into the MS-manager, and then analysed by using image analysis techniques. The data are reduced to a list containing the *m/z* value and retention time of each ion.

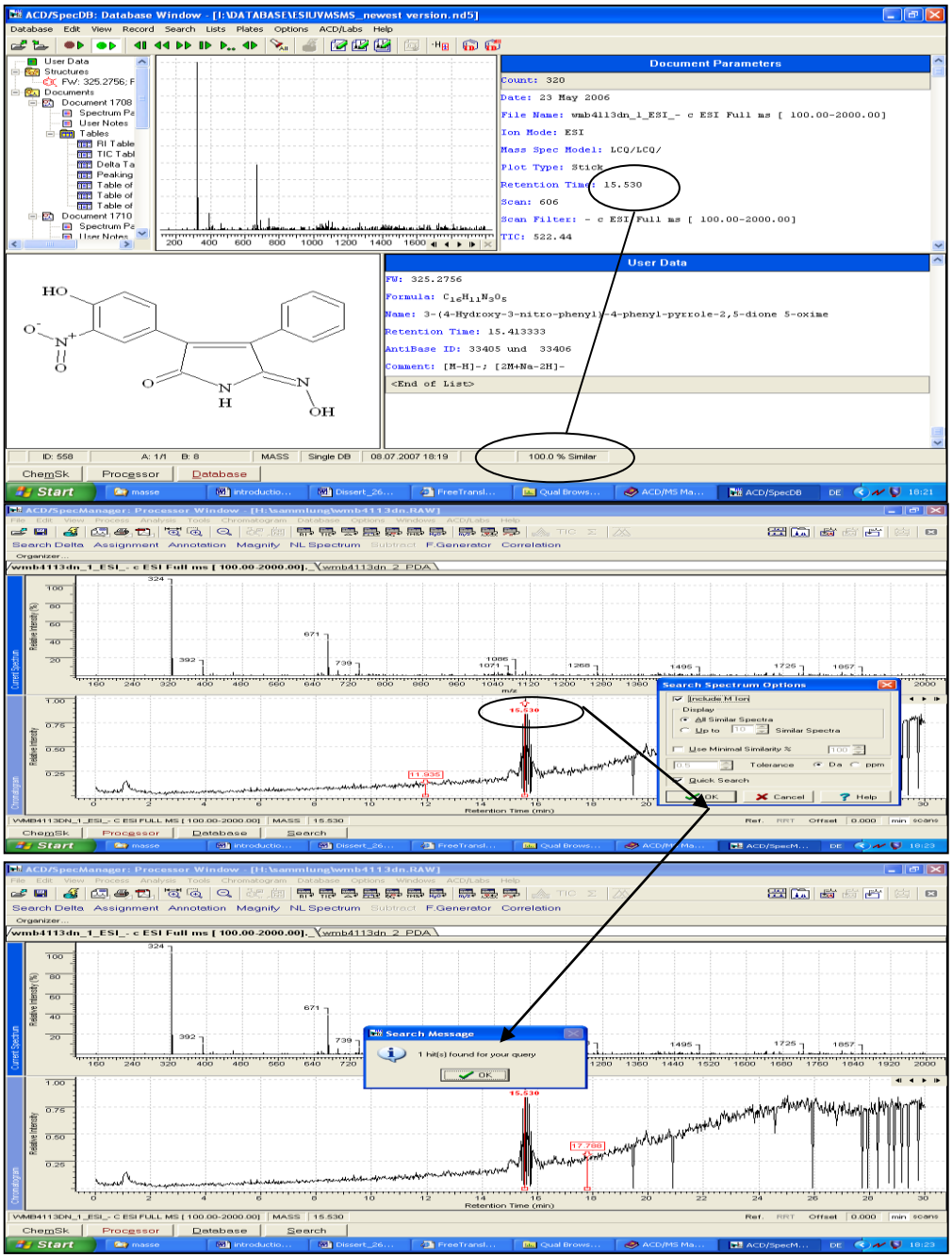


Figure 8: Search of aqabamycin in the ACD-database (the same spectrum gives as expected 100 % similarity)

The ion lists or the spectrum are then compared in pairs to compute a sample similarity index between two spectra: this index is based on the number of ions common to both samples and is scaled from 0 to 1 (100%). Due to the specificity of the fragmentation patter, is to be expected that identity of same samples will give a similarity of 100 %. It is to be noticed, however, that the ionisation gives different *quasi*moleculare ions such as $[M+H]^+$ and $[M+Na]^+$, and sometimes the intensity of the dimer peak and the background is higher than that of the monomer ion examined. Comparison of both spectra from pure samples indicates that the similarity index should be higher than 0.5 for identical samples (Figure 8).

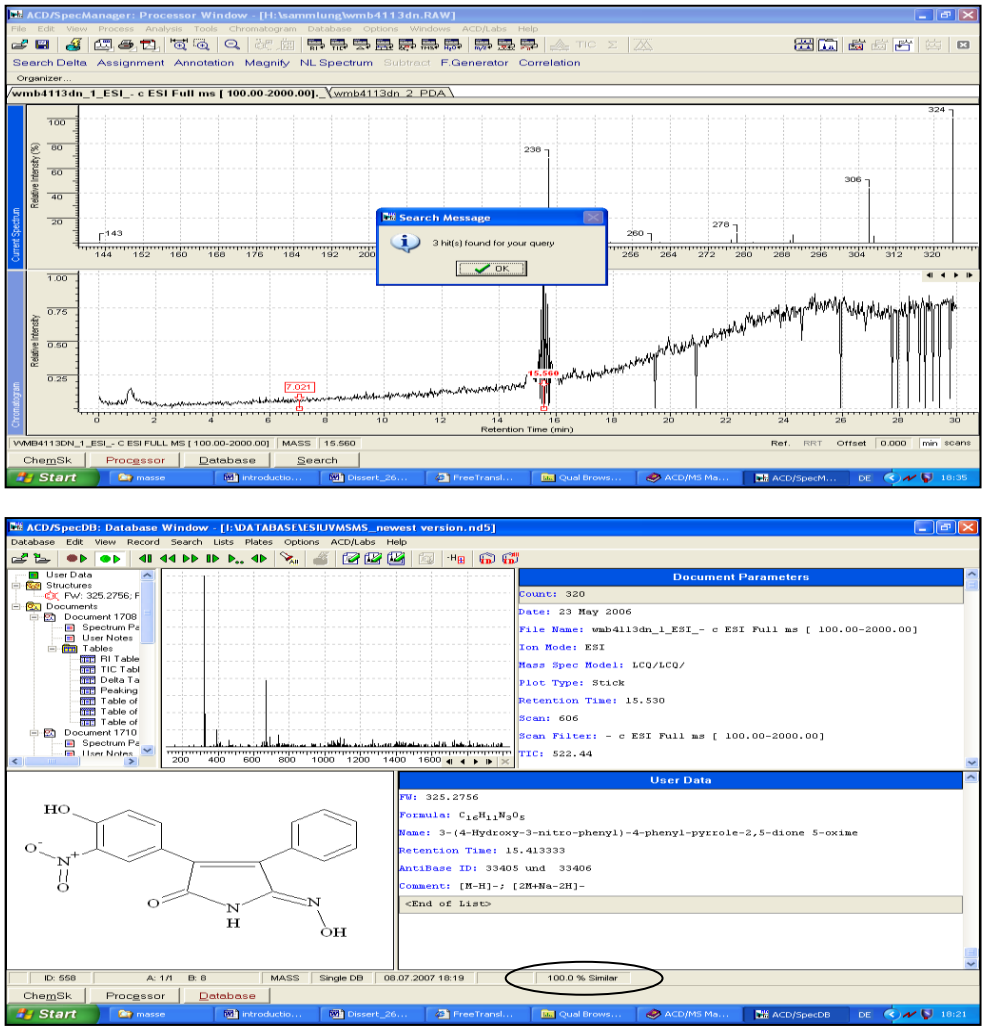


Figure 9: The use of the ACD-database for dereplication

By searching which another spectrum, 3 hits with different similarities of 100, 17.50 and 16.16% were found, where that of 100% was again that of aqabamycin.

The information gained by the ACD database can also be used to judge the chemical diversity of natural extract samples, which is one approach to determining the quality of libraries being used for drug discovery *via* high throughput screening. The combination of the received data led in most cases to a quick identification of the single substances by means of the HPLC-UV-ESI MS/MS (Figure 9).

For the dereplication of crude extracts, the same conditions were used for the measurement as for pure compounds. All signals must be examined, even if it is not easily discernible from the background in the mass chromatogram: It is then better then to enlarge the chromatogram, as the intensity of the main compound in the crude extract can decrease the signal of the trace components. For a compound, which cannot be detected by ESI, the available UV chromatogram can be helpful for supplementary information by searching in another database.

The importance to include also crude extracts into the database is to limit the repetitive fermentation of similar or even identical bacterial strains. All occurring signals, if not already existing in the database, must be included, so that identification of metabolites (similarity factors around between 50 - 100%) in a second crude extract should indicate the similarity of these extracts and may so be used for their dereplication.

If a substance cannot be identified, this may have various reasons like:

- Not yet included in the database
- The compound can not be ionised by ESI and therefore no spectrum is obtained
- The peak intensity of the dimers and/or its isotopomers is in spite of the related collision energy (ISCID) substantially higher than that of the monomers
- Finally, the substance may be unknown until now

To confirm this statement, the UV absorption and the molecular mass must be checked and afterwards searched in furthers databases like AntiBase, the Chemical Abstracts, or the Dictionary of Natural Products.

4 Investigation on selected strains

4.1 General techniques

4.1.1 Collection of strains

The strains for this investigation were obtained *via* cooperations with various microbiological groups. The terrestrial strains with code names AdM 02 were isolated by Prof. H. Anke from soil samples provided by Prof. A. de Meijere. Streptomycetes with numbers like B8722 were obtained from the strain collections of E. Helmke at the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven, and strains with code named GW14/1869 came from the Laboratory I. Grün-Wollny, respectively. Further strains were isolated from the Mediterranean Sea and the Red Sea. The organisms were described at the beginning temporarily by colour, morphology, the presence of mucus etc. The precise taxonomy will usually be determined later.

4.1.2 Pre-screening

From the received strains, 30% were usually able to produce metabolites with bioactivity or other interesting properties. To select these strains, a so-called pre-screening was 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 are then used to inoculate 1-L Erlenmeyer flasks containing 250 ml medium, followed by incubation on a rotary shaker at 28 °C. The culture broth is 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-UV-ESI MS/MS measurements as described above.

4.1.3 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 us to reach this aim at the earliest stages of separation, and is therefore economically very important.

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 reproducible. A spot of the crude extract is separated by TLC with a mixture of e.g. CH_2Cl_2/MeOH. The developed TLC plate is visualized under UV light and interesting zones are further localized by exposure to spray reagents. In our group, anisaldehyde/sulphuric acid, Ehrlich's reagent, sulphuric acid and 2N NaOH are the most widely used ones.

- Anisaldehyde/sulphuric acid gives different colour reactions with many structural elements (glycosides, steroids, terpenes, macrolides and phenols).
- Ehrlich's reagent is a specific reagent used to determine indoles and some other nitrogen containing compounds; indoles turn pink, blue or violet, or brown for pyrroles and furan. Anthranilic acid derivatives change to yellow.
- 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 a colour change to yellow with base.
- Tin(II)-chloride/hydrochloric acid/ 4-dimethylamino-benzaldehyde is used for nitro compounds and gives yellow to deep yellow or orange spots. This reaction uses the reduction of the nitro group to the amino group and the formation of Schiff's bases.

4.1.4 Pharmacological and Biological Assays

It is evident that in order to screen a crude extract for bioactive substances, an appropriate biological test is needed. In that case, all bioassays should have high capacity, sensitivity, low cost, and must give rapid answers. In our group the crude extract is screened using the agar diffusion test with a few Gram-positive and Gram-negative bacteria and fungi such as *Escherichia coli*, *Streptomyces viridochromogenes (*Tü 57), *Bacillus subtilis, Staphylococcus aureus*, *Mucor miehei (*Tü 284), *Candida albicans.* The microalgae *Chlorella vulgaris*, *Chlorella sorokiniana, and Scenedesmus subspicatus* are used as test organisms to screen for phytotoxicity. In parallel, the cytotoxic activity was evaluated against brine shrimps (*Artemia salina*) and nematodes (*Caenorhabditis elegans*). 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), Oncotest (Freiburg) and later at the Institute of Biotechnology and Active Agent Research (Kaiserslautern).

4.1.5 Cultivation and scale-up

The cultivation and scale-up steps are carried out only after both primary screenings. 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 always 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 flasks or in a fermentor. A pre-culture of 2 L is to be used for the inoculation.

After harvesting, the culture broth is mixed with Celite and filtered under pressure. The water phase, which contains highly polar compounds like sugars, certain polyhydroxy acids, amino acids and many peptides can be submitted to extraction with ethyl acetate. However it is highly recommended to use a solid phase extraction with special adsorber resins (mostly Amberlite XAD-16 or Mitsubishi DIAION

HP20) due to the fact that is not harmful and reduces considerably the costs for solvents, than the extraction with a solvent of higher polarity like water-saturated ethyl acetate or even methanol. The mycelium is extracted with ethyl acetate and acetone. The organic phases are evaporated to dryness and the remaining crude extract used for separations.

4.1.6 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 rates 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.

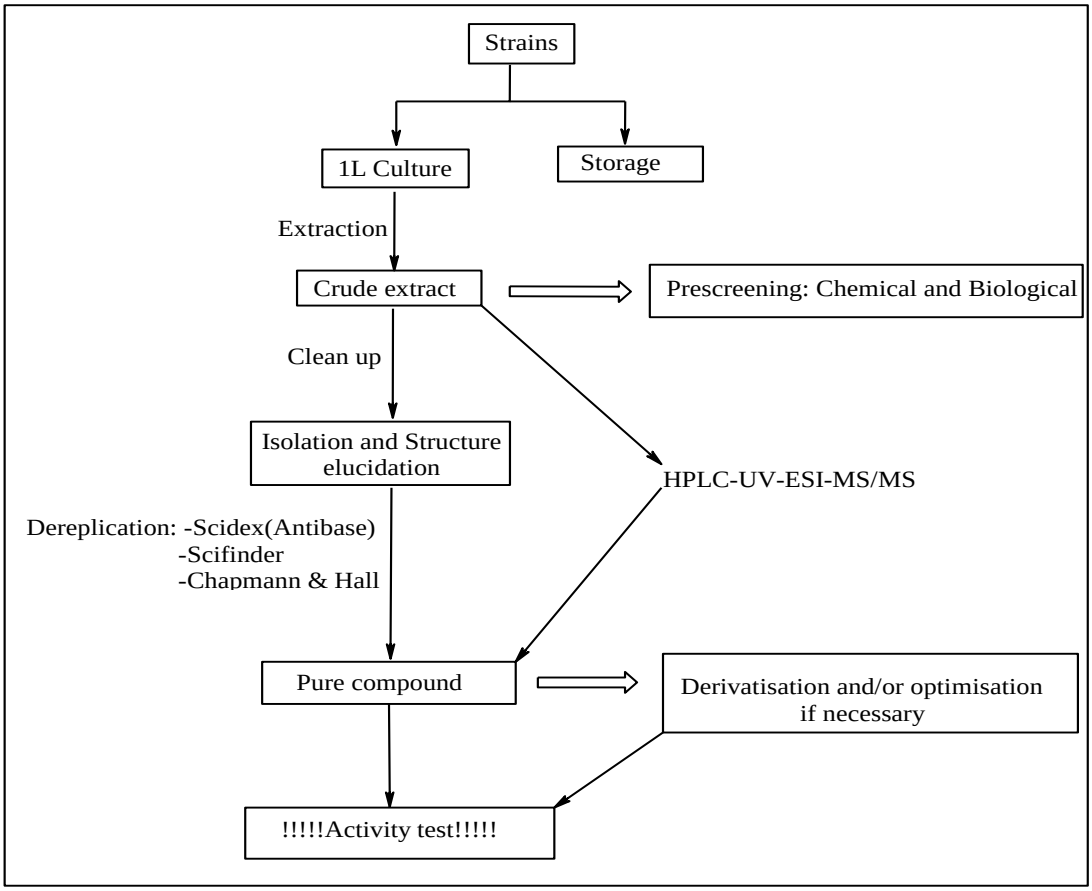


Figure 10: General screening of the selected strains

5 Some Strains from Marine and other Origins

5.1 *Pseudoalteromonas* Strain T 268

The strain T268 was isolated from the intestine of the Antarctic krill *Euphausia superba;* it was chosen because of its high antibacterial and antifungal activities. The culture on M1 agar plate showed transparent, light brown colonies. The cultivation of the strain, followed by work-up and purification was done at the Institute for Biotechnology and Active Agent Research (Kaiserslautern). 5 pure compounds were obtained for analysis.

5.1.1 3-Methylthiopropanoic acid

Compound **20** was isolated as colourless oil. It was UV active at 366 nm and showed on TLC after spraying with anisaldehyde/sulphuric acid no colour reaction. The ^{1}H NMR spectrum exhibited one broad signal at δ 10.15, no signals were seen in the aromatic range, two triplets integrating each two protons at δ 2.65 (J = 8.3 Hz) and 2.51 (J = 8.3 Hz) attributed to two connected methylene groups and one singlet at δ 2.05 were present in upfield region.

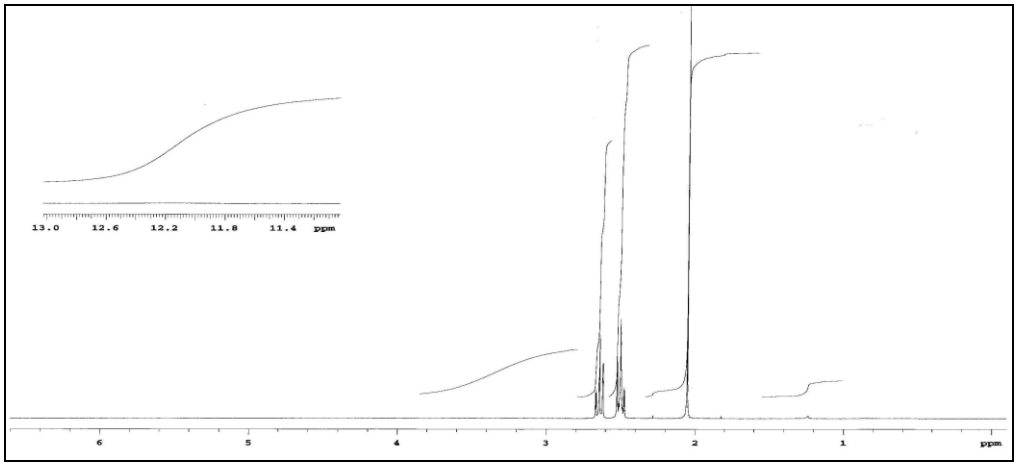


Figure 11: ^{1}H NMR (300 MHz) spectrum of 3-methylthiopropanoic acid (**20**) in DMSO-d_6

The ^{13}C NMR spectrum showed only four signals: a carbonyl at δ 172.9, two methylene carbons at δ 34.0, 28.5 and a methyl at δ 14.6. The search in AntiBase proposed 3-methylthiopropanoic acid (**20**). The complete agreement of the spectroscopic data with reference values available in our team confirmed this suggestion.

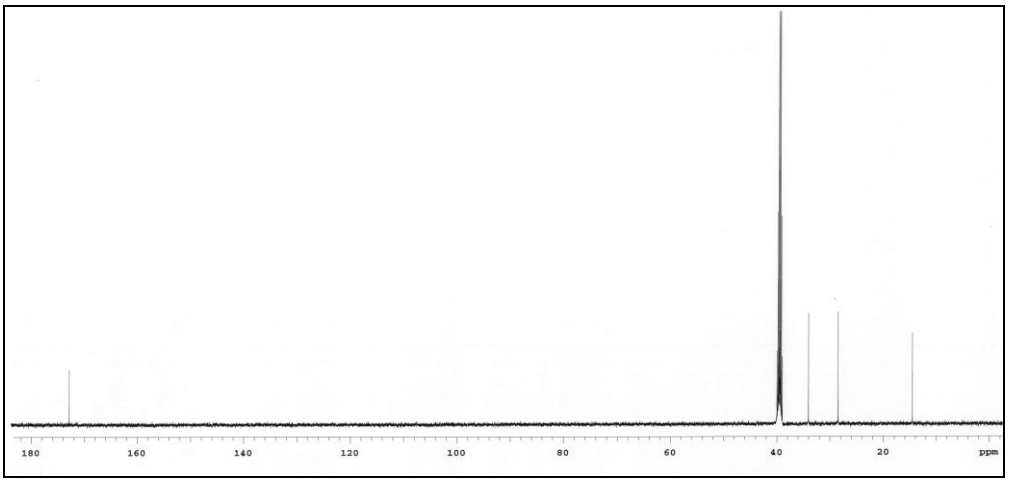
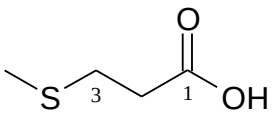


Figure 12: ^{13}C NMR (125 MHz) spectrum of 3-methylthiopropanoic acid (**20**) in DMSO-d_6



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3-Methylthiopropanoic acid (**20**) is a product of the demethylation of dimethylsulfonium propionate (DMSP), which after cleavage to dimethylsulfoxide (DMSO), plays an important biogeochemical role in the global sea-to-land transfer of sulphur.[60] The biosynthesis of the 3-methylthiopropanoic acid (**20**) occurs through the transamination and subsequent decarboxylation of the keto acid of methionine.[61] 3-Methylthiopropanoic acid is the reduced form of the 3-methylthioacrylic acid, which has already been isolated from the same strain cultivated in different media. In the year 1970 Arima *et al.* showed that both compounds can be isolated from *Streptomyces* incubated in a medium containing DL-methionine.[62]

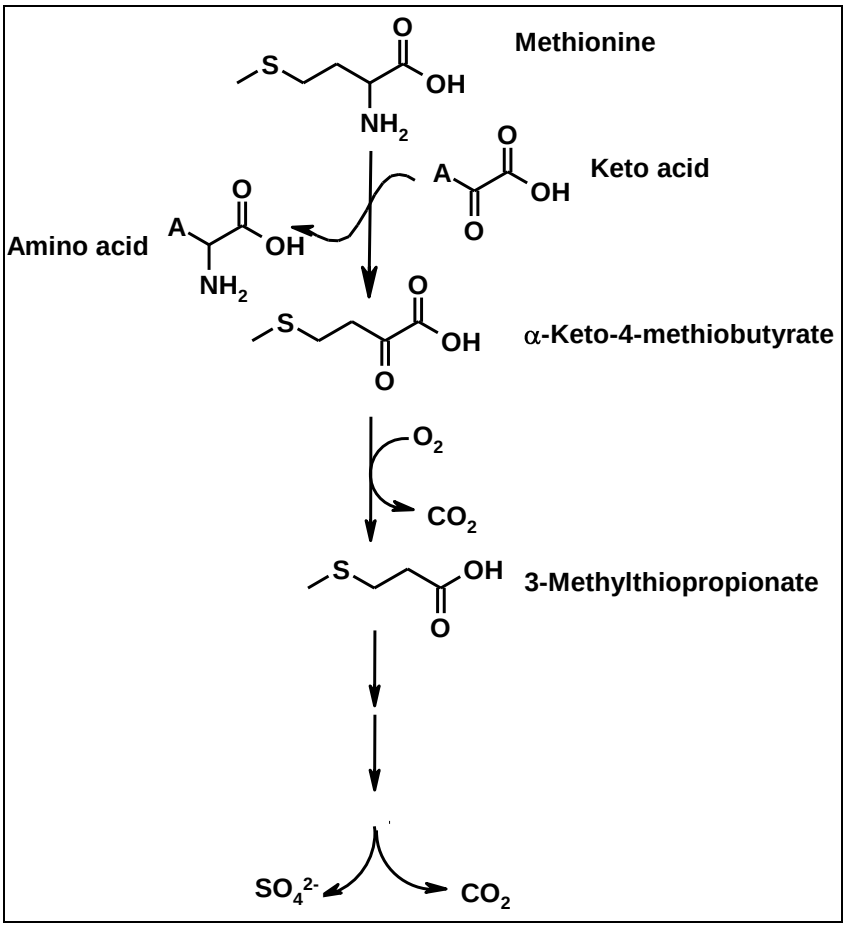


Figure 13: Metabolic path for the catabolism of methionine *via* transamination [61]

3-Methylthiopropanoic acid (**20**) was the only substance from this strain that showed a weak nematicidal activity against *Meloidogyne incognita* with LD50 of 50 µg/ml and LD90 of 70µg/ml, but *Caenorhabditis elegans* was not affected up to 100 µg/ml.

5.1.2 Homogentisic acid and Homogentisic acid methyl ester

Compound **21** was separated as white substance. The polar compound gave an uncommon green colour reaction with anisaldehyde/sulphuric acid, and EI MS indicated a molecular ion at 168 Dalton. The search in AntiBase with the mass delivered 27 hits and the search in the MS Nist Database gave 3 hits. The proton NMR spectrum indicated in the aromatic range two broad signals at δ 10.51 and 8.56 attributed to two exchangeable protons, two doublets at δ 6.58 and δ 6.53 and one doublet of doublet at δ 6.46 for a 1,2,4-trisubstituted benzene moiety. In the aliphatic range one

singlet at δ 3.77 can be assigned to a methylene group. The substructure search in AntiBase with the ^{1}H NMR spectrum and the mass indicated 3 hits.

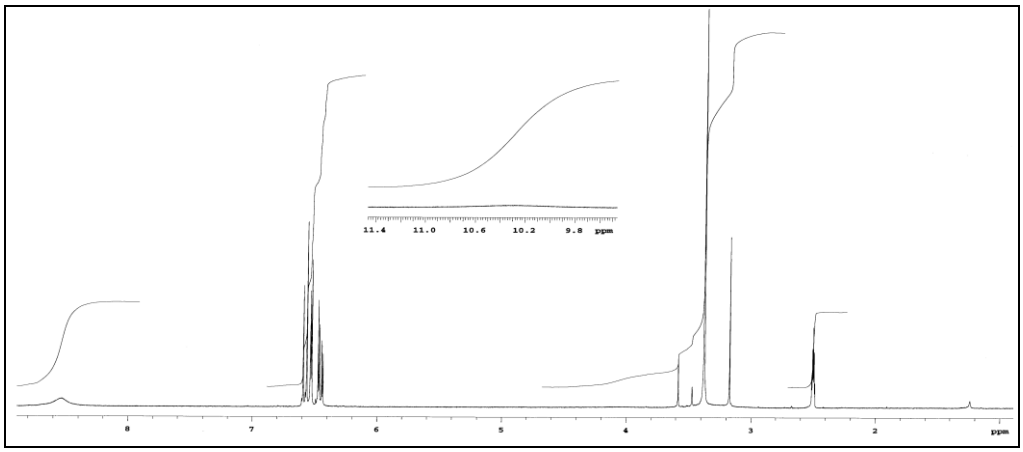
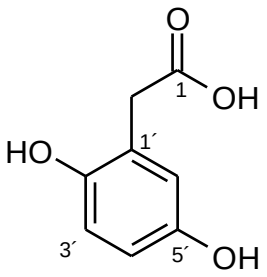


Figure 14: ^{1}H NMR spectrum (300 MHz) of homogentisic acid (**21**) in DMSO-d_6

Comparison of the proton NMR spectrum with theses hits matched with homogentisic acid (**21**).

Homogentisic acid is a derivative of the gentisic acid (**22**) and is formed as intermediate in phenylalanine or tyrosine metabolism. [63] It is reported that the presence of homogentisic acid (**21**) in the urine is a symptom of the illness Alkaptonuria (black urine disease), a rare inherited genetic disorder of tyrosine metabolism. [64]



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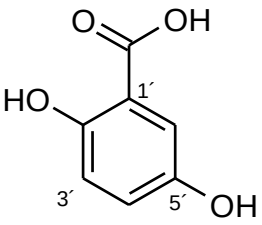

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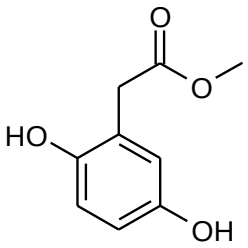
Obtained as white powder, compound **23** indicated in the EI MS a molecular ion at *m/z* 182. Its high-resolution delivered the molecular formula $C_9H_{10}O_4$. The Δm = 14 between **23** and **21** was attributed to a methyl group and suggested that compound **23** is just the methyl ester of **21**, i.e. methyl homogentisate. It was first obtained by hydrolysis with methanol in hydrochloric acid from Phaseoloidin, which was isolated from the seeds of the plant *Entada phaseoloides*. [65]



23

Homogentisic acid (**21**) exhibited phytotoxic activities on the root of the monocotyledonous and dicotyledonous seeds with 333 µg/ml and 67 µg/ml, respectively.

5.1.3 Euphamycin A

The electron impact (EI) mass spectrum showed a molecular peak at *m/z* 306 for the light pale yellow oily compound **25**. The HREIMS delivered the molecular formula $C_{11}H_{14}O_4S_3$. The ^{1}H NMR spectrum displayed one broad signal at δ 8.38, one singlet at δ 3.86 with an intensity of two protons and three other singlets integrating for three protons each at δ 2.36, 2.32 and 2.25 suggesting that they may be aromatic methyls or *S*-methyls, as the chemical shift of *O*-methyls is in the range of 3.00 and 4.00.

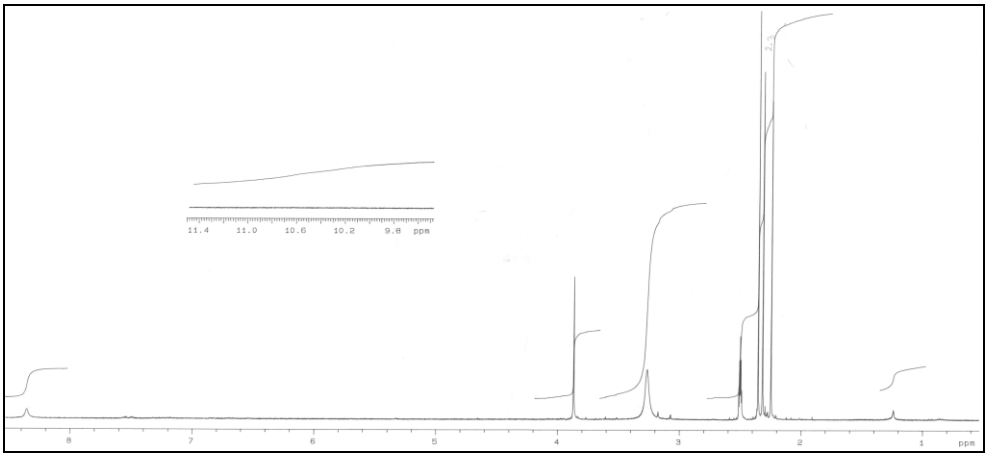


Figure 15: ^{1}H NMR spectrum (600 MHz) of euphamycin A (**25**) in DMSO-d_6

The ^{13}C NMR spectrum of compound **25** exhibited one carbonyl carbon at δ 172.2, six quaternary carbons at δ 127.8, 126.6, and 124.5 (2 C) including two carbons bearing oxygen at δ 151.8 and 149.6. Additionally, one methylene group at δ 35.2 and three methyl signals at δ 18.8, 18.6 and 17.6 were observed.

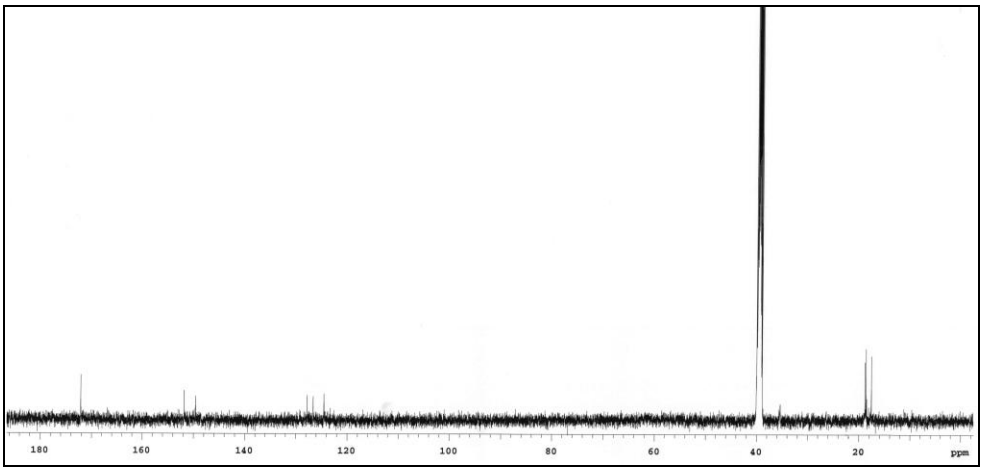
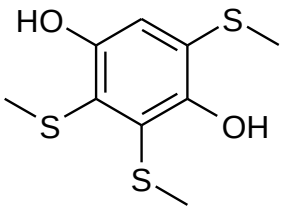


Figure 16: ^{13}C NMR spectrum (125 MHz) of compound **25** in DMSO-d_6

The search in AntiBase, the Dictionary of Natural Product and the Chemical Abstracts by considering the NMR data, molecular mass and formula gave no hits, suggesting compound **25** as a new metabolite. A second search using only NMR sub-structures delivered similarities with compound T-1801-A (**24**), a compound isolated from *Pseudomonas* sp. sc-1801.[66]



24

The 2D NMR experiments, namely HSQC, HMBC and the 1D NOESY were measured. The HMBC spectrum indicated a correlation of the methylene singlet at δ 3.86 to the carbonyl at δ 172.2, the oxygenated sp^2 at ẟ 149.6 as well as to the quaternary carbons at ẟ 127.8 and 124. 6 and gave fragment I.

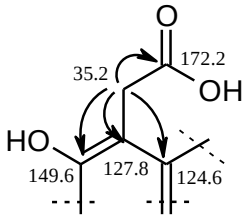


Figure 17: HMBC correlations in fragment I of compound **25**

Comparison of data with those of compound T-1801-A (**24**) confirmed the chemical shifts of the methyl carbons as *S*-methyls; they showed individual long range correlations to δ 124.6, 126.6 and 124.6, respectively.

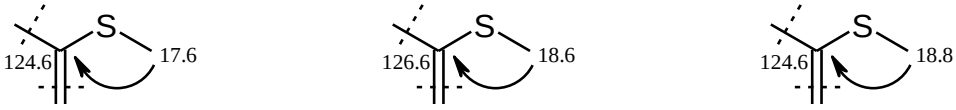


Figure 18: S-methyl fragments of compound **25**

The connectivity between the four fragments was difficult to find out. The interpretation of the 1D-NOESY delivered the following evidences: irradiation of the methylene group at δ 3.86 delivered only one weak NOE effect with the thiomethyl signal at 2.25, so that obviously only one SMe group is in *o*-position to the methylene group; the other *o*-position must be occupied by OH. Similarly, irradiation of the methyl signal at δ 2.25 increased slightly the methylene signal, however, did not influence any of the other two SMe signals: It follows, that an OH group must be next to this SMe group, so that **25** is the only possible structure.

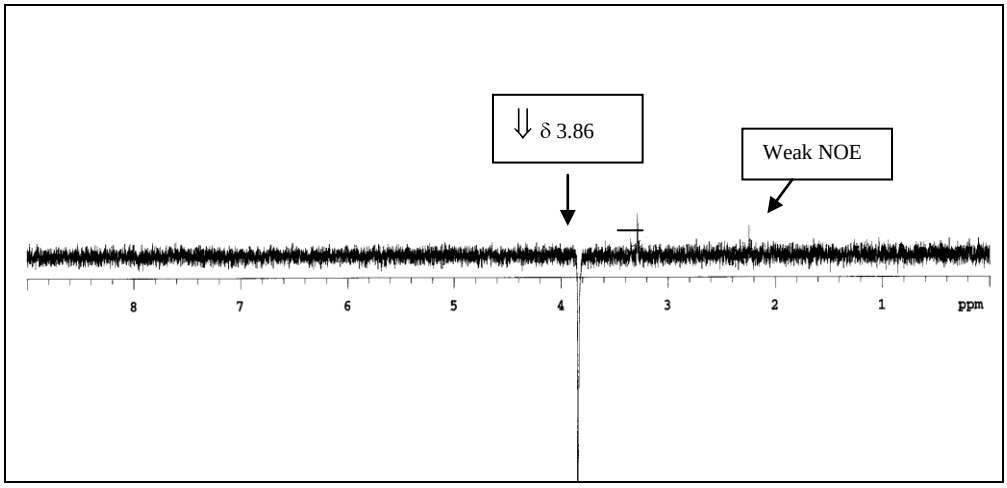
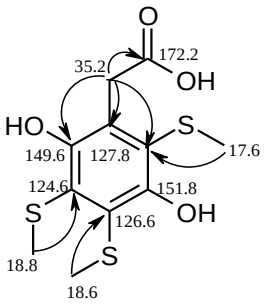


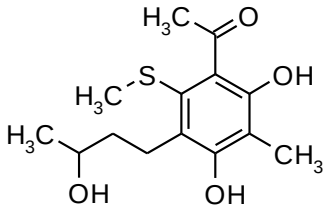
Figure 19: 1D-NOESY spectrum (600 MHz) of the proton at δ 3.86 in DMSO-d_6

This information, in addition to the comparison with data from T-1801-A (**24**) suggested the structure **25** of a tris-thiomethyl-homogentisic acid, for which the name euphamycin A was given. Like the hydroquinone T-1801-A (**24**), which is reported to have antimicrobial and antifungal activities, euphamycin A (**25**) showed activities against *Bacillus subtilis*, *Bacillus brevis*, *Micrococcus luteus*, *Escherichia coli*, *Paecilomyces variotii* and *Phytophthora infestans*. As for the antitumour antibi-

otic resorthiomycin [67] (**26**), it showed also cytotoxic activities against L1210, Jurkat, MDA-MB-321, MCF-7, and Colo-320 cells (Table 26).



25

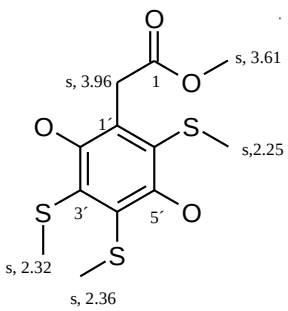


26

5.1.4 Euphamycin B

The temperature and solvent sensitive compound **27** was isolated as pale oil. It showed a UV absorbing spot at 254 nm with gave a yellow colour after spraying with anisaldehyde/sulphuric acid. The EI mass spectrum showed a molecular peak at *m/z* 320 and its high-resolution delivered the molecular formula $C_{12}H_{16}O_4S_3$. The ^{1}H NMR spectrum was very similar to that of compound **27** with the methylene singlet at δ 3.86 and the three *S*-methyls at δ 2.36, 2.32, 2.25. The major difference was the additional singlet of three protons at δ 3.61 attributed to a methoxy group.

The molecular mass of compound **27** in EI spectrum indicated a mass 14 unit higher than that of compound **25** confirming the presence of a methoxy group as indicated in the ^{1}H NMR spectrum. No hits were found in an AntiBase search, consequently compound **27** was deduced as the methoxy derivative of compound **25**; it is also a new natural product, which was named euphamycin B. Due to the small amount and the high sensitivity of the compound, the 2D NMR spectra could not be measured. Euphamycin B showed activity against Gram-positive bacteria.



27

5.2 Strain T48

The TLC of strain T48 extracts indicated distinctive UV active zones, which showed multiple colour reactions with anisaldehyde/sulphuric acid. The biological screening of the crude extract showed antimicrobial activity against different test organisms, and indicated cytotoxicity.

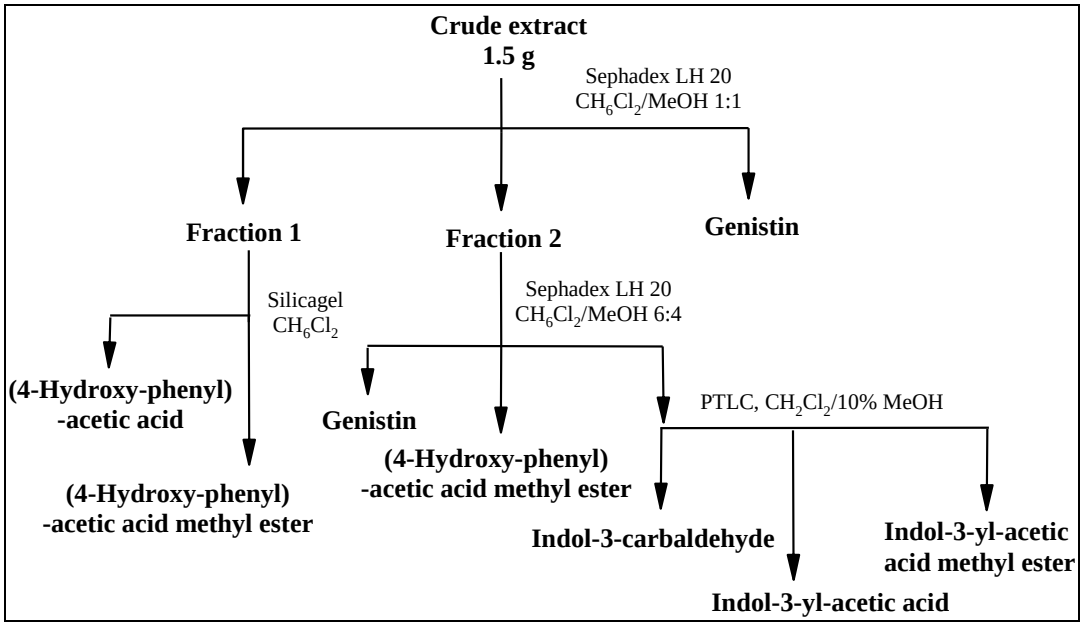


Figure 20: Work-up scheme of the strain T48A

5.2.1 Genistin

Compound **28** was obtained as white to light yellow powder, which was easily soluble in DMSO. ESI MS showed a *quasi*molecular ion at *m/z* 431 $[M-H]^-$. The proton NMR spectrum of the compound measured in DMSO-d_6 exhibited in the aromatic region nine protons among which two were exchangeable and appeared at δ 12.82 for a chelated proton and δ 9.51, one singlet at δ 8.41, two doublets at δ 7.41 (J = 8.8 Hz) and 6.82 (J = 8.7 Hz) each with the intensity of two protons attributed to a *para* disubstituted benzene ring. In addition, two protons in *meta* position at δ 6.72 (d, J = 1.3 Hz) and 6.45 (d, J = 1.3 Hz) were seen. The aliphatic region displayed a set of protons due to a sugar moiety in the range of δ 3.75-3.18, and the anomeric proton signal appeared at δ 5.10 (d, J = 1.8 Hz). Two exchangeable protons at δ 5.38 and 4.58 were also visible.

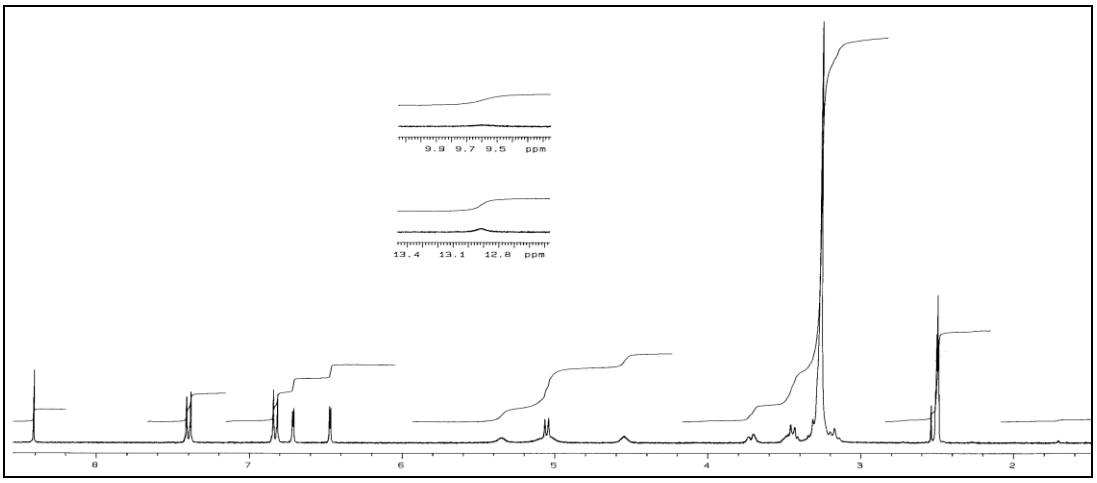


Figure 21: ^{1}H NMR spectrum (300 MHz) of genistin (**28**) in DMSO-d_6

The ^{13}C NMR spectrum of the compound displayed 21 carbons signals, which were sorted as seven quaternary carbon signals including one carbonyl at δ 180.5, seven sp^2 methines, five sp^3 methines and one methylene. From the ^{13}C NMR data two aromatic hydroxyl groups (δ 162.9, 157.4) and two other aromatic sp^2 carbon attach to the oxygen (δ 161.6, 157.2) can be derived.

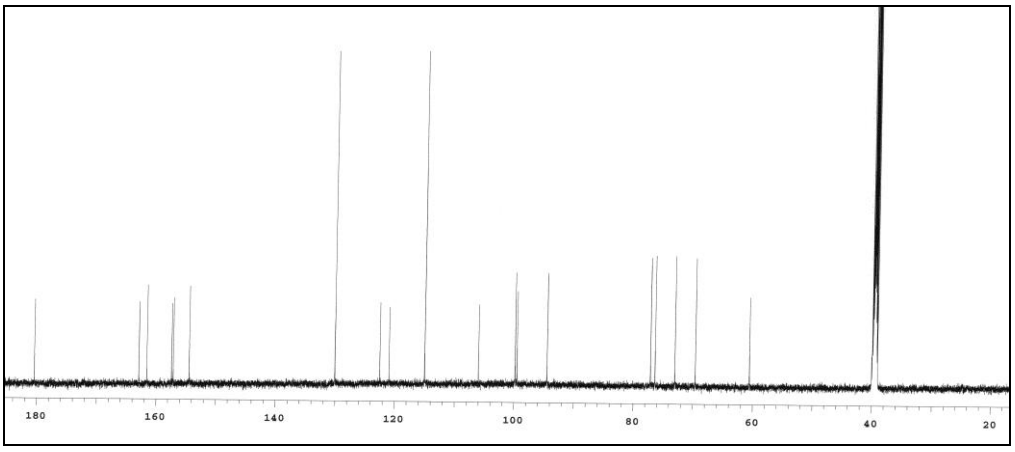


Figure 22: ^{13}C NMR spectrum (125 MHz) of genistin (**28**)

The H,H COSY spectrum confirmed the presence of *meta* and *para* disubstituted aromatic systems suggested by the ^{1}H NMR spectrum.

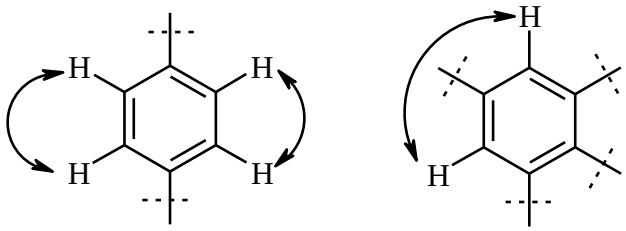
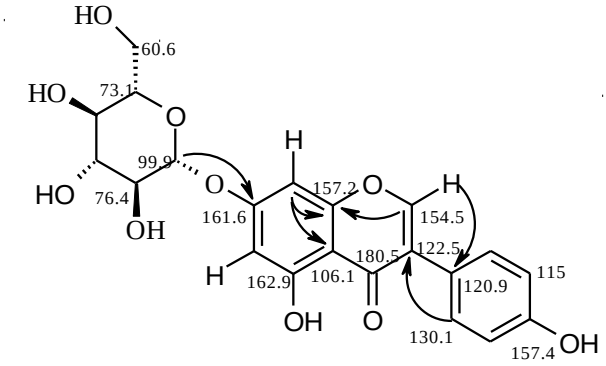


Figure 23: Fragments in genistin (**28**)

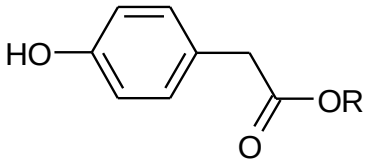
The search in AntiBase, [68] the Dictionary of Natural Products and the Chemical Abstracts, [69] with the mass and the molecular formula delivered three proposals, and compound **28** matched with the data of the isoflavone genistin. This glycoside of genisteine is very predominant in plants. Genistein-7-glucoside is known to stimulate estrogen dependent breast cancer cell growth *in vivo.* [70] Compound **28** might be new from bacteria; however, it is more plausible that it came from the malt extract of the nutrient broth.



28

5.2.2 4-Hydroxyphenyl-acetic acid

The white compound **29a** showed on the TLC a light red colour with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed one broad signal at δ 10.40 for an exchangeable proton, two doublets integrating for each two protons and characteristic of a *para* disubstituted benzene ring at δ 7.10 and 6.72, in addition one methylene singlet at δ 3.40. The search in AntiBase indicated compound **29a** as 4-hydroxyphenyl-acetic acid, a very common substituent in bacteria.



29a: R=H

29b: R= CH_3

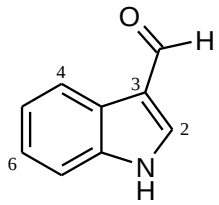
5.2.3 4-Hydroxyphenylacetic acid methyl ester

Compound **29b** was obtained as white solid and showed a red colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed the same signals as for compound **29b** but it differs by the presence of one methoxy signal at δ 4.90 and the disappearance of the broad signal for an exchangeable proton at δ 10.40 in **29a.** The methylene signals were seen at δ 3.65. The ESI MS spectrum gave a *quasi*-molecular ion at *m/z* 165 $[M-H]^-$. The search in AntiBase with the ^{1}H NMR spectrum and the molecular mass proposed the compound as (4-hydroxyphenyl)acetic acid methyl ester (**29b**). The ^{13}C NMR spectrum gave 8 signals and the comparison of the data and the literature confirmed the structure.

5.2.4 Indol derivatives

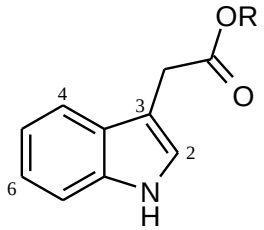
Fraction 2 was chromatographed on Sephadex LH-20 (CH_2Cl_2/40%MeOH) to give 3 subfractions. Subfraction 3 was chromatographed by PTLC (CH_2Cl_2/10% MeOH) and gave compounds **30**, **31a**, and **31b**. Compound **30** exhibited an UV absorbing band, which turned orange with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum indicated a singlet at δ 9.89, which could be assigned to an aldehyde proton, two doublets δ 8.18 and 7.45 with a coupling constant J = 3.0 Hz, one multiplet at δ 7.23, and finally a singlet at δ 8.12, all those four signals were characteristic for an indole moiety substituted at 3-position.

The ESI mass spectrum determined the *quasi*molecular ion at *m/z* 144 $[M-H]^-$. A search in AntiBase fixed the compound **30** as indolyl-3-carbaldehyde, which was finally confirmed by direct comparison with authentic spectra.



30

Compound **31a** showed an UV absorbing band, which turned to orange and violet with anisaldehyde like compound **30**. The ^{1}H NMR spectrum exhibited one singlet at δ 10.50 for the exchangeable proton and, the aromatic displayed five protons at δ 7.54 (d, *J* = 7.8 Hz), 7.38 (d, *J* = 7.9 Hz), 7.22 (d, *J* = 1.5 Hz), 7.08 (t, *J* = 8.1 Hz), 6.98 (t, *J* = 8.1 Hz), the major difference with compound **30** being the presence of one methylene group at δ 3.62. All those signals were assigned to an indole system substituted at position 3 with acetic acid. The search in AntiBase and comparison with reference data identified compound **31a** as indol-3-yl-acetic acid.



31a: R = H

31b: R = Me

Compound **31b** was found to be the methyl ester of compound **31a** based on its ^{1}H NMR spectrum, which indicated as major difference the presence of one singlet attributed to a methoxy signal at δ 3.65. The ESI MS spectrum gave a *quasi*molecular ion at *m/z* 212 $[M+Na]^{+}$. A search in AntiBase with the molecular mass and ^{1}H NMR spectrum identified compound **31b** as indol-3-yl-acetic acid methyl ester (**31b**), which is often isolated in our group. Comparison with reference data confirmed the structure.

Compounds **31a** and **31b** are reported to have a function as growth factor (auxin) in plants as well as in bacteria. [71,72] These important hormones induce a prolongation and acceleration of growth of higher plants (plant growth regulator) at lower concentration; at a higher concentration the process is stopped, and the auxins are toxic. Compound **31a** plays also an important role in a number of plant activities

like development of the embryo, leaf formation, phototropism, gravitropism, fruit development, abscission, and root initiation [72]. Indol-3-acetic acid can also be used as fungicide for the protection of plants. Otherwise, synthetic auxin derivatives like 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram) are known as herbicides. Indol-3-acetic acid is biosynthesised in the plants from tryptophane.

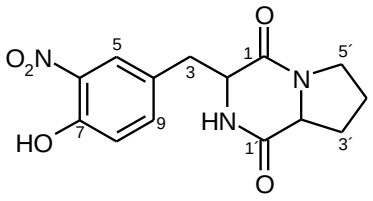
5.3 *Salegentibacter holothuriorum* T436

The Strain T436 was isolated from the Arctic ice in the Eastern Weddell Sea. Our attention in this strain was evoked by its high antibacterial and antifungal activity and the huge metabolic potency. Due to its characteristic and its capacity to produce a wide number of nitro compounds, [56] we re-fermented it now. The strain T436 grew after two days from plating on M1 and showed in the first 3-5 days circular, beige colonies with shiny smooth surfaces. The taxonomy was identified on the basis of 16 S-RNA as S*alegentibacter holothuriorum*.

The crude extract showed in the TLC (CH_2Cl_2/MeOH 9:1) a series of yellow zones in polare as well as in the apolare range, which showed no colour reaction which anisaldehyde. Several new nitro and chloro derivates were isolated now, which were different from those isolated in the first fermentation, [56] and their structures were established on the basis of NMR data, MS data, and other spectroscopic method and by comparison with known and the previously isolated compounds.

In the first fermentation, 24 aromatic nitro compounds and nitroindoles were isolated: [56,73] Pyriculamide [nitro-*cyclo* (tyrosylprolyl)] (**32**), 3'-nitro-daidzein (**33**) and 3',5'-dinitro-genistein (**34**), 2,6-dinitro-4- (2'-nitroethenyl)phenol (**35**), *N*- (4-hydroxy-3-nitrophenylethyl)acetamide (**36a**), *N*-(3,5-dinitro-4-hydroxyphenylethyl)-acetamide (**36b**), 3,5-dinitro-4-hydroxyphenyl-propionic acid (**37**a), 3,5-dinitro-4-hydroxyphenyl-2-chloro-propionic acid methyl ester (**37b**), 3,5-dinitro-4-hydroxyphenyl-ethylchloride (**38a**), 2-(4-hydroxy-3-nitrophenyl)ethanol (**38b**), 4-hydroxy-3-nitrophenyl-acetic acid (**39a**), 3,5-dinitro-4-hydroxyphenyl-acetic acid methyl ester (**39b**), 4-hydroxy-3-nitrophenyl-acetic acid methyl ester (**39c**), 4-hydroxy-3-nitrophenyl-propionic acid (**40**), 2-nitro-N_β-acetyltryptamin (**41a**), 6-nitro-N_β-acetyl tryptamine (**41b**) und 7-nitro-N_β-acetyltryptamin (**41c**); salegentipyrrol A (**42a**), and salegentipyrrol B (**42b**).

The second fermentation produced similar compounds: 3-(4-hydroxy-3,5-dinitrophenyl)propionic acid (**37b**), 4-hydroxy-3-nitrophenyl-propionic acid (**40**) and some new nitro compounds 3-(4-hydroxy-3,5-dinitrophenyl)propionic acid methyl ester (**37c**), dinitro-tyrosol (**38c**)**,** 4-hydroxy-3,5-nitrophenyl-acetic acid (**39d**), 3'-nitrogenistein (**43**), 2-hydroxy-3-(4-hydroxy-3-nitrophenyl)propionic acid methyl ester (**45**).



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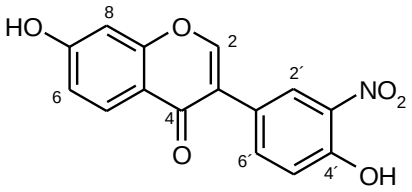

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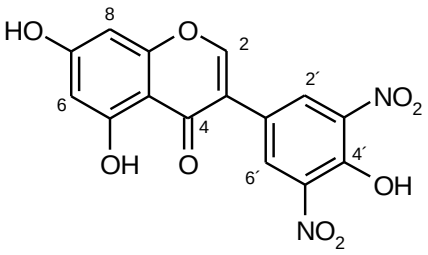

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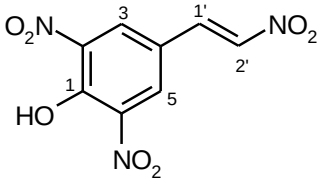
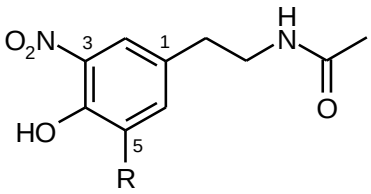
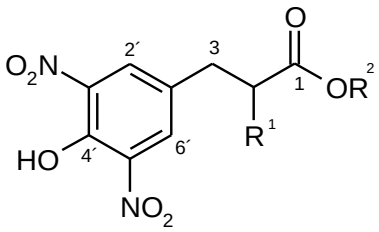


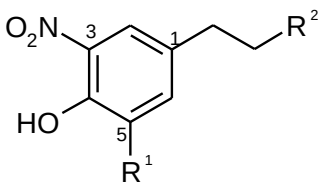
35



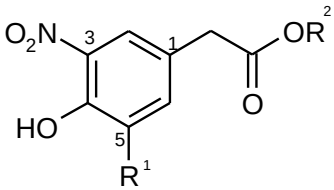
36a: R = H
36b: R = NO^2



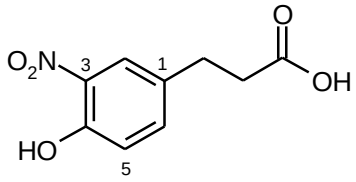
37a: R^1 = H, R^2 = H
37b: R^1 = Cl, R^2 = Me



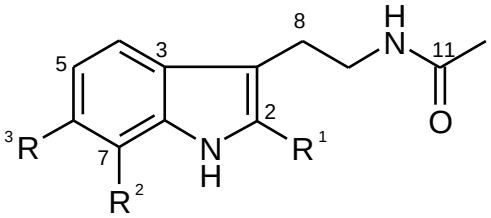
38a: R^1= NO_2, R^2 = Cl
38b: R^1= H, R^2 = OH
38c: R^1= NO_2, R^2 = OH



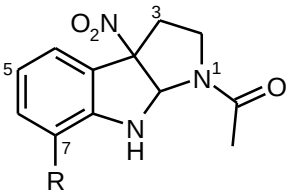
39a: R^1 = H, R^2 = H
39b: R^1 = NO_2, R^2 = Me
39c: R^1 = H, R^2 = Me
39d: R^1 = NO_2, R^2 = H



40



41a: $R^1 = NO_2$; $R^2 = R^3 = H$
41b: $R^1 = R^3 = H$; $R^2 = NO_2$
41c: $R^1 = R^2 = H$; $R^3 = NO_2$



42a: R = H
42b: R = NO_2

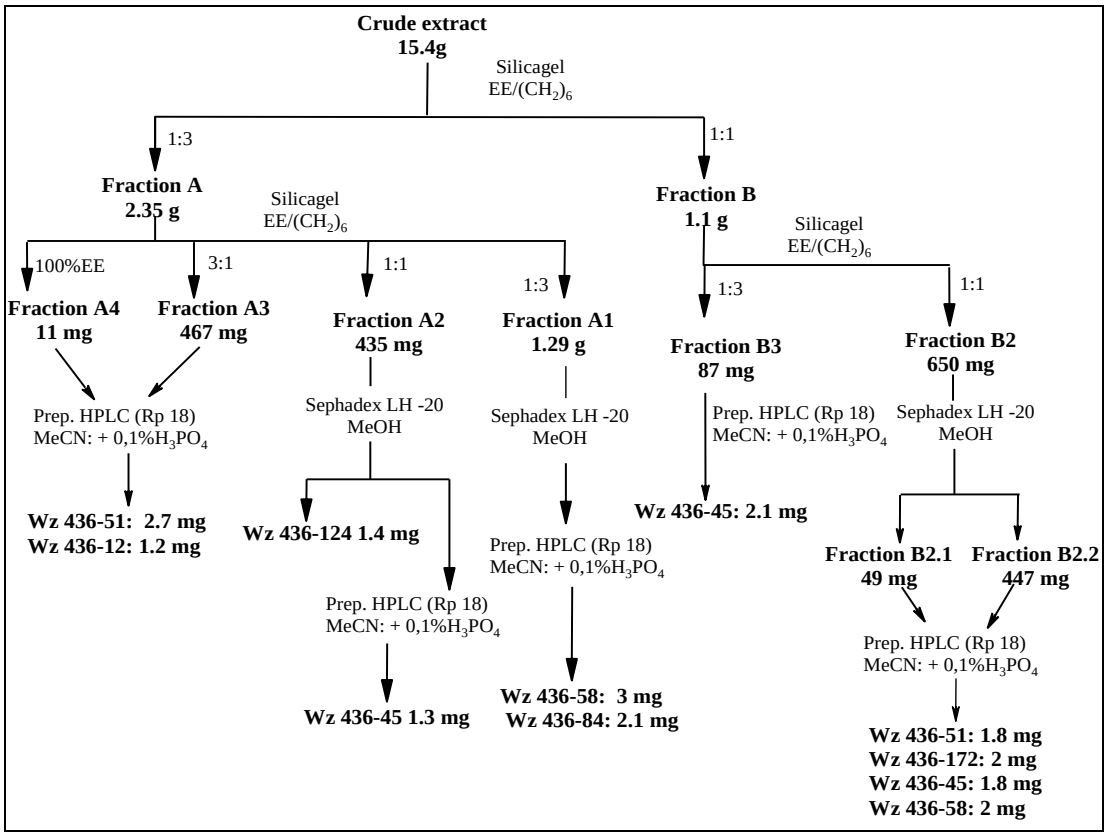


Figure 24: Work-up scheme of *Salegentibacter holothuriorum* T436

5.3.1 3´-Nitrogenistein

The yellow UV active solid **43** showed in the ^{1}H NMR spectrum six signals in the aromatic range, which were attributed due to the coupling constants to a 1,2,4-trisubstituted aromatic ring, two protons in *meta* position at δ 6.18 and 6.28 and at the end one singlet at δ 8.15.

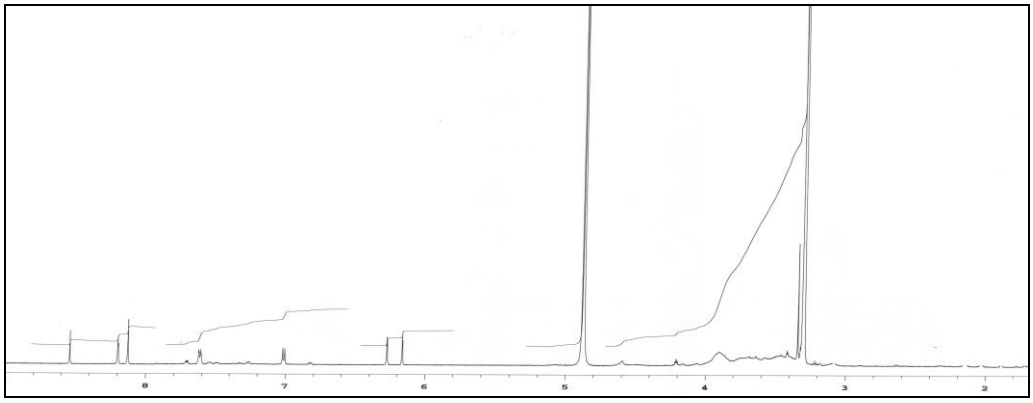


Figure 25 : ^{1}H NMR spectrum (600 MHz) of 3-nitrogenistein (**43**) in MeOH-d_4

Compound **43** showed by (-)-ESI MS a *quasi*molecular ion at *m/z* 314 $[M-H]^-$. The search in AntiBase with the proton data and the molecular mass gave 3´-nitrogenistein (**43**). The fragmentation by LC/MS/MS/MS with a collision energy of 35 eV showed characteristic elimination of HNO_2 and two hydroxy groups. The fragmentation at higher collision energy (45 eV) gave only one daughter ion with the maximum abundance at *m/z* 297 due to the loss of an OH group. By decreasing the collision energy (35 eV), the abundance of product ions increased [*m/z* 297 (100), 280 (90), 267 (70)] (Figure 26).

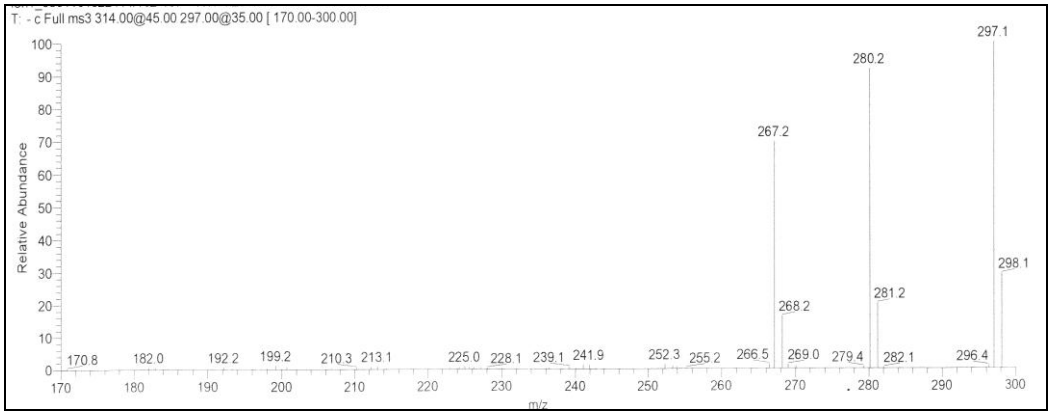


Figure 26: (-)-ESI LC/MS/MS spectrum of 3'-nitrogenistein (**43**)

The structure was confirmed by LC-ESI MS/MS by comparison with the published spectrum from the synthetic product [74].

It is reported that the loss of those 3 fragments (two OH and HNO_2) is untypical for nitro compounds; a proposed fragmentation scheme for this special case is shown in Figure 27 [74,75].

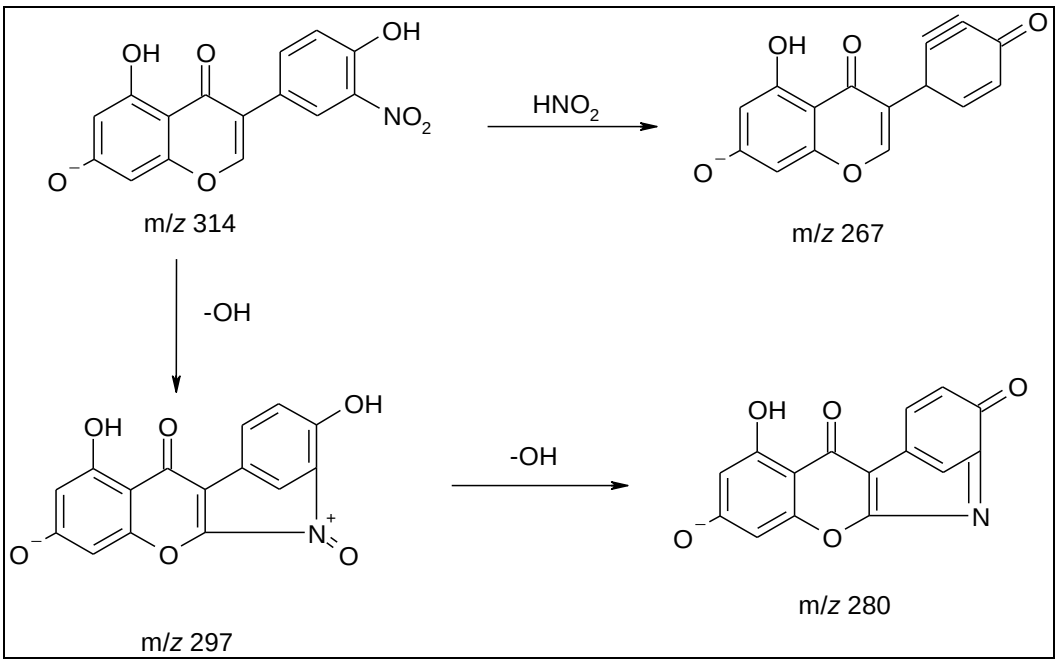
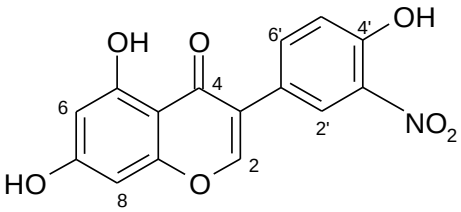


Figure 27: Proposed mechanism for the elimination of HNO_2 and two OH groups from 3'-nitrogenistein (**43**) (*m/z* 314 for $[M-H]^-$)

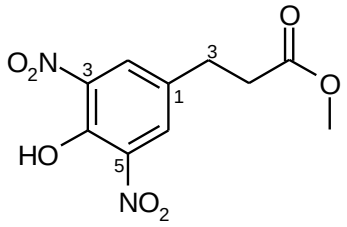


43

Nitrogenistein had been isolated previously from a genetically engineered *Streptomyces* sp. K3. [76] It showed no significant antimicrobial or phytotoxic activities. Among the compounds isolated from this strain, it was however, the only one with a mild cytotoxicity against L1210 and the Jurkat cells.

5.3.2 3-(4-Hydroxy-3,5-dinitrophenyl)propionic acid methyl ester

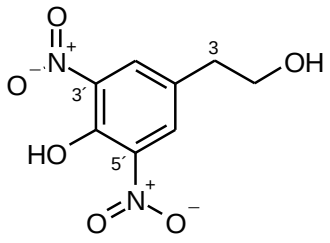
The yellow compound **37c** was obtained from fractions A2 and B3. The ESI MS spectrum gave a molecular mass of 270 Dalton and the high resolution delivered the molecule formula $C_{10}H_{10}N_2O_7$. The UV-spectrum shows the same maximum as compound **37b**. The search in AntiBase gave 3-(4-hydroxy-3,5-dinitrophenyl)propionic acid (**37b**). Comparison with the spectra and mass led me to conclude that compound **37c** was the methyl ester of compound **37b** which was previously isolated from the same strain. It was impossible to interpret the proton spectrum, because the substance seemed to decompose with time. Due to the small amount of material (0.7 mg) there was no possibility to purify it further.



37c

5.3.3 3,5-Dinitro-tyrosol

The orange compound **38c** showed the same UV absorption as compounds **38a** and **38b**. It was isolated from fraction A3 and B2. Its ^1H NMR spectrum was almost the same as that of compound **38a**. Both methylene signals, however, were shifted downfield. ESI and HRESIMS gave a molecular mass of 228 Dalton and a molecular formula $C_8H_7N_2O_6$. Compound **38c** is the new 3,5-dinitro-4-hydroxyphenyl-ethanol (dinitro-tyrosol). The chloro derivate **38a** had been isolated previously from the same strain.



38c

Due to its small amount it was not possible to measure the 2D NMR spectra for all compounds. The structures were elucidated by means of ^{1}H NMR, APCI, EI, ESI and HRESIMS and finally comparison was made with the derivatives isolated previously from the same strain.

5.3.4 4-Hydroxy-3, 5-dinitrophenylacetic acid

Compound **39d** was isolated as orange solid from fraction A1 and showed yellow fluorescence under UV at 366 nm; it gave no colour reaction with anisaldehyde. The ^{1}H NMR spectrum showed only two singlets, one at δ 8.21 for two symmetrical aromatic protons and the other one at δ 3.60 with the intensity of two protons, which belong to a methylene group.

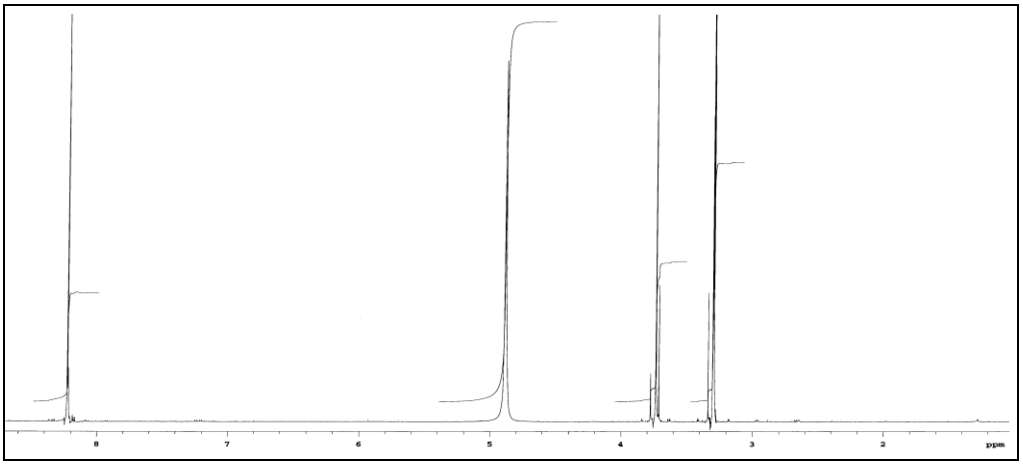
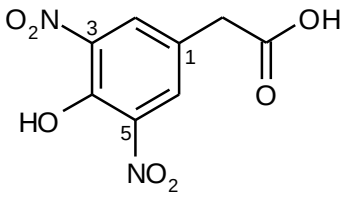


Figure 28: ^{1}H NMR spectrum (600 MHz) of compound **39d** in MeOH-d_4

From the (-)-ESI MS spectrum a *quasi*molecular ion at *m/z* 241 $[M-H]^-$ can be identified and the HR-ESI MS gave the molecular formula $C_8H_6N_2O_7$. The search in the database with the substructure produced many hits including the 4-hydroxy-3,5-dinitrophenyl-acetic acid methyl ester (**39b**), which was isolated from the same strain cultivated in another medium. [56] By comparison of their proton data, it could be observed that they differed only in one signal at δ 3.70 (attributed to a methylene group) and the absence of the methoxy signal. Comparison also of the UV spectra showed absorption at 432 nm for a dinitro-substituted phenyl ring. The search in AntiBase with the proton NMR substructure and the molecular mass gave no hits. Interpretations of 1D NMR und MS spectra delivered the free acid corresponding to com-

pound **39b.** Due to the small amount it was not possible to measure the 2D NMR spectra.



39d

5.3.5 2-Hydroxy-3-(4-hydroxy-3-nitrophenyl)propionic acid methyl ester

The orange compound **45** was isolated from fraction B2 and showed yellow fluorescence under UV at 366 nm. Its ^{1}H NMR spectrum revealed in the aromatic region characteristic signals for a 1,2,4-trisubstituted aromatic ring at δ 7.98, 7.52 und 7.13, one methine multiplet at δ 4.38, which is supposed to be near an hetero atom, one methoxy group at δ 3.70 and in addition two doublets of doublets for a methylene group at δ 3.10 and 2.98 were visible.

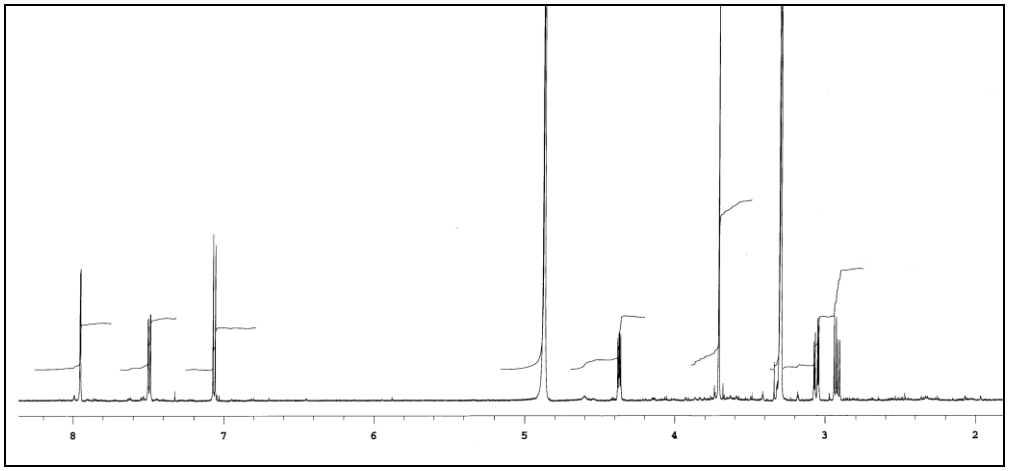
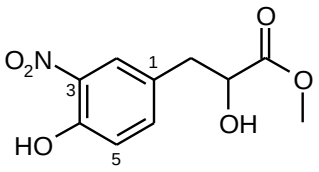


Figure 29: ^{1}H NMR spectrum (600 MHz) of compound **45** in MeOH-d_4

The (-)-APCI mass spectra gave a *quasi*molecular ion at *m/z* 240. The search in AntiBase gave one hit, which was already described in the literature. [56] Comparison with the literature confirmed compound **45** to be 2-hydroxy-3-(4-hydroxy-3-nitrophenyl)propionic acid methyl ester.



45

6 Terrestrial Streptomycetes

6.1 Terrestrial *Streptomyces* sp Ank 2

The strain was obtained from slant agar and exhibited in the biological pre-screening activity against *Bacillus subtilis*, *Mucor miehei, Escherichia coli*, *Streptomyces viridochromogenes, Staphylococcus aureus*, *Chlorella vulgaris* and *Chlorella sorokiniana* and strong cytotoxicity against *Artemia salina*.

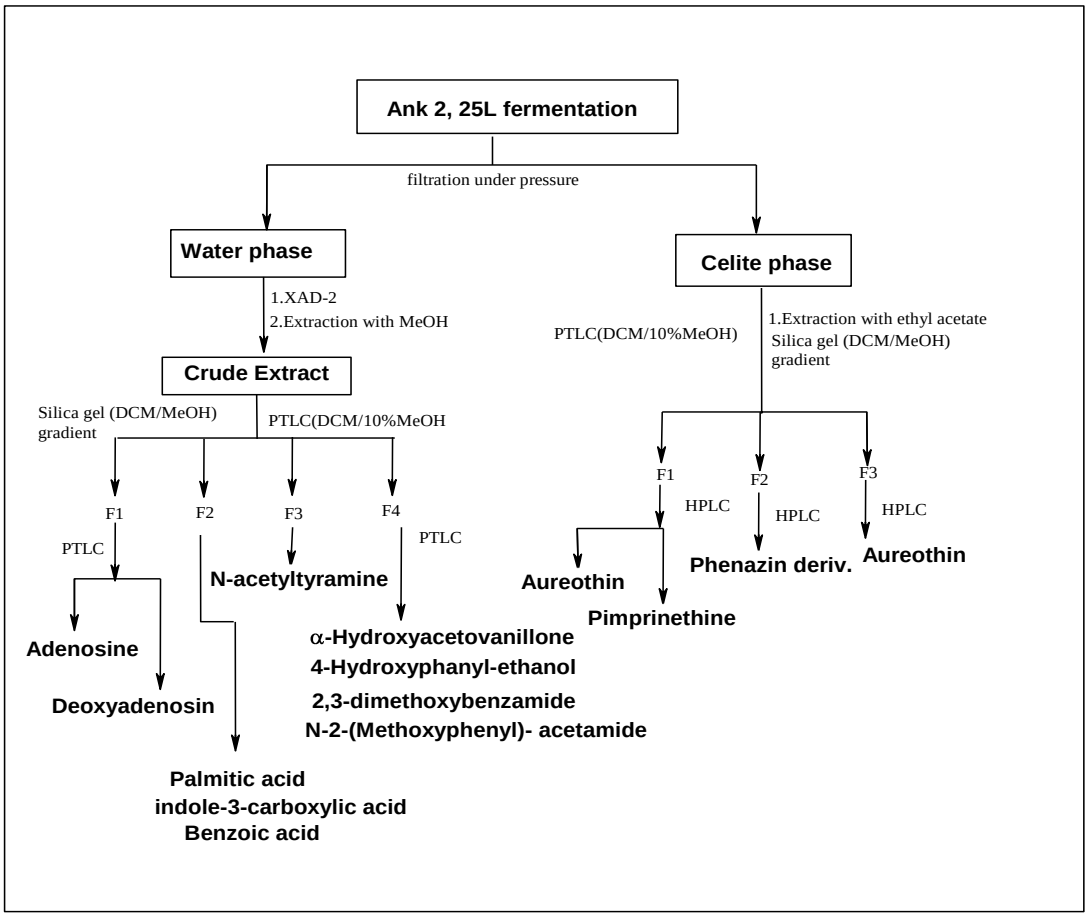


Figure 30: Work-up scheme of the *Streptomyces sp*. Ank 2

On a preparative scale, the strain Ank 2 was cultivated in M_2 medium for 5 days, the culture broth was mixed with celite and filtration afford the water phase and my-

celium which were extracted separately. The crude extract was chromatographed on silica gel using a dichloromethane/methanol gradient.

6.1.1 9-Hydroxymethyl-4-methoxyphenazine-1-carboxylic acid methyl ester

Obtained as pale oil from the purification of fraction 2 by preparative HPLC, compound **46** gave a yellow colouration with anisaldehyde/sulphuric acid.

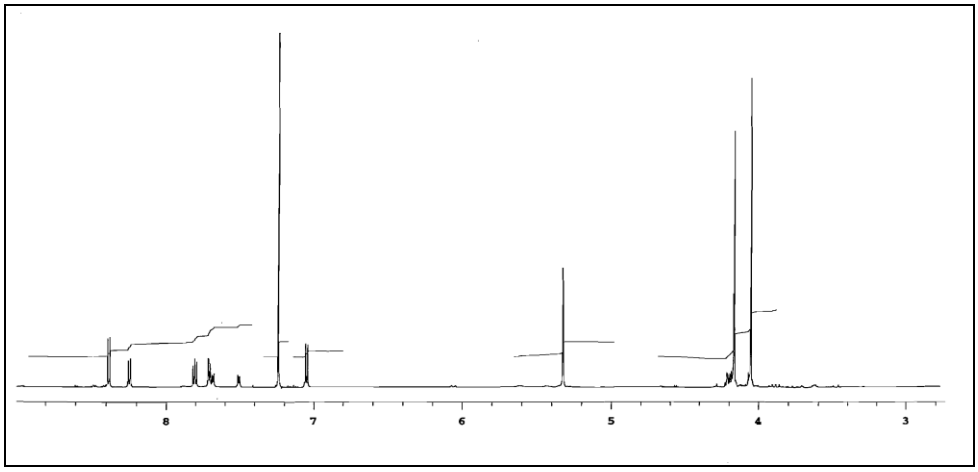


Figure 31: ^{1}H NMR spectrum (600 MHz) of Phenazine derivative **46** in $CDCl_3$

The ^{1}H NMR spectrum in $CDCl_3$ was very simple and showed and aromatic ABC system characterised by two broad doublets and a doublet of doublet at δ 8.28. In addition, two doublets of *ortho*-coupling protons appeared at δ 8.41 and 7.07. The aliphatic region delivered only three singlets attributed to a methylene connected with oxygen at δ 5.35 and two methoxy groups at δ 4.19 and 4.08.

The molecular mass was deduced to be *m/z* 298 from (+)-ESI MS, which showed the *quasi*molecular ion at *m/z* 321 $[M+Na]^+$; the molecular formula was found to be $C_{16}H_{14}N_2O_4$. The H,H COSY spectrum confirmed the presence of the two following fragments (Figure 32).

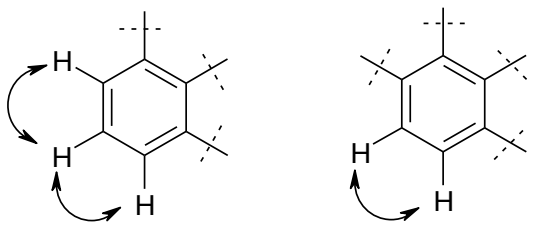


Figure 32: H,H COSY spectrum of compound **46**

The ^{13}C NMR spectrum indicated the presence of 5 sp^2 methines, one methylene, two methoxy and 8 quaternary carbons including a carbonyl group. The HMBC spectrum indicated a correlation between the doublet at δ 7.74 and the methylene carbon at δ 64.2; further correlations were visible between the methylene protons, the doublet at δ 8.28 to the carbons at δ 128.6 and 140.6. In addition, the triplet and the doublet at δ 8.28 showed correlations to the quaternary carbon at δ 144.0. All these observations suggested that the methylene group should be connected to the ring containing the ABC system delivering the sub-structure I.

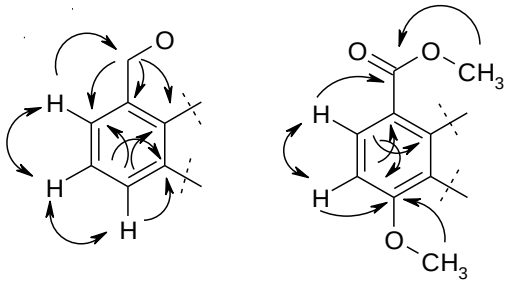
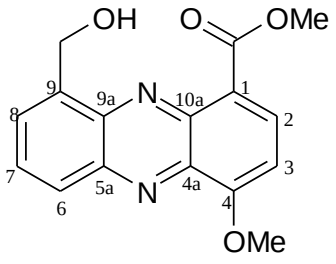


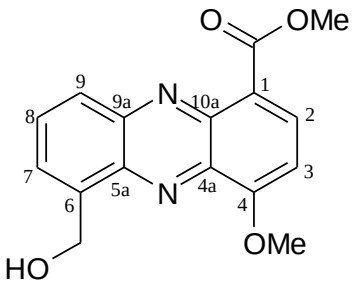
Figure 33: H,H COSY (↔)and HMBC (→) correlations of sub-structures I and II

The methoxy signal at δ 4.08 and the doublet at δ 8.41 (2-H) indicated a correlation to the carbonyl at δ 166.4 while the two *ortho* protons (2-,3-H) as well as the methoxy protons at δ 4.19 (4-OCH_3) showed a coupling to the quaternary carbon at δ 158.2 (C-4) suggesting that the two methoxy groups belong to the same ring where one appears as ester function delivering the sub-structure II.

Both substructures together result in $C_{16}H_{13}O_4$, so that two nitrogen atoms and one hydrogen as well as two double bond equivalents are missing. Due the absence of correlations between the substructures, two alternative structures **46a** and **46b** had to be discussed. Another way to distinguish between both is the use of ^{15}N-^{13}C coupling constant to assign the position of the CH_2OH on the aromatic ring at position C9 or C6. Due to the small amount of material, also a NOESY spectrum did not furnish a satisfactory and interpretable spectrum to solve this problem.

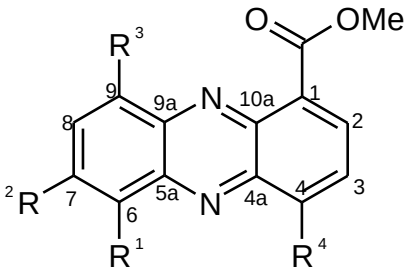


46a



46b

Both structures gave no hit in AntiBase and the Chemical Abstracts, pointing to a new metabolite. A search in the literature delivered only related compounds like griseoluteic acid-6-methyl ester (**46c**), compounds **46d** and **46e**. Although substituents were different, the comparison of the ^{13}C NMR data from substructure I and II those of **46d** and **46c** from the literature [77] led to the identification of the search compound as a phenazine derivative **46 a** or **b**.



46

	46a	**46b**	**46c**	**46d**	**46**
R^4	OMe	OMe	H	OMe	H
R^1	H	CH_2OH	OMe	H	H
R^2	H	H	H	H	OMe
R^3	CH_2OH	H	CH_2OH	H	CH_3

Natural phenazines were isolated as secondary metabolites primarily from *Pseudomonas, Streptomyces* and a few other bacterial genera from soil or marine habitats. [78] Their biological properties include antibiotic, antitumor, antimalaria, and antiparasitic activities, for example the first antibiotic griseolutein reported 1951 by Umezawa *et al.*[79] is a phenazine.

Table 2: ^{13}C NMR data of compound **46a**, **46b** and other phenazine derivates

C	**46a**	**46b**	**46c**[a]	**46d**[77]	**46e**[77]
1	122.0	122.0	132.9	122.5	132.2
2	135.1	135.1	130.7	134.7	129.9
3	105.2	105.2	129.4	105.2	129.0
4	158.2	158.2	135.4	158.4	132.3
4a	134.0	134.0	142.0	136.0	142.6
5a	144.0	144.0	136.0	142.0	145.5
6	131.1	131.1	154.8	130.1	102.3
7	129.7	129.7	109.7	131.0	161.9
8	128.6	128.6	123.9	131.4	125.8
9	138.9	138.9	141.0	130.4	139.9
9a	140.6	140.6	140.5	143.9	140.9
10a	142.0	142.0	142.3	142.2	138.6
CH_3	-	-	-	-	17.2
CH_2OH	64.2	64.2	63.9	-	-
COO	166.4	166.4	167.9	166.7	168.0
OCH_3	56.6-52.3	56.6-52.3	56.9-52.5	56.9-52.4	55.8-52.5

[a:] simulated data

The biosynthesis of phenazines has been traced to the shikimate pathway, with two molecules of a monomeric precursor pairing.[80] In this shikimate pathway, it appears that either anthranilate or an intermediate such as 2-amino-2-deoxyisochorismic acid (ADIC) must be the branch point.[81] Which is responsible for assembly of the tricycle phenazine core to yield phenazine-1-carboxylic acid (PCA) (Figure 34), the common precursor of strain-specific modifying enzymes that introduce additional functional groups on the heterocyclic ring. These substituents are largely responsible for differences in the physical and chemical properties of the individual phenazines and hence, their biological activity. [82]

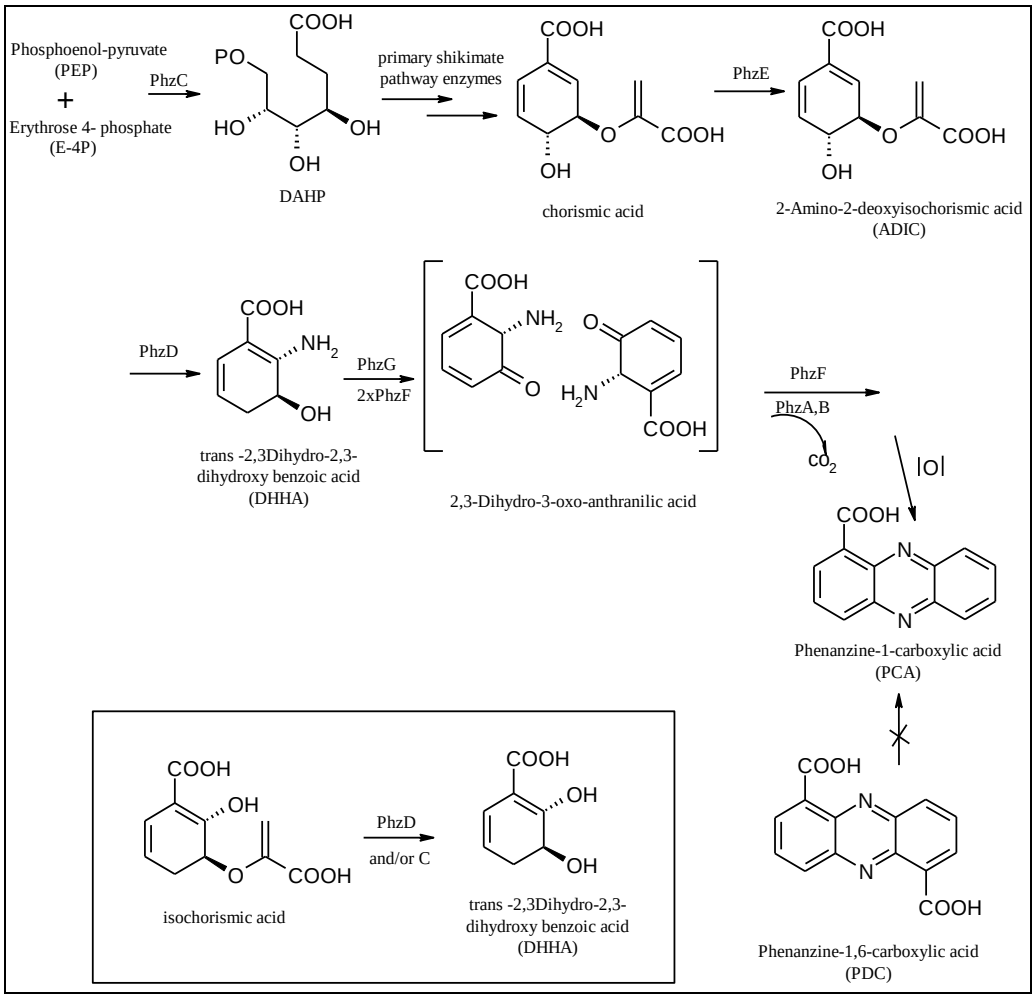


Figure 34: Proposed biosynthetic pathway for phenazine-1-carboxylic acid

6.1.2 *N*-(2-Methoxyphenyl)-acetamide

The purification of fraction 3 by PTLC followed by HPLC delivered compound **47** as colourless oil; the molecular mass was deduced to be *m/z* 165. The ^{1}H NMR spectrum exhibited only four aromatic protons as two doublets of doublets and two triplets of doublets, respectively, at δ 8.38, 6.83, 7.04 and 6.98 indicating four consecutives protons. In addition, a broad singlet of an exchangeable proton at δ 7.80, a methoxy (δ 3.84) and a methyl group at δ 2.20 were seen.

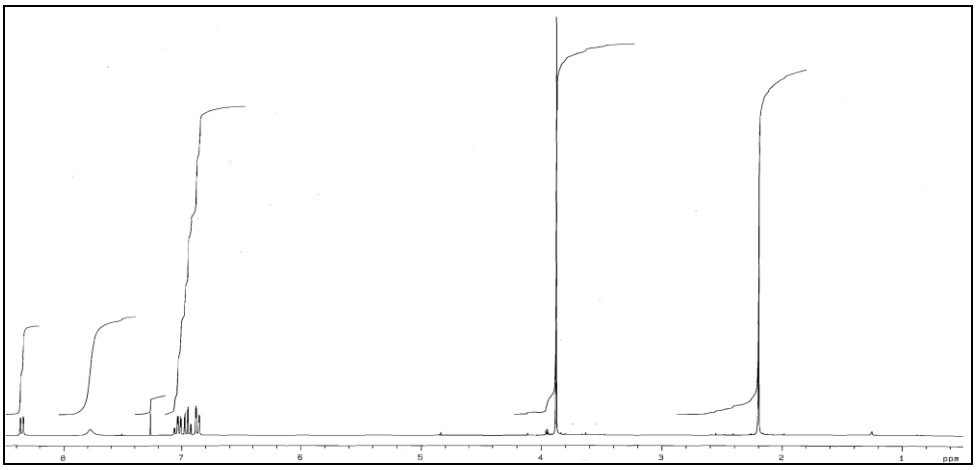


Figure 35: ^{1}H NMR spectrum (300 MHz) of metabolite **47** in $CDCl_3$

The ^{13}C NMR spectrum indicated only nine carbon signals including a carbonyl of an amide at δ 168.1, two quaternary carbons at δ 147.6 and 127.6, four methines, a methoxy and a methyl carbon.

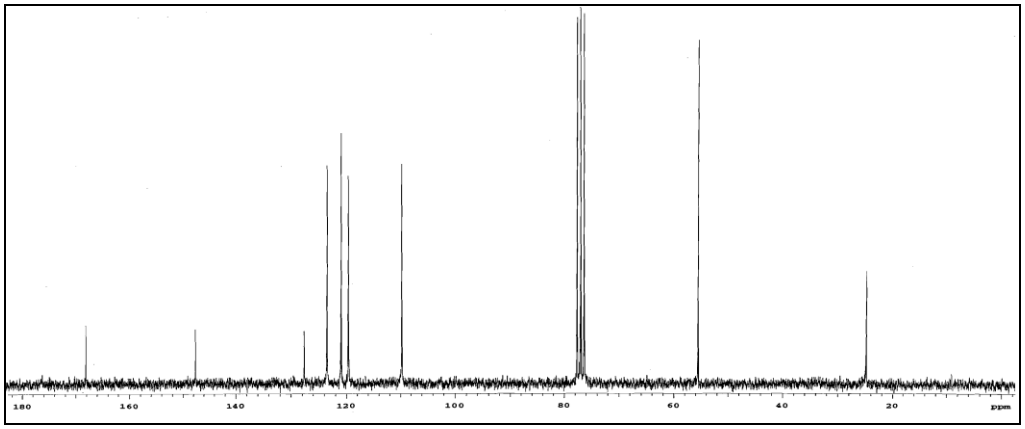
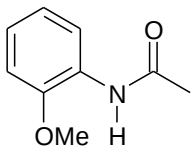
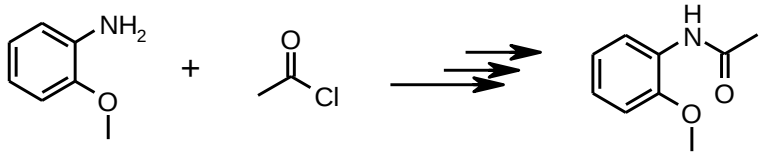


Figure 36: ^{13}C NMR spectrum (300 MHz) of metabolite **47** in $CDCl_3$

By comparison with published data, this compound was finally deduced as *N*-(2-methoxyphenyl)-acetamide (**47**), which was already reported as synthetic product,[83] but is described here for the first time from nature.



47



Scheme 1[83a]: Synthesis schema of *N*- (2-methoxyphenyl)-acetamide (**47**)

6.1.3 2,3-Dimethoxy-benzamide

The colourless solid **48** showed a blue colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum indicated in the aliphatic region two singlets at δ 3.94 and 3.91. Two broad singlets of acidic protons at δ 7.91 and 5.96, in addition signals of three consecutive protons at δ 7.72 (dd), 7.18 (t) and 7.09 (dd) were observed.

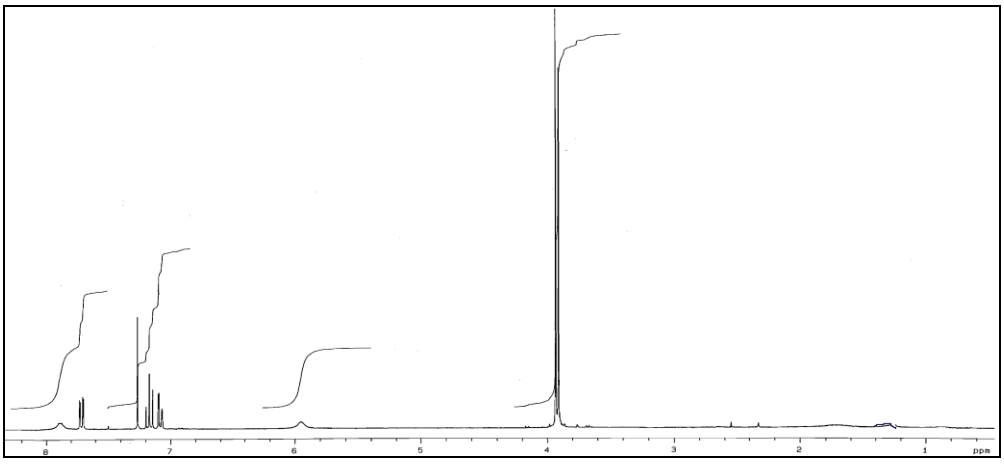


Figure 37: ^{1}H NMR spectrum (300 MHz) of of metabolite **48** in $CDCl_3$

The ^{13}C NMR spectrum showed nine carbon signals including one carbonyl of an amide or acid at δ 167.0 and two methoxy groups at δ 61.9 and 56.1. The compound was easily identified as 2,3-dimethoxybenzamide (**48**) firstly described from nature here.

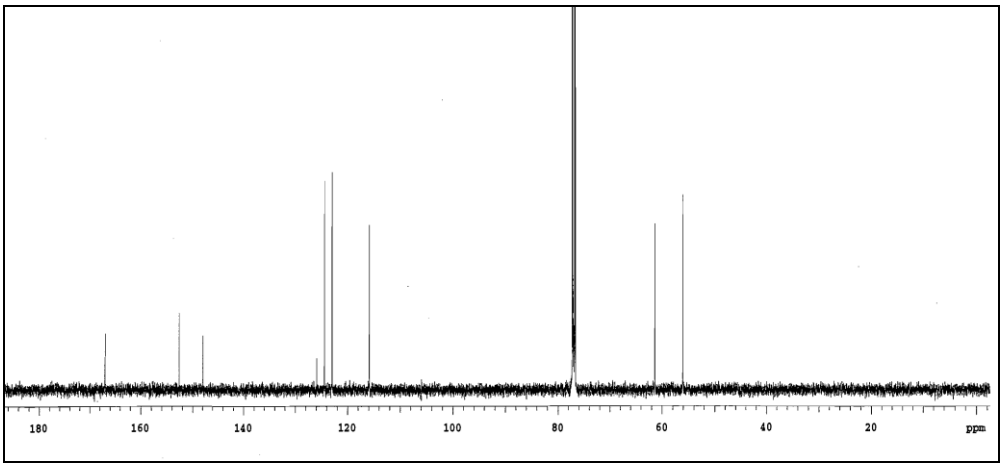
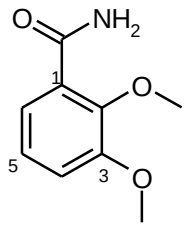


Figure 38: ^{13}C NMR spectrum (75 MHz) of 2,3-dimethoxybenzamide (**48**) in $CDCl_3$



48

6.1.4 2-Hydroxy-(4-hydroxy-3-methoxyphenyl)-ethanone

The sample was obtained as a colourless inseparable mixture of **49** and a minor component **48**, and showed a yellow colour reaction with anisaldehyde/sulphuric acid. The ESI mass spectrum as well as HPLC MS of the mixture indicated two *qua-si*molecular ions at *m/z* 183 $[M+H]^+$ for **48** and 181 $[M-H]^-$ for **49** The ^1H NMR spectrum showed also signals of a mixture, but after subtracting the signals of **49** it was easy to identify a 1,3,4-trisubstituted benzene ring, a methylene connected to oxygen and a methoxy group.

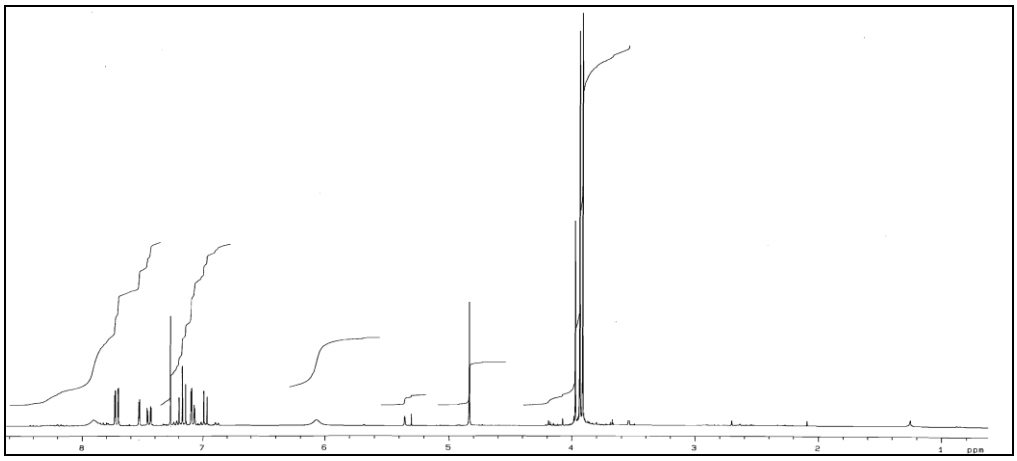


Figure 39: ^{1}H NMR spectrum (300 MHz) of **48** and **49** in $CDCl_3$

The ^{13}C NMR spectrum indicated nine peaks, including signal at δ 196.7, which can be attributed to a conjugated ketone.

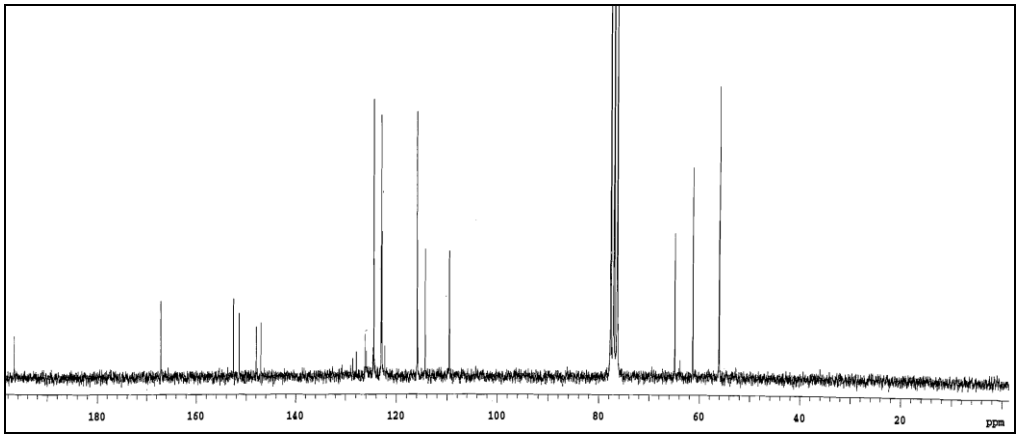
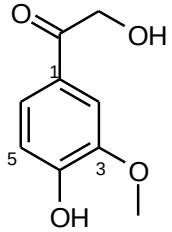


Figure 40: ^{13}C NMR spectrum (75 MHz) of **48** and **49** $CDCl_3$

The substructure search in AntiBase delivered no results pointing for a new compound, which was finally determined as 2'-hydroxy-3-methoxy-4-hydroxy-acetophenone (**49**), which was previously isolated from the cell culture of *Solanum khasianum* [84].



49

2-Hydroxy-(4-hydroxy-3-methoxyphenyl)-ethanone (**49**) is known to induce wounds. α-Hydroxy derivatives of acetovanillone have the capability to activate the virulence genes of *Agrobacterium tumefaciens.* [85]

6.1.5 Aureothin

Fractions 1 and 3 from the mycelia gave after separation by preparative RP-HPLC a fairly large amount of the yellow compound **50** (100 mg). The molecular mass was determined to be 397 Dalton from the (+)-ESI MS. Due to the odd number of the molecular mass, the formula must contain an odd number of nitrogen atoms. (+)-HRESIMS of the molecular peak resulted in the molecular formula $C_{22}H_{23}NO_6$.

The ^{1}H NMR spectrum showed in the aromatic range 2 doublets at δ 8.21 (J = 8.8 Hz) and 7.41 (J = 8.7 Hz), with the intensity of two for a *para*-disubstituted benzene ring, two singlets at δ 6.38 and 6.23 for the conjugated aryl part, one triplet at δ 5.17 corresponding to one methine proton, two AB methylene signals at δ 4.87 and 3.05, one of them seem near oxygen. Finally one methoxy group and three methyl groups at δ 3.99, 2.06, 2.05 and 1.87, respectively, were identifiable.

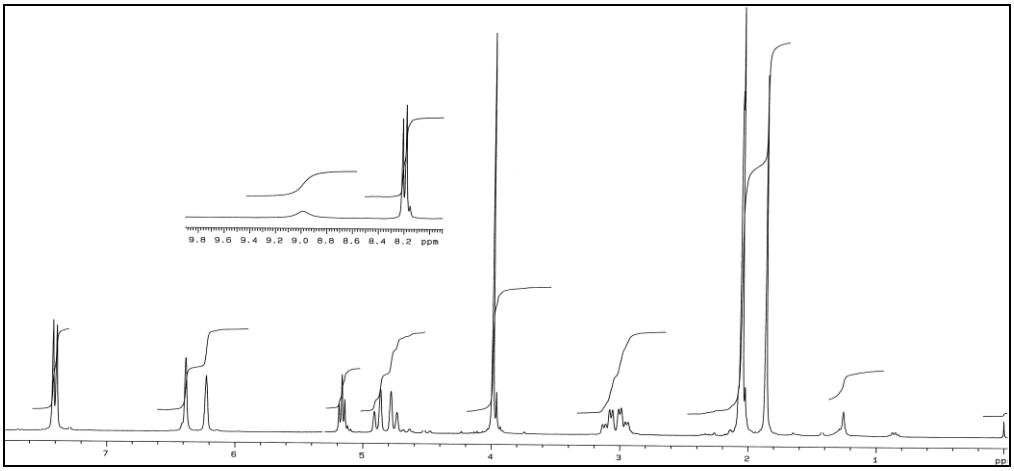


Figure 41: ^{1}H NMR spectrum (300 MHz) of aureothin (**50**) in $CDCl_3$

In the ^{13}C NMR spectrum, seven signals were revealed in the aliphatic range between δ 73.6 and 69.8, one carbonyl at δ 181.2, two quaternary connected to oxygen at 162.7 and 155.0, each two aromatic methine carbons at δ 129.5 and 123.5, which belonged to the *para*-disubstituted benzene ring, two methines at δ 128.2 and 124.1 and six other quaternary signals between δ 144.2 and 100.0 (Figure 42).

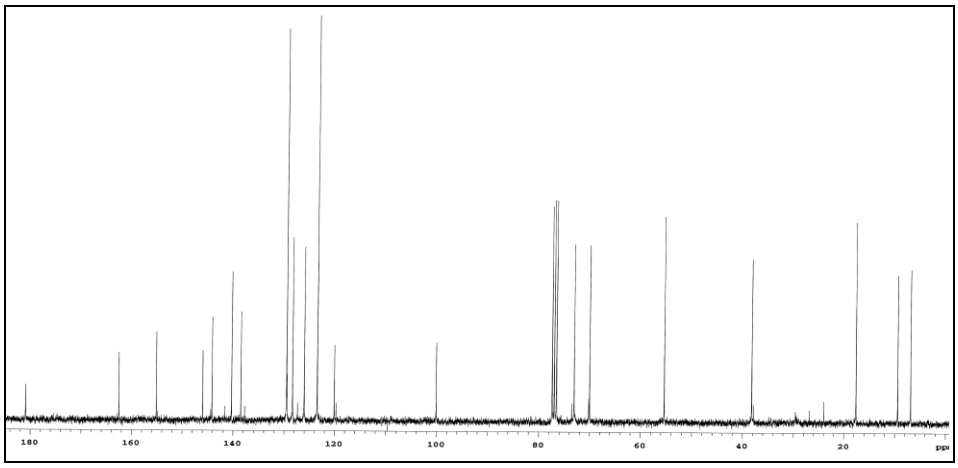
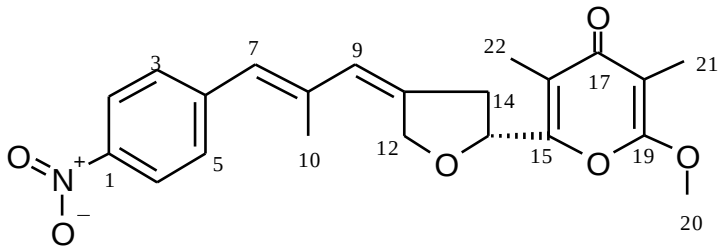


Figure 42: ^{13}C NMR spectrum (300 MHz) of aureothin (**50**) in $CDCl_3$

A search in AntiBase with the ^{1}H NMR, ^{13}C NMR, MS and HR MS data delivered aureothin and the comparison of the spectra confirmed the structure.



50

Aureothin (**50**) has cytotoxic, antifungal, antitumor and pesticidal activities [86]. The biosynthesis of aureothin (**50**) required the arrangement of the polyketide backbone and two tailoring reactions (introduction of the furan oxygen and then methylation of the pyrone ring). The order of the late steps in the aureothin biosynthesis was finally elucidated in 2006 by Hertweck *et al.* [87] by using mutational analysis of regiospecific γ-pyrone methyltransferase (Aurl), isolation and structure elucidation of novel metabolites and by biotransformation experiments.

All the news compounds **46, 47, 48** were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57) and *Escherichia coli*, the fungi *Mucor miehei* and *Candida albicans*, and the micro algae *Chlorella vulgaris, Chlorella sorokiniana and Scenedesmus subspicatus* in the agar diffusion test at a concentration of 40 μg/paper and showed no biological activity. Obviously, the activity of the strain was due to aureothin.

6.1.6 Pimprinethine

Compound **51** was separated as colourless needles from the fraction 1 after HPLC. (+)-ESI MS gave a molecular mass of 213 Dalton for the *quasi*molecular ion ($[M+H]^+$) and its high resolution delivered the formula $C_{13}H_{12}N_2O$. The ^1H NMR spectrum indicated the presence of an ethyl group at δ 1.49 (3H) and 2.99 (2H), a 3-substituted indole ring with two multiplets at δ 7.30, two doublets at δ 7.45 and 7.79 one singlet at δ 7.59 and one exchangeable proton at δ 8.38 and one singlet at δ 8.84.

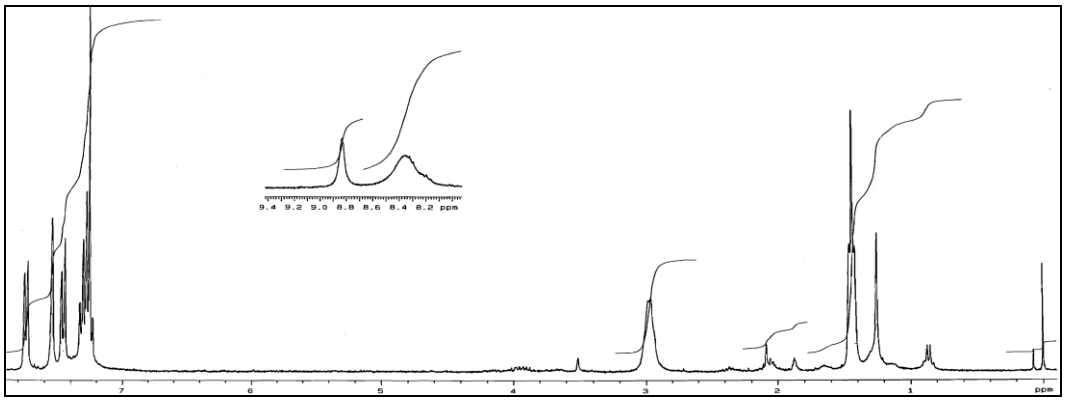
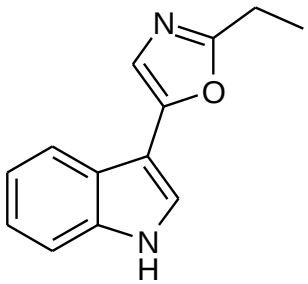


Figure 43: ^1H NMR spectrum (300 MHz) of pimprinethine (**51**) in $CDCl_3$

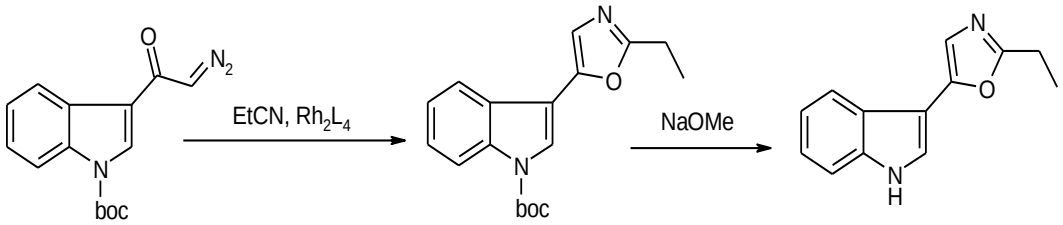
The ^{13}C NMR spectrum gave 13 carbon signals including two carbonyls at δ 174.8 and 150.6. The search in the database with the molecular mass, formula and the comparison of the proton NMR spectrum with the literature indicated that the search compound was pimprinethine (**51**).



51

The structure of pimprinethine (**51**) was determined 1981 by spectroscopic and X-ray analysis. [88] Pimprinethine belongs to group of simple microbial indole alkaloids (oxazolylindole alkaloid), which can be considered as disguised tryptamine derivates. Pimprinethine can be obtained in a two step reaction catalysed with rho-

dium (II) of *N*-Boc-3-diazoacetylindol with the appropriate nitrile followed by removal of the protecting Boc group. [89]



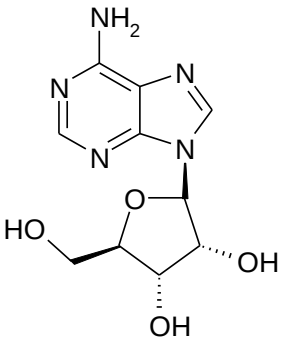
Scheme 2: Synthesis of pimprinethine **51**

6.1.7 Trivial compounds

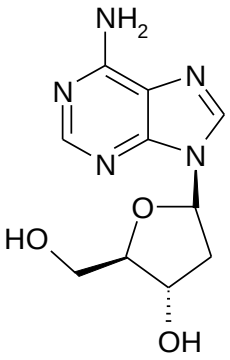
Adenosine and Deoxyadenosine

The colourless solid compounds **52** and **53** were isolated from fraction I as a mixture exhibiting an UV absorbing band, which turned to green-blue with anisaldehyde/sulphuric acid. After comparison of our spectral data of the above compounds with the literature, as well as with the spectra in our collection, this led us to identify the compounds as adenosine (**52**) and 2'-deoxyadenosin (**53**).[90]

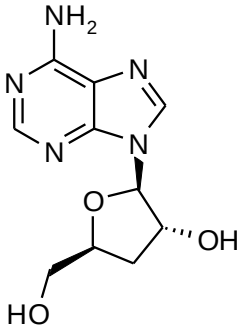
The related 3'-deoxyadenosin (also called cordycepin) (**54**) is one of the first adenine-nucleoside analogues, which was isolated from cultures of the fungus *Cordyceps militaris*. [90b] Cordycepin is a strong inhibitor of RNA synthesis showing cytostatic activity. [91] In contrast, 2'-deoxyadenosin is an essential base of DNA. Adenosine is an endogenous nucleoside occurring in all cells of the body. Adenosine plays an important role in biochemical processes such as synthesis of nucleic acids and proteins, photosynthesis, muscle contraction and intracellular signal transduction (cAMP). Adenosine is a potent anti-inflammatory agent, acting at its four G-protein coupled receptors. Tropical treatment of adenosine to foot wounds in diabetes mellitus has been shown in lab animals to drastically increase tissue repair and reconstruction. Adenosine, a ubiquitous neuromodulator, and its analogues have been shown to produce 'depressant' effects in animal models believed to be relevant to depressive disorders, while adenosine receptor antagonists have been found to reverse adenosine-mediated depressant effects.[92]



52



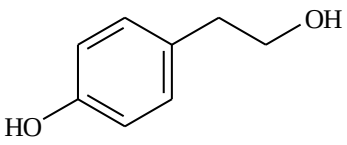
53



54

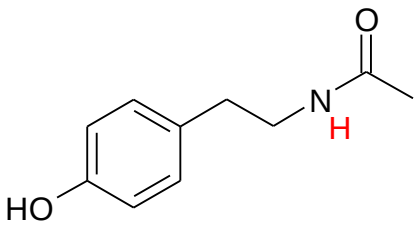
4-Hydroxyphenylethanol and *N*-Acetyl-tyramine

The structure of the colourless compound **55** was confirmed by direct comparison of the proton NMR spectrum with that of an authentic sample as 4-hydroxyphenylethanol (**55**), which has often been isolated in our group. [93,94]



55

N-Acetyl-tyramin (**56**) was isolated as a colourless solid from fraction 3 after silica gel chromatography of an UV absorbing zone. The EI mass spectrum determined the molecular mass to be 179 Dalton. The mass ion exhibited an expulsion of an acetamide group to give a base peak at *m/z* 120. *N*-acetyl tyramine was isolated 1959 from the mother liquor of the antibiotic holomycin, a metabolite of *Streptomyces griseus.* [95] The compound plays an important role in the transformation of hydroxyquinones to quinones in the metamorphosis of spiders [96] and is an antitumor agent. [97]



56

6.2 Terrestrial *Streptomyces* sp. AdM5

The dark red extract of a terrestrial *Streptomyces* strain AdM5 exhibited UV absorbing spots on TLC, which developed a characteristic pink colour reaction upon spraying with anisaldehyde /sulphuric acid. The strain was fermented on a 25 L scale using yeast, malt and glucose (YMG) medium and worked up under usual conditions.[98] Separation using various methods such as silica gel column chromatography, PTLC buffered with sodium acetate and reversed phase HPLC, delivered indol-3-carboxylic acid, phenyl acetic acid (**57**) and phenyl acetamide (**58**) Column chromatography on aluminium oxide gave two prodigiosins (**59**, **61**) in a good yield; they were easily identified by comparison of the NMR data with those reported in the literature.

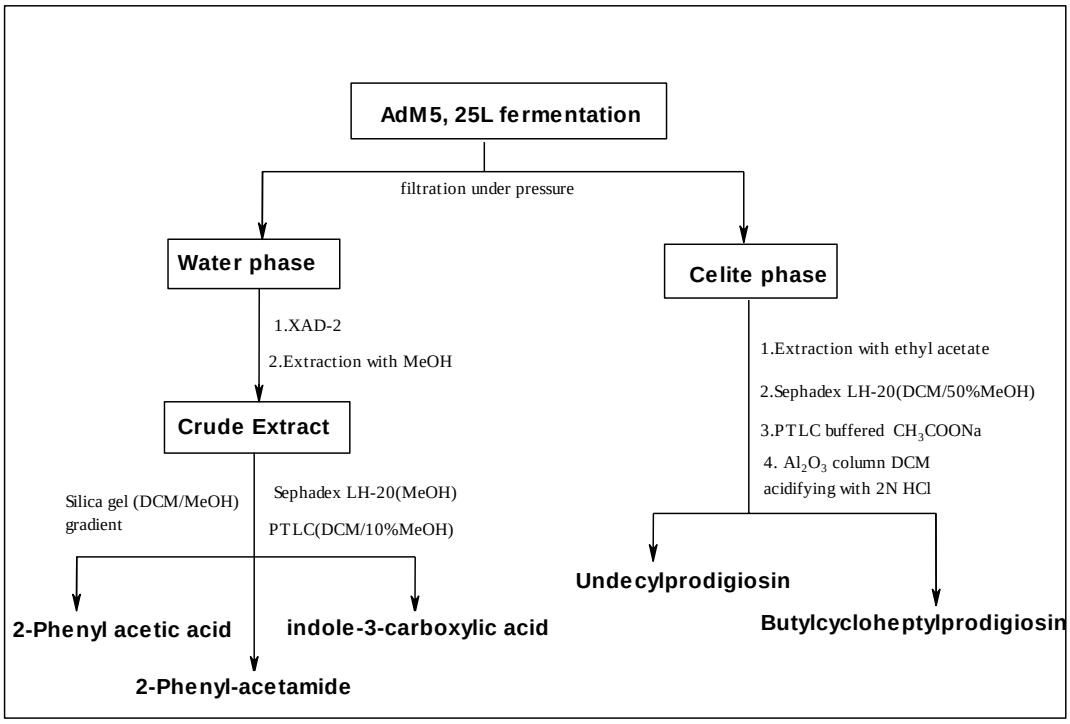
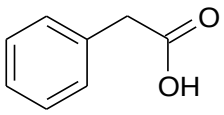


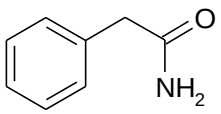
Figure 44: Work-up scheme of the strain *Streptomyces sp*. ADM5

6.2.1 Phenyl acetic acid and Phenyl acetamide

The compound **57** showed on TLC an UV absorption of 254 nm. It exhibited no colour reaction after spraying with anisaldehyde/sulphuric acid. Based on the ^{1}H NMR spectrum and the EI MS-fragmentation pattern, the substance could be easily identified as phenyl acetic acid.



57



58

The pale yellow crystalline **58** showed the same UV absorption and also no colouration with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **58** was also similar to that of **57**, but the EI mass spectrum indicated the molecular ion to be *m/z* 135: The mass difference of Δm = 1 between both compounds suggested that compound **58** may be the amide of **58**. This assumption was confirmed by comparison of the data with the literature. Compound **58** was frequently isolated in our team since 1988.

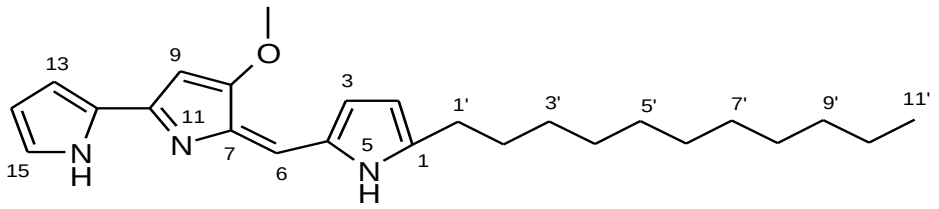
6.2.2 Prodigiosins

Prodigiosins are typical secondary metabolites only appearing in the later stages of bacterial growth. [99] They belong to the family of naturally occurring polypyrroles [100] and are red pigments firstly isolated from a culture of *Serratia marcescens*. [101,102] They are only found in restricted groups including streptomycetes. They are known to exhibit a wide range of activities such as potent cytotoxicity against various human cancer cell lines, immunosuppressive activity [103] and are potent antimalarial agent. [104]

Prodigiosins are red in acid and yellow in base. Their chromatographic separation is difficult. The preparative thin-layer chromatography in dichloromethane/5% methanol disclosed three major spots: a faster moving orange spot, a middle polar elongated slightly orange spot and a slower moving, thin pink spot.

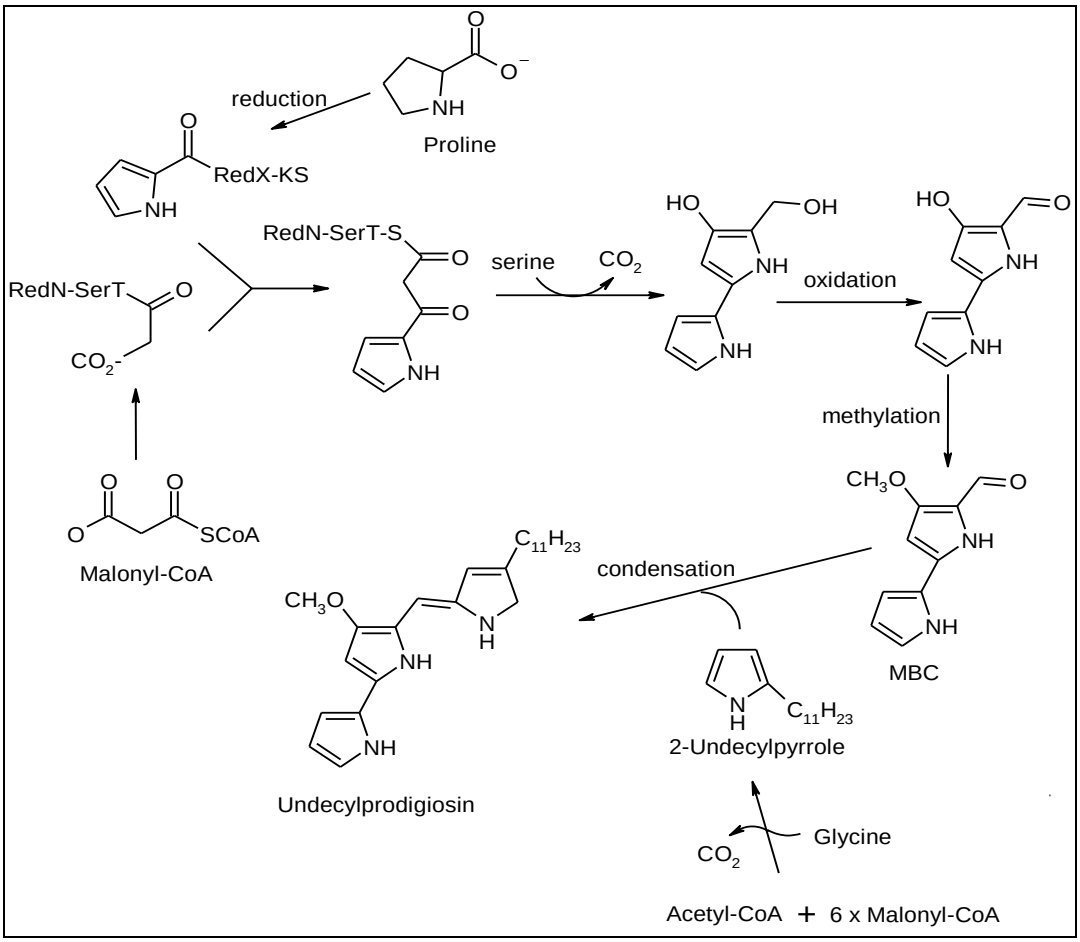
Undecylprodigiosin

The pH dependently coloured compound **59** was chromatographed using Sephadex LH-20, buffered PTLC and aluminium oxide. The orange crystals showed UV absorption and gave a deep pink colouration with anisaldehyde/sulphuric acid. The molecular ion of **59** was deduced from EI MS to be *m/z* 393. A search in AntiBase, [68] with the ^{1}H-NMR data and the molecular mass delivered one answer pointing to undecylprodigiosin (**59**). Comparison with the literature data [105] confirmed the structure.



59

Undecylprodigiosin is a frequently occurring compound and has recently been re-isolated from a sponge-derived actinomycete *Saccharopolyspora* sp. and exhibited significant cytotoxic activities against 5 cancer cell lines: P388, HL-60, A549, BEL-7402, and SPCA4.[105] The biosynthesis of undecylprodigiosin is formed by the condensation of 4-methoxy-2,2'-bipyrrole-5-carbaldehyde (MBC) with 2-undecylpyrrole (Scheme 3).



Scheme 3: Biosynthesis of undecylprodigiosin **59** with 2-undecylpyyrole as precursor

Butylcycloheptylprodigiosin

Compound **61** was obtained as a red brown solid, showed a UV-absorption at 366 nm and gave a pink colour reaction with anisaldehyde/sulphuric acid. The molecular mass of **61** was deduced from the EI MS and ESI MS to be *m/z* 391.

The ^{1}H NMR spectrum of **61** in $CDCl_3$ indicated in the offset range two broad singlets at δ 13.85, 9.06 attributed to the NH of the pyrrole rings. Three aromatic singlets at δ 7.10, 6.49 and 6.09, two doublets of doublets at δ 6.98, 6.38 and one triplet at δ 7.30 were attributed to a monosubstituted pyrrole system of a prodigiosin. The aliphatic region indicated between δ 3.10 and 1.20 further multiplets attributed to methine and methylenes. Additionally a 3H singlet at δ 4.02 and a triplet at 0.92, respectively, indicated the presence of a methoxy group as well as a methyl connected to a methylene in the structure of **61**. An unexplainable multiplet signal appeared at δ 15.4, however, was shifted to δ -1.54 in a second correct measurement. This signal is a characteristic fingerprint for *meta*-pyrrolophanes and was already reported as one of the protons of the *ansa* chain forming a rigid 10-membered ring. [106] The unusual upfield shift is due to the anisotropy cone of the aromatic pyrrole nucleus. [106,107]

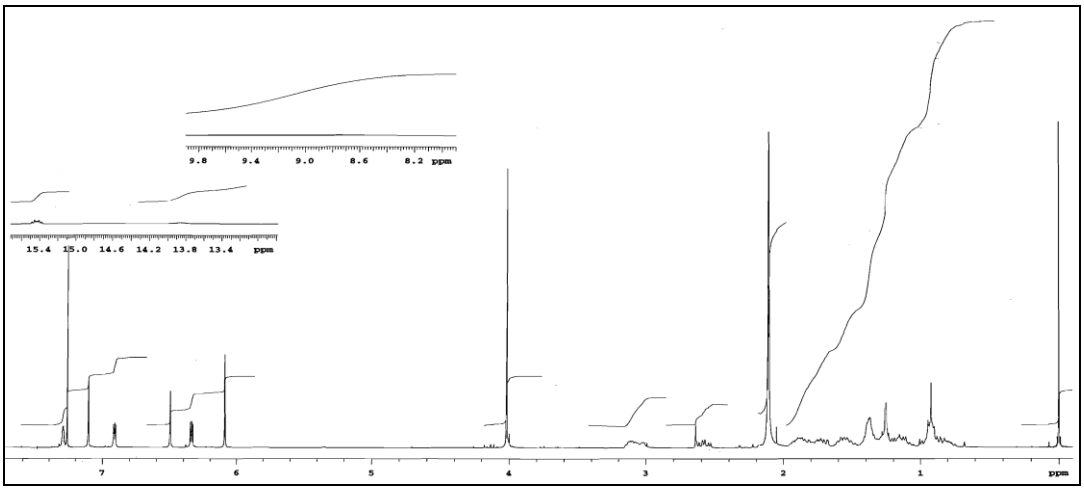
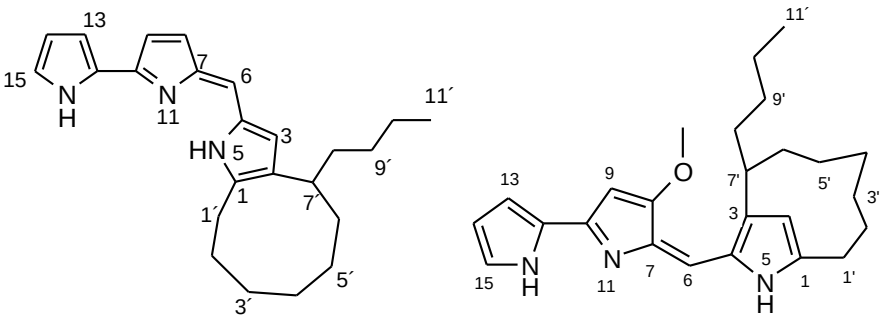


Figure 45: ^{1}H NMR spectrum (300 MHz) of butylcycloheptylprodigiosin (**61**) in $CDCl_3$

A substructure search delivered five hits, among them butylcycloheptylprodigiosin (**61**) as possible structure. [106] Streptorubin B (**60**) was first isolated by Gerber and co-workers. [108] The structure of streptorubin was revised 1991 by Laatsch *et al.*

[106] into the *ortho*-anneleted butylcycloheptylprodigiosin (**61**). Comparison with data from the total synthesis published by Wasserman *et al.* [109] as well as from the natural product [106] further confirmed the identity.



60 **61**

Figure 46: Formation of Undecylprodigiosine **59** and oxidative cyclisation to **61**

Some studies *in vivo* have shown that **61** acts in synergy with cyclosporin A, FK 506 or rapamycin, which are the dominant drugs in immunosuppressive treatments. The prodigiosins constitute important new lead compounds in the search for supplementary drugs to prevent allograft rejection. These findings have attracted interest in this class of pyrrole alkaloids, as evident from many recent studies mapping their biological profiles in great detail. [107]

6.3 Terrestrial *Streptomyces* sp GW 4723

During our screening programme of terrestrial *Streptomyces* sp. for bioactive principles, the strain GW 4723 drew our attention due to its anti-bacterial, anti-algal and anti-fungal activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, activity, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*, *Mucor miehei* and *Candida albicans* respectively, and mainly the dark colour of the culture broth. In the chemical screening on TLC, in addition to the fats and fatty acids, several yellow bands were observed: some turned violet with dilute sodium hydroxide while others remained unchanged. The latter exhibited a green fluorescence under 366 nm and a light green colour reaction after spraying with anisaldehyde/sulphuric acid. The crude extract was separated as indicated in Figure 47. The scale-up of this strain was started by S. Fotso during his dissertation [110] and was subsequently completed in this work.

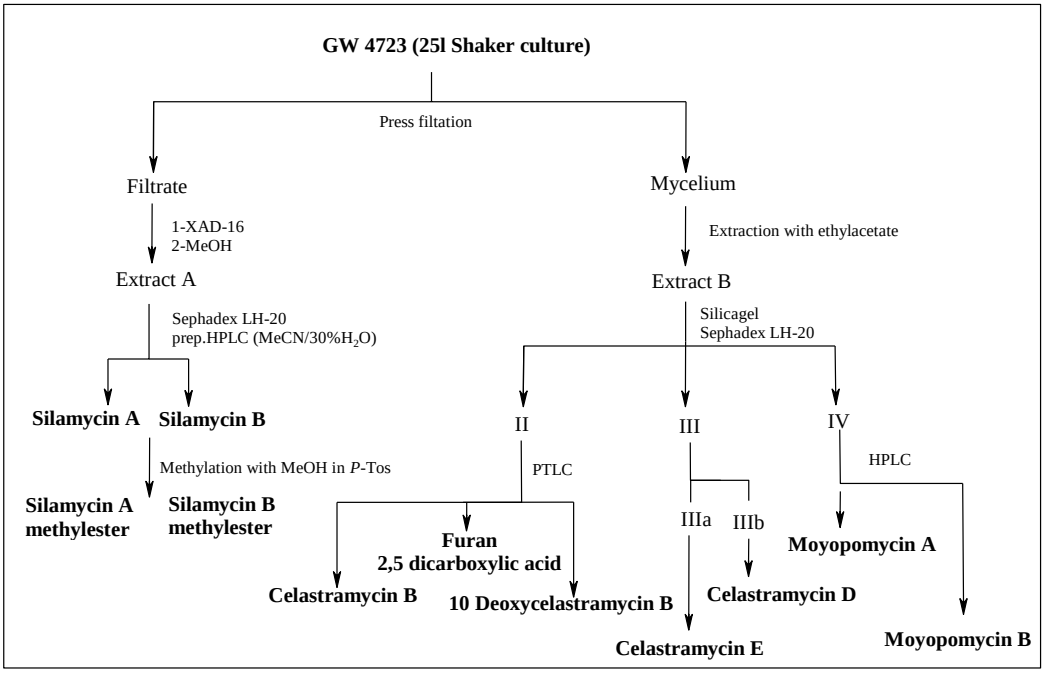


Figure 47: Work-up scheme of the isolate GW 4723

6.3.1 Silamycin A and B

Compounds **62a** and **62b** were obtained as colourless solids and exhibited a blue colour on spraying with anisaldehyde/sulphuric acid. They were easily soluble in $CDCl_3$, MeOH-d_4, DMSO-d_6 and pyridine and insoluble in water. ESI MS of **62a** showed a *quasi*molecular ion at *m/z* 677 $[M-H]^-$ and EI MS indicated the molecular mass to be 678 Dalton. The molecular mass of **62b** was determined to be 664 Dalton, indicating a difference of Δm = 14 between **62a** and **62b** corresponding to a methylene group. HRESIMS established the molecular formula of **62a** and **62b** as $C_{36}H_{54}O_{12}$ and $C_{35}H_{52}O_{12}$, respectively, each containing ten double bond equivalents.

The ^1H NMR spectrum of **62a** measured in DMSO-d_6 exhibited in the aromatic region four proton signals, among which two were exchangeable and appeared at δ 9.31 and 7.76, and two sp^2 signals were at δ 6.81 (d, 1.3 Hz) and 5.72 (d, 1.3 Hz). The aliphatic region was more complex and displayed a set of protons due to a sugar moiety, including an anomeric proton signal at δ 4.90 (d, 1.8 Hz), whose coupling constant indicated an α-glycosyl linkage.[111] In addition, signals of three methoxy and five methyl groups were visible, and multiplets of six methylene groups appeared between δ_H 0.95-1.90.

A search in AntiBase,[68] the Dictionary of Natural Product and the Chemical Abstracts,[69] with the mass and the molecular formula delivered only some triterpenes which did not match with the proton data of **62a**.

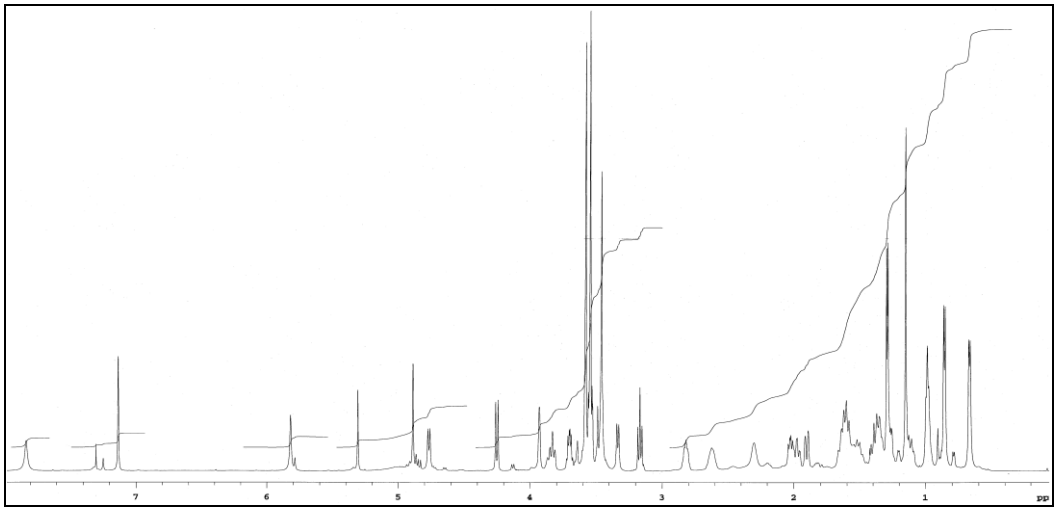


Figure 48: ^{1}H NMR spectrum (600 MHz) of silamycin A (**62a**) in $CDCl_3$

The ^{13}C NMR/APT spectrum of **62a** displayed seven quaternary carbon signals including two carbonyls, four sp^2 and one Cq-O sp^3 (δ_c 84.4). Additionally fifteen methine and six methylene, three methoxy and five methyl signals were distinguished. The combination of the ^{1}H and ^{13}C NMR data revealed that compound **62a** possessed three hydroxyl groups.

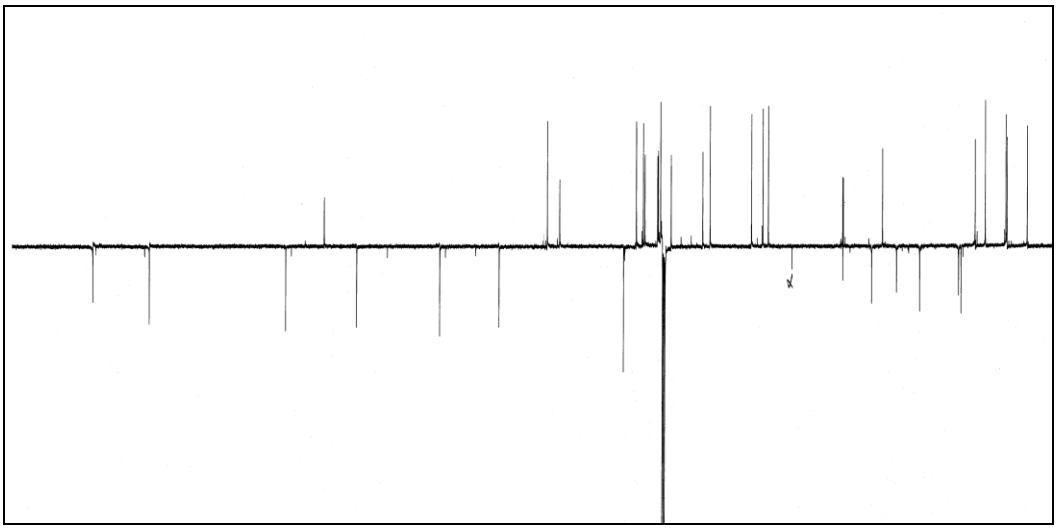


Figure 49: APT NMR spectrum of silamycin a (**62a**)

The interpretation of the H,H COSY experiments of **62a** indicated consecutive couplings from the anomeric proton at δ 4.90 (δ 97.7) till to the methyl doublet at δ 1.30 (δ 17.6) of a desoxyhexose. The HMBC spectrum displayed cross peaks of three methoxy groups with the carbon signals of C-2´, 3´ and 4´, and an important correlation from H-1 to C-5´ at δ 67.8, resulting in a 2,3,4-tri-*O*-methyl-6-deoxy-α-glucopyranoside as substructure (Figure 50), which was also confirmed by the presence of the peak at *m/z* 471 [M - trimethoxy sugar] on (-)-ESI MS/MS.

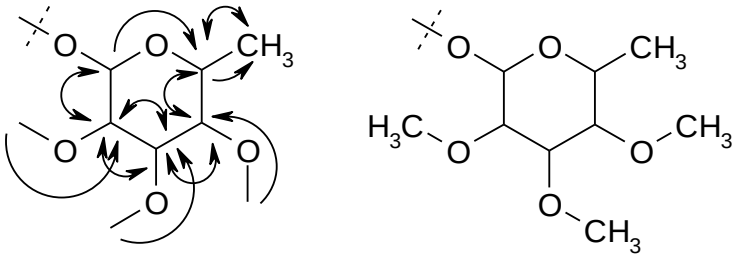


Figure 50: H,H COSY (↔) and HMBC (→) correlations of the sugar moiety (substructure I) of silamycin A (**62a**)

The structure of compound **62a** was extended from substructure I. The methylene group at δ 1.80, 1.42 (δc 38.0) showed in the COSY spectrum couplings with the methine protons at δ 3.83 (δ_C 69.1) and δ 3.91 (δ_C 77.9); the latter indicated HMBC correlations to the carbons atoms at δ 75.3 (C-16) and 69.1 (C-12). The important correlations in the HMBC spectrum of the methine proton at δ 4.18 (16-H) with the carbon atom δ 69.1 (C-12) and the correlation of the anomeric proton at δ 4.90 (1´-H) with the carbon signal at δ 77.9 (C-14) indicated the connection site with the aglycon as shown in substructure II (Figure 51)

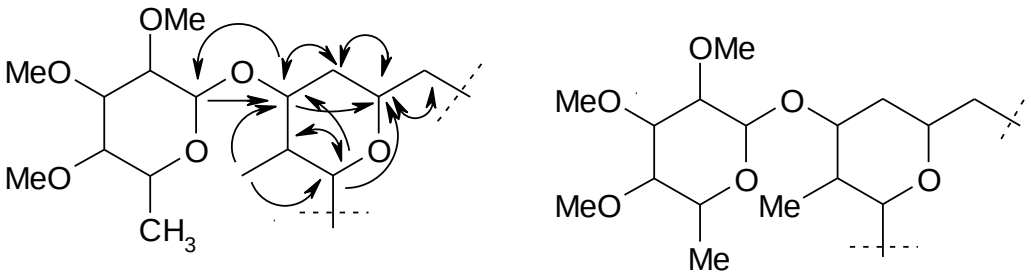


Figure 51: H,H COSY (↔)and HMBC (→) correlations of substructure II

Two oxymethines 4-H, 8-H, a methylene group 6-H_2 and the methyl singlet 7-CH_3 displayed HMBC correlations to the quaternary sp^3 carbon at δ 84.4 (7-C); in addition, 8-H (δ_H 3.09/ 78.6) and the 6-H_2 (δ_H 1.30; 1.80 / 43.9) showed a correlation to the methyl singlet δc 19.5 (7-CH_3). This and further correlations delivered sub-structure III with a methyl-tetrahydrofurane unit (Figure 52).

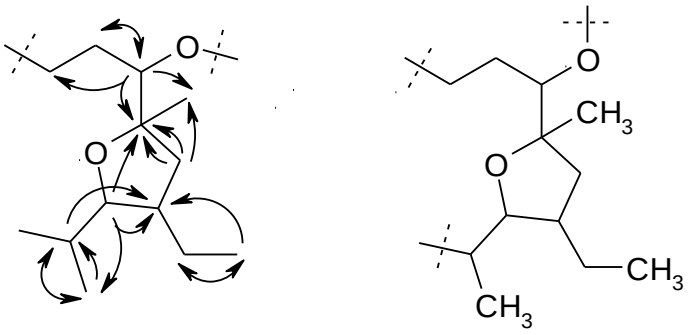


Figure 52: Selected H,H COSY (↔) and HMBC (→) correlations of substructure III

The substructures II and III were connected by the COSY coupling between the methylenes at δ_H 1.42/1.80 (11-H_2) and δ_H 1.52/1.30 (10-H_2) delivering substructure IV ($C_{28}H_{50}O_8$).

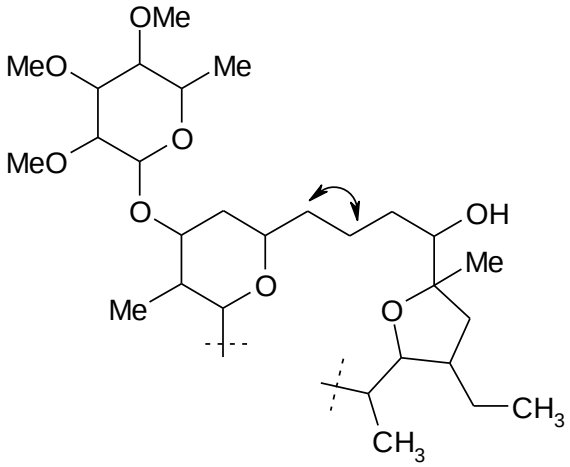


Figure 53: Substructure IV of silamycin A (**62a**) with the connecting H,H COSY (↔) coupling.

The silamycin A contains in addition six quaternary carbons at δ 182.1, 171.0, 147.6, 133.0, 118.7 and 107.5, two methine protons with a coupling constant of 1.3 Hz at δ 6.81 (δ_c 138.6) and 5.72 (δ_c 95.8) and two acid protons (OH). The HMBC

correlations of the remaining fragment $C_8H_4O_4$ indicated the following evidences: In addition to their long-range (^4J) coupling in the H,H COSY spectrum, the methines 1-H and 19-H indicated a correlation with the carbonyl signal at δ 171.0, the methines at δ 4.18 (16-H), 5.72 (19-H) and the acid proton at δ 9.31 correlated with the carbon atoms at δ 182.1 (C-22), 147.6 (C-21), and 107.5 (C-17). Further correlations were seen from the methines at δ 6.81 (1-H), 5.72 (19-H) and 4.18 (16-H) to the carbon signal at δ 133.0 (C-18) and from the acidic proton at δ 9.31, 16-H and 1-H to the carbon at δ 107.5 (C-17). Only the acid proton at δ 9.31 showed a coupling with the second carbonyl signal at δ 182.1.

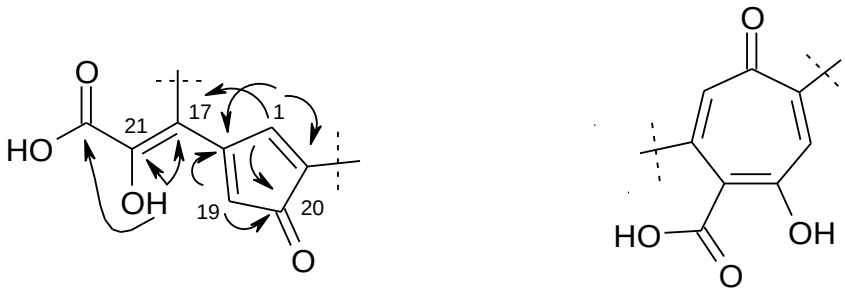
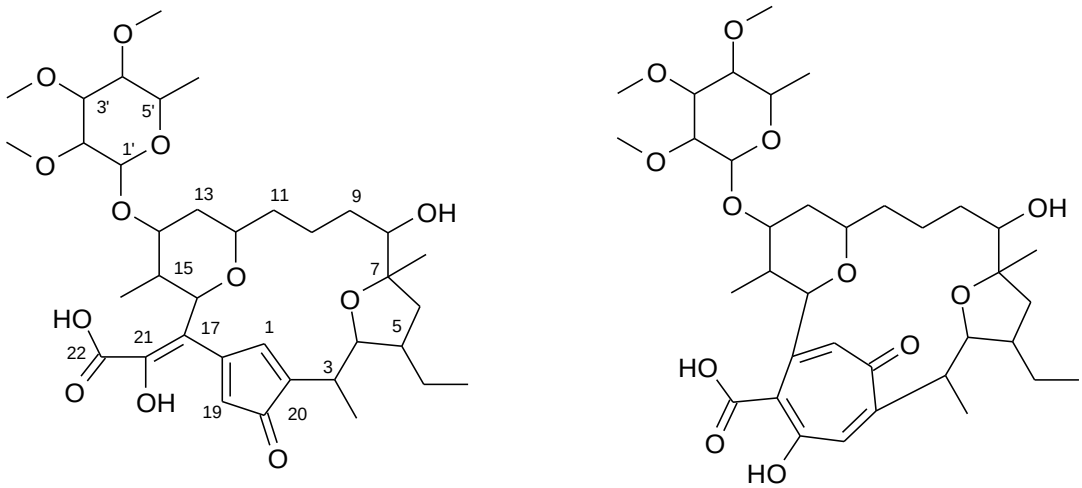


Figure 54: Alternative substructures V of silamycin a (**62a**)

The connection of substructures IV and V was done through the interpretation of the HMBC correlations from the 3-CH_3, 1-H, 3-H and 4-H protons to the quaternary sp^2 carbon at δ 118.7, in addition the 3-H proton displayed another coupling with the carbon atom δ 138.6 (C-1), leading to two possible structures C and D (**62a**).



C D

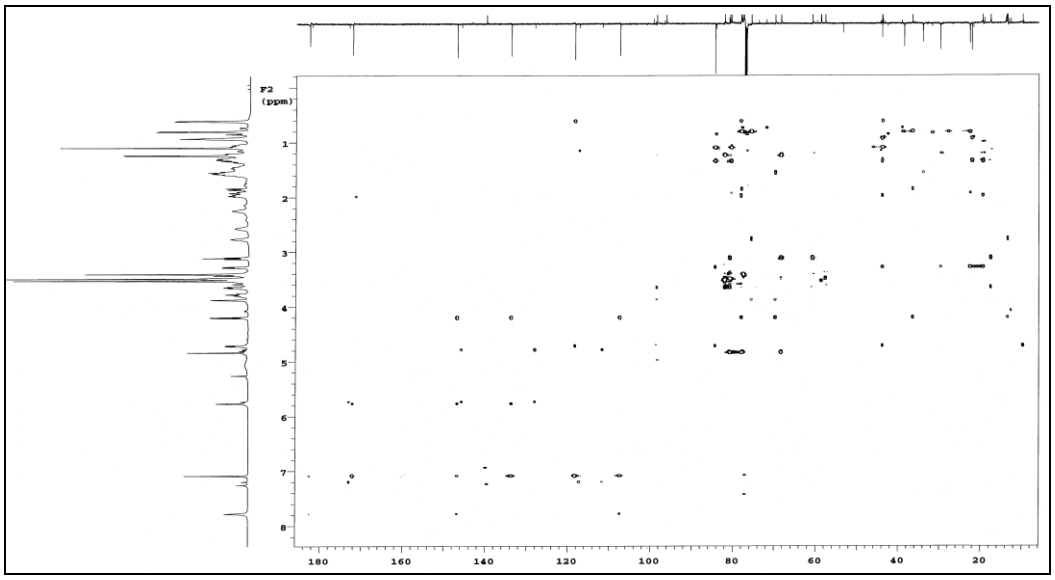


Figure 55: HMBC spectrum of silamycin A (**62a**)

The structure D was ruled out due to the absence of many HMBC correlations e.g: the methine at δ 5.72 (δ_c 95.8) did not show a ^2J signal to the carbonyl signal at δ 182.1, and would indicate a ^4J correlation to the carbonyl at δ 172.0. In addition many HMBC correlations in structure C could be explained by ^2J and ^3J coupling, therefore compound (**62a)** was identified to have structure C.

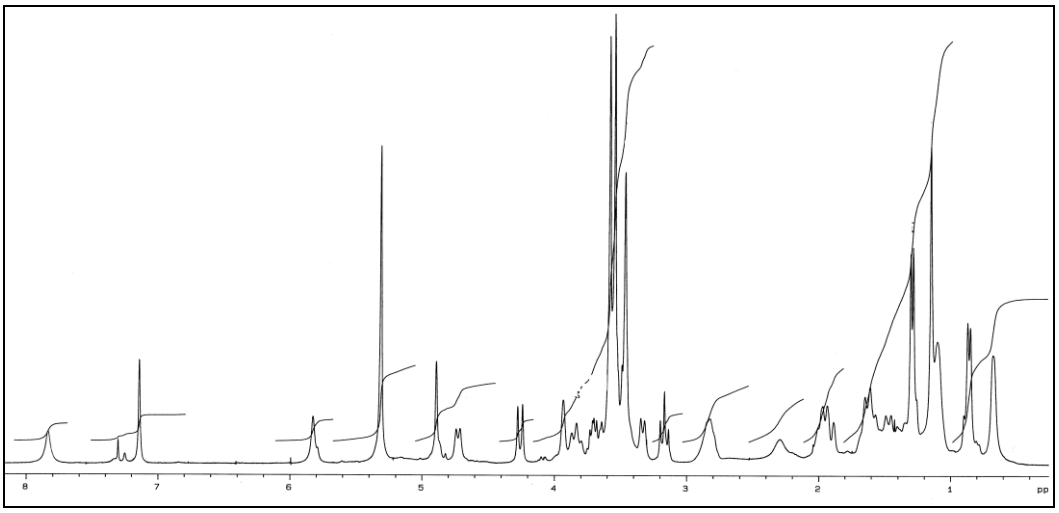
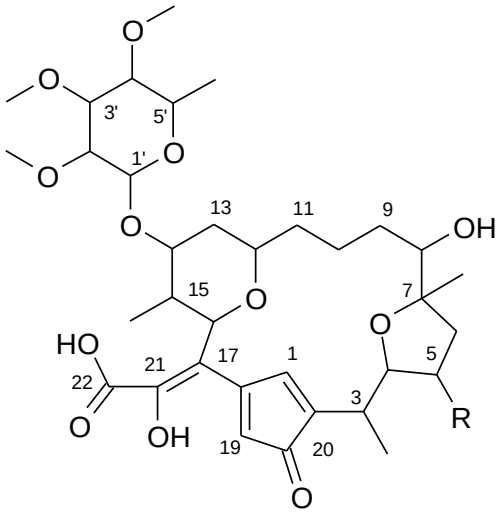


Figure 56: ^{1}H NMR spectrum of silamycin B (**62b**)

The major difference between **62a** and **62b** was the absence of a methylene group in **62b**. This was confirmed by the molecular formula, and the presence of a methyl doublet at δ 1.02 in **62b** instead of a methyl triplet in **62a** at δ 0.95. The struc-

ture of **62b** was finally determined by interpretation of HMBC and comparison of the data with those of **62a**.



62a: R = Et

62b: R = Me

The relative stereochemistry of compound **62a** was determined by the interpretation of the NOESY spectrum which showed cross signals between the pair of methines 16-H (δ_H 4.18) and 12-H, 4-H and 5-H, further signals were also seen between protons 14-H and 15-H, resulting in Fig. 57. The lack of resolved *J* coupling of 3'-H did not allowed the determination of the sugar stereochemistry.

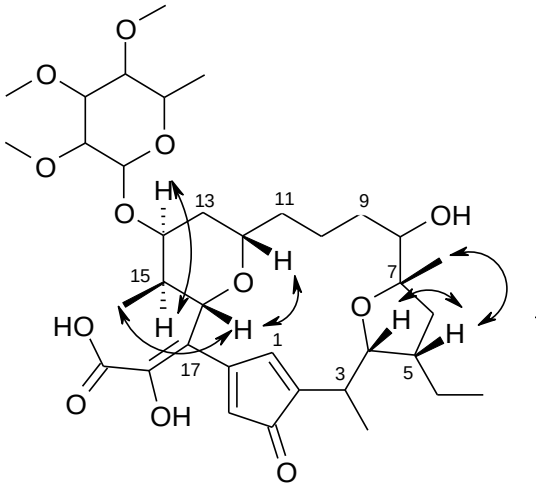


Figure 57: Significant NOESY correlations in **62a** and **62b**

Table 3: ^{1}H (300 MHz, *J* in Hz) and ^{13}C (75 MHz) NMR data of silamycin A (**62a**) and B (**62b**) in $CDCl_3$

No	**62a**	**62b**	**62a**	**62b**
1	7.13 (br s)	7.15 (br s)	139.7	138.8
2	-		118.3	118.3
3	2.21 (brq, 2.1)	2.31 (brs)	43.9	44.0
3-Me	0.69 (d, 6.3)	0.68 (br s)	9.8	9.5
4	4.77 (d, 9.3)	4.73 (d, 9.0)	78.2	78.5
5	2.63 (m)	2.85 (m)	43.9	36.5
5-$CH_2$$CH_3$	1.36 (m)	-	22.1	-
5-CH_2CH_3	0.99 (t, 7.0)	1.06 (d, 7.1)	13.7	13.5
6	2.00, 1.38 (m)	2.00, 1.40 (m)	43.8	46.1
7	-	-	84.4	84.3
7-Me	1.16 (s)	1.13 (s)	19.5	19.3
8	3.35 (d, 8.9)	3.36 (d, 9.1)	80.5	80.5
9	1.23, 1.10 (m)	1.20 (m)	29.7	29.7
10	1.52 (m)	1.58 (m)	22.5	22.5
11	1.90, 1.60 (m)	1.86, 1.66 (m)	38.5	38.5
12	3.81 (br t, 11.0)	3.91 (m)	69.8	69.8
13	1.62, 1.36 (m)	1.70 (m)	33.9	33.9
14	3.94 (d, 2.5)	3.98 (m)	78.0	78.0
15	2.83 (m)	2.97 (m)	36.5	36.0
15-Me	0.87 (d, 6.7)	0.86 (d, 6.3)	13.5	13.5
16	4.25 (d, 11.0)	4.26 (d, 10.9)	75.7	75.7
17	-	-	107.4	107.4
18	-	-	146.8	146.8
19	5.85 (br s)	5.83 (br s)	96.2	96.2
20	-	-	182.6	182.6
21	-	-	133.8	133.8
22	-	-	172.4	172.2
1’	4.90 (d, 1.2)	4.89 (br s)	98.5	98.5
2’	3.58 (m)	3.58 (m)	77.6	77.6
2’-OMe	3.48 (s)	3.47 (s)	58.7	58.7
3’	3.52 (m)	3.58 (m)	80.8	80.8
3’-OMe	3.56 (m)	3.55 (m)	57.7	57.7
4’	3.17 (t, 9.2)	3.18 (t, 9.2)	82.0	82.1
4’-OMe	3.59 (s)	3.58 (s)	60.8	60.8
5’	3.71 (m)	3.71 (m)	68.4	68.4
6’	1.30 (d, 6.2)	1.29 (d, 6.0)	17.6	17.6
OH	7.83 (br s)	7.84 (br s)	-	-

Table 4: ^{1}H (300 MHz, *J* in Hz) and ^{13}C (75 MHz) NMR data of silamycin A (**62a**) in DMSO-d_6

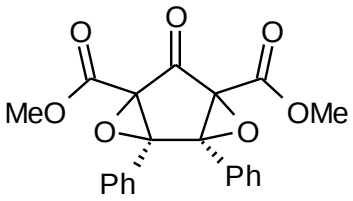
No	^{1}H	^{13}C	No	^{1}H	^{13}C
1	6.81 (d, 1.3)	138.6	15-Me	0.80 (d, 6.7)	13.4
2	-	118.3	16	4.18 (d, 10.8)	75.3
3	2.16 (q, 6.7)	43.4	17	-	107.5
3-Me	0.56 (d, 6.7)	9.3	18	-	133.0
4	4.59 (d, 9.8)	77.6	19	5.72 (d, 1.3)	95.8
5	2.54 (m)	43.1	20	-	171.0
5-CH_2CH$_3$	1.50, 1.30 (m)	21.7	21	-	147.7
5-CH$_2$$CH_3$	0.95 (t, 7.2)	13.5	21-OH	9.31 (s)	-
6	1.80, 1.30 (m)	43.9	22	-	182.1
7	-	84.4	1’	4.90 (d, 1.8)	97.7
7-Me	1.04 (s)	19.5	2’	3.53 (br t, 2.5)	76.6
8	3.09 (d, 9.2)	78.6	2’-OMe	3.36 (s)	57.9
9	1.80, 0.95 (m)	29.0	3’	3.36 (m)	80.6
10	1.52, 1.30 (m)	22.3	3’-OMe	3.34 (s)	56.4
11	1.80, 1.42 (m)	38.0	4’	3.04 (t, 9.4)	81.1
12	3.83 (br t, 11.0)	69.1	4’-OMe	3.42 (s)	59.5
13	1.50, 1.30 (m)	33.7	5’	3.62 (br dd, 9.3, 6.4)	67.8
14	3.91 (m)	77.9	6’	1.30 (d, 6.2)	17.6
15	2.72 (m)	36.1	OH	7.76 (br s)	-

For further confirmation of the structures, methylation of **62**a and **62**b using diazomethane was attempted. This gave, however, only a complex mixture of decomposition products without the properties of the parent compound, with respect to the behaviour on TLC after spraying with anisaldehyde/sulphuric acid.

Silamycin A (**62a**) and B (**62b**) were finally methylated using MeOH with *p*-toluene sulphonic acid as catalyst, which delivered compounds **64a** and **64b**, respectively. The proton NMR spectrum of **64b** was very similar to that of **62b** except for the presence of two additional methoxy groups at δ_H 3.80 (δ_c 56.3) and 3.65 (δ_c 60.0) in **64b**. In addition an important change was observed in the chemical shift of

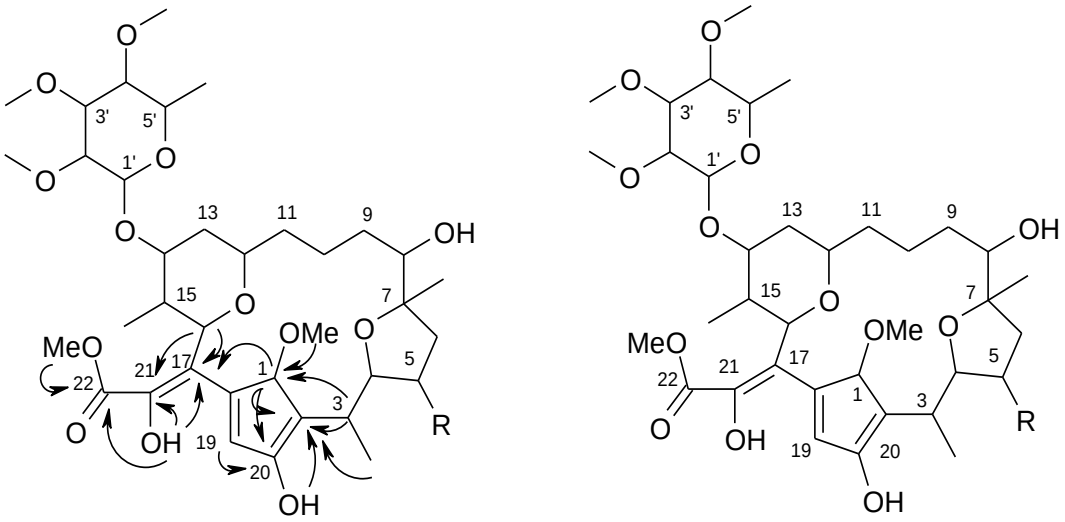
^{1}H (1-H) from δ 7.15 (δ 138.8) in **64b** to δ 5.41 (1H, δ 78.4) in **64b** indicating the reduction of the double bond to single bond. The ^{13}C NMR spectrum indicated the presence of 37 carbon signals including signals of 5 methoxy groups, three of which were attributed to the trimethoxy sugar, and the remaining two were provided by methylation.

Here too, differences were observed in the chemical shifts of the two carbonyl signals: They appeared at δ 182.6 and 172.4 in **62b** and were replaced by signals at δ 149.1 (C-22) and 151.5 (C-20) in structure **64b**. In addition, the shift of C-1 changed from δ 38.8 in **62b** to 78.4 in structure **64b**. The HMBC spectrum of **64b** indicated a correlation of the 1-OCH_3 (δ 3.52), the 3-H and the 20-OH to C-1, additionally, 1-H, 3-H, 4-H, 3-Me and 20-OH displayed correlations to δ 110.9 (C-2). Furthermore, 1-H and 19-H showed correlation to δ 151.5 (C-21) confirming the presence of an α,β-unsaturated carbonyl in the structure of **62b**. Further HMBC correlations were observed between the 22-OCH_3 (δ 3.80), the 21-OH (δ 5.48) and the carbon at δ 149.1 (C-22). It was very surprising to find the chemical shift of an ester carbonyl at δ 149.1, but a similar value was observed for the ester carbonyl at δ 141.0 in compound **63** [112]



63

The structure of compound **64a** and **64b** were elucidated as indicated in the Figure 58 below.

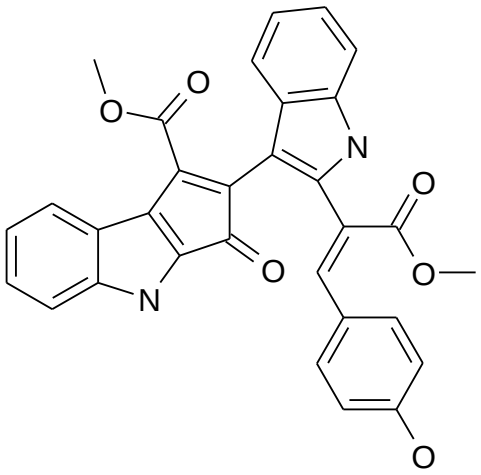


64a: R = Et

64b: R = Me

Figure 58: Selected HMBC correlations in 1-methoxysilamycin A (**64a**) and B (**64b**) methyl esters

Silamycin A (**62a**) and B (**62b**) are the first members of this 19-membered unusual cyclopentendienone derivatives connected to a α-hydroxyacrylic acid. Natural products from microorganisms possessing a cyclopentendienone unit are very rare, to my knowledge only scytonine (**65**) [113] and nostodione A (**66**) [114] are comparable.



65

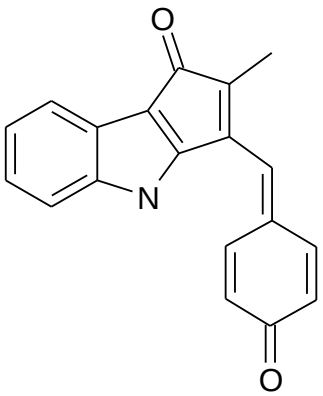


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Table 5: ^{1}H (300 MHz) and ^{13}C (150 MHz) NMR data of silamycin B (**62b**) (75 MHz) and 1-methoxysilamycin B methyl ester (**64b**) in $CDCl_3$

No	**62b**	**64b**	**62b**	**64b**
1	7.15 (br s)	5.41 (br s)	138.8	78.4
1-OMe	-	3.65 (s)	-	60.0
2	-	-	118.3	110.9
3	2.31 (brs)	2.21 (q, 7.1)	44.0	42.0
3-Me	0.68 (br s)	0.62 (d, 7.1)	9.5	10.2
4	4.73 (d, 9.0)	4.61 (d, 9.6)	78.5	79.5
5	2.85 (m)	2.83 (m)	36.5	36.6
5-CH_3	1.12 (d, 7.0)	1.06 (d, 7.1)	13.5	13.6
6	2.00, 1.50 (m)	2.00, 1.40 (m)	46.1	46.5
7	-	-	84.3	84.2
7-Me	1.13 (s)	1.13 (s)	19.3	19.5
8	3.36 (d, 9.1)	3.33 (d, 9.1)	80.5	81.1
9	1.20 (m)	1.40,1.10 (m)	29.7	29.9
10	1.58 (m)	1.62 (m)	22.5	22.8
11	1.86, 1.66 (m)	2.00, 1.60 (m)	38.5	39.2
12	3.91 (m)	3.85 (m)	69.8	70.8
13	1.70 (m)	1.62 (m)	33.9	34.0
14	3.98 (m)	3.94 (m)	78.0	78.6
15	2.97 (m)	2.85 (m)	36.5	36.9
15-Me	0.86 (d, 6.3)	0.86 (d, 6.3)	13.5	14.0
16	4.26 (d, 10.9)	4.51 (d, 10.9)	75.7	76.8
17	-	-	107.4	118.6
18	-	-	133.8	121.1
19	5.83 (br s)	6.24 (s)	96.2	93.2
20	-	-	172.4	151.5
20-OH	-	4.86 (s)	-	-
21	-	-	146.8	138.4
21-OH	-	5.52 (s)	-	-
22	-	-	182.6	149.1
22-OMe	-	3.80 (s)	-	56.3
1’	4.89 (br s)	4.91 (d, 1.7)	98.5	98.9
2’	3.58 (m)	3.60 (m)	77.6	77.8
2’-OMe	3.47 (s)	3.45 (s)	58.7	58.9
3’	3.58 (m)	3.50 (m)	80.8	81.2
3’-OMe	3.55 (m)	3.47 (s)	57.7	57.7
4’	3.18 (t, 9.2)	3.15 (t, 9.4)	82.0	82.2
4’-OMe	3.58 (s)	3.53 (s)	60.8	61.0
5’	3.71 (m)	3.73 (dd, 9.3, 6.4)	68.4	68.5
6’	1.29 (d, 6.0)	1.27 (d, 6.3)	17.6	17.6
OH	7.84 (br s)	-	-	-

6.3.2 Moyopomycin A and B

Compound **67**a was obtained as a yellow solid with a green fluorescence under UV light at 366 nm and a yellow spot on TLC, which turned greenish on spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **67a** (in $CDCl_3$) was very simple: In the aliphatic region, it exhibited only three methoxy signals at δ 3.75, 3.44, 3.11 and a signal at δ 2.94, which may be attributed to an heteroatom bound methyl group. The aromatic region displayed one H/D exchangeable proton at δ 9.24 as a broad singlet. In addition, five *sp*^{2} protons appeared as four doublets of doublets and a doublet at δ 7.74, 7.59, 7.54, 7.50 and δ 7.21, respectively.

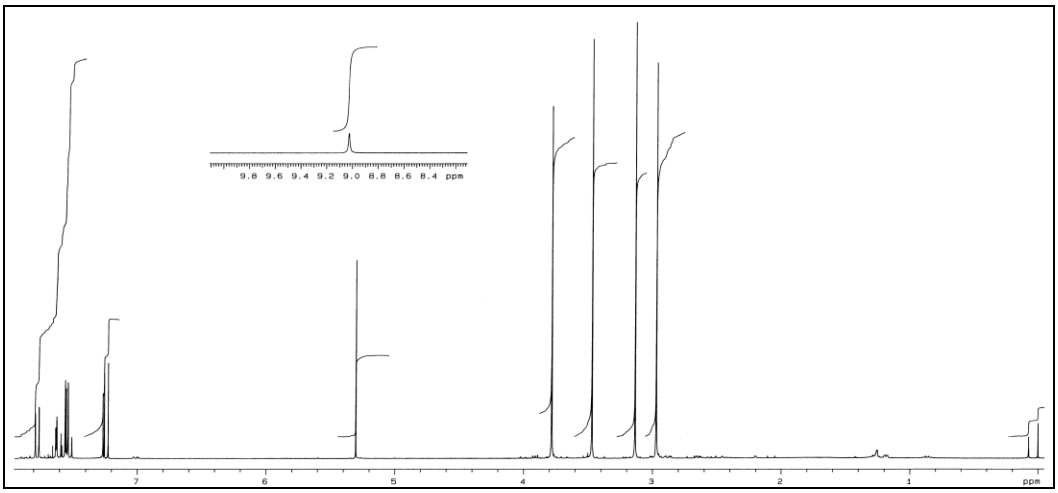


Figure 59: ^{1}H NMR spectrum (300 MHz) of compound **67a** in $CDCl_3$

The ^{13}C NMR spectrum (in $CDCl_3$) showed 24 resolved signals, which were classified as three carbonyls at δ 194.5, 171.3 and 167.0, five *sp*^{2} methine carbons, nine quaternary *sp*^{2} carbons, and three quaternary oxygenated *sp*^{3} carbons; the first two may be attributed to ketal, *hemi*-ketal or *hemi*-aminal carbon atoms. Three methoxy signals at δ 55.2, 55.0 52.3 and an *N*-methyl carbon at δ 25.0 by (APT NMR) completed the spectrum.

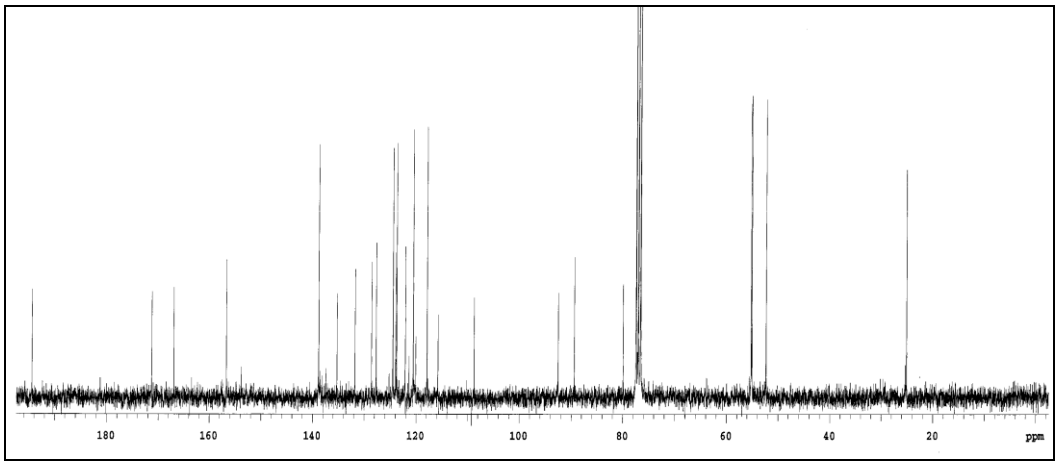


Figure 60: ^{13}C NMR spectrum (75 MHz) of moyopomycin A (**67a**)

The EI mass spectrum indicated with peaks at *m/z* 549, 551, 553 and 555 the pattern of a trichlorinated compound, and (-)-ESI MS showed peaks at *m/z* 548 and 550 $[M-H]^{-1}$ confirming the presence of an halogen atom. The molecular formula was determined as $C_{24}H_{18}N_3O_6Cl_3$ by (-)-HRESIMS indicating 16 double bond equivalents. The search in AntiBase revealed no hit pointing compound **67a** to be a novel natural product.

The assignment of the shift values was established by the HSQC spectrum, and analysis of the H,H COSY spectrum gave two partial structures (Figure 61).

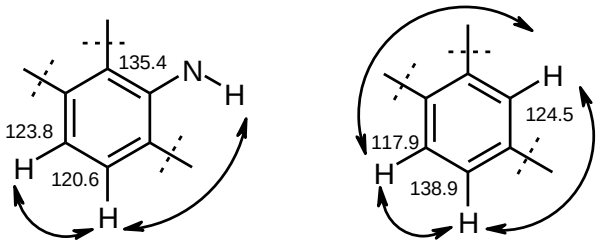
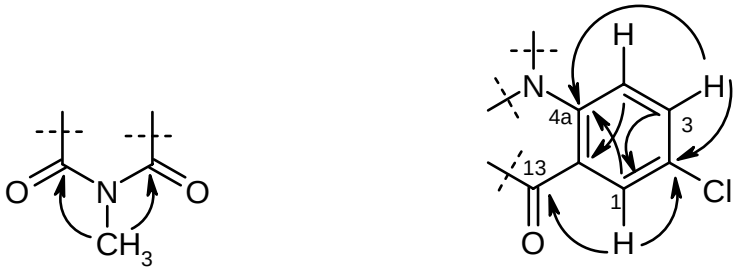


Figure 61: Partial structures of moyopomycin A (**67a**) from the H,H COSY (↔) spectrum

Interpretation of the long range couplings observed in the HMBC spectrum indicated the following evidences: the *N*-methyl signal at δ 2.94 (s) showed cross peaks to the carbonyls at δ 171.3 and 167.0. The proton signals at δ 7.54 (δ 138.9, 3-C) and δ 7.59 (δ 124.5, 1-C) indicated correlations to the carbon atoms at δ 156.8 (4a-C) and 127.7 (2-C). In addition, a cross peak was seen from 1-H (δ 7.59) to the carbonyl

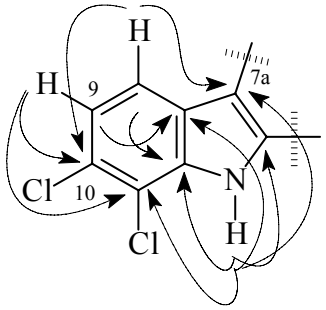
carbon at δ 194.5 (13-C) and a quaternary carbon at δ 138.9. Another correlation was observed from 1-H to C-13a (δ 122.1) leading to the fragments I and II (Figure 62).



I II

Figure 62: Fragments I and II of **67a** from HMBC spectrum

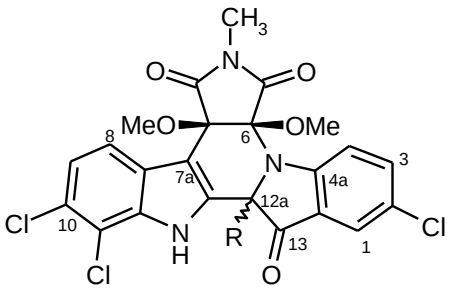
Further correlations were observed in the HMBC spectrum between the acidic proton (N-H) at δ 9.24 and 8-H at δ 7.74 (δc 120.6) to the quaternary carbon atoms at δ 135.4 (C-11a) and 108.7 (C-7a). Both protons at 7.74 (δ 120.6) and 7.21 (δ 123.8), respectively, showed cross peaks to the carbon atom at δ 128.6 (C-10), furthermore the NH proton and the proton at 7.21 (9-H, δc 123.8) exhibited correlation to a carbon atom at δ 124.0. With these data, fragment III (Figure 63) was constructed.



III

Figure 63: HMBC (→) correlations of fragment III

The fragments I, II and III possess 13 double bond equivalents and 18 carbon atoms. The six remaining carbons atoms were three methoxy groups and three quaternary carbons, which must be connected to the fragments in a way to generate 3 rings. This resulted in structure **67a**, for which the name moyopomycin A is suggested.



67a: R = OCH_3

67b: R = OH

The minor component **67b** was obtained as yellow solid as well. It presented the same properties as **67a** under UV light and on TLC after spraying with anisaldehyde/sulphuric acid.

Also the proton NMR spectrum of compound **67b** was very similar to that of **67a**. It exhibited in the aromatic region an H/D exchangeable proton at δ 9.28 (*br* s) and five sp^2 protons representing a 1,2,4-trisubstituted aromatic system and a 1,2,3,4-tetrasubstituted aromatic ring. The major difference appeared in the aliphatic region with the presence of an acidic proton at δ 4.84 (*br* s) and only the signals of two methoxy groups at δ 3.76, 3.13 instead of three as in **67a.**

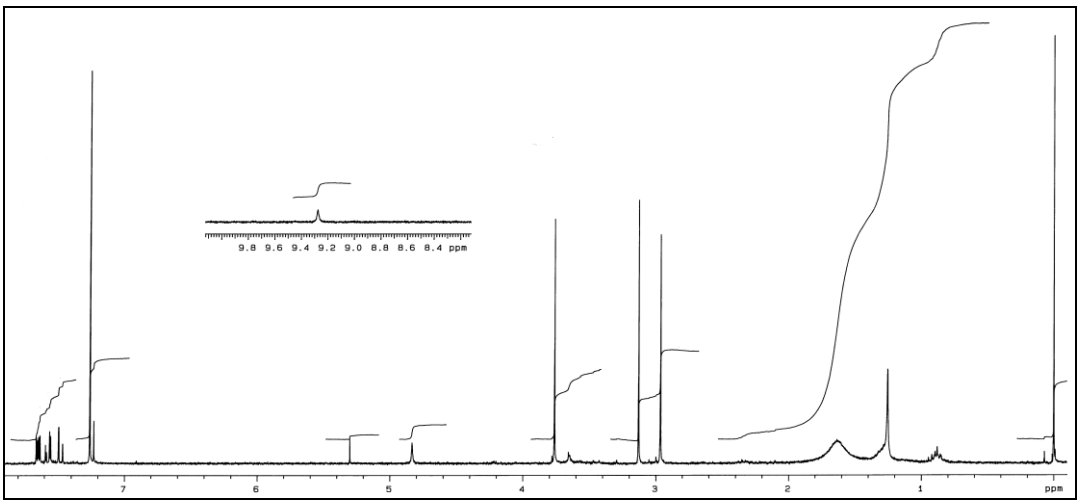


Figure 64: ^{1}H NMR spectrum (300 MHz) of moyopomycin B (**67b)** in $CDCl_3$

The ^{13}C and APT NMR spectra of **67b** showed also similarities with that of **67a** and indicated 23 carbon signals. Here too the major difference was the absence of a signal which appeared at δ 52.3 in **67a**. (-)-HRESIMS delivered the molecular formula $C_{23}H_{16}N_3O_6Cl_3$ confirming also the absence of one methoxy group. The struc-

ture of **67b** was deduced by comparison of the data with those of **67a** and further compounds from the literature: Compound **67b** was elucidated as the demethyl derivative of **67a** and the name moyopomycin B was proposed.

The relative configuration of **67a** and **67b** was determined by interpretation of the NOESY spectrum, which showed cross peaks between 4-H and 6-OMe and also cross peaks between 6-OMe and 7-OMe.

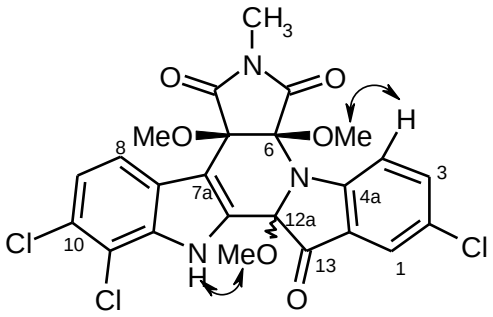


Figure 65 : NOESY correlations in **67a**

Table 6: ^{13}C NMR (150 MHz) data of moyopomycin A (**67a**) and B (**67b**)

C_{No}	**67a**	**67b**	C_{No}	**67a**	**67b**
1	124.5	125.3	8	120.6	120.1
2	127.7	128.4	9	123.8	124.0
3	138.9	138.6	10	128.6	128.8
4	117.9	117.7	11	115.8	112.1
4a	156.8	155.9	11a	135.4	135.7
6	92.5	89.1	12a	131.8	133.1
6-OMe	55.2	55.6	12b	89.6	84.1
7	80.0	80.8	12b-OMe	52.3	-
7a	108.7	94.6	13	194.5	193.0
7b	124.0	123.8	13a	122.1	121.7
7-OMe	55.0	55.1	6’	167.0	171.2
N-Me	25.0	25.3	7’	171.3	172.0
N-H	-	-			

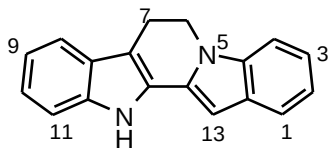
Table 7: ^{1}H NMR (600 MHz) data of moyopomycin A (**67a**) and B (**67b**) in $CDCl_3$ (δ; *J* in Hz)

H_{No}	**67a**	**67b**	H_{No}	**67a**	**67b**
1	7.59 (dd, 2.3, 0.5)	7.65 (dd, 2.3, 0.5)	8	7.74 (dd, 8.6, 0.6)	7.66 (dd, 8.6, 0.6)
2	-	-	9	7.21 (d, 8.6)	7.25 (d, 8.8)
3	7.54 (dd, 9.0, 2.3)	7.57 (dd, 2.3, 9.0)	10	-	-
4	7.50 (dd, 9.0, 0.5)	7.49 (dd, 9.0 0.5)	11	-	-
4	-	-	11a	-	-
6	-	-	12a	-	-
6-OMe	3.75	3.76 (s)	12b	-	-
7	-	-	12b-OMe	3.44 (s)	-
7a	-	-	13	-	-
7b	-	-	13a	-	-
7-OMe	3.11 (s)	3.13 (s)	6’	-	-
N-Me	2.94 (s)	9.28 (s)	7’	-	-
N-H	9.24 (s)	9.28 (s)			

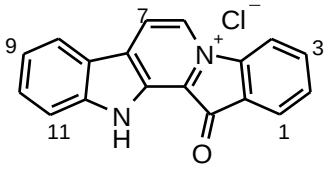
Table 8: Physicochemical properties of moyopomycin A (**67a**) and B (**67b**)

	67a	**67ab**
Appearance	Yellow solid	Yellow solid
R_f (CH_2Cl_2/5 % MeOH)	0.83	0.52
Molecular formula	$C_{24}H_{18}O_6\ N_3Cl_3$	$C_{23}H_{16}O_6\ N_3Cl_3$
(-)-ESI MS: *m/z* (%)	548 ($[M-H]^{-1}$, 26), 550 ($[M_2-H]^-$, 26), 1119 ($[2M+Na-H]^{-1}$, 50), 1121 ($[2M+Na-2H]^{-1}$, 50)	534 ($[M-H]^{-1}$, 96), 536 ($[M-H]^-$, 100), 538 (26) 1093 ($[2M+Na-2H]^{-1}$,88)
EI MS: *m/z* (%)	549 (16), 551 (15), 553 (3), 555 (4), 520 (98), 518 (100)	-
(-)-HRESIMS	548.018982 ($[M-H]^{-1}$) (calcd. 548.017763)	534.003267 ($[M-H]^{-1}$) (calcd. 534.002113)
IR (KBr): ν_{max} cm^{-1}	3524, 2926, 2351, 2284, 1725, 1634, 1464, 1436, 1385, 1304, 1179, 1113, 1079, 1020, 806, 792	3535, 2932, 1723, 1635, 1465, 1385, 1115, 1022, 876, 792
UV/VIS (MeOH): λ_{max} (log ε)	376 (3.44), 302 (3.84), 233 (4.5)	372 (3.12), 300 (3.67), 236 (4.43)

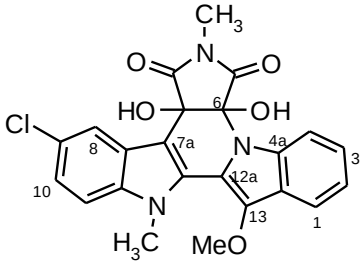
Moyopomycin A (**67a**) and B (**67b**) are bis-indole natural products and new members with the rare pentacyclic 12*H*-pyrido[1,2-a:3,4-b']diindole system (**68**). In the last 30 years, a variety of indolecarbazoles has been isolated from natural sources, [115] and because of their diverse structures and different biological activities, they became a very interesting research topic. Indolo[3,2-*a*]carbazoles or indolo[3,2-*c*]carbazoles like the natural BE-54017 (**70**) have structures closely related to **67a** and **67b**, and were also isolated from *Streptomyces* sp.[116] Also included are homofascaplysin A, B and C isolated from the Fijian sponge *Fascaplysinopsis reticulata* [117] and later synthesized by Gribble *et al.*, [118] and fascaplysin (**69**) isolated as quaternary salt from Fijian sponge *Fascaplysinopsis* sp. Berquist [119] was the first member of this group and was also synthesized by Gribble *et al.*[120] Fascaplysin (**69**) and Homofascaplysin are reported to be exclusively from marine origin [121] Recently some brominated fascaplysins have been isolated and their cytotoxicities were reported.[121]



68



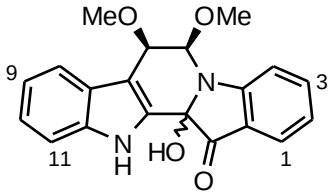
69



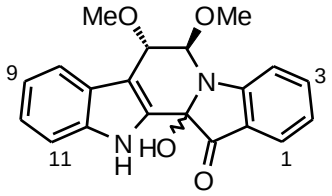
70

Fascaplysin (**69**) is reported to inhibit the growth of microbes such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*. It also shows strong activity against the murine leukaemia L1210 [119], and recently it has been demonstrated that fascaplysin interferes with the elements of the cell cycle machinery by inhibiting the cycle-dependent kinase 4 (cdk4) [122] and by interacting with DNA [123]. Further reports on the reactivity of **69** delivered a mixture of two

stereoisomers [123] **71a** and **71b**. Compound BE-54017 (**70**) shows also activity against P388 murine leukaemia cells. [116]



71a



71b

6.3.3 Celastramycin B

Compound **72a** was obtained as yellow solid, which on thin layer chromatography turned violet in contact with some drops of 2N NaOH indicating a *peri*-hydroxy quinone chromophore. The proton NMR spectrum ($CDCl_3$) exhibited in the aromatic region two *ortho*-coupled protons at δ_H 7.79 (dd, J = 0.5, 8.1 Hz), 7.63 (d, J = 8.1 Hz), a doublet at δ 7.25 (d, J = 0.7 Hz) and two hydroxy protons at δ_H 12.24 (d, J = 0.5 Hz) and 12.20 (s). More ever the presence of two methines was seen, one of them probably connected to an oxygen atom, the second one coupled with a methyl and a methylene group, respectively. The (+)-ESI mass spectrum showed peaks at *m/z* 767 $[2M+Na]^+$ and an isotope peak at *m/z* 769, indicating the presence of a chlorine atom in **72a.**

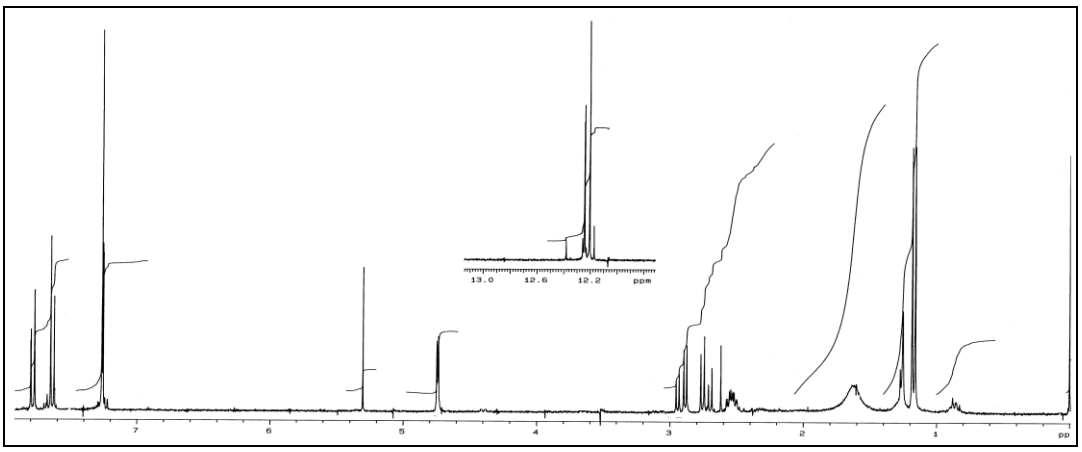
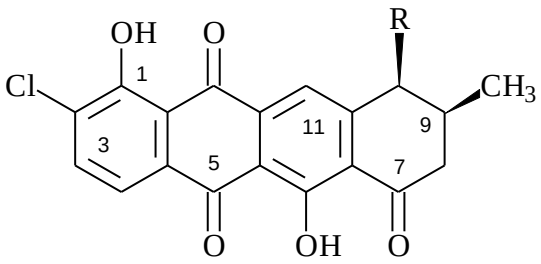


Figure 66: ^1H NMR spectrum (300 MHz) of celastramycin B (**72a**) in $CDCl_3$

The latter was confirmed by the HRESIMS, which delivered the molecular formula $C_{19}H_{13}O_6Cl$. The ^{13}C NMR spectrum indicated 19 signals as demand by the formula, whereby the signals at δ 197.2, 192.4 and 181.7 were suspected to be due to

an α,β-unsaturated ketone and to carbonyls of the quinone system, respectively. The sub-structure search in AntiBase with the NMR data led to celastramycin B (**72a**) as possible structure, which was already reported from *Streptomyces* MaB-QuH-8 [124] and exhibited antibiotic activity against *Mycobacterium vaccae* and *Bacillus subtilis*.



72a: R = OH

72b: R = H

10-Deoxycelastramycin B (**72b**), which was also obtained as yellow solid, showed similarities with celastramycin B (**72a**) in its physical and chemical properties. The ^{1}H NMR spectrum ($CDCl_3$) was very similar to that of **72a** with two chelated hydroxy protons. The *ortho*-coupled protons were shifted slightly upfield from δ_H 7.25 in **72a** and gave a singlet in **72b** instead of a doublet. The major difference appeared in the aliphatic region where the signal due to the methine proton connected to oxygen disappeared and the presence of two methylene groups and a CH_3CH-fragment was evident. The ^{13}C NMR spectrum of **72b** exhibited also 19 carbon signals as in **72a**, which were sorted according to APT to three carbonyl groups, three sp^2 methines, 9 quaternary sp^2 atoms, one aliphatic methine, two methylenes and one methyl carbon atom.

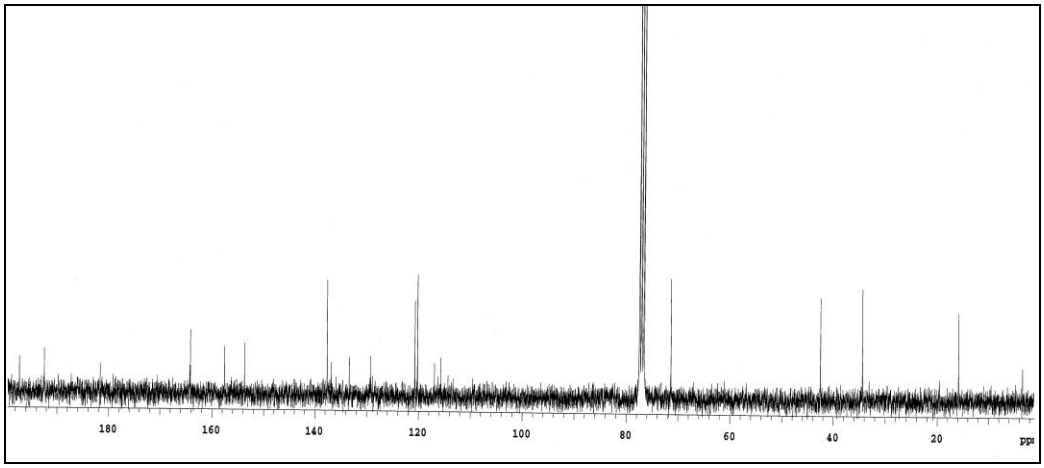


Figure 67: ^{13}C NMR spectrum (75 MHz) of celastramycin B (**72a**) in $CDCl_3$

Through the EI- and (-)-ESI MS, the molecular mass was deduced to be *m/z* 356. The difference Δm = 16 between **72a** and **72b** can be attributed to the loss of one oxygen atom, an assumption which was confirmed by HRESIMS which gave the molecular formula $C_{19}H_{13}O_5Cl$. Interpretation of the 2D NMR spectra (see Figure 68) confirmed the structure of **72b** as 10-deoxy celastramycin B, which is reported here as a novel natural product.

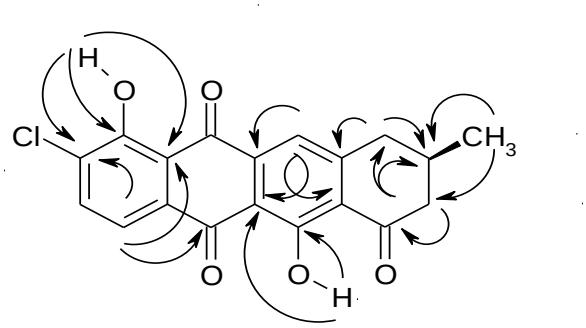


Figure 68: HMBC correlations of 10-deoxy-celastramycin B (**72b**)

6.3.4 Celastramycin D

Celastramycin D **73** was obtained as yellow powder which did not dissolve in water and DMSO, but was slightly soluble in $CDCl_3$ and CD_2Cl_2. The aromatic part of the proton NMR spectrum of **73** with sharp singlets of two chelated phenolic hydroxy groups and two *ortho* protons was also similar to that of **72a** and **72b**. However, a downfield shift of H-11 was noticed from δ 7.25 in **72a, b** to δ 7.90 in **73**, suggesting a strong electron acceptor in the neighbourhood. In addition, another sp^2 proton appeared as a quartet at δ 6.98 (J = 1.5 Hz) and a unique signal in the aliphatic region of a methyl doublet at δ 2.17 (J = 1.5 Hz) suggesting a CH_3-C=CH- fragment in **73**.

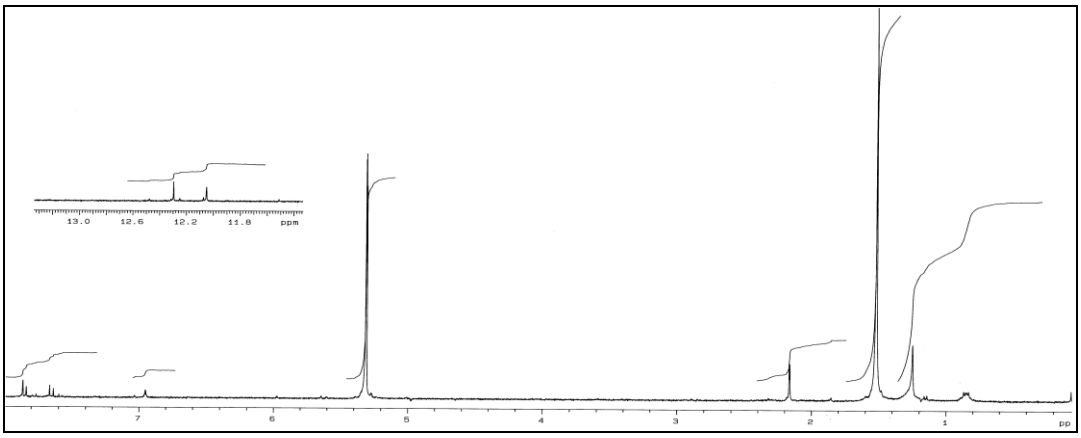
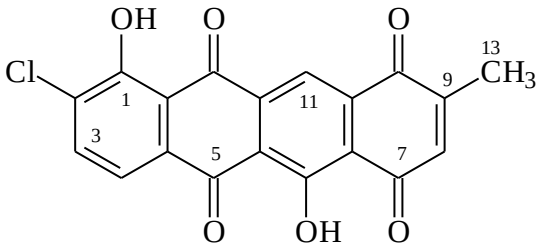


Figure 69: ^{1}H NMR spectrum (300 MHz) of celastramycin D (**73**) in CD_2Cl_2

EI and (-)-ESI MS indicated two pairs of peaks at *m/z* 368/370 and 367/369, respectively, and the molecular mass was deduced to be *m/z* 368. The molecular formula was obtained from HRESIMS ($C_{19}H_9O_6Cl$) indicating four hydrogen atoms less than in **72b** and containing 15 double bond equivalents. As in **72b**, the major difference had occurred in the last ring, and interpretation of the HMBC spectra led to structure **73** for which we propose the name celastramycin D.



73

6.3.5 Celastramycin E

Obtained as orange solid, celastramycin E (**74a**) is yellow in solution and moderately soluble in most organic solvents. The proton NMR spectrum in CD_2Cl_2 with the chelated hydroxy groups, the two doublets and the singlet is similar to that of celastramycin D (**73**) in the aromatic region. A major difference was the absence of the doublet of H-8, which appeared in **73** at δ 6.98. Besides the methyl signal, which appeared at δ 2.17 as doublet in **73** and as singlet in **74a**, an additional methyl singlet at δ 2.78 in compound **74a** was obvious, which may be attributed to an aromatic or heteroatom bound methyl.

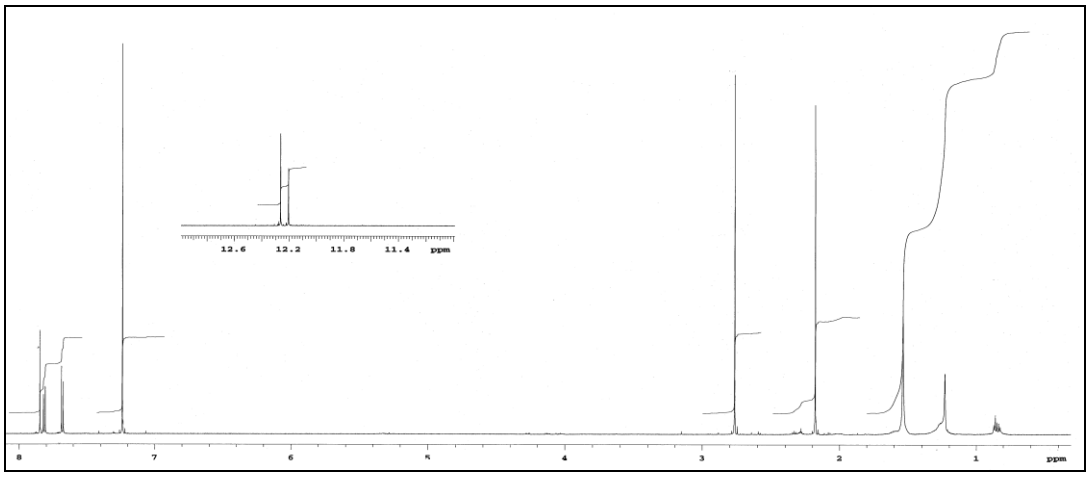


Figure 70: ^{1}H NMR spectrum (600 MHz) of celastramycin E (**74a**) in $CDCl_3$

The carbon NMR spectrum exhibited the signals of 20 carbons atoms. In addition to the carbonyl at δ 192.5 (5-C), quinone carbonyls appeared at δ 181.1 and 180.7, and a further carbonyl was seen at δ 178.3. The EI and (-)-ESI MS spectra delivered the molecular mass *m/z* 414, and HRESIMS gave the molecular formula $C_{20}H_{11}O_6ClS$. The search in AntiBase delivered no hits. Interpretation of the 2D NMR spectrum indicated that compound **74a** possessed the same fragment A as celastramycin B (**72a**), 10-deoxy-celastramycin B (**72b**) and celastramycin D (**73**), the major difference may have occurred in the last ring.

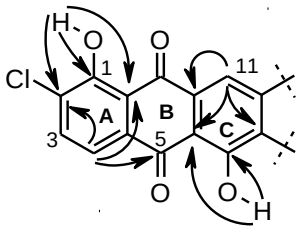


Figure 71: HMBC correlation of fragment A in celastramycin E (**74a**)

Cross peaks were seen in the HMBC spectrum between H-11 (δ 7.85), the methyl at δ 2.18 and the carbonyl at δ 178.3. Both methyls showed also correlation to the carbon atom at δ 155.6 leading to fragment B containing a thiomethyl group. The connection of both fragments gave two possible structures **74a** and **74b** (Figure 73).

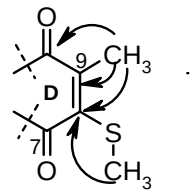
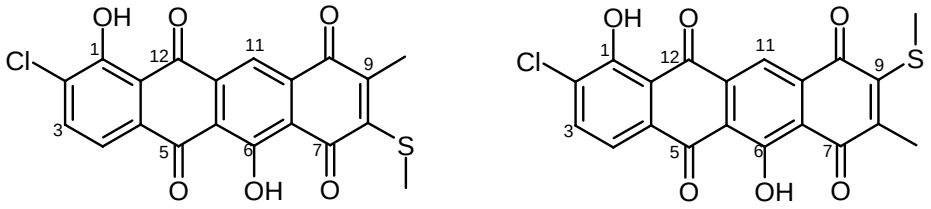


Figure 72: HMBC correlation in fragment B of celastramycin E (**74a**)



74a **74b**

Figure 73: Alternative structures of celastramycin E (**74a**)

The structure of celastramycin E was finally assigned to be **74a** due to the HMBC correlation of H-11 and the methyl at δ 2.18 with the carbonyl at δ 178.3. It follows that all these compounds may be derived from the same biosynthetical precursor followed by modifications like oxidation and thiomethylation of ring D.

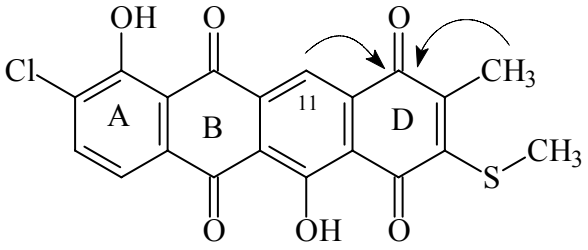
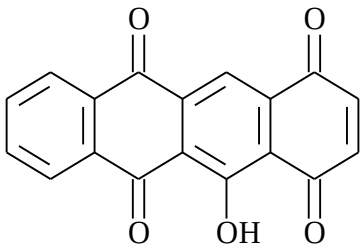


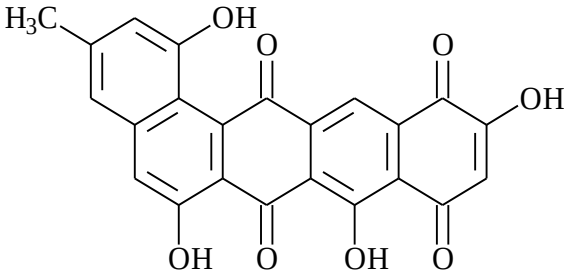
Figure 74: Important HMBC correlations in **74a**

The 1,4,6,11-naphthacenetetrone chromophore **75** as in **73** and **74a** is very rare in natural products, which may be due to an easy reduction of the terminal benzoquinone system to the more stabile dihydroxy-tetracenquinone. To my knowledge only the compound BE-45985X (**76**), so far has been isolated from the culture of *Streptomyces* sp. A45985 [125] and is reported to inhibit *in vitro* the mouse leukaemia cells P388 with IC50 of 5.0 µg/mL. The stability of **74a** may be due to the thiomethyl group, which lowers the oxidation potential. Celastramycin D (**73**) and E (**74a**) are new members of this group. Celastramycin E (**74a**) is also the first example

of a tetracycline containing the thiomethyl group. Several benzoquinones and 2-methyl-3-thiomethyl-1,4-naphthoquinone have been synthesized and exhibited activity against fungi and yeasts.[126]



75



76

6.4 Terrestrial *Streptomyces* sp. GW 14/1869

The terrestrial S*treptomyces* sp GW 14/1869 showed in the agar diffusion test no activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris* and *Scenedesmus subspicatus*. TLC of the crude extract showed some UV absorbing bands, which after spraying with anisaldehyde/sulphuric acid revealed many coloured spots. The 25-liter shaker culture of the strain was incubated in M_2-medium at 28 °C for 4 days. The pre-separation of the crude extract was done by flash column chromatography using silica gel. The final purification by HPLC and Sephadex LH-20 resulted in four compounds (Figure 75). One of them, celastramycin B (**72a**) was already isolated from strain GW 4723 and is described above.

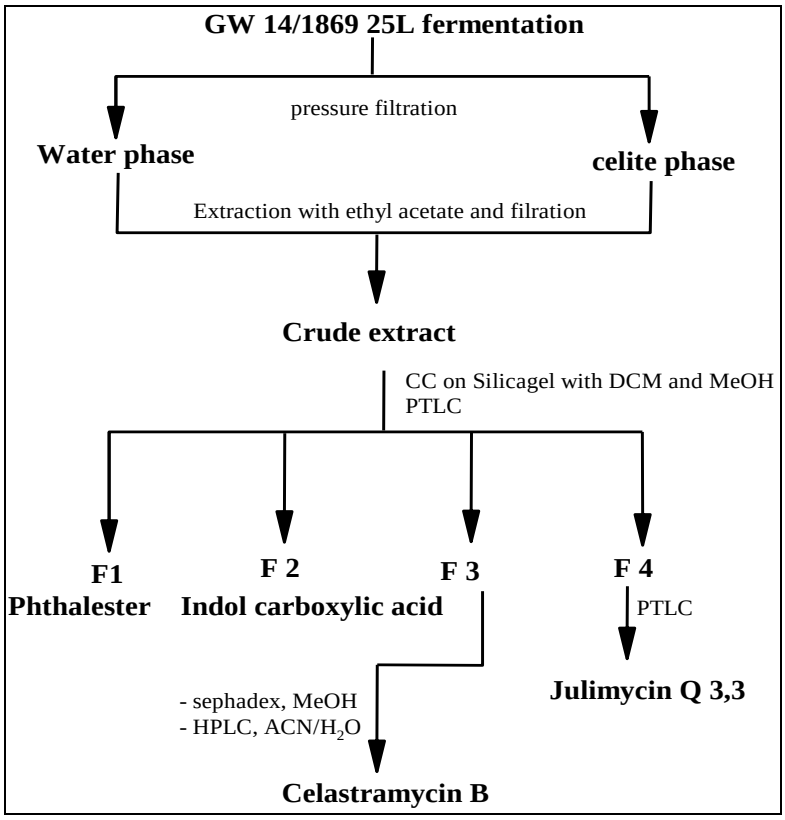


Figure 75: Work-up scheme of the strain *Streptomyces sp*. GW14/1869

6.4.1 Julimycin Q3,3

The yellow orange major compound **77** was isolated from fraction IV. Its colour changed from orange to pink with 2N NaOH indicating a *peri*-hydroxyquinone. Its ^{1}H NMR spectrum in DMSO-d_6 showed two aromatic doublets at δ 7.41 and 7.21 (d, J = 8.2 Hz) characteristic for *ortho*-couplings. In the aliphatic region the presence of a 1H singlet at δ 5.92 and a 1H multiplet at δ 5.60 indicated that these methine groups were attached to an oxygenated carbon. Two broad signals at δ 5.51 and 5.20 were attributed to H/D exchangeable protons. The presence of a CH_3CH-O fragment was characterised by a multiplet at δ 5.60 and a doublet at δ 1.41, respectively. Furthermore, one methine doublet at δ 3.01, a methylene AB system at δ 2.45, and three methyl groups, which appeared as two singlets at δ 1.65, 1.40 and a doublet at δ 1.39 were observed.

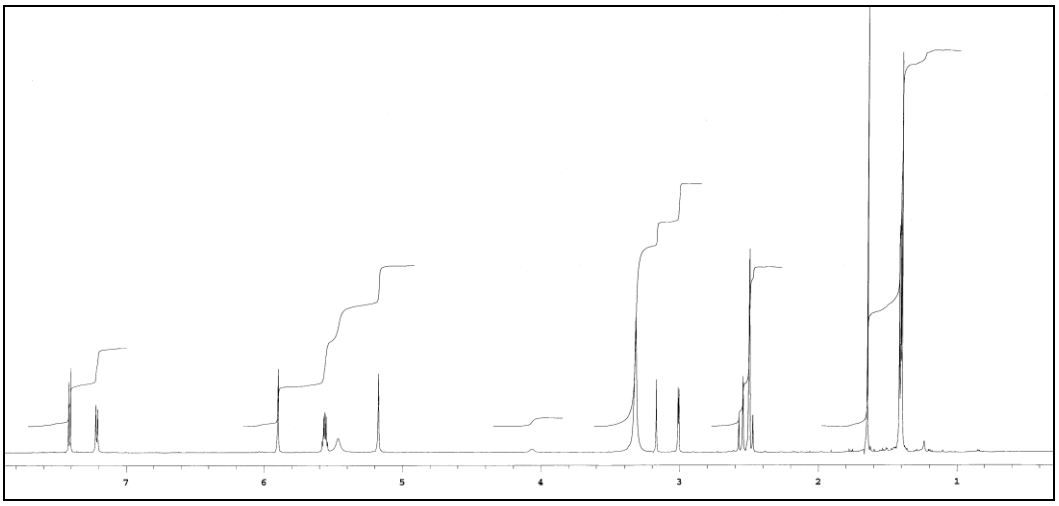


Figure 76: ^{1}H NMR spectrum (300 MHz) of julimycin Q3,3 (**77**) in DMSO-d_6

The ^{13}C NMR spectrum displayed 19 signals for three methyls, one methylene, three methine, two aromatic methine, four aromatic and three aliphatic quaternary carbons, one carbonyl at δ 169.6, and two carbonyls of a ketone at δ 199.4 and 189.9.

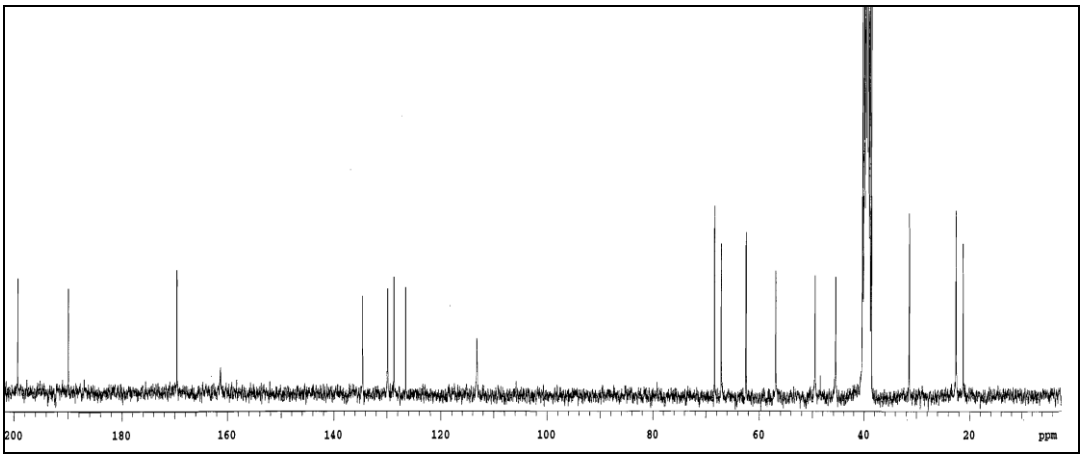


Figure 77: ^{13}C NMR spectrum (75 MHz) of julimycin Q3,3 (**77**) in DMSO-d_6

The (+)-ESI MS gave a *quasi*molecular ion at *m/z* 773 $[M+Na]^+$ and HRESIMS of the *quasi*molecular ion delivered the molecular formula $C_{38}H_{38}O_{16}$. The mass and NMR data of compound **77** suggest that it is a dimmer. The H,H COSY spectrum confirmed the presence of the doublets in *ortho* position, and the fragment CH_3CHCH.

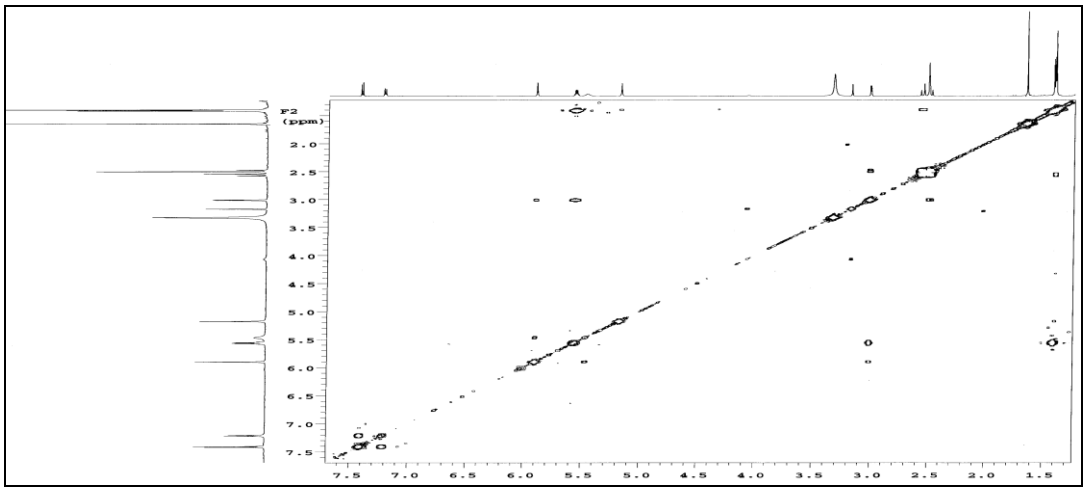


Figure 78: H,H COSY spectrum (300 MHz) of julimycin Q3,3 (**77**) in DMSO-d_6

The HMBC spectrum was of pivotal importance. The doublet at δ 7.21 as well as the doublet at δ 3.01 indicated correlations to the carbonyl at δ 189.9. The strong C,H heteronuclear coupling of 7-H (δ 7.40), 10-H (δ 5.92) to the carbon 9-C (δ 161.3) proved that the phenolic hydroxyl group must be near to 10-H and 10-OH.

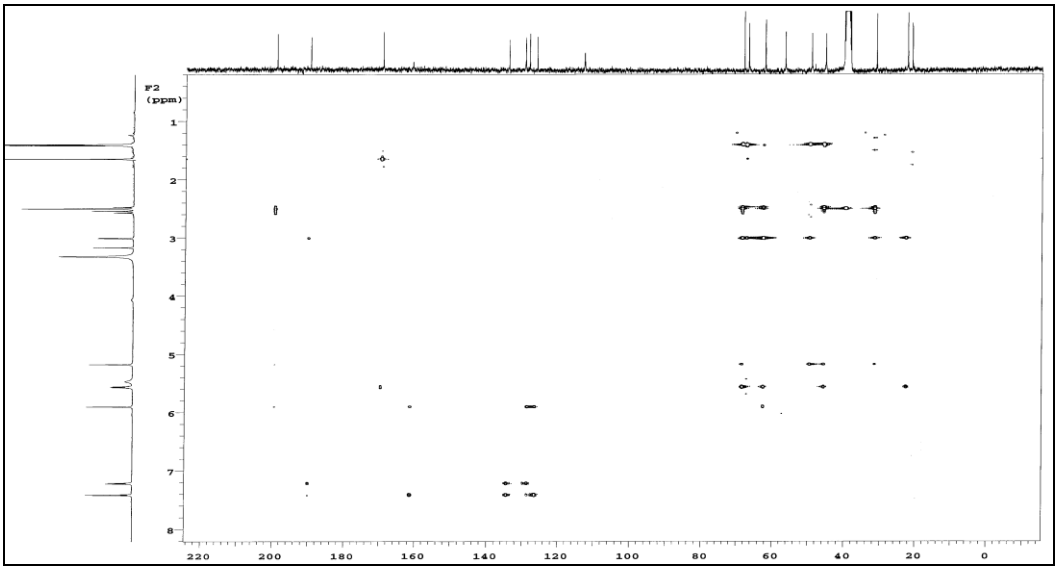
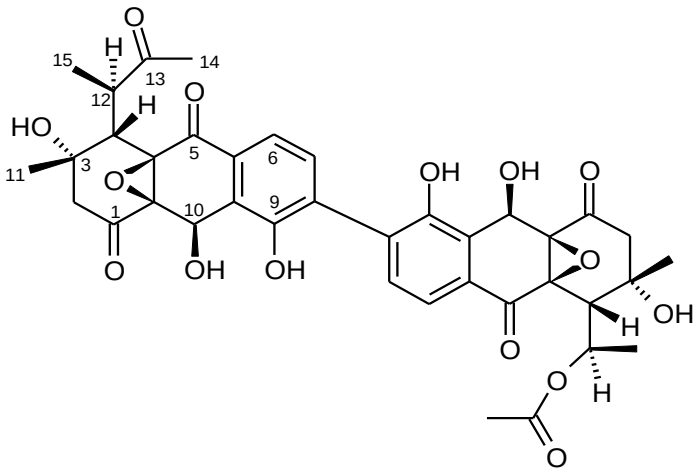


Figure 79: HMBC spectrum (300 MHz) of julimycin Q3,3 (**77**) in DMSO-d_6

Careful interpretation of the 1D and 2D NMR data in combination with the molecular formula led to the dinaphthoquinone julimycin Q3,3 (**77**), which was already reported from *Streptomyces shiodaensis.*[127] The relative stereochemistry of **77** was obtained by interpretation of the NOESY spectrum and confirmed the structure of

compound **77** as julimycin Q3,3. It showed activity against *Chlorella vulgaris* in the agar diffusion test (20 mm inhibition zone).



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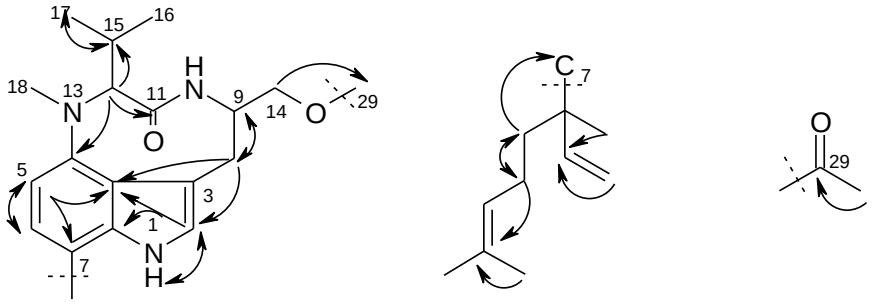
6.5 Terrestrial *Streptomyces* sp. AdM02

In the agar diffusion test, the crude extract of the strain AdM02 showed antibacterial activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* at concentrations of 50 μg per paper disk. 80 Erlenmeyer flasks each containing 250 ml of yeast/malt extract/glucose (YMG) medium were inoculated from agar plates and incubated for 3 days at 30 °C. The culture broth was mixed with *ca.* 1 kg Celite and separated by pressure filtration. The mycelial cake was extracted three times with ethyl acetate and acetone and the culture broth adsorbed on XAD-16. The combined extracts exhibited UV absorbing spots on TLC, which developed a dark colour reaction upon spraying with anisaldehyde/sulphuric acid. Chromatographic separations were performing using various methods such as silica gel column chromatography and reversed phase HPLC.

6.5.1 Lyngbyatoxin A acetate and its Homologue

Compound **78a** was obtained as colourless oil; it gave a red colour reaction with anisaldehyde/sulphuric acid. The proton NMR spectrum indicated in the aromatic region two *ortho* protons at δ 6.98 and 6.50 (H-5, 6) and one singlet at δ 6.82, those three protons are attributed to a disubstituted indole ring. The aliphatic region showed two methyl doublets (δ 0.66 and 0.96) and the methine signal of an isopropyl

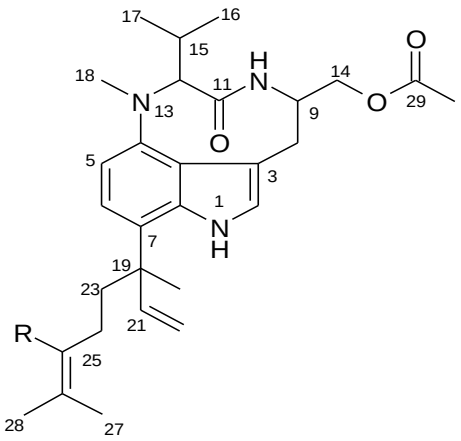
group, and two methyl singlets connected to a double bond at δ 1.65 and 1.38. The ^{13}C NMR spectrum showed 9 quaternary signals including 2 carbonyls, 6 olefinic signals, and 14 aliphatic carbon signals, in total 29 carbon signals (Table 9). (+) ESI MS indicated the *quasi*molecular ion at *m/z* 480 $[M+H]^+$ and HRESIMS gave the molecular formula $C_{29}H_{41}N_3O_3$. A search in AntiBase delivered no hits. From the 2D NMR (HMBC, HSQC and H,H COSY) measurements, the substructures of indolactam V [128] (I), a 1,5-dimethyl-1-vinyl-4-hexenyl (linalyl) side chain (II) connected to the indolactam at position C-7, and an acetyl fragment (III) connected at position C-14 were derived (Figure 80).



I II III

Figure 80: HMBC and COSY correlations in substructures I, II and III

Further searches with Scifinder (Chemical Abstracts) indicated compound **78 a** to be lyngbyatoxin A acetate firstly synthesized [129] from lyngbyatoxin A. Lyngbyatoxin A was previously isolated from the animal sea hare *S. longicauda* [130] and it exhibited potent activity against the cancer cell lines A-549 (IC_{50} 0.05 μg/ml).[130]



78a: R = H

78b: R = CH_3

Table 9: ^{1}H and ^{13}C NMR data of lyngbyatoxin A acetate (**78a**) and the homologue **78b** in $CDCl_3$.

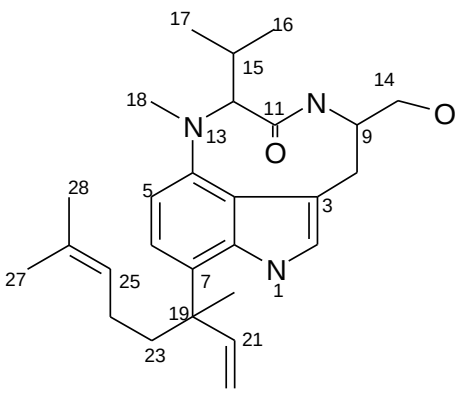
	^{1}H (300 MHz)		^{13}C (125 MHz)	
Position	**78a**	**78b**	**78a**	**78b**
1-NH	8. 02 (br s)	8.20 (s)	-	-
2	6.82 (br s)	6.80 (s)	121.2	120.8
3	-	-	113.2	112.9
3a	-	-	118.5	116.8
4	-	-	146.3	145.4
5	6.50 (d, 8.3)	6.54 (d, 8.0)	106.2	107.1
6	6.98 (d, 8.0)	7.01 (d, 7.8)	120.4	122.6
7	-	-	123.3	117.7
7a	-	-	137.6	137.6
8	3.06 (m)	3.05 (m)	34.4	34.1
	3.22 (d, 17.6)	3.18 (dd, 17.2,7.5)		
9	4.52 (m)	4.58 (m)	53.0	53.3
10-NH	6.29 (br s)	-	-	-
11-CO	-	-	173.2	174.0
12	4.31 (d, 10.1)	4.33 (d, 10.3)	71.3	70.2
14	3.97 (dd, 8.5, 11.4)	4.01 (dd, 8.4,11.4)	65.9	65.7
	4.22 (dd, 3.7, 11.4)	4.18 (m)		
15	2.60 (m)	2.60 (m)	28.6	28.4
16	0.66 (d, 6.8)	0.70 (d, 6.7)	19.4	19.5
17	0.93 (d, 6.1)	0.92 (d, 6.0)	21.6	21.6
18-N*CH*$_3$	2.92 (s)	2.92	32.9	33.1
19	-	-	43.4	40.1
20	1.44 (s)	1.45	24.7	25.2
21	6.21 (dd, 6.92)	6.11, dd, 6.7)	148.9	148.2
22	5.31 (d, 12.8)	5.09 (d, 12.7)	112.2	112.8
	5.36 (d, 14.9)	5.19 (d, 13.90)		
23	1.80-2.00 (m)	1.60 (m)	38.1	35.6
		1.85 (m)		
24	1.54 (m)	1.46 (m)	23.1	26.6
	1.96 (m)	1.82 (m)		
25	5.06 (m)	-	124.5	138.9
25-*CH*$_3$	-	1.29 (s)	-	27.8
26	-	-	131.5	132.7
27	1.65 (s)	1.29 (s)	25.6	27.8
28	1.38 (s)	1.45 (s)	17.3	18.6
29-CO	-	-	171.2	171.0
30 CO*CH*$_3$	2.09 (s)	2.08 (s)	20.8	20.9

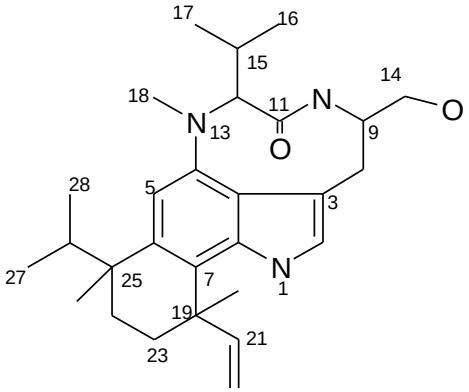
[a]Multiplicity; [b]coupling constants in [Hz]

(+)-ESI MS of **78b** delivered a $[M+H]^+$ ion at *m/z* 494. The mass difference of 14 between **78a** and **78b** indicated the presence of an additional methylene group in **78b**. Close examination of the NMR spectrum of **78b** indicated great similarities with **78a**, the difference between both spectra is that the 25-H which appears as multiplet in **78a** at δ 5.06 was replaced by a singlet of the methyl protons at δ 1.29 in **78b**. This observation suggested a possible substitution at the sp^2 carbon (C-25) in **78a,** which was confirmed by the observed Δm between **78a** and **78b**. Compound **78b** was identified as 25-methyllyngbyatoxin A acetate (**78a**), which was reported as synthetic product but has not been isolated from nature. Lyngbyatoxin A acetate (**78a**) and its homologue **78b** belong to the teleocidin family and are tumour promoting agents. [129, 130] They are reported here for the first time from bacteria.

6.5.2 Teleocidins A and B

Compounds **79** and **80** were isolated as yellow and white crystals, respectively, from the mycelial extract after chromatography on Sephadex LH-20. The proton NMR spectra of these compounds indicated similarities in the aromatic and aliphatic regions and differed only by the presence of a doublet at δ 6.50 (6-H) in compound **79**.This doublet coupled in *ortho*-position with the proton 5-H. This signal is absent in compound **80** due to the ring closure. (+)-ESI MS of **79** indicated the *quasi*-molecular ion at *m/z* 438 $[M+H]^+$ and (+)-HRESIMS gave the molecular formula $C_{27}H_{40}N_3O_2$. Compound **80** indicated a *quasi*molecular ion at *m/z* 452 $[M+H]^+$ and (+)-HRESIMS gave the molecular formula $C_{28}H_{42}N_3O_2$. The molecular formulas of both compounds implied 9 double bond equivalents. The search in AntiBase delivered teleocidin A1 (**79a**) and A2 (**79b**) and teleocidin B1 (**80**a) and B2 (**80b**), respectively, which are isomers. Teleocidins are often examinated in the literature, therefore the separation was not continuing.

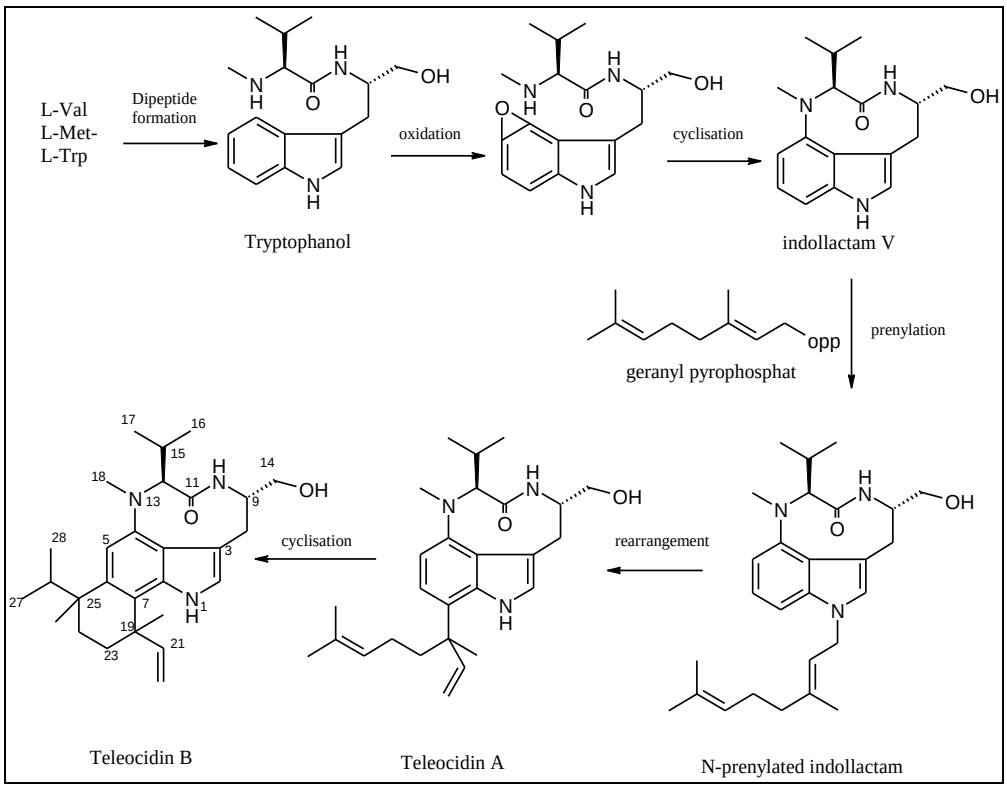




79a: (19R) **80a**: (19R, 25S)

79b: (19S) **80b**: (19S, 25R)

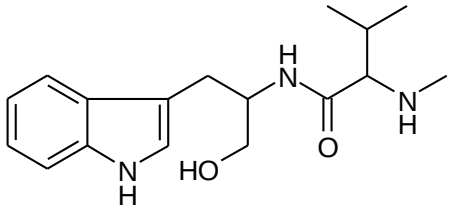
According to Scheme 4, teleocidin B (**80**) is obtained by the intramolecular cyclisation of the monoterpenoid side chain after methylation at position 25 of teleocidin A (**79**).[132b,c]



Scheme 4: proposed biosynthesis of **79** and **80**

6.5.3 *N*-Methyl-L-valyl-tryptophanol (valindolmycin)

Compound **81** was isolated as a colourless solid. The ^{1}H NMR spectrum showed in the aromatic region the characteristic fingerprint of a 3-monosubstituted indole. The aliphatic region showed signals between δ 4.40 and 0.98. The search in Anti-Base gave valindolmycin.



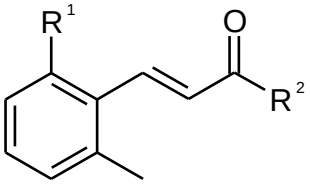
81

N-Methyl-L-val-tryptophanol is the precursor in the biosynthesis of indolactam V [131], and is also the precursor in the biosynthesis of teleocidin [132] and this may explain its presence in this strain (Scheme 4).

6.5.4 2-Hydroxy-6-methyl-cinnamic acid

Compound **82a** was obtained as a white solid, which showed on TLC an orange colour after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **82a** in MeOH-d_4 exhibited two doublets at δ 7.89 and 6.82 typical for a *trans* double bond. A triplet at δ 7.08 and two doublets at δ 6.87 and 6.83 were attributed to a 1,2,3-trisubstituted aromatic ring system, which was confirmed by the H,H COSY couplings. In the aliphatic region, only a 3H singlet at δ 2.40 characteristic of an aromatic methyl group was observed. The EI mass spectrum showed a molecular ion at *m/z* 178, and the HRESIMS delivered the molecular formula $C_{10}H_{10}O_2$.

The ^{13}C NMR spectrum exhibited 10 signals including a carbonyl at δ 172.1, three quaternary carbons, 5 sp^2 methines and a methyl carbon. Compound **82a** was identified as 2-hydroxy-6-methylphenylcinnamic acid and the structure was confirmed by HMBC correlations; this compound is reported here for the first time.



82

	R^1	R^2
82a	OH	OH
82b	OCH_3	OH
82c	OCH_3	NH_2
82d	H	NH_2

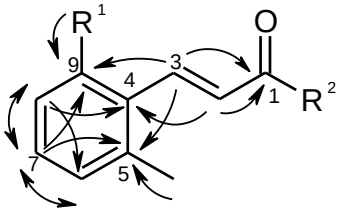


Figure 81: Selected HMBC (→) and COSY (↔) correlations in compounds **82b-c**

6.5.5 2-Methoxy-6-methyl-cinnamic acid

The EI mass spectrum of compound **82b** gave a molecular ion at *m/z* 192, which delivered the molecular formula $C_{11}H_{12}O_3$ by HREIMS. The ^{1}H NMR spectra of **82b** showed a *trans* double bond signal, the trisubstituted aromatic system and the aromatic methyl and was very similar to those of **82a**, the major difference being the presence of a methoxy group at δ 3.86 in **82b**.

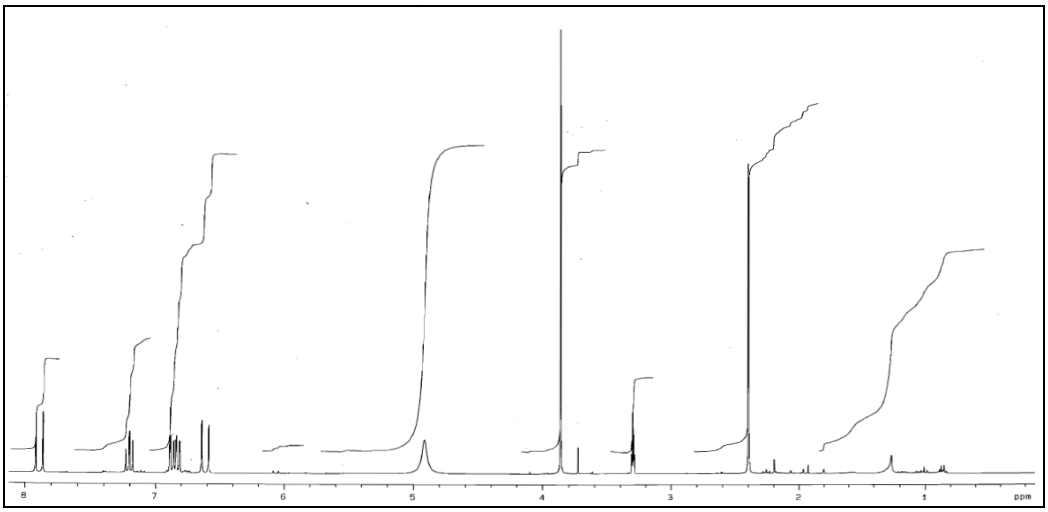


Figure 82: ^{1}H NMR spectrum (300 MHz) of 2-methoxy-6-methylcinnamic acid (**82b**) in MeOH-d_4

The ^{13}C NMR spectrum confirmed the presence of eleven carbon atoms as demanded by high resolution MS.

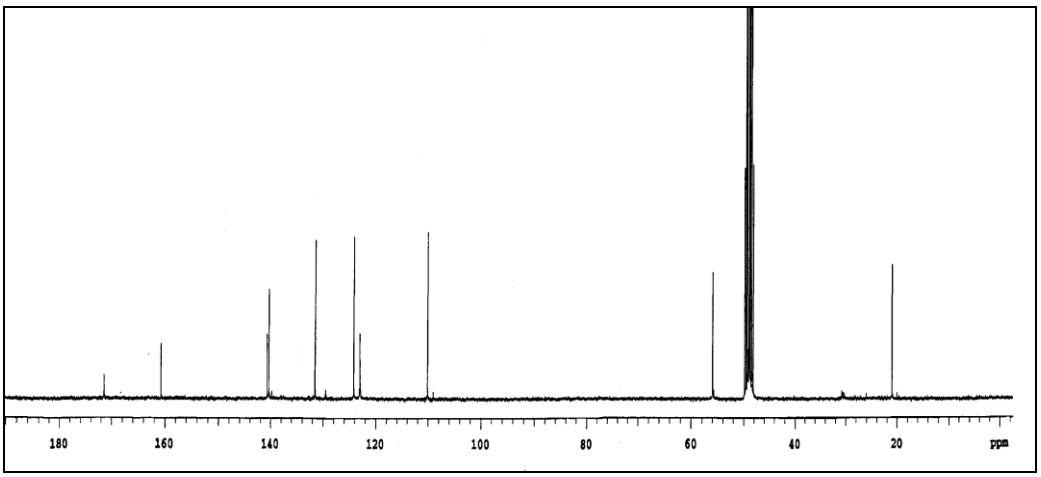


Figure 83: ^{13}C NMR spectrum (75 MHz) of 2-methoxy-6-methylphenylcinnamic acid (**82b**) in MeOH-d_4

The HMBC spectrum indicated a correlation of the methoxy group to the quaternary carbon at δ 160.6, confirming the methoxy group in position C-9.

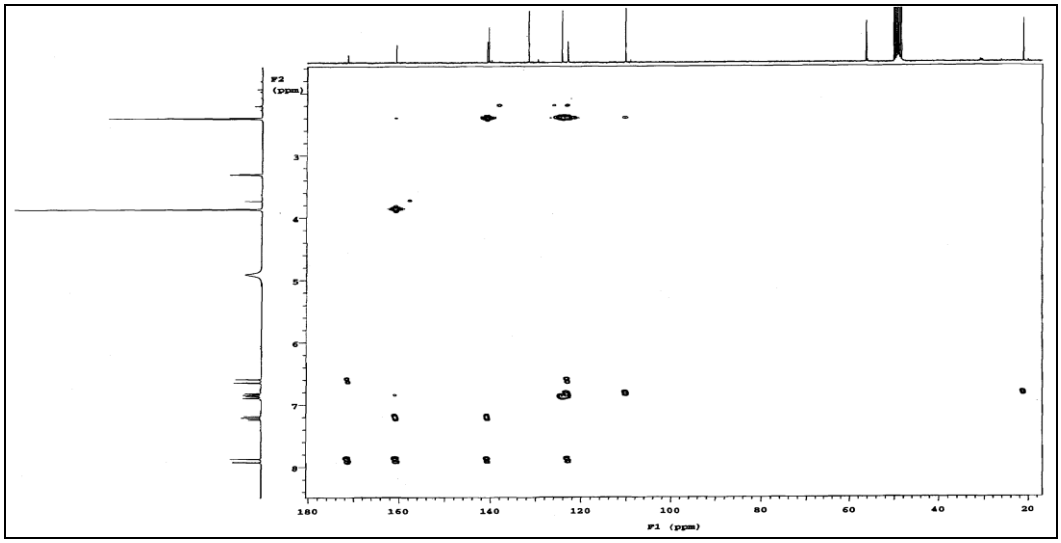


Figure 84: HMBC spectrum (300 MHz) of 2-methoxy-6-methylphenylcinnamic acid (**82b**) in MeOH-d_4

Further HMBC correlations (Figure 81) confirmed the structure of the new compound as 2-methoxy-6-methyl-cinnamic acid (**82b**). The related trans-2,6-dimethoxycinnamic acid was previously isolated from the plant *Taxus floridana*.[133]

6.5.6 2-Methoxy-6-methylcinnamide

Compound **82c** was obtained as white solid with a molecular ion at *m/z* 191 in the EI mass spectrum. The molecular formula $C_{11}H_{12}NO_2$ was derived by HREI MS. The ^1H and ^{13}C NMR data of **82c** (Table 10) were very similar to those of **82a** and **82b**. The major difference was the replacement of an oxygen by a nitrogen atom in **82c**, which indicated the presence of an amide group instead of an carboxylic acid in **82c**. Compound U77863 (**82d**) is closely related to **82c** and was isolated from *Streptomyces griseoluteus* by Herper *et al.*[134] It showed antiinvasive and antimetastatic activities versus K-1735-M2 and B16-F10 murine melonomas.[135] Compound **82c** is also a new natural product.

Table 10: ^1H (300 MHz); δ (*J*, Hz) and ^{13}C NMR (75 MHz) data of **82a, b** and **c** in MeOH-d_4.

	^1H			^{13}C		
No	**82a**	**82b**	**82c**	**82a**	**82b**	**82c**
1	-	-	-	172.1	171.5	172.2
2	6.82 (d,16.2)	6.62 (d, 16.2)	6.82 (d, 16.0)	121.3	123.0	123.5
3	7.89 (d,16.2)	7.89 (d, 16.2)	7.78 (d, 16.2)	140.8	140.4	136.9
4	-	-	-	122.4	122.9	125.2
5	-	-	-	140.8	140.8	140.6
5- CH_3	2.39 (s)	2.40 (s)	2.41 (s)	20.9	20.9	21.0
6	6.87 (d, 8.3)	6.88 (d, 8.3)	6.87 (d, 8.0)	114.9	110.1	110.1
7	7.06 (t, 7.9)	7.21 (t, 7.9)	7. 18 (t, 8.0)	131.4	131.6	131.0
8	6.83 (d, 7.5)	6.83 (d, 7.5)	6.83 (d, 8.0)	122.9	124.2	124.2
9	-	-	-	159.2	160.8	160.6
9-OCH_3	-	3.86 (s)	3.86 (s)	-	55.9	55.9

6.5.7 Heramide

Compound **83a** was obtained as a yellowish solid with a blue fluorescence under UV at 366 nm; it gave a yellow colour reaction with anisaldehyde/sulphuric acid. The molecular ion peak of **83a** was deduced from (+)-ESI HR mass spectrum to be *m/z* 253.15467, corresponding to the molecular formula $C_{13}H_{20}N_2O_3$. The ^1H NMR spectrum of **83a** in MeOH-d_4 indicated two doublets of doublets of intensity six at δ 1.00 and one methine signal at δ 2.14, typical for an isopropyl fragment, the singlet

of a methyl group connected to a double bond at δ 1.57, and in addition signals of four methylene groups at δ 2, 35 (12-H_2), 1.94 and 1.64 (3-H_2), 2.35 and 2.13 (4-H_2) and δ 3.41, 3.05 (5-H_2). The NMR spectrum in DMSO-d_6 indicated the presence of two H/D exchangeable protons by signals at δ 6.00 and 10.14.

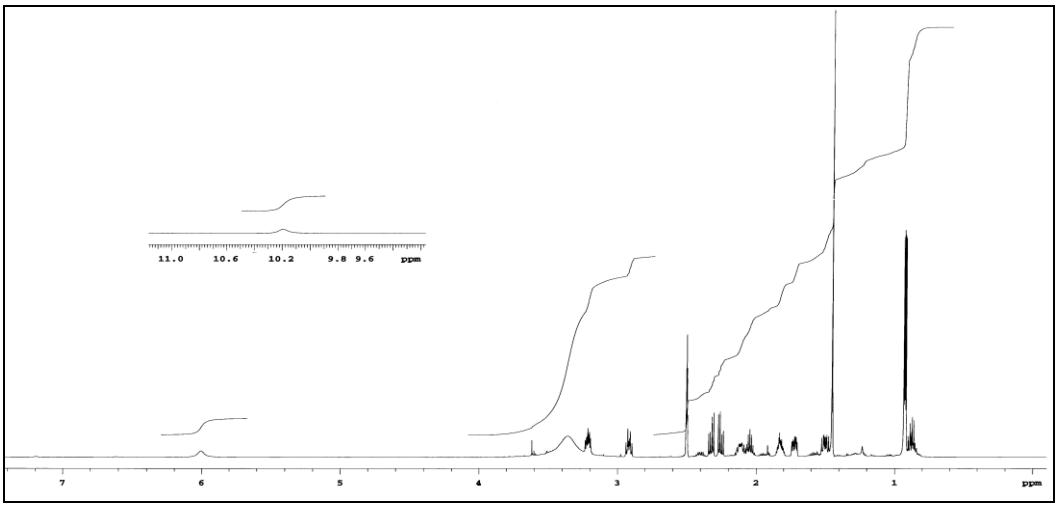


Figure 85: ^{1}H NMR spectrum (300 MHz) of heramide (**83a**) in DMSO-d_6

The ^{13}C NMR spectrum of indicated 13 carbon signals including three sp^2 carbon signals at δ 201.7, 172.9 and 169.5 which may be attributed to a conjugated ketone, acid or amide, respectively. The spectrum showed two quaternary carbons at δ 99.2 and 97.6, and signals due to four methylenes (δ 48.2, 46.5, 33.8, 27.7), one methine (δ 27.2) and three methyls (δ 22.7, 22.6, 7.1).

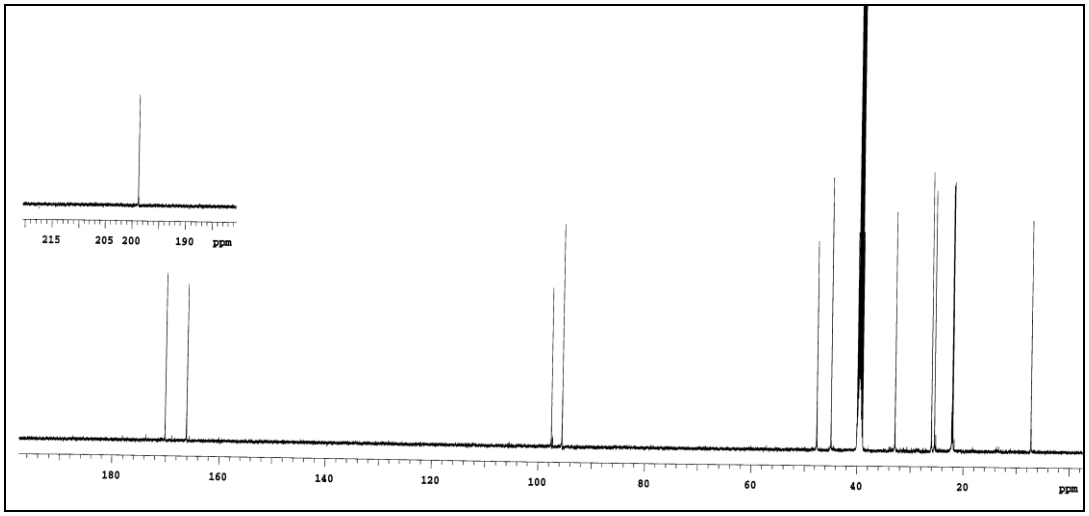
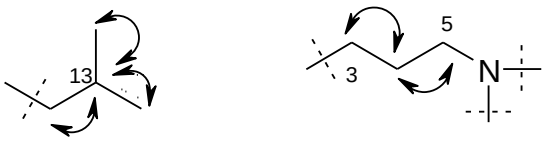
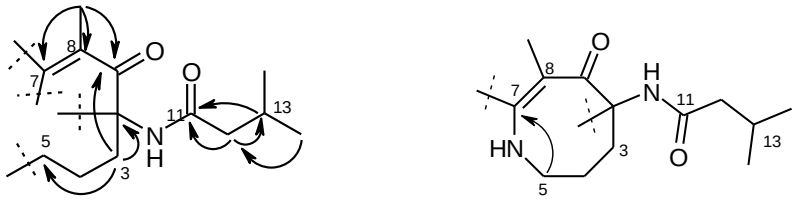


Figure 86: ^{13}C NMR Spectrum (75 MHz) of heramide (**83a**) in DMSO-d_6

A search in AntiBase[68] and the Dictionary of Natural Products (DNP)[69] with the ^{1}H and ^{13}C NMR data, the molecular mass and the molecular formula delivered no answer, indicating **83a** to be probably a new natural product. The interpretation of the H,H COSY spectrum confirmed the presence of an isopropyl group connected to a methylene group as well as a three methylene fragment as the only sub-structures.



In the HMBC spectrum, the methylene protons at δ 1.94 and 1.64, as well as the methine δ 27.2 (C-13) and the methylene δ 2.35 (C-12) protons of the isobutyl fragment showed a correlation with the carbonyl at δ 172.9 (C-9). In addition, correlations of both methyls of the isobutyl group with the methylene (δ 2.35, 12-H_2) were visible. The methylene and the methyl at δ 1.64 (3-H_2) and 1.58 (8-CH_3), respectively, showed correlations to the carbonyl at δ 201.7 (C-11). Further HMBC correlations were observed between the methylene signals at δ 3.41/3.03 (5-H_2) and the methyl at δ 1.58 (8-CH_3) to the carbon at δ 169.5 (C-7). Finally, the 8-CH_3 exhibited a correlation with the quaternary carbon at δ 99.2 (C-8), and of 3-H_2 with the signal at δ 97.2 (C-2). Careful interpretation of these HMBC correlations delivered the substructure I.

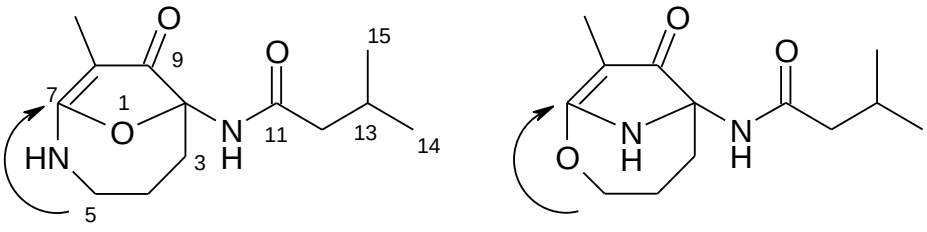


I II

Figure 87: HMBC (→) correlation of the substructures I and II in **83a**

The substructure I (Figure 87) contains already $C_{13}H_{19}N_2O_3$ and three double bond equivalences. With respect to the molecular formula of **83a**, only one acidic proton, one oxygen, one nitrogen and two double bond equivalences were remaining. In respect of the chemical shift of 5-H_2 (3.03/3.41), this methylene group cannot be bound to oxygen, and due to the correlation of the 5-H_2 to the carbon signal at δ

169.5 (C-7) substructure II (Figure 87) was found. The chemical shift at δ 97.2 (C-2) for a quaternary sp^3 carbon was explained by a cyclisation *via* oxygen. The possibility of structure **83b** was consequently excluded and the structure **83a** was finally confirmed by the fact that in the ^{13}C NMR spectrum no signal for an oxymethylene group was visible. Compound **83a** was named heramide.



83a **83b**

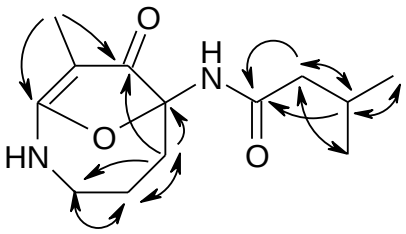
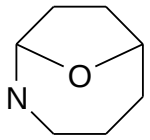


Figure 88: Selected HMBC (→) and COSY (↔) correlations in **83a**

Heramide (**83a**) possesses a complex bicyclic ring system consisting of a furanone and a [1,3]oxepane. According to the literature, 6-deoxymanzamine X and manzamine X isolated from the sponges *Haliclona* and *Xestospongia* sp.[136,137] are the only natural products containing the 9-oxa-2-aza-bicyclo[4.2.1]nonane ring (**84**), however, similar ring systems were observed in synthetic products.[138]



84

To further support structure **83a**, the mass-spectrometric fragmentation pattern was investigated. The EI MS afforded a base peak at *m/z* 139 and several other peaks (167). The ESI MS/MS presented the fragments at m/z 169, 151, whose fragments formulas were identified through HRESI MS/MS (Figure 89).

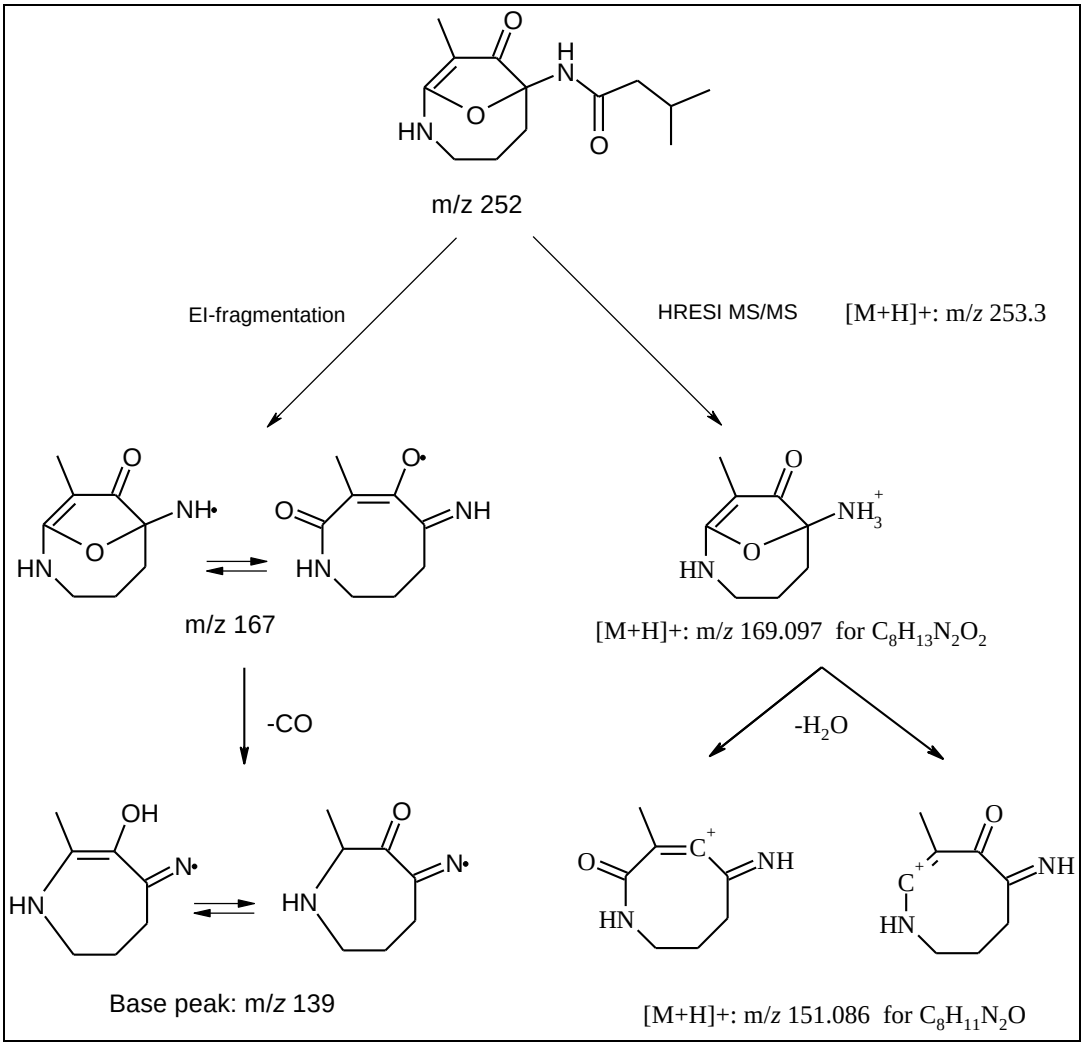


Figure 89: Proposed EI and HRESI MS/MS fragmentations of compound **83a**

Retrosynthetically, heramide (**83a**) can be deduced from propionic acid and ornithine. Both acids could afford the keto-diamino acid intermediate by means of an ester condensation. Similar compounds are reported in the literature [139] as precursor in the enzymatic synthesis of the roast smelling 2-propionyl-1-pyrroline (R = CH_3) (Figure 90[139]) Subsequent lactam formation should yield an ozacinone derivative, which under oxidative conditions might form heramide (**83a**).

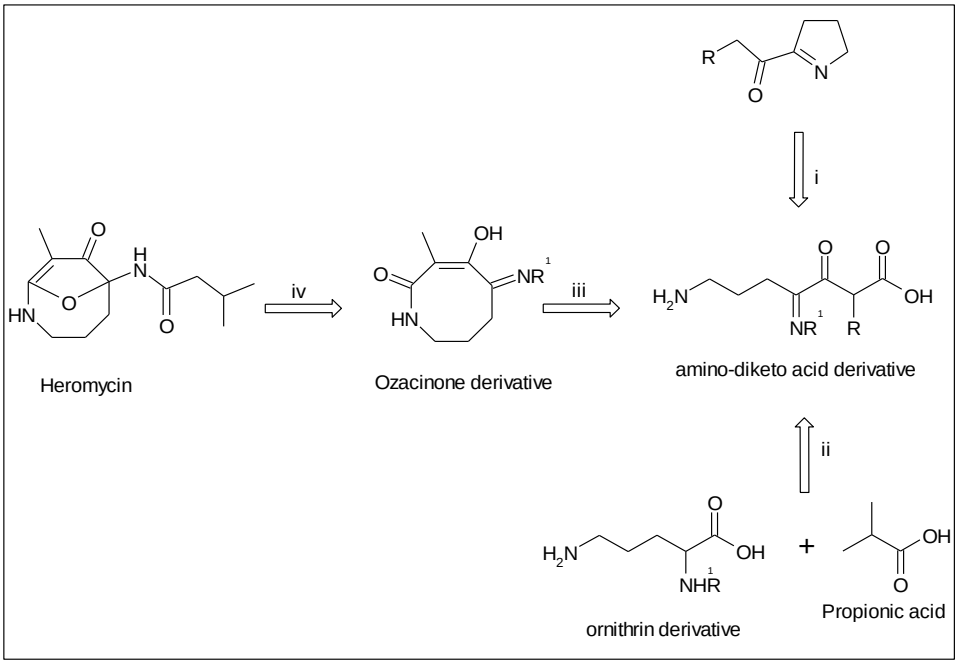


Figure 90: Retrosynthesis of heramide (**83a**) i: Retrosynthesis from aminodiketo acid derivative [139], ii: ester condensation; iii: lactamization; iv: aminal cleavage

Table 11: ^{1}H (300 MHz, *J* in Hz) and ^{13}C NMR (75 MHz) data of heramide (**83a**)

No	$^{1}H^{a}$	$^{1}H^{b}$	$^{13}C^{a}$
2	-	-	97.6
3	1.94 (ddd, 9.8, 6.5, 3.3); 1.64 (m)	1.74 (m) 1.55 (m)	33.8
4	2.13 (m); 2.35 (m)	1.83 (m)	27.7
5	3.41 (ddd, 11.2, 7.7, 3.7) 3.03 (m)	3.20 (ddd, 11.8, 7.2, 4.4); 2.93 (m)	48.2
6-NH	-	6.00 (s)[c]	
7	-	-	169.5
8	-	-	99.2
8-CH_3	1.57 (s)	1.46 (s)	7.1
9	-	-	201.7
10-NH	-	10.14 (s)[c]	-
11	-	-	172.9
12	2.35 (m)	2.30 (dd, 14.0, 7.2)	46.5
13	2.14 (m)	2.07 (m)	27.2
14	1.00 (dd, 6.6, 1.5)	0.93 (dd, 6.6, 1.7)	22.7
15	1.00 (dd, 6.6, 1.5)	0.93 (dd, 6.6, 1.7)	22.6

[a)] in MeOH-d_4; [b)] in DMSO-d_6; [c)] assignment may be interchangeable

6.5.8 3-Hydroxy-5-hydroxyaminisochroman-1-one

Compound **85a** was obtained as a colourless solid, which showed on TLC a blue fluorescent under UV 366 nm and pink colour after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **85a** in MeOH-d_4 exhibited two doublets of doublets at δ 7.45 (J = 8.1, 1.5 Hz) and 6.84 (J = 7.8, 1.5 Hz) and one triplet at δ 6.49 (J = 7.9 Hz), attributed to a 1,2,3-trisubstituted aromatic ring system and confirmed by the H,H COSY couplings. The doublet of doublet of an oxymethine signal at δ 5.40 (J = 2.4, 4.3 Hz) indicated a correlation to the methylene group at δ 3.49 and 3.25 (J = 2.4, 11.9 Hz). The proton NMR spectrum in MeOH-d_4 indicated only six protons, suggesting three further acidic protons.

The ^{13}C NMR spectrum exhibited 9 signals including a carbonyl at δ 172.0, three quaternary carbons, three sp^2 methines, one methylene carbon and a methine carbon. The interpretation of the HMBC spectrum showed the correlations of a proton at δ 7.45 (8-H) and the methylene group at δ 3.25/3.49 (4-H_2) to the quaternary carbon at δ 139.0 (C-4a). The methylene group gave also the correlation to the hemiacetal carbon at δ 90.5 (C-3). This methine (3-H) showed a H,H COSY correlation to the methylene group. The three aromatic protons (6-, 7- and 8-H) showed correlations to the quaternary carbons C-5, C-4a and C-8a. Finally, 8-H exhibited a correlation to the carbonyl carbon at δ 172 (C-1), which is the only correlation to this signal. The information from all the NMR data (1D, 2D) delivered the substructure depicted in Figure 91.

The CI mass spectrum showed a *quasi*molecular ion at *m/z* 196 ($[M+H]^+$), and HREI MS delivered the molecular formula $C_9H_9NO_4$. Compound **85a** contained 6 double bond equivalences. From the substructure, one oxygen, one nitrogen, 2 hydogen atoms and one double bond equivalence are missing. Comparison of the ^{1}H data from compounds **85a** and **85c** showed similarity, so that C-5 seems to be substituted with an amino group. The missing oxygen can only be assigned to the amine group to form an oxime. So the structure of compound **85a** was finally elucidated as 3-hydroxy-5-hydroxyaminisochroman-1-one (Figure 92).

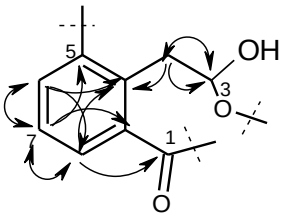
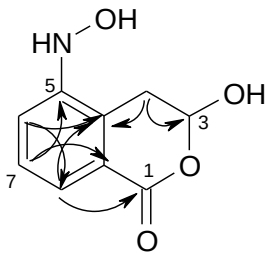


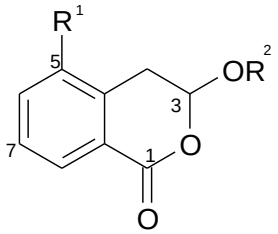
Figure 91: Sub-structures of **85a** from H,H COSY (↔) and HMBC (→) correlations.

Table 12: ^{1}H (300 MHz, *J* in Hz) and ^{13}C NMR (125 MHz) data of **85a** in MeOH-d_4.

No	^{1}H	^{13}C
1	-	172.0
3	5.40 (dd, 2.4, 4.3)	90.5
4	3.49 (dd, 2.4, 11.9) 3.25 (dd, 4.3, 11.9)	45.7
4a	-	139
5	-	143.0
6	6.84 (dd, 7.8, 1.5)	121.4
7	6.49 (t, 7.8)	116.1
8	7.45 (dd, 8.1, 1.5)	125.3
8a	-	113.0

Compounds possessing the isochroman chromophore were isolated mainly from fungi and rarely from bacteria. They possessed moderate antifungal activity. [140] The new compound **85a** may be derived from **85b** by reduction of the nitro group, **85b** and **85c** are reported as intermediates in the synthesis of 5-nitroisocoumarins and methylindoles. [141,142]





85a: R^1 = NHOH, R^2 = H

85b: R^1 = NO_2, R^2 = CH_3

85c: R^1 = NH_2, R^2 = CH_3

Figure 92: HMBC (→) correlations in the structure **85**

Table 13:[1]H NMR data comparison (*J* in Hz) of **85a** in MeOH-d_4 and **85c** in $CDCl_3$.

No	**85a**	**85c**[141]
3	5.40 (dd, 2.4, 4.3)	5.36 (t, 4)
3-CH_3	-	3.53 (s)
4	3.49 (dd, 2.4, 11.9) 3.25 (dd, 4.3, 11.9)	3.05 (dd, 4.0, 15.0) 2.73 (dd, 4.0, 15.0)
4a	-	-
5	-	-
6	6.84 (dd, 7.8, 1.5)	6.80 (dd, 8.0, 2.0)
7	6.49 (t, 7.8)	7.10 (t, 8.0)
8	7.45 (dd, 8.1, 1.5)	7.48 (dd, 8.0, 2.0)
8a	-	-

6.5.9 *N*-(2-methoxyphenyl)-benzamide

Purification of fraction 3 by PTLC followed by HPLC delivered compound **86** as colourless crystals; the molecular mass was deduced to be *m/z* 227. The [1]H NMR spectrum exhibited only nine aromatic protons as four doublets (δ 8.08, 7.98, 7.97, 7.60) and five triplets, respectively, at δ 7.58, 7.54, 7.20, 7.10 and 6.98 suggesting the presence of two spins system with four consecutives protons for the first one (a aromatic ring) and five consecutives protons for the second spin system, in addition a methoxy group was visible (δ 3.90).

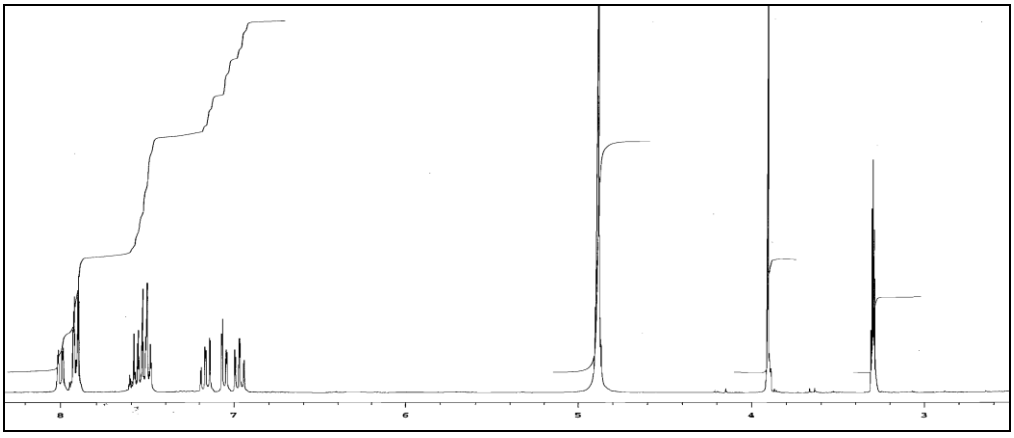
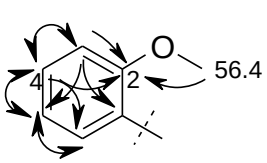


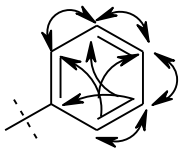
Figure 93: [1]H NMR spectrum (300 MHz) **86** in MeOH-d_4

The [13]C NMR spectrum indicated only fourteen carbon signals including a carbonyl of an amide at δ 168.1 (C-7), three quaternary carbons at δ 152.0, 136.1 and

128.0, nine methines at δ 133.0-121.6 and a methoxy carbon at δ 56.4. The H,H COSY spectrum confirmed the presence of two spin systems as derived from the ^{1}H NMR spectrum. The HMBC spectrum indicated the correlations of the methoxy signal together with the doublet at δ 8.08 (3-H) to the carbon at δ 152.0 (C-2), indicating that the methoxy group should be located in the disubstituted aromatic system. From these interpretations, two different aromatic rings are resulting: a 1,2-disubstituted (I) and a mono substituted benzene rings (II).



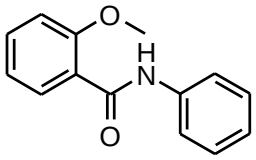
I



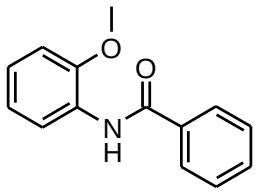
II

Figure 94: Fragments I and II from the COSY and HMBC spectra of **86**

The HMBC spectrum showed no correlation to the carbonyl group at 168.1 so that including fragments I and II, two plausible structures can be derived: *N*-(2-methoxyphenyl)-benzamide (**86a**) und 2-(methoxy-*N*phenyl)-benzamide (**86b**).

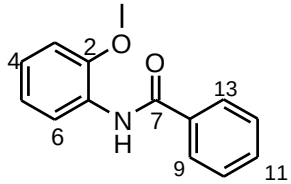


86a



86b

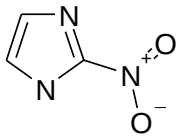
Both structures are known from the synthesis. [143,144] The preparation of *N*- (2-methoxyphenyl)-benzamide was repeated (**86b**) according to the literature [144] by reacting benzoyl chloride with 2-methoxyaniline. Comparison of the proton spectra identified compound **86** as *N*- (2-methoxyphenyl)-benzamide, which is described here for the first time as natural product.



86

6.5.10 Azomycin

Compound **87** was isolated as a yellow powder. The ^{1}H NMR spectrum of **87** in DMSO-d_6 exhibited one singlet at δ7.22 with the intensity of 2 and one broad singlet for an exchangeable proton at δ14.60. EI MS gave a molecular ion at 113 Dalton. By searching in the MS database NIST, compound **87** was identified as azomycin, which was already isolated in 1954. Azomycin (**87**) is an antimicrobial antibiotic firstly isolated from a strain of *Nocardia mesenterica*.[145]

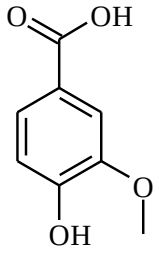


87

6.5.11 Trivial compounds

Vanillic acid

Compound **88** was isolated as white powder. The UV active compound showed on TLC a light blue colour reaction which anisaldehyde. Its proton NMR spectrum showed only four signals: at δ 7.58 (d, *J* = 1.5 Hz), 7.40 (dd, *J* = 8.6, 1.5 Hz), 6.88 (d, *J* = 8.6 Hz) for a 1,3,4-trisubstituted benzene ring and a singlet at δ 3.92 attributed to a methoxy group. The search in AntiBase gave 4-hydroxy-5-methoxy-benzoic acid as a plausible structure. Comparison of the ^{1}H NMR data with the spectra in our spectra collection confirmed the assignment.



88

6.6 Terrestrial *Streptomyces* sp AdM19

The crude extract of the terrestrial *Streptomyces* sp. AdM19 obtained from a 1-L culture exhibited biological activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus and Chlorella sorokiniana*. 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. The strain was cultivated in M_2 medium for five days. The cultured broth was filtered, the water phase adsorbed on XAD-16 and eluted with methanol, while the mycelium was extracted with ethyl acetate. The combined crude extracts (8 g) were worked up as indicated in Figure 95.

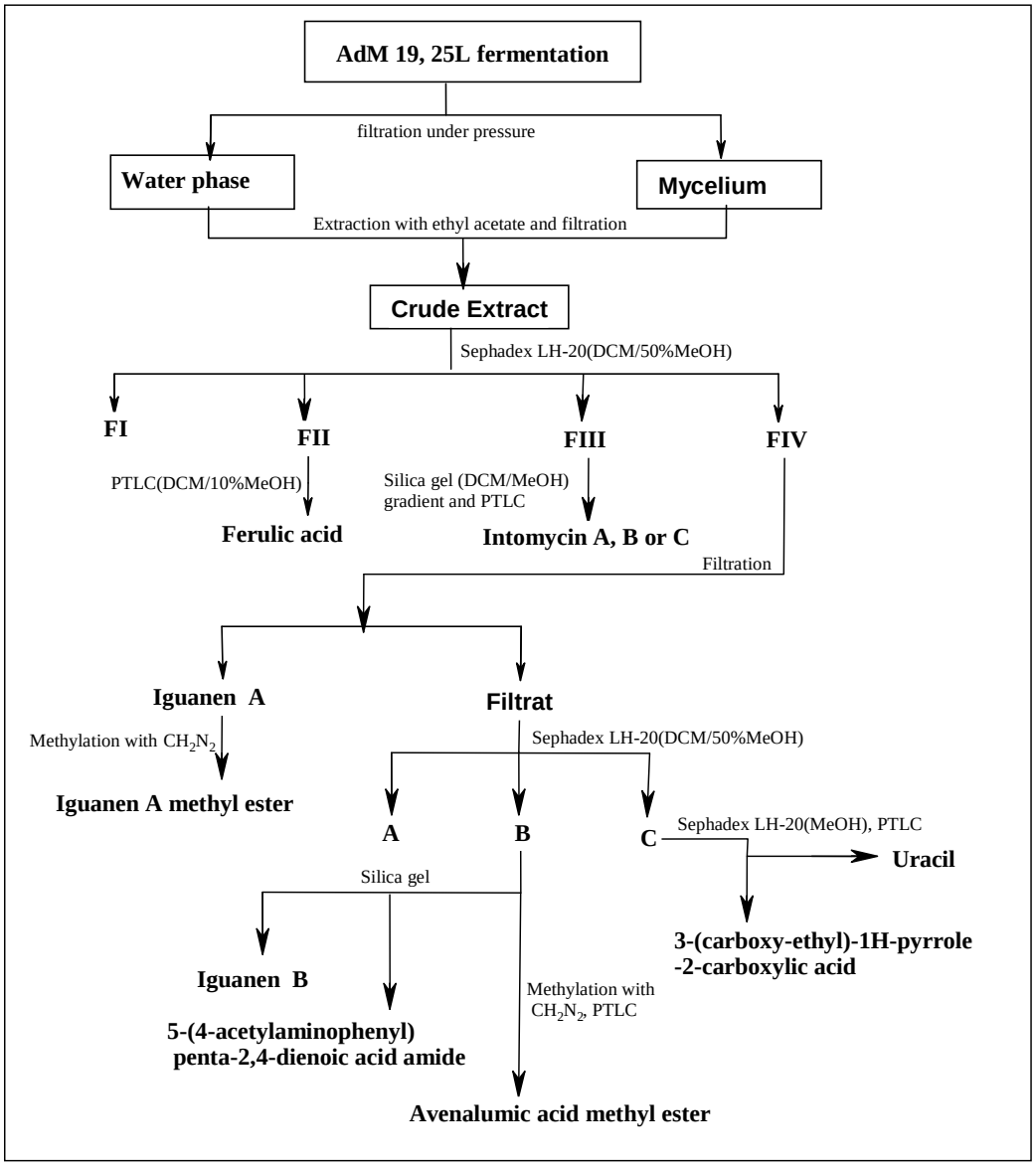


Figure 95: Work-up scheme of the strain *Streptomyces sp*. AdM 19

6.6.1 3-(Carboxy-ethyl)-1*H*-pyrrole-2-carboxylic acid

The compound **89** was obtained as a white powder, which gave a yellow colour reaction with anisaldehyde/sulphuric acid on TLC. The proton NMR spectrum was very simple and exhibited in the sp^2 region one exchangeable proton at δ 11.32 and two triplets at δ 6.80 and 6.02. The aliphatic region indicated only signals due to two methylene groups as triplets at δ 2.93 and 2.46. The carbon NMR spectrum indicated 8 carbon signals among which two at δ =173.9 and 162.0 were due to carbonyls, two sp^2 methines (δ 121.8, 110.0), two quaternary carbons (δ 118.5, 130.0) and two methylene groups (δ 34.7, 22.0). The molecular mass of 183 Dalton was deduced from the *quasi*molecular ion *m/z* 206 $[M+Na]^+$ and the HRESIMS gave $C_8H_9NO_4$ as molecular formula. The H,H COSY spectrum indicated only two fragments: the coupling between both methylene protons and one between the two sp^2 protons. Due to the small coupling constant as well as their appearance as a triplet, which can be explain by the correlation with an acidic NH proton, the aromatic protons were attributed to a pyrrole ring.

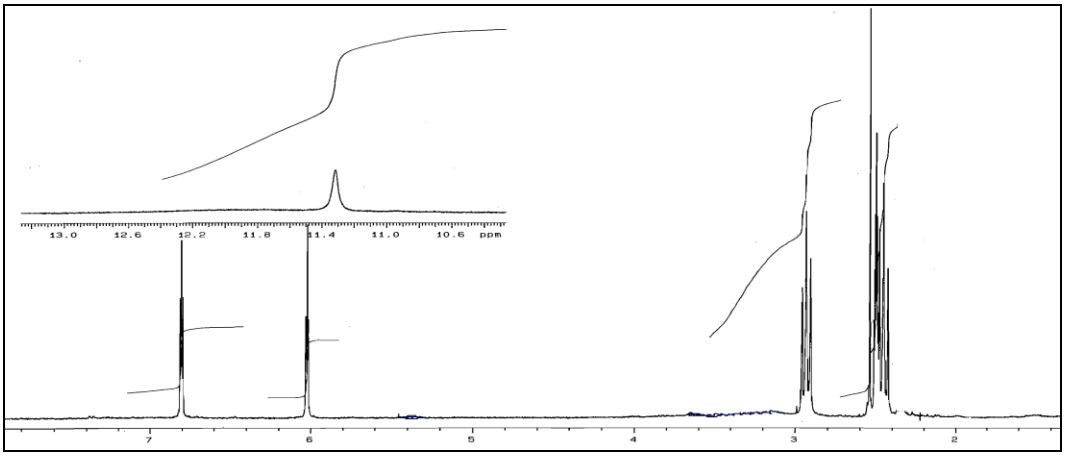


Figure 96:[1]H NMR spectrum (300 MHz) of compound **89** in DMSO-d_6

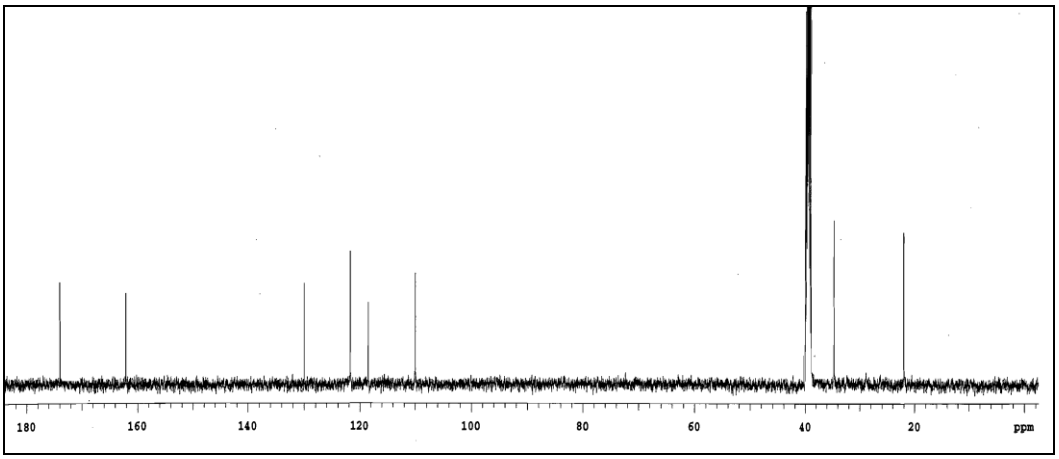


Figure 97:[13]C NMR (75 MHz) spectrum of **89** in DMSO-d_6

The HMBC spectrum indicated cross couplings between both methylene protons (1´,2´-H_2) to the carbon at δ 173.9 (C-3´), in addition the two sp^2 protons (4,5-H) and both methylenes (1´,2´-H_2) correlated to the carbon at δ 130.0 (C-3). Further correlations were seen between the methylene protons (1´-H_2), the sp^2 methine (4-H) to the carbon (C-2). The interpretation of all these data coupled with MS gave 3-(carboxyethyl)-1*H*-pyrrole-2-carboxylic acid (**89**) as new natural product. Compound **89** was already reported as synthetic product by Mizuno,[146] unfortunately without spectroscopic data. Comparison with simulated data confirmed the structure.

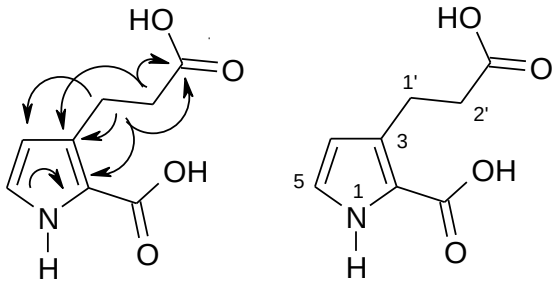


Figure 98: Selected HMBC correlations in **89**

6.6.2 Avenalumic acid and the methyl ester

The methylation of sub-fraction B with diazomethane followed by PTLC delivered compound **90a**, which was obtained as yellow powder. It exhibited a red spot on TLC after spraying with anisaldehyde/sulphuric acid. The proton NMR spectrum indicated a signal due to a methoxy group at δ 3.67, the sp^2 region indicated signals of *ortho*-coupled protons of an aromatic system at δ 7.40 and 6.78, and a doublet of a *trans* double bond at δ 6.00 (J = 15.1 Hz). In addition three protons of a double bond

at δ 7.37, 7.00, 6.92 and an acid proton at δ 9.78 were observed. EI MS indicated the molecular mass at *m/z* 204 and the HREIMS delivered the molecular formula $C_{12}H_{12}O_3$.

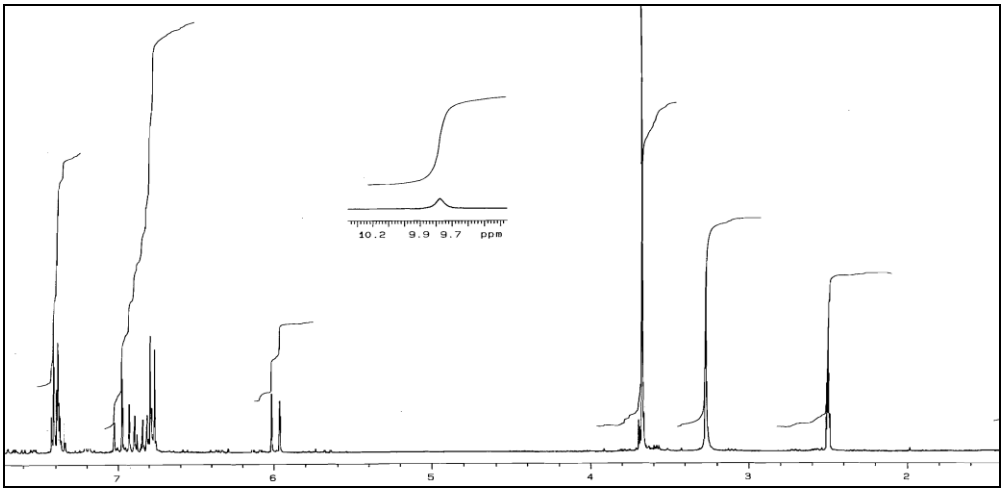


Figure 99: ^{1}H NMR spectrum (300 MHz) of **90** in DMSO-d_6

The ^{13}C NMR spectrum was in accordance with the HREIMS and indicated twelve carbon signals among which was a carbonyl atom at δ 166.7.

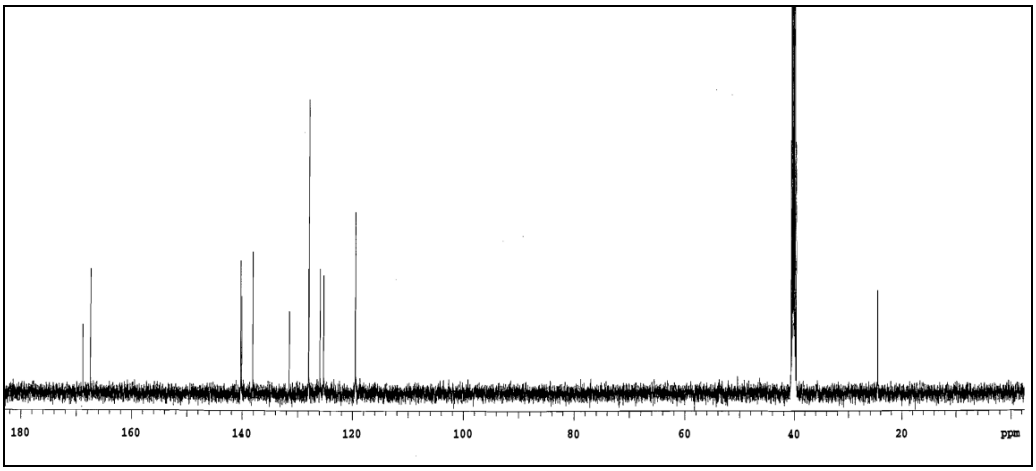
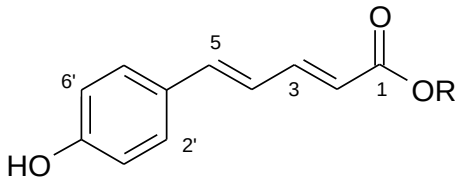


Figure 100: ^{13}C NMR spectrum (75 MHz) of **90a** in DMSO-d_6

A search in AntiBase with the molecular formula delivered no hits. A search in the Dictionary of Natural Products and Chemical Abstract delivered avenalumic acid methyl ester (**90a**) and psilotic acid [147] previously reported from oat grout, hulls [148,149] and *Psilotum nodum* respectively. Avenalumic acid (**90b**) is reported to inter-convert to the 2Z,4E (psilotic acid) and the 2E,4E isomer, with the latter being pre-

dominant. They are homologues of *p*-hydroxy-coumaric acid. This is the first time that compound **90b** is reported from microorganisms.



90a: R = CH_3

90b: R = H

6.6.3 5-(4-Acetylaminophenyl)penta-2,4-dienamide

The yellowish compound **91a** showed also a yellow spot on TLC after spraying with anisaldehyde/ sulphuric acid. Beside some minor differences, the proton NMR spectrum indicated similar signals to compound **90b** both in the aromatic and aliphatic the region.

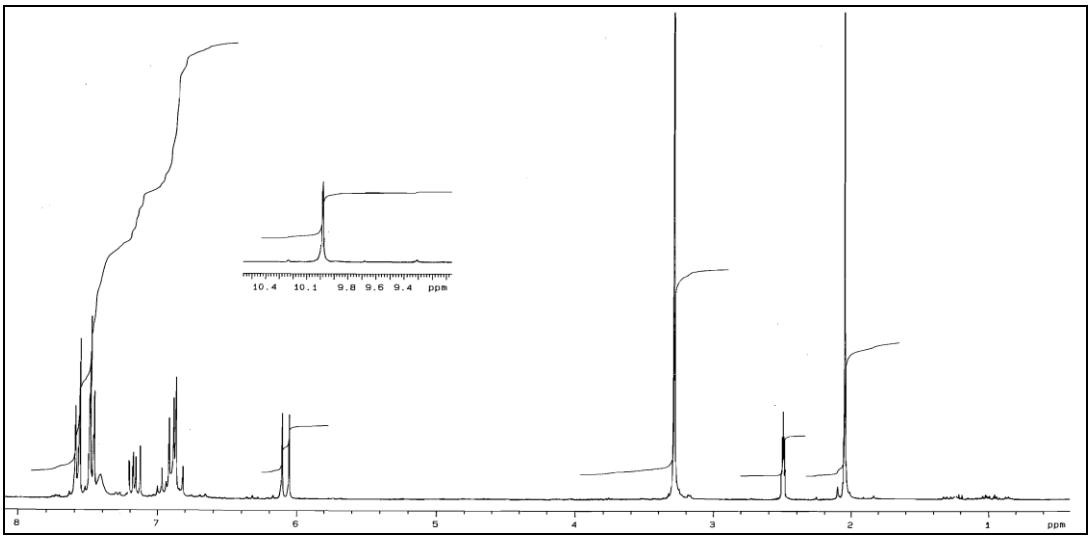


Figure 101:^{1}H NMR spectrum (300 MHz) of compound **91a** in DMSO-d_6

ESI MS indicated the *quasi*molecular ion at *m/z* 253 $[M+Na]^+$ and HRESIMS (231.11237 $[M+H]^+$) gave the molecular formula $C_{13}H_{14}N_2O_2$ for compound **91a**. In addition to the two carbonyls of an amide and/or ester at δ 168.9 and 167.5, the carbon NMR spectrum indicated eleven signals, which were assigned by APT to 8 sp^2 methines, two quaternary, and one methyl carbon.

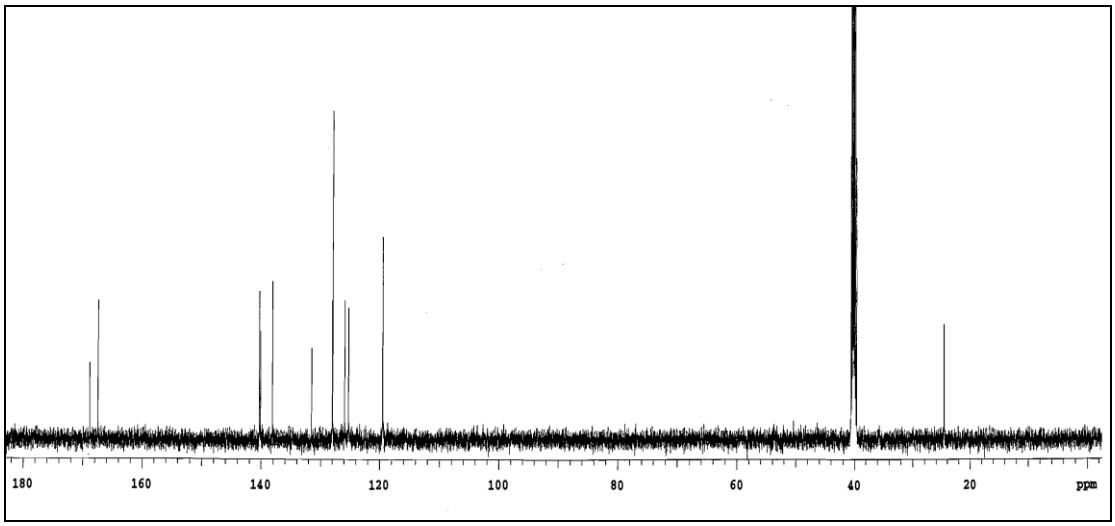


Figure 102: ^{13}C NMR spectrum (75 MHz) of compound **91a** in DMSO-d_6

As a sub-structure search in AntiBase delivered no hits, 2D spectra (H,H COSY and HMBC) were recorded.

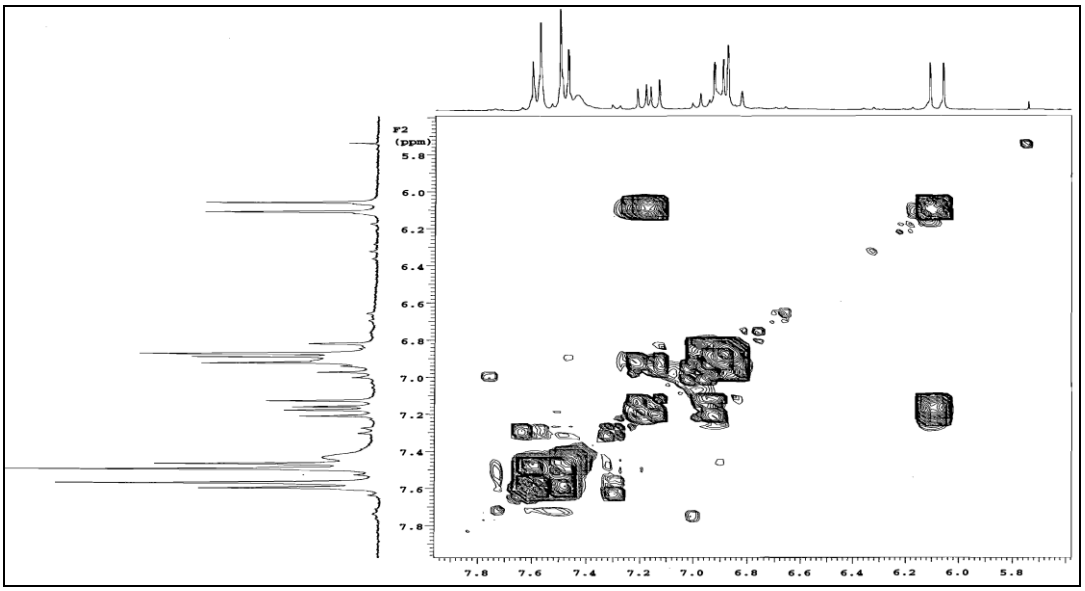


Figure 103:H,H COSY spectrum of compound **91a** in DMSO-d_6

The interpretation of the HMBC spectrum of **91a** indicated the following evidence: the methyl proton at δ 2.05 and the acidic proton at δ 10.00 indicated correlation to the carbonyl at δ 168.9 (*CO*CH$_3$) while the acidic proton showed also a cross coupling to the carbon of the *para* disubstituted benzene ring at δ 119.5 (C-3´, -5´); the doublet at δ 6.08 and the doublet of doublet of the *trans* double at δ 7.19 indicated correlations to the carbonyl at δ 167.3. The structure of compound **91a** was

deduced as 5-(4-acetylaminophenyl) penta-2,4-dienoic acid amide (**91a**) possessing the same configuration (2*E*,4*E*) as in **90a**.

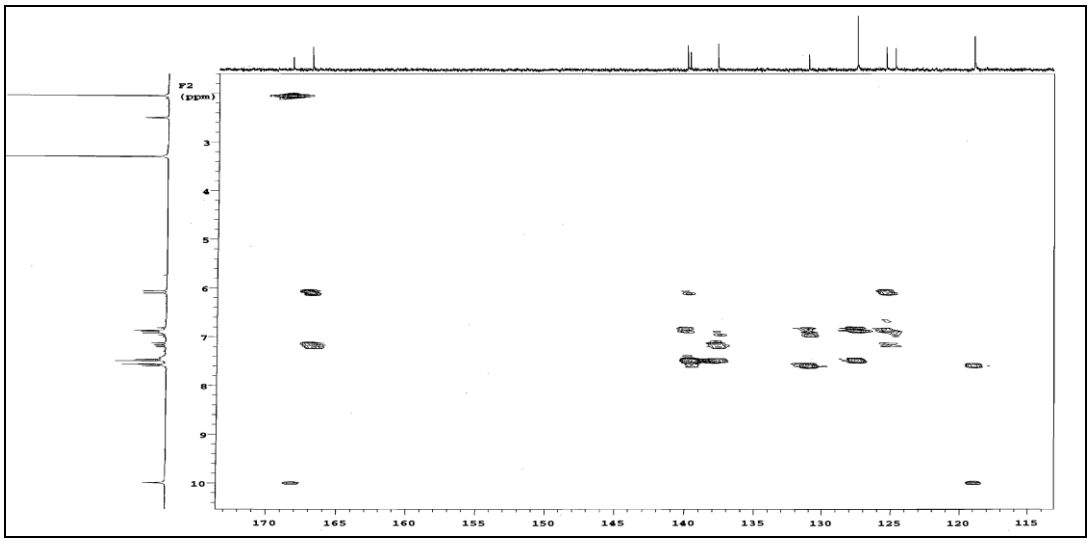


Figure 104:HMBC spectrum of **91a** in DMSO-d_6

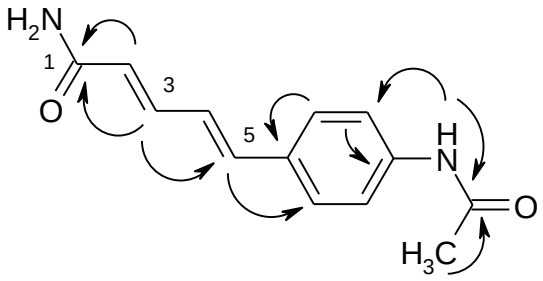
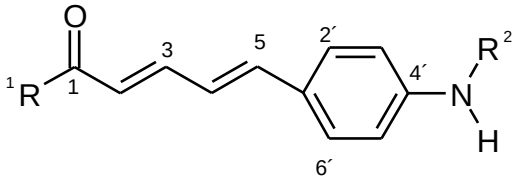


Figure 105: Selected HMBC correlations in **91a**

Compound **91a** is a derivative of 5-(4-aminophenyl)penta-2,4-dienamide (**91b**) previously reported from *Streptomyces* sp.[150]

Compound **91c** was obtained as white solid, and gave a yellowish to greenish colour reaction with anisaldehyde/sulphuric acid. The proton NMR spectrum in DMSO-d_6 was very similar to that of **91a**, the only difference being the presence of an exchangeable proton at δ 12.20. (-)-ESI MS indicated a *quasi*molecular ion at *m/z* 230 $[M-H]^-$ and HRESIMS delivered the molecular formula $C_{13}H_{13}NO_3$. With respect of the molecular formula, compound **91c** should be an acid related to **91a**. This was confirmed by methylation of **91c** using diazomethane, which delivered the methoxy derivative **91d**.



	R^1	R^2
91a	NH_2	$COCH_3$
91b	NH_2	H
91c	OH	$COCH_3$
91d	OCH_3	$COCH_3$
91e	OH	H

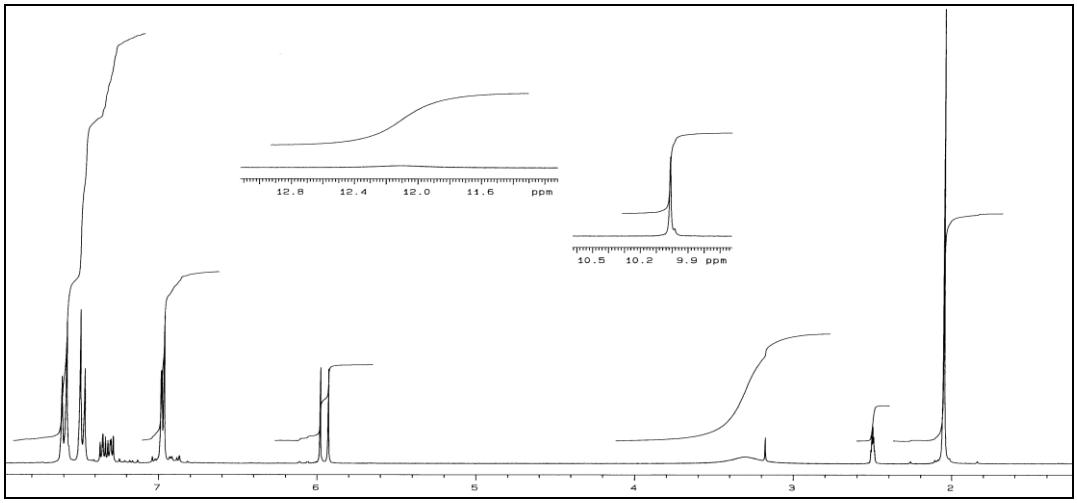


Figure 106: ^{1}H NMR spectrum (300 MHz) of **91c** in DMSO-d_6

The ^{13}C NMR spectrum indicated also 13 carbon signals as in **91a** but here some slight differences were observed. The structure of **91c** was deduced as (2*E*, 4*E*)-5-(4-acetylaminophenyl)penta-2,4-dienoic acid which is also a derivative of **91e** also reported from *Streptomyces* sp.[150]

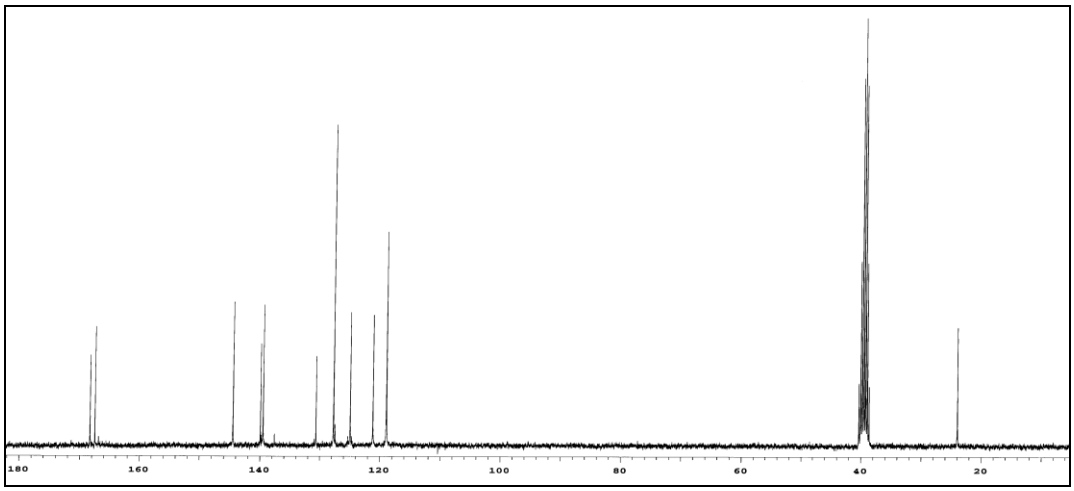


Figure 107:[13]C NMR spectrum (125 MHz) of **91c** in DMSO-d_6

6.6.4 Iguanen A

Compound **92** was obtained as yellow powder; it gave a red spot with anisaldehyde/sulphuric acid. The sp^2 region of the NMR spectrum was similar to those of **91a-c**, with the protons of the *para* disubstituted benzene ring at δ 7.61 and 7.51, and the protons of the double bond side chain. In addition there were three protons as triplet with small coupling constants (J = 2.3 Hz); and two methylene groups giving triplets similar to those of **89** were also observed, suggesting the presence of a pyrrole ring in **92a**. The (-)-ESI MS spectrum delivered the *quasi*molecular ion at *m/z* 309 $[M-H]^-$, and HRESIMS delivered the molecular formula $C_{18}H_{18}N_2O_3$. A search in AntiBase delivered no hits.

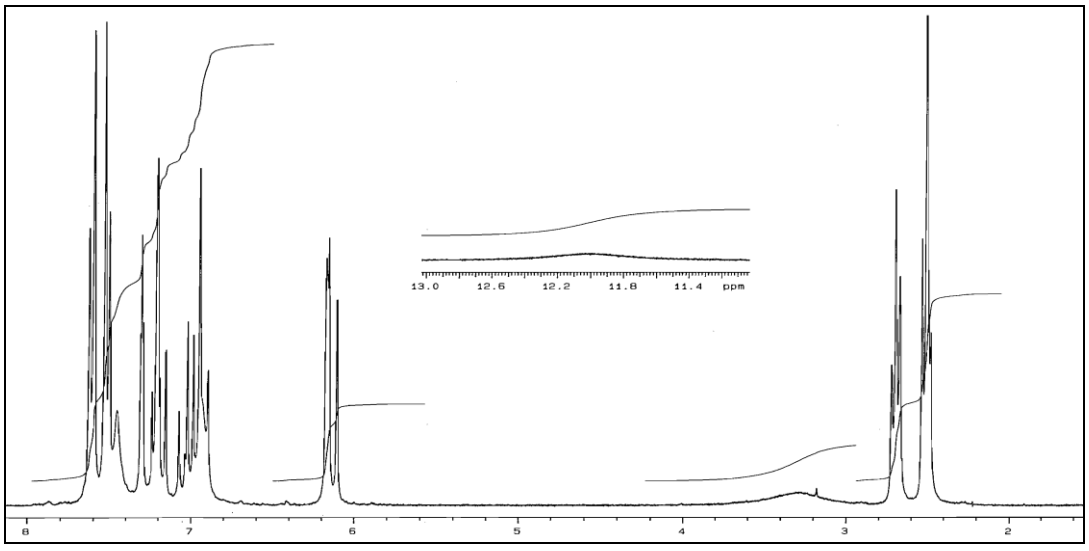


Figure 108:[1]H NMR spectrum (300 MHz) of iguanen a **92a** in $CDCl_3$

The ^{13}C NMR spectrum was in accordance with the high resolution and indicated in addition to two carbonyl groups at δ 173.0 and 166.0, two methylene, 11 sp^2 methines and two quaternary carbons.

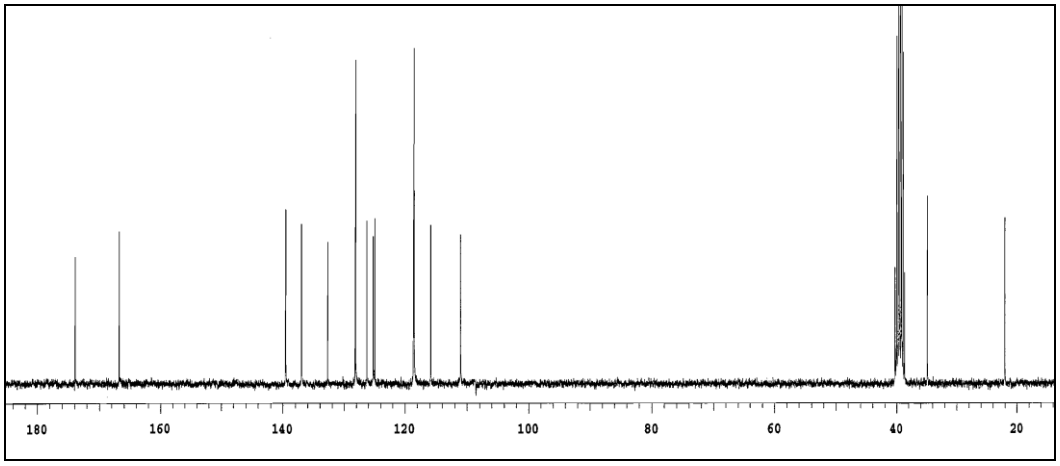


Figure 109: ^{13}C NMR spectrum (125 MHz) of iguanen a **92a** in $CDCl_3$

The HMBC spectrum indicated the correlation of both methylene groups to the carbonyl at δ 173.0 (C-1´´), the three triplets of the pyrrole ring and both methylenes showed cross peaks to the quaternary carbon at δ 125.0 (C-3). Further correlations were seen between the methylene at δ 2.70 (2´´-H_2), the two triplets of the pyrrole ring at δ 7.30 (5-H) and 7.20 (2-H) to the carbon at δ 111.1 (C-4). In addition the *trans* double bond protons at δ 6.13 (d, 15.0 Hz, 4´´´-H) and 7.18 (dd, 15.3; 10.4 Hz, 3´´´-H) indicated couplings to the carbonyl at δ 166.0 (C-5´´´).

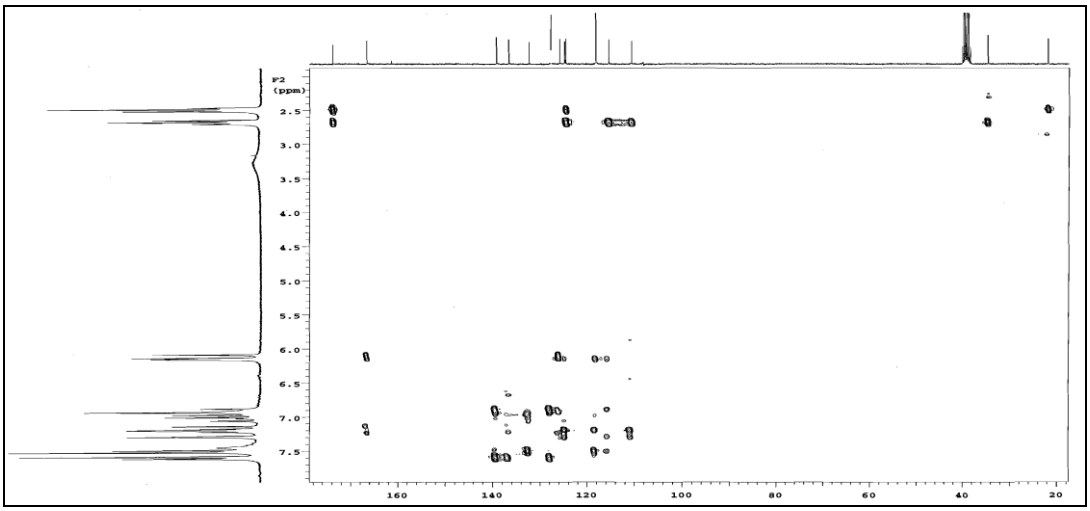


Figure 110: HMBC spectrum of compound iguanen a **92a** inDMSO-d_6

Table 14: ^{1}H (*J* in Hz, 300 MHz) and ^{13}C NMR (75 MHz) data of iguanen A **92a** and its methyl ester **92b** in DMSO-d_6

	92a		**92b**	
No	^{1}H	^{13}C	^{1}H	^{13}C
2	7.20 (br t, 2.5)	115.9	7.21 (br t, 2.6)	116.0
3	-	125.0	-	125.3
4	6.17 (m)	111.1	6.13 (m)	111.0
5	7.30 (t, 2.3)	118.5	7.30 (t, 2.6)	118.6
1’	-	139.6	-	139.5
2’	7.51 (d, 8.6)	118.6	7.52 (d, 8.7)	118.7
3’	7.61 (d, 8.6)	128.3	7.61 (d, 8.7)	128.2
4’	-	132.8	-	132.8
5’	7.61 (d, 8.6)	128.3	7.61 (d, 8.7)	128.2
6’	7.51 (d, 8.6)	118.6	7.52 (d, 8.7)	118.7
1’’	-	173.0	-	172.9
2’’	2.70 (t, 7.2)	34.9	2.71 (br t, 7.2)	34.6
3’’	2.50 (t, 7.2)	22.1	2.61 (br t, 7.2)	22.0
OH	12.00 (s)	-	-	-
OCH_3	-	-	3.61, (s)	51.1
1’’’	6.90 (d, 15.3)	137.0	6.92, (d, 15.3)	137.0
2’’’	7.00 (dd, 15.3, 10.4)	126.3	7.05 (dd, 15.3, 10.4)	126.3
3’’’	7.18 (dd, 15.3, 10.4)	139.5	7.17 (dd, 14.9, 10.4)	139.6
4’’’	6.13 (d, 15.0)	125.2	6.13 (d, 14.9)	124.6
5’’’	-	166.0	-	166.8

The structure was further confirmed by preparation of its methyl ester **92b** using diazomethane. Compound **92a** was finally deduced as 3- (1- (4- (1E,3E)-carbamoyl-butan-1,3-dienyl)phenyl]-1*H*-pyrrol-3-yl)propionic acid, for which the name iguanen A is proposed.

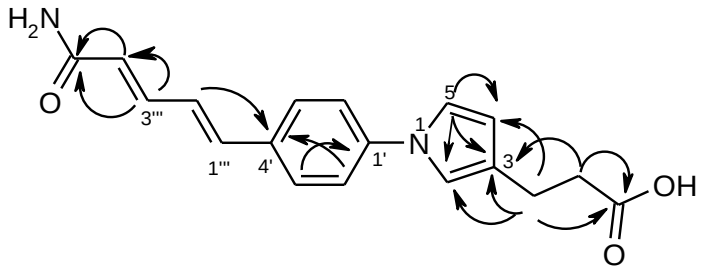
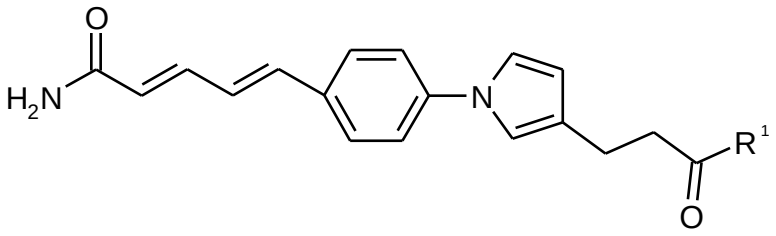


Figure 111: Selected HMBC correlations in iguanen A **92a**

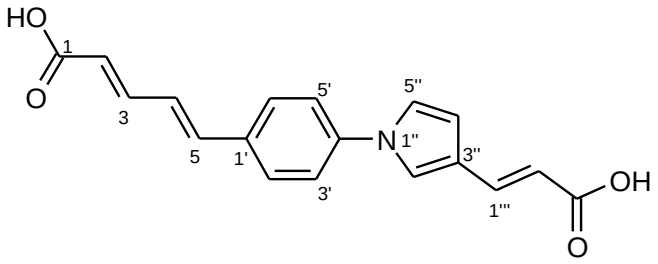


	R^1
92 a	OH
92b	OCH_3

Compounds possessing a 1phenyl-1*H*-pyrrole unit are very rare in nature, but were widely synthesised, because of their pharmacological activities, such as inhibitor of glucogen synthases kinase 3ß.[151]

6.6.5 Iguanen B

Compound **93** was isolated as a yellow powder with similar chemical and physical properties as **92a**. The proton NMR spectrum in DMSO-d_6 showed the signals of the 1*E*,3*E*-diene side chain, and also signals for a *para* disubstituted benzene ring were observed. The signals due to the monosubstituted pyrrole were also visible at δ 7.87, 7.50 and 6.70. The major difference between **92a** and **93** was observed in the aliphatic region, where the methylene triplets of **92a** at δ 2.70 and 2.50 were missing. Instead of the latter, two doublets of a *trans* double bond appeared at δ 6.16 and 7.51. The (-)-ESI mass spectrum indicated the *quasi*molecular ion at *m/z* 308 $[M-H]^-$ and HRESIMS delivered the molecular formula $C_{18}H_{15}NO_4$. Comparison of the data with those of **92a** delivered (2E,4E)-5-(4-[3-((E)-2-carboxy-vinyl)pyrrol-1-yl]phenyl)penta-2,4-dienoic acid (**93**), for which the name iguanen B is proposed.



93

Iguanen A (**92a**) and B (**93**) belong to the rare groups of natural products containing the 1phenyl-1*H*-pyrrole skeleton to my knowledge only four natural products: (+)-neopyrrolomycin, [152] bhimamycins D, F and G [153] have been reported from microbial source possessing this unit.

6.6.6 Intomycin A

Compound **94** was isolated from fraction III. It showed UV fluorescence and gave grey colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum indicated signals of 2 aromatic singlets at δ 8.20 und 6.98, five olefinic protons at δ 6.80-5.80, three methyl groups at δ 1.80, 1.10 and 0.98, a methylene at δ 3.58 and a methine group at δ 4.60. Moreover one broad signal was seen at δ 7.05 indicating the presence of an exchangeable proton.

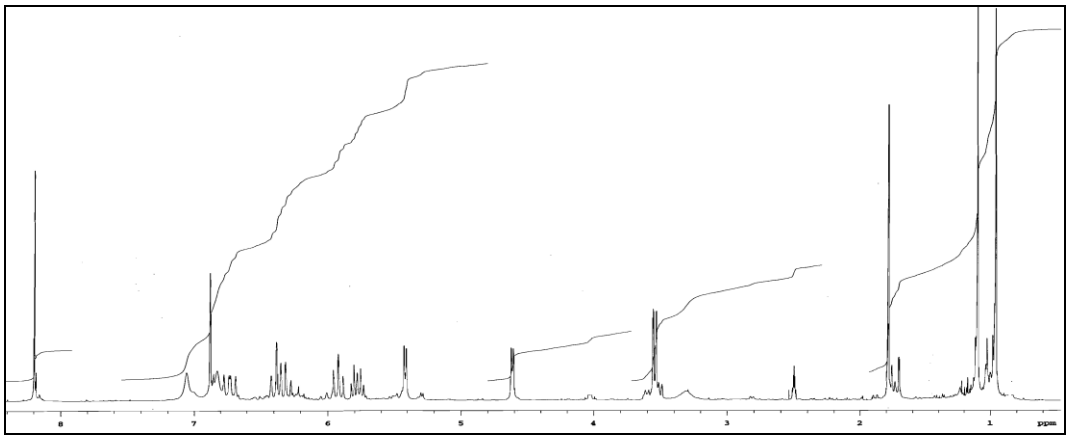


Figure 112: ^{1}H NMR spectrum (300 MHz) of intomycin **94** in $CDCl_3$

In the ^{13}C NMR spectrum 16 carbons were displayed and sorted as one carbonyl at δ 179.0, four quaternary carbons at δ 151.9, 151.1, 140.0 and the aliphatic carbon at δ 45.4, one methylene carbon at δ 28.2, seven methines and three methyl carbons.

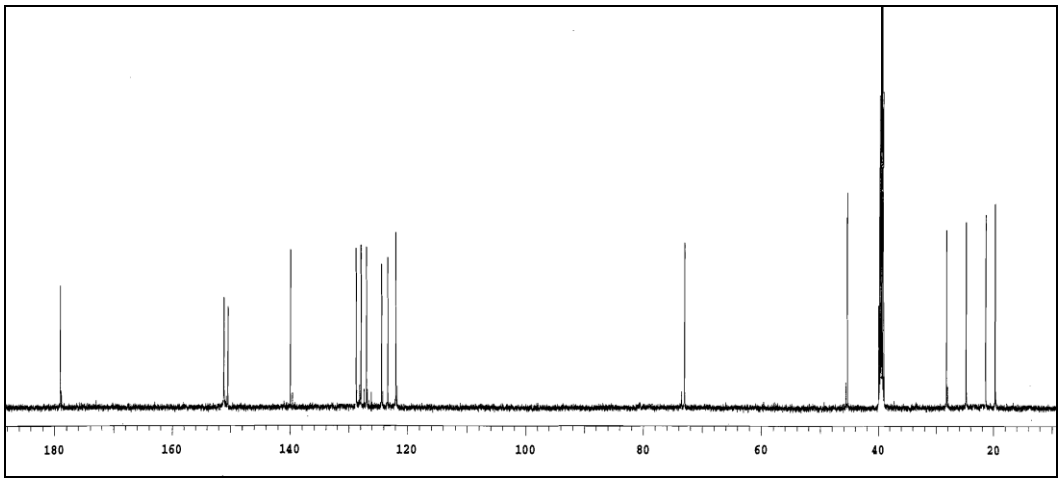


Figure 113: ^{13}C NMR spectrum (75 MHz) of intomycin **94** in $CDCl_3$

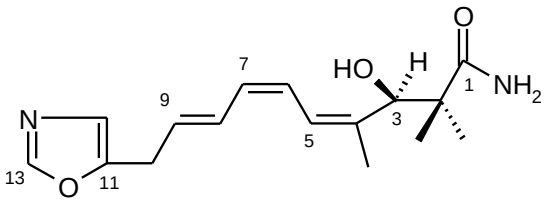
The ESI mass spectrum showed a molecular mass of 290 Dalton. The search in AntiBase gave 3 matches: inthomycin A (**94a**), inthomycin B (**94b**) and inthomycin C (**94c**). They all belong to group of oxazole trienes and were isolated in 1990 from *Streptomyces sp.*[154]

Table 15: ^{13}C NMR (DMSO-d_6, 125 MHz) data of inthomycin A **94a**

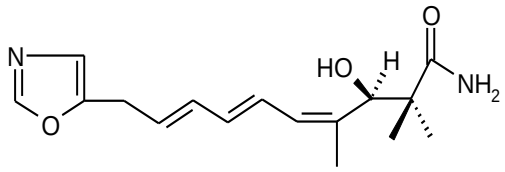
	94a (exp.)	**94a** [154]	**94b**[154]	**94b**[154]
1	179.0	181.1	181.1	180.8
2	45.4	45.4	45.4	45.8
2-CH_3	21.4	22.1	22.2	22.4
2-CH_3	24.8	26.3	26.3	26.5
3	73.1	75.4	75.8	83.7
4	140.0	140.6	139.9	140.0
4-CH_3	19.8	19.8	19.6	13.4
5	123.3	124.7	129.9	129.1
6	124.4	124.9	128.2	128.2
7	127.0	128.4	132.2	132.7
8	127.9	129.0	134.2	134.2
9	128.8	129.7	129.0	128.7
10	18.2	29.3	n.d	n.d
11	151.9	151.6	151.6	151.6
12	121.9	123.1	123.1	123.1
13	151.1	151.6	151.6	151.6

(n.d. = not defined)

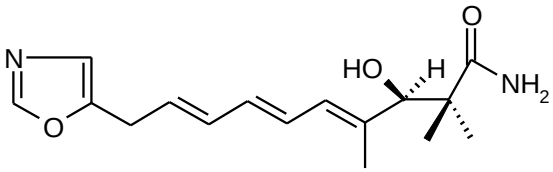
After comparison from experimental data from ^{13}C NMR data, compound **94a** appeared to be inthomycin A. Inthomycins are known to possess fungicide, herbicide activities, [155] and are growth inhibitor of radish seedlings.[156]



94a



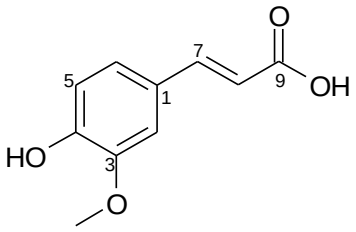
94b



94c

6.6.7 Ferulic acid

The UV active compound 95 was obtained as pale yellow crystals and showed on TLC a light blue colour reaction with anisaldehyde. Its proton NMR spectrum showed seven signals: at δ 7.25 (d, *J* = 1.5 Hz), 7.10 (dd, *J* = 8.6, 1.5 Hz), 6.80 (d, *J* = 8.6 Hz), which is the patter of a 1,3,4-trisubstituted aromatic ring . At δ 6.38-7.50 there were signals of an olefinic double bond in *trans* configuration; a singlet at δ 3.80 was attributed to a methoxy group and a broad signal at δ 9.80 for exchange-able proton. Substructure searches in AntiBase gave 4-hydroxy-3-methoxycinnamic acid (**95**) as a plausible structure. (-)-ESI MS showed a *quasi*molecular mass of 193 for $[M-H]^-$ and comparison of the ^1H proton NMR and ESI MS data with the spectra in our spectra collection confirmed the assignment.



95

Ferulic acid (**95**) was often isolated from leaves and seeds of many plants. It has activity against many cancer types,[157] including those of the stomach, colon, breast, prostate, liver, lung and tongue. It serves as a precursor in the production of useful aromatic compounds like vanilline.[158] Ferulic acid is an antioxidant and neutralizes free radicals (superoxide, nitric oxide and hydroxyl radical) which could cause oxidative damage of cell membranes and DNA.[159]

6.7 Terrestrial *Streptomyces* sp. AdM 21

The extract of the terrestrial *Streptomyces* isolate AdM 21 exhibited UV absorbing spots on TLC, which developed a red colour reaction upon spraying with anisaldehyde/sulphuric acid. AdM 21 showed strong activity against *Bacillus subtilis*, *Mucor miehei*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Streptomyces viridochromogenes* in the agar diffusion test at concentrations of 100 µg/ml per paper disk and cytotoxicity against brine shrimp. The strain was fermented in a 25 l scale on a yeast extract/malt extract/glucose (YMG) medium and worked up under usual conditions.[98] The strain was chromatographed on Sephadex LH-20 as indicated in Figure 114 and delivered a complex mixture of antimycin A components [180] with masses between *m/z* 436 and 578, *cis*-cyclo(leucylprolyl), [160] a mixture of palmitic acid and homologues, polyhydroxybutyric acid (sPHB), [161] and the new urauchimycin D. Additionally, a new derivative of isoprekinamycin [162] identified as 1,6,7-trihydroxy-3-methylbenzo[a]fluoren-11-one (**99**) is reported here for the first time from a natural source.

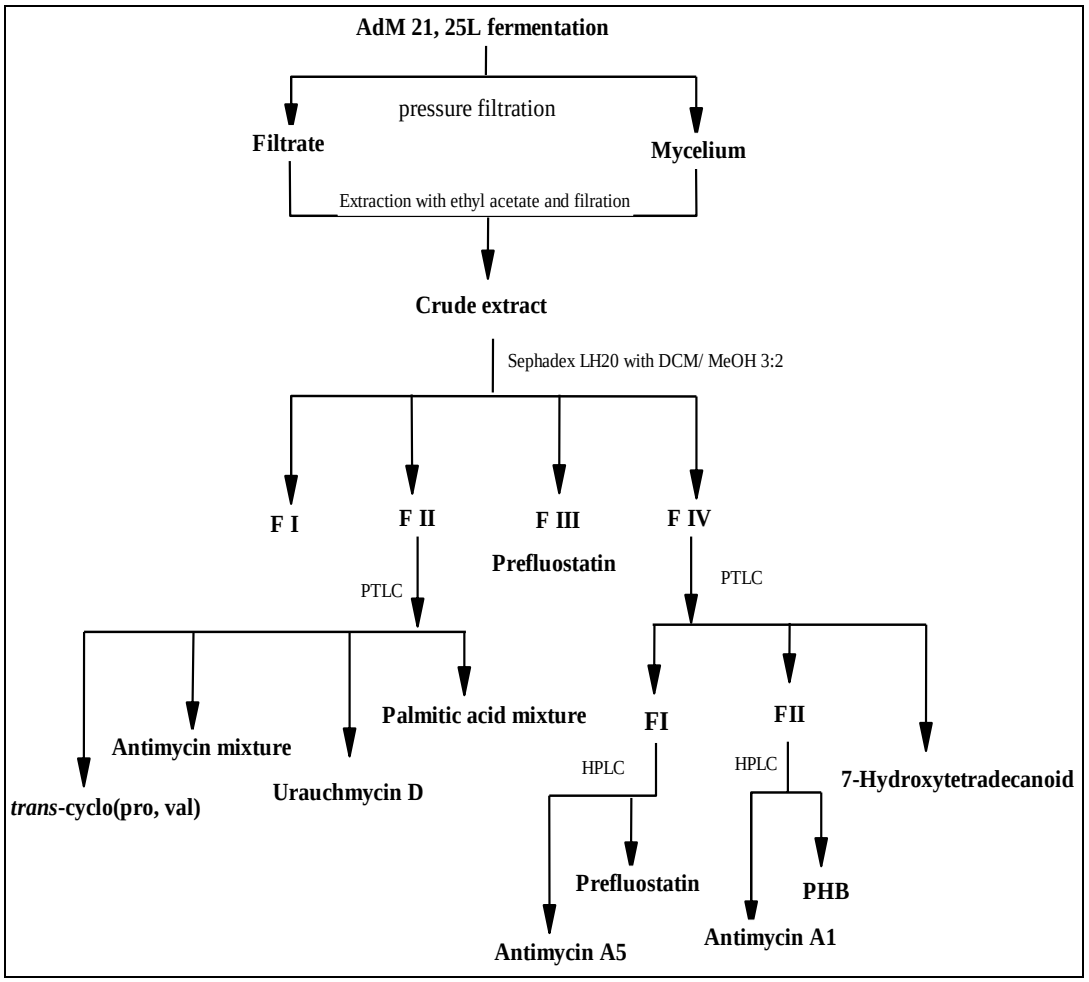


Figure 114: Work-up scheme of the strain *Streptomyces sp*. AdM 21

6.7.1 *Trans*-cyclo(prolyl-valyl)

Compound **96** was obtained as a colourless powder. The ^{1}H NMR spectrum showed in the aliphatic region a triplet at δ 4.10 (1H), a methane signal as doublet of doublet at δ 4.00, a multiplet between δ 3.60 - 3.50 (2H), a multiplet of six protons in the range of δ 2.40 - 1.80 and a ddd-signal at δ 1.50 (1H). Additionally, it depicted two methyl doublets at δ 1.00 (3H, *J* = 6.5 Hz) and 0.96 (3H, *J* = 6.5 Hz) and an acidic proton as a broad signal at δ 5.92.

A substructure search in AntiBase [68] with the NMR data led to *trans*-cyclo-(prolyl-valyl) (**96**) as a possible structure.

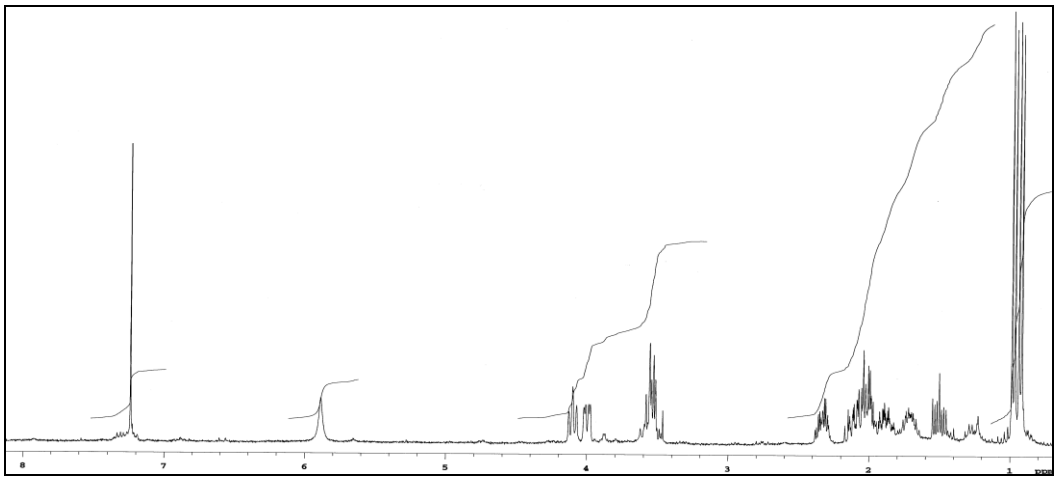
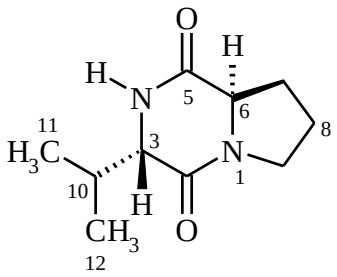


Figure 115: ^{1}H NMR spectrum (300 MHz) of *trans*-cyclo(prolyl-valyl) (**96**) in $CDCl_3$



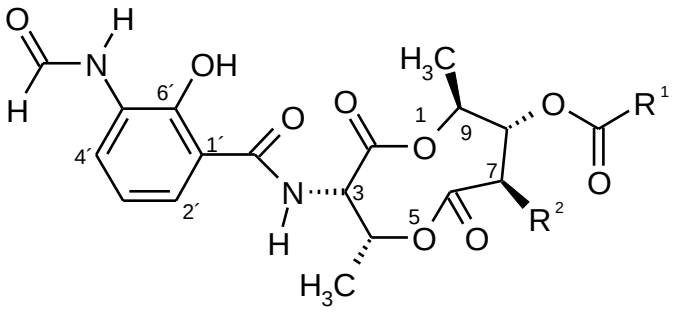
96

The structure **96** was finally confirmed by comparison of the NMR data with the literature.[163] Compound **96** belongs to the group of diketopiperazines which are very frequently isolated as secondary metabolites from microorganisms in our group. All stereoisomers of *trans*-cyclo(Pro,Val) have been reported.[163] Thus stereochemistry of the compound **96** was not determined.

6.7.2 Antimycin mixture 1

TLC of the fraction II containing antimycins showed under UV at 366 nm a strong blue fluorescent spot, which did not give any colouration on spraying with anisaldehyde/sulphuric acid. Column chromatography on silica gel followed by PTLC delivered the compound **97**. The ^{1}H NMR spectrum of **97** 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 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 1.00-0.80. The complexity of the spectrum and the ratio of the integrals suggested a mixture of similar compounds antimycin A_1b, A_2b and A_3b.



97

Antimycins	R^1	R^2
A_1	CH $(CH_3)CH_2CH_3$	$(CH_2)_5CH_3$
$A1_b$	$-CH_2CH$ $(CH_3)_2$	$-(CH_2)_5CH_3$
A_2	$CH_2CH_2CH_3$	$(CH_2)_5CH_3$
A_{2b}	$-CH$ $(CH_3)_2$	$-(CH_2)_5CH_3$
A_3	CH $(CH_3)CH_2CH_3$	$CH_2CH_2CH_2CH_3$
A_{3b}	$-CH_2CH$ $(CH_3)_2$	$-(CH_2)_3CH_3$
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 (+)-ESI mass spectrum indicated a mixture of three components by $[M+Na]^+$ ions corresponding to the molecular masses 520, 534 and 548. A search in AntiBase with these MS-data led to the identification of **97** as antimycin A-complex (antimycin A_1b, A_2b and A_3b).

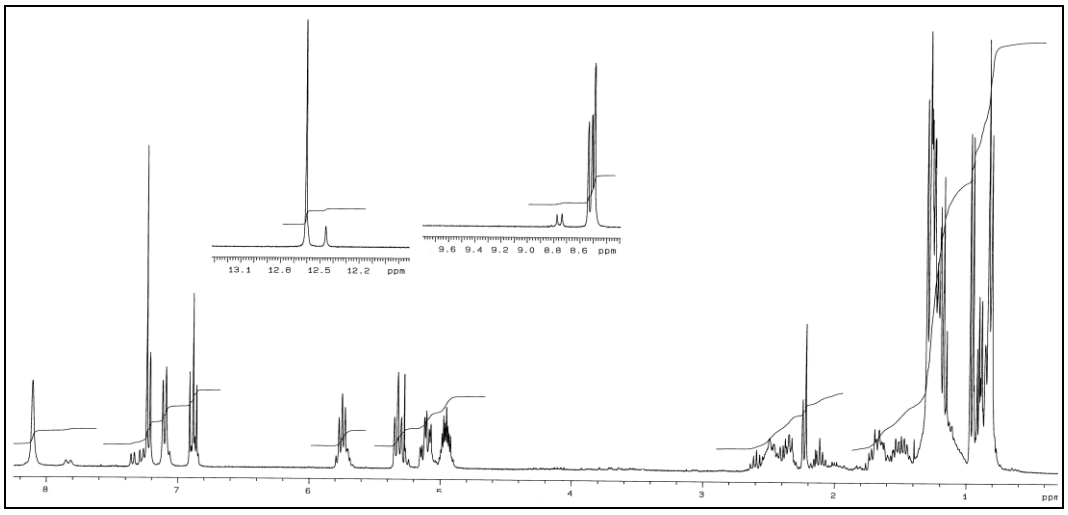


Figure 116: ^{1}H NMR spectrum (300 MHz) of antimycin A mixture (**97**) in $CDCl_3$

6.7.3 Urauchimycin D

The (+)-ESI mass spectrum of compound **98a** gave a *quasi*molecular ion at *m/z* 417 ($[M+Na]^+$), which delivered the molecular formula $C_{18}H_{22}N_2O_8$ by HRESIMS. This compound was obtained as a yellowish solid which gave a greenish colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR data (

Table 16) in the range of $\delta > 1.0$ suggesting that this compound belonged to the group of urauchimycins/antimycins as well. At high field, three methyl doublets were visible (δ 1.45, 1.29 and 1.25).

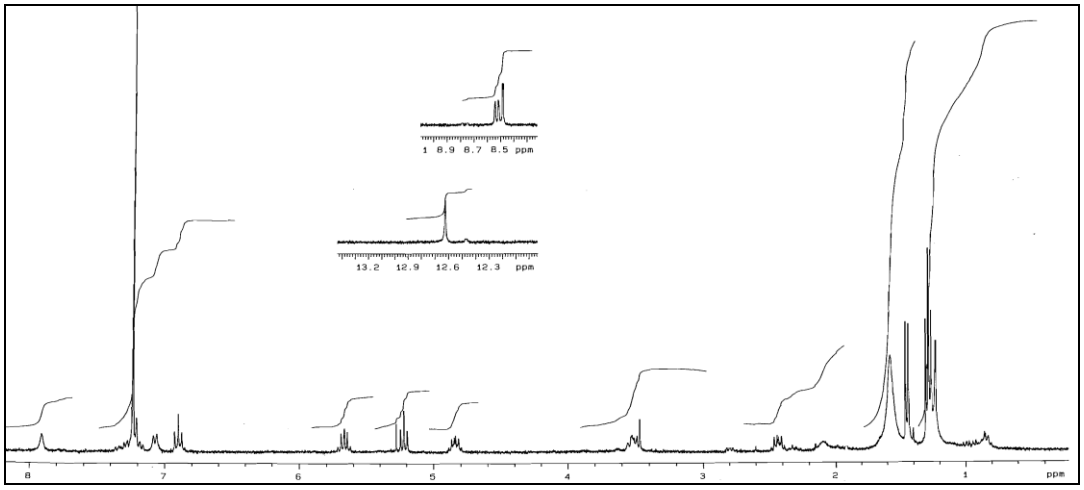


Figure 117: ^{1}H NMR spectrum (300 MHz) of urauchmimycin D (**98a**) in $CDCl_3$

Table 16: ^{1}H (*J* in Hz) and ^{13}C NMR (125 MHz) data of urauchimycins C (**98b**) and D (**98a**) in $CDCl_3$.

	^{1}H		^{13}C	^{13}C
Position	**98a**	**98b**[164]	**98a**	**98b**[164]
2			170.1	170.1
3	5.22 (t, 7.3)	5.25 (t, 7.3)	53.5	53.7
4	5.70 (m)	5.71 (m)	70.4	70.7
6			174.1	173.8
7	2.40 (m)	2.31 (d, 7.0)	45.3	53.7
8	2.5 (m)	3.61 (t, 10.0)	77.9	77.3
9	4.83 (m)	4.95-4.80 (m)	77.4	77.0*
9-CH_3	1.45 (d, 6.3)	1.46 (d, 6.0)	18.4	18.3
10 (NH)	7.08 (d, 8.2)	7.10 (d, 7.0)		
11			169.4	169.4
12			112.5	112.6
13			150.6	150.6
13-OH	12.60 (s)	12.65 (s)		
14			127.4	127.4
14 (NH)	7.91 (s)	7.93 (s)		
14 (NH*CH*O)	8.48 (d, 1.6)	8.51 (d, 1.0)	158.9	159.1
15	8.53 (dd, 1.3, 7.7)	8.55 (dd, 8.0, 1.0)	124.8	124.8
16	6.90 (t, 8.3,16.4)	6.93 (t, 8)	118.9	119.0
17	7.80 (d)	7.25-7.22 m	120.1	120.2
18	1.25 d (6.5)	1.31 (d, 7)	14.5	15.0
19		1.80-1.60 (m)		22.1
20	1.29 (d ,6.5)	0.94 (t, 7)	14.1	11.6
21				
21- $(CH_3)_2$				

[a]Multiplicity; [b]coupling constants in [Hz]; *under solvent peak.

The H,H COSY and HMBC spectra (Figure 118) indicated a correlation of the methyl group at δ 1.29 (C-19) with the methine carbon at δ 45.3 (C-7), the carbonyl

signal at δ 174.1 (C-6) and the methine at δ 77.9 (C-8), confirming the methyl to be in position C-7.

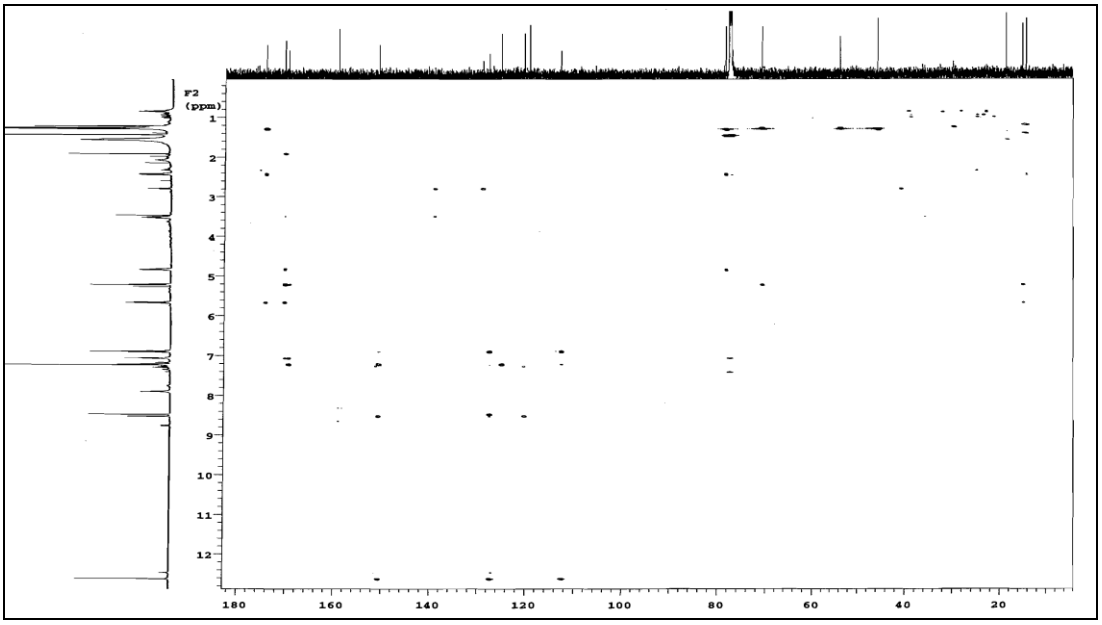


Figure 118:HMBC spectrum of Urauchimycin D (**98a**) in $CDCl_3$

According to the H,H COSY and HMBC data, there was a methyl group at C-7 resulting in **98a**, instead of an ethyl group as in **98b**. Further HMBC correlations (Figure 118) confirmed the structure of the new compound **98a** as a further member in the series of homologous urauchimycins; it was named urauchimycin D (**98**).

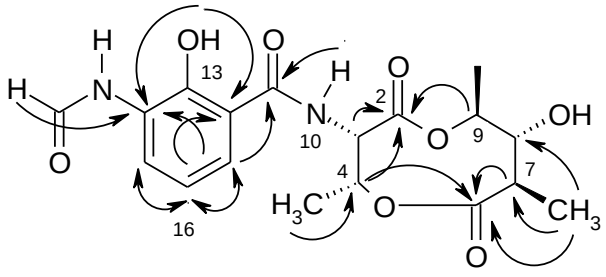
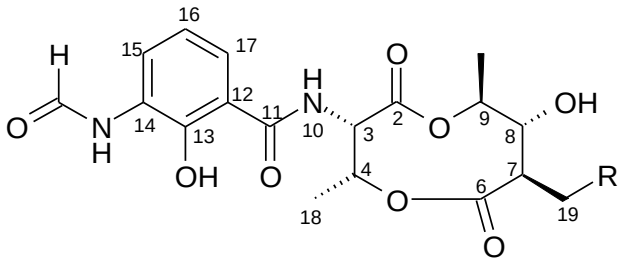


Figure 119: Selected HMBC (⟶) and H,H COSY (⟷) correlations of urauchimycin D (**98a**)

The antimycins were first isolated from a *Streptomyces* sp. in 1949.[165] The structure determination was completed in 1961[166] and the absolute configuration was established by Kinoshita *et al.*[167] in 1972. They form a group of about 30

closely related lactolide antibiotics with the skeleton of **98**. Their common feature is a 9-membered ring of an α-substituted ß,γ-dihydroxyvaleric acid and threonine, wherein the nitrogen is acylated with 3-formylamino salicylic acid. The antimycins differ in the nature of their alkyl residue at C-7 and of the oxygen substituent at C-8. Although first identified as antibiotics and used commercially as fungicides,[168] more recently antimycins 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.[168]

Acylation of the 8-hydroxy group modulates the strong antifungal, antiviral and antitumor activities. These acylated antimycins inhibit the mitochondrial ATP-production and are therefore not effective against bacteria that do not have mitochondria. Antimycins have also been reported to inhibit the oxidation of NADH.[169,180] They are occasionally used in fruit cultivation as antifungals and can be applied in fish-breeding to kill sick specimens.[170] The relatively low stability of the agent allows new stock to be used after just a few days.[171]



98

Urauchimycin group with the new member **98a**

	R		R
98a:	H	**98e**	CH $(CH_3)CH_2CH_3$
98b:	CH_3	**98f**	$(CH_2)_4CH_3$
98c:	$(CH_2)_2CH_3$	**98g**	$(CH_2)_2CH\ (CH_3)_2$
98d:	$CH_2CH\ (CH_3)_2$		

Urauchimycins are antimycin derivatives with a free 8-OH group, e.g. deisovalerylblastmycin (**98c**), [172] urauchimycin A (**98e**), urauchimycin B [173] (**98d**), and

the related kitamycins A (**98f**) and B (**98g**)[174]. Only weak antifungal properties were reported due to the free hydroxy group at C-8.[173] The urauchimycin C [164] (**98b**) and D (**98a**) were inactive as well against *Candida albicans* and *Mucor miehei* in the agar diffusion test at concentrations of 25 μg per paper disk. Expectedly, they were also inactive against *Escherichia coli* and *Staphylococcus aureus*.

6.7.4 Prefluostatin

Compound **99** was obtained as a purple solid, which showed on TLC a brown colour after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **99** exhibited in DMSO-d_6 a singlet at δ 11.46 attributed to a chelated hydroxy group, however, the expected typical colour change of *peri*-hydroxyquinones with diluted sodium hydroxide was very weak. In the aromatic region, a triplet and two doublets were attributed to an 1,2,3-trisubstituted aromatic system and confirmed by H,H COSY couplings. Two further doublets indicated protons in *meta*-position. In the aliphatic region, only a 3H signal of an aromatic methyl group was present.

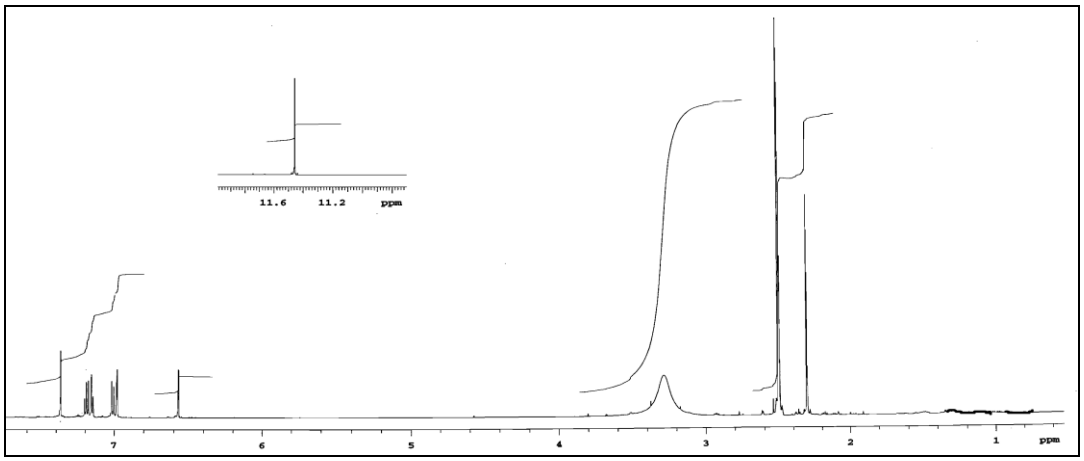


Figure 120: ^{1}H NMR spectrum (300 MHz) of prefluostatin (**99**) in DMSO-d_6

The (+)-ESI and (-)-ESI mass spectra showed *quasi*molecular ions at *m/z* 606 ($[2M + Na]^+$) and 291 ($[M-H]^-$) for a molecular mass of *m/z* 292, and HRESIMS delivered the molecular formula $C_{18}H_{13}O_4$. The ^{13}C NMR spectrum exhibited 18 signals including a carbonyl at δ 197.9, ten quaternary carbons, six sp^2 methines and the methyl carbon.

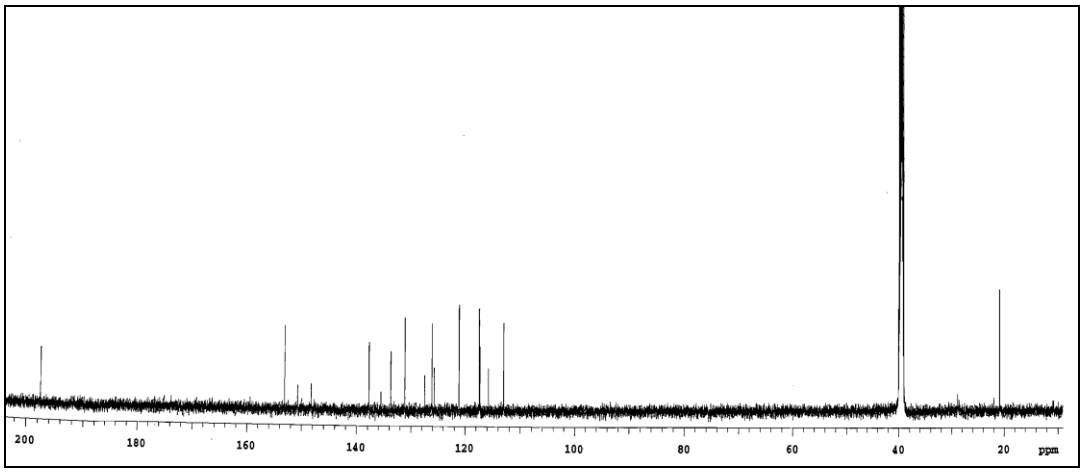


Figure 121: ^{13}C NMR spectrum (75 MHz) of prefluostatin (**99**) in DMSO-d_6

The HMBC spectrum showed a correlation of the methyl group (C-12) with the quaternary carbon at δ 137.8 (C-3), the methine carbons at δ 113.4 (C-2) and 117.4 (C-4) respectively. This confirmed that both ^{1}H singlets at δ 6.98 (4-H) and 6.80 (2-H) were due to protons in *meta* position (Figure 122). Overlapping correlations with cross signals of 5-H indicated finally a 1,6-dihydroxy-naphthalene. Among others, the HMBC correlation between the proton signal at δ 7.15 (10-H) and the carbonyl signal at δ 197.9 indicated a 4-hydroxyinden-1-one. Two ways to connect both fragments are possible, one yielding the skeleton of prekinamycin (**100**) [175] and momofulvenone A,[176] the other delivering structure **99**. A long-range coupling between 5-H and C-6b confirmed the structure latter, for a ^{4}J coupling in **99** is more plausible than the corresponding ^{5}J coupling in a benzo[b]fluorenone of type **100**.

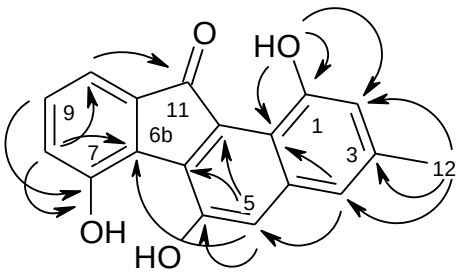
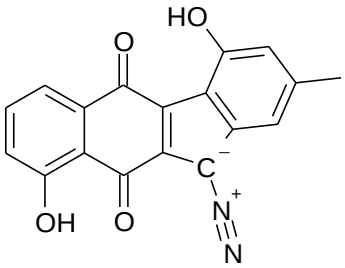


Figure 122: Structure and selected HMBC correlations of prefluostatin (**99**)

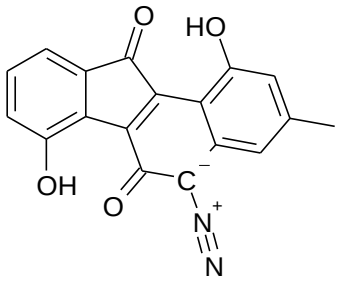
Compound **99** was previously obtained during synthetic studies on kinamycins [162] in a reaction using rhodium acetate induced deazotisation of isoprekinamycin (**101**), a minor metabolite from *Streptomyces murayamaensis.*[177] However, it is found here for the first time in nature. Our ^{13}C NMR data were identical with the

reported values within the error limits, [162] although the proton shifts showed some deviations (Table 17).

The kinamycins and the aromatised prekinamycin (**100**) are related, as the fluostatins [178] and compound **99**, for which therefore the name prefluostatin is suggested. Isoprekinamycin (**101**) or other kinamycins have not been found in *Streptomyces* sp. However, a search of AdM21 by systematic variations of the fermentation conditions may make sense.



100



101

Table 17: ^{1}H and ^{13}C NMR data of prefluostatin (**99**) in DMSO-d_6.

	^{1}H (300 MHz)		^{13}C (75 MHz)	
position	**99**	ref.[162]	**99**	ref[162]
1	-	-	153.1	154.8
1-OH	11.42 (br s)	11.42 (br s)		
2	6.80 (s)	6.41 (s)	113.4	111.7
3	-	-	137.8	138.7
3-CH_3	2.30 (s)	2.25 (s)	20.1	21.0
4	6.98 (s)	6.82 (s)	117.4	117.1
4a	-		127.5	127.2
5	7.38 (s)	7.08 (s)	121.1	121
6-OH	-	3.34 (br s)	148.3	-
6a	-	-	135.7	136.7
6b	-	-	125.8	126.2
7-OH	-	3.34 (br s)	150.7	153.1
8	7.01 (d, 8.1 Hz)	6.78 (d, 8.1 Hz)	126.1	126.9
9	7.19 (t, 7.11 Hz)	7.01 (t, 7.6 Hz)	131.1	130.7
10	7.15 (d, 6.98 Hz)	6.90 (d, 6.9 Hz)	117.3	115.1
10a	-	-	133.7	133.6
11	-	-	197.5	198.4
11a	-	-	137.8	138.3
11b	-	-	115.8	114.9

Although the diazo group is believed to be responsible for the antibacterial and weakly antitumoral properties [179] of kinamycins, compound **99** showed as well moderate activity in the agar diffusion test against *Bacillus subtilis* and *B. brevis* (11 mm inhibition zone), *Mucor miehei* (11 mm), *Escherichia coli* (11 mm) and *Staphylococcus aureus* (18 mm) at concentrations of 20 µg per paper disk. Weak cytotoxic activities towards MCF-7 cells and HeLa S3 cells were observed with LD_{50} ranging between 20 and 30 µg/ml. Jurkat and Hep G2 cells were only affected at higher concentrations (LD_{50} 50 µg/ml).

6.8 Terrestrial *Streptomyces* sp. Ank 5

The extract of the terrestrial streptomycete isolate Ank 5 exhibited UV absorbing spots on TLC, which developed a characteristic red colour reaction upon spraying with anisaldehyde/sulphuric acid. Extracts of Ank 5 showed strong activity against *Staphylococcus aureus, Candida albicans* and *Streptomyces viridochromogenes* in the agar diffusion test at concentrations of 20 mg per paper disk and a higher cytotoxicity against brine shrimp. The strain was fermented in a 25 L scale on a yeast extract/malt extract/glucose (YMG) medium and worked up under usual conditions.[98]

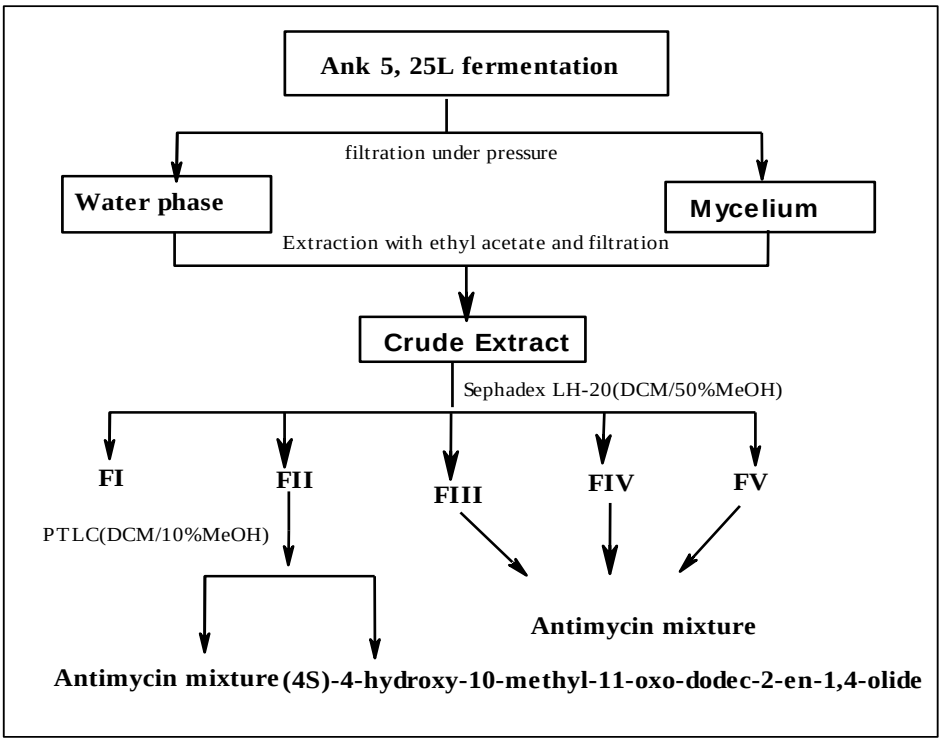


Figure 123: Work-up schema of the strain *Streptomyces sp*. Ank5

The crude extract was chromatographed on Sephadex LH-20 as indicated in the Figure 123 and delivered a complex mixture of antimycin A components [180] with masses between *m/z* 506 and 578, and (4S)-4-hydroxy-10-methyl-11-oxododec-2-en-1,4-olide.

6.8.1 Antimycin mixture 2

Column chromatography on Sephadex LH 20 (CH_2Cl_2/MeOH 3:2) followed by PTLC (CH_2Cl_2/10 % MeOH) delivered again a mixture of antimycin **97**, which was previously discussed in strain AdM 21. The difference between this two mixtures was the addition of others antimycins in mixture 2. The (+)-ESI mass spectrum of mixture 2 indicated six $[M+Na]^+$ ions corresponding to the molecular mass 506, 520, 534, 548, 562 and 574. A search in AntiBase with these data led to the identification of antimycins A-complex (antimycin A_1b, A_2b, A_3b and A_1, A_2, A_3).

6.8.2 (4*S*)-4-Hydroxy-10-methyl-11-oxododec-2-en-1,4-olide

Compound **102** was obtained as oil from fraction 2. 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 simple ^{1}H NMR spectrum showed in the sp^2 region only two 1H signals at δ 7.48 and 6.16 (dd, J = 5.6, 1.7 Hz). The small coupling constants between the olefinic protons suggested that they belongs to a small ring; at δ 5.03 (m) appeared a 1H signal as multiplet, which was assigned to an oxymethine proton. The aliphatic region delivered a methine quartet at δ 2.54 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 finally a multiplet in the range of δ 1.70-1.20 was assigned to ten protons of a long methylene chain. Thus, **102** was identified as a metabolite with a branched chain.

The ESI MS indicated the molecular mass at *m/z* 224. From a search in AntiBase using the substructure and the molecular mass, the compound was identified as (4*S*)-4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**102**), which had previously been isolated in our group by Mukku.[181]

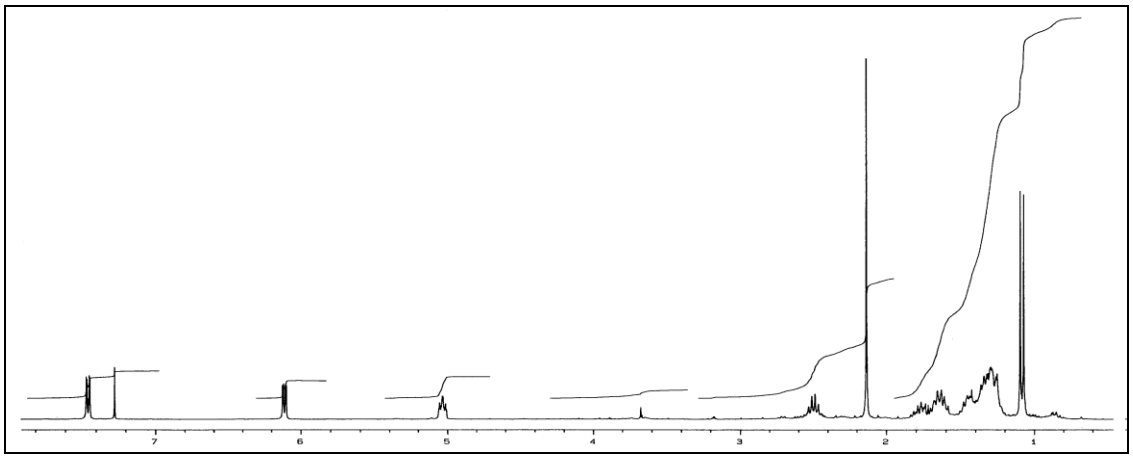
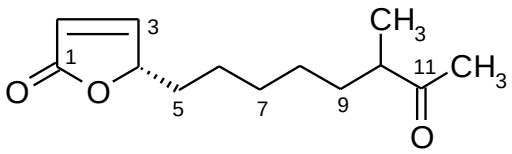


Figure 124: ^{1}H NMR spectrum (300 MHz) of **102** in $CDCl_3$



102

Butenolides (mainly but-2-enolides) are common natural products from plants, fungi, bacteria and animals.[182] Their saturated analogues act as signalling substances in bacteria [183] and enhance the spore formation in streptomycetes or induce metabolite production.[184] They are inactive in antibacterial tests.

6.9 Marine *Vibrio* sp. WMBA1-4

In a screening of marine bacteria from the surface of the soft coral *Sinularia polydactyla* from the Red Sea (Aqaba, Jordan) for the production of antibiotics, we identified a *Vibrio* strain WMB4 as a producer of metabolites with antibacterial and cytotoxic activity. A series of new nitro substituted maleimide derivatives, of which three were maleimide oxim derivates, and a new azirine derivate were isolated. Their structures were established on the basis of NMR data, MS data, and other spectroscopic methods and by comparison with known compounds. Most of these compounds showed antibacterial activity against Gram positive bacteria (*Micrococcus luteus, Bacillus subtilis, and Bacillus brevis*), cytotoxic activity against breast cancer, colorectal cancer, mouse lymphocytes leukaemia and Jurkat-T cell leukaemia.

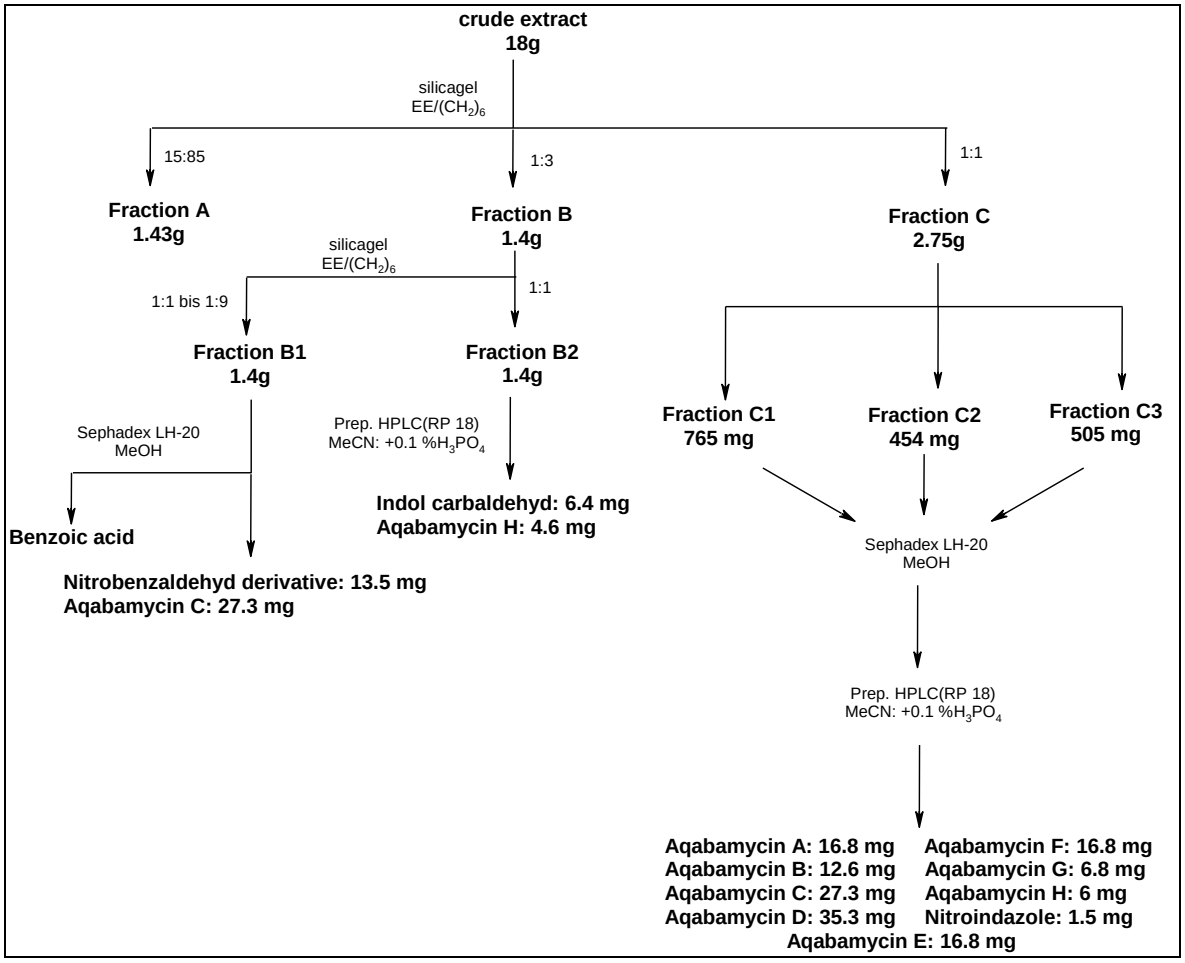


Figure 125: Work-up scheme 1 of the strain *Vibrio sp*.WMB41-4

6.9.1 Aqabamycin A

Compound **103a** was obtained as a yellow solid, whose colour remained unchanged on spraying with anisaldehyde/sulphuric acid. The proton NMR spectrum indicated signals in the aromatic region: a pair of doublets of a *para* disubstituted benzene ring at δ 7.32 and 6.72. In addition two multiplets integrating for three and two protons were seen at δ 7.36 and 7.42, which were attributed to a phenyl ring based on the H,H COSY spectrum.

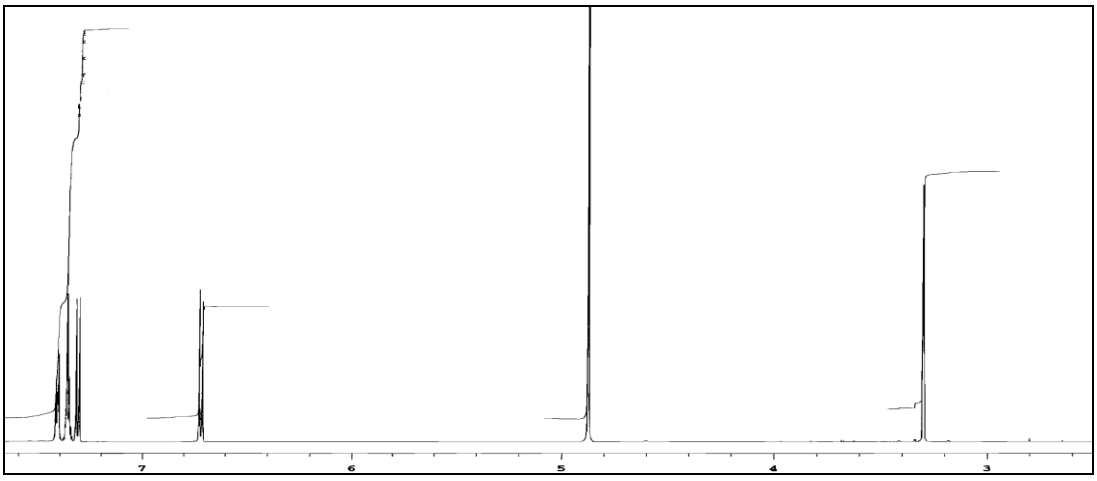


Figure 126: ^{1}H NMR spectrum (600 MHz) of aqabamycin A (**103a**) in MeOH-d_4

The (+)-ESI mass spectrum indicated *quasi*molecular ions at *m/z* 264 $[M-H]^-$, 528.7 $[2M-H]^-$, and HRESIMS gave the molecular formula $C_{16}H_{11}NO_3$. A search in AntiBase delivered no hits, pointing to a new compound.

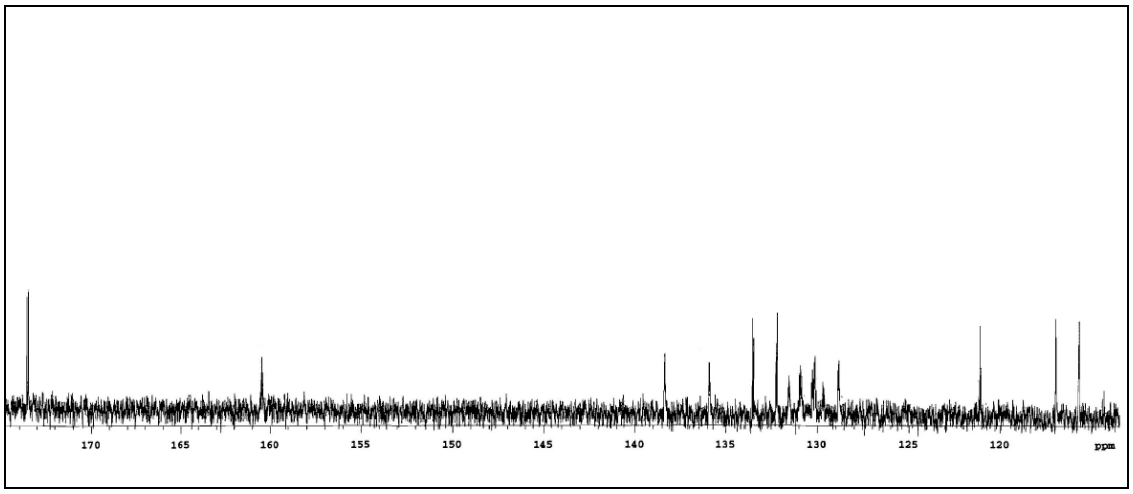
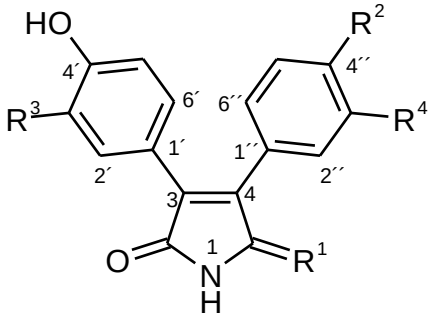


Figure 127: ^{13}C NMR spectrum (125 MHz) of aqabamycin A (**103a**) in MeOH-d_4

The ^{13}C NMR spectrum indicated 16 carbon signals as requested by the high resolution MS, including two carbonyls of an acid or amide at δ 173.6 and 173.5 (C-1, 5), four quaternary sp^2 carbons (C-1´, 3, 1´´, 4) including an oxygenated sp^2 carbon at δ 160.5 (C-4´) and nine sp^2 methines.



103

	R^1	R^2	R^3	R^4
103a	O	H	H	H
103b	O	OH	H	NO_2
103c	O	H	NO_2	H
103d	O	OH	NO_2	NO_2
103e	NOH	H	NO_2	H
103f	NOH	OH	NO_2	NO_2

Table 18: ^{1}H (600 MHz) data of compounds **103a-c**

Position	**103a**[a]	**103 b**[b]	**103c**
1-NH	-	11.02 (s)	10.78 (s)
2´	7.32 (d, 8.8)	6.78 (d, 8.8)	8.42 (d, 2.2)
3´	6.72 (d, 8.8)	7.35 (d, 8.8)	-
4´	-	-	-
4´-OH	-	9.91 (br s)	7.60 (s br)
5´	6.72 (d, 8.8)	7.35 (d, 8.8)	7.10 (d, 8.8)
6´	7.32 (d, 8.8)	6.78 (d, 8.8)	7.61 (dd, 8.8, 2.2)
1´´	-	-	-
2´´	7.42 (m)	8.29 (d, 2.2)	7.42 (m)
3´´	7.36 (m)	-	7.42 (m)
4´´	7.36 (m)	-	7.42 (m)
5´´	7.36 (m)	7.09 (d, 8.8)	7.42 (m)
6´´	7.42 (m)	7.61 (dd, 8.7, 2.2)	7.42 (m)

a) MeOH-d_4 b) DMSO-d_6 c) $CDCl_3$

The HBMC spectrum confirmed the presence of two spins systems with the correlation of both *ortho* protons (C-5´, 6´) to the oxygenated carbon at δ 160.5 (C-4´) and correlations within the phenyl ring. The two spin systems contained twelve carbon signals and one hydroxyl group encounting for 8 double bond equivalents and nine sp^2 methines. The remaining 4 double bond equivalences were attributed to two carbonyls, a ring and a double bond. The combination of all these information led finally to the structure 3-(4-hydroxyphenyl)-4phenyl-pyrrol-2,5-dione, for which the name aqabamycin A (**103a**) is suggested.

6.9.2 Aqabamycin B

The compound **103b** presented similar chemical properties as **103a**. The proton NMR spectrum was similar to that of **103a** with *para* disubstituted benzene ring, two doublets and a doublet of doublet, respectively, at δ 8.29 (d, *J* = 2.2 Hz), 7.09 (d, *J* = 8.8 Hz) and 7.61 (dd, *J* = 8.7, 2.2 Hz) attributed to a 1,2,4-trisubstituted aromatic ring.

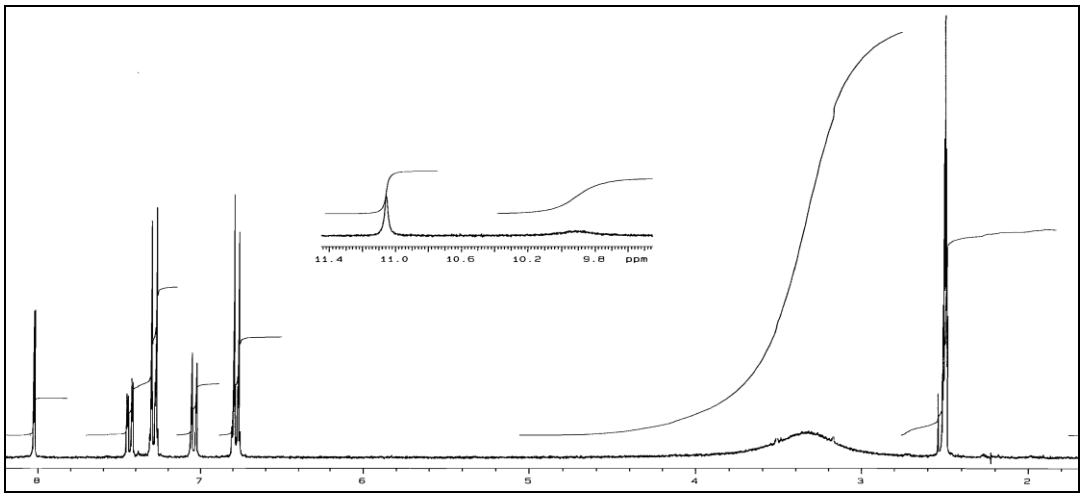


Figure 128: ^{1}H NMR spectrum (600 MHz) of aqabamycin B (**103b**) in DMSO-d_6

The molecular mass was deduced from the (+)-ESI MS spectrum to be 326 Dalton. The molecular formula $C_{16}H_{10}N_2O_6$ derived from HRESIMS indicated 13 double bond equivalences, one more than in **103a**. The difference in the molecular formula from ΔMF = HNO_3 between **103a** and **103b** could be interpreted as additional OH and NO_2 groups.

The ^{13}C NMR spectrum was similar to that of **103a** and indicated also 16 carbon signals as expected by the high resolution and exhibited also no aliphatic signals. It exhibited two carbonyl signals δ 173.3 and 173.1, two oxygenated sp^2 carbons at δ 160.8 (C-4´) and 156.2 (C-4´´) instead of one as in **103a**. The HSQC spectrum revealed the presence of seven sp^2 methines.

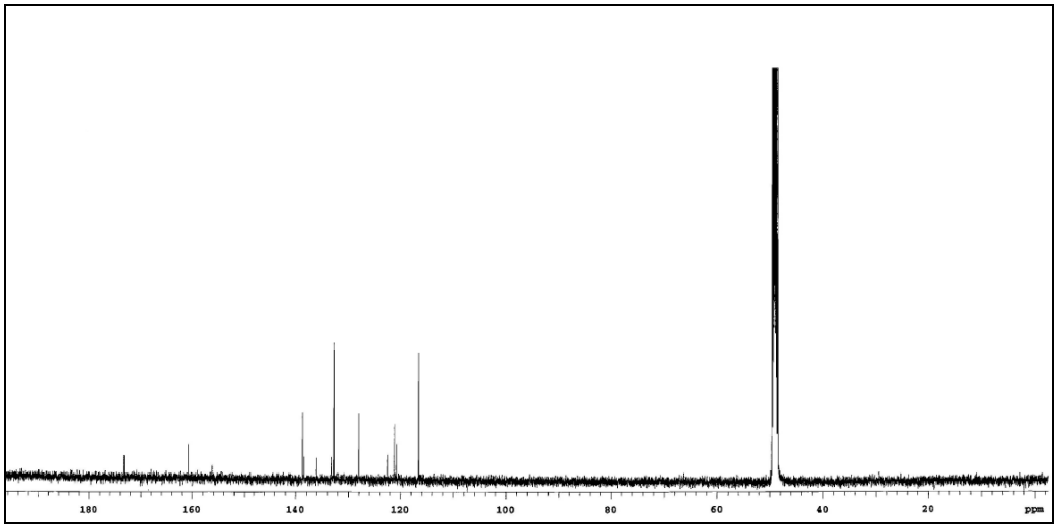


Figure 129: ^{13}C NMR spectrum (125 MHz) of aqabamycin B (**103b**) in DMSO-d_6

The H,H COSY spectrum confirmed the two fragments derived from the proton NMR spectrum.

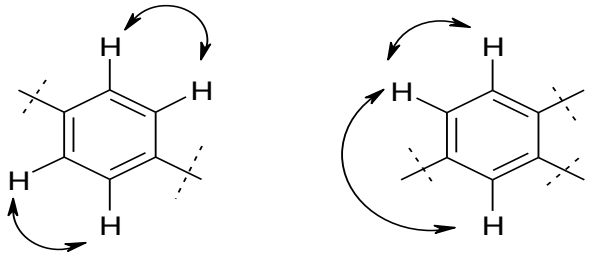


Figure 130: H,H COSY couplings in fragments of aqabamycin B (**103b**)

The HMBC spectrum indicated correlations of the protons from the *para* disubstituted benzene ring to the carbon at δ 160.8 (C-4´) as well as of the protons of the 1,2,4-trisubstituted aromatic ring to the carbon at δ 156.2 (C-4´´). Furthermore, the doublet of the *para* disubstituted benzene ring at δ 7.35 and the doublet at δ 8.29 (J = 2.2 Hz) showed couplings to the quaternary carbon at δ 138.5. The colour reaction with tin(II)-chloride/hydrochloric acid/4-dimethylaminobenzaldehyde delivered a orange colour, which is typical for the reduction of a nitro group to the amine and its

further reaction to an azomethine. Both doublets of the trisubstituted system coupled with the carbon at δ 136.1 fixing the connection of the nitro group.

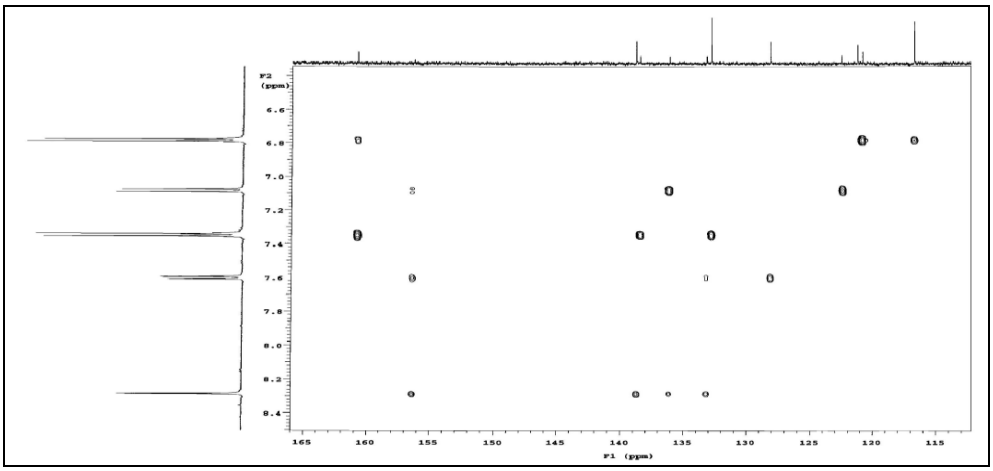


Figure 131: HMBC spectrum of aqabamycin B (**103b**) in DMSO-d_6

The structure of **103b** was finally deduced as 3-(4-hydroxy-3-nitrophenyl)-4-(4-hydroxphenyl)pyrrol-2, 5-dione (**103b**) and the name aqabamycin B proposed.

6.9.3 Aqabamycin C

Obtained also as a yellow solid, compound **103c** exhibited similar physico-chemical properties as **103a** and **103b**. The ^{1}H and ^{13}C NMR spectra of **103c** were similar to those of **103b** with the 1,2,4-trisubstituted aromatic system, the major difference being the absence of the protons of the second *para* disubstituted benzene ring, which was present in **103a** and **103b**. Instead, an unsubstituted benzene ring was present in **103c** suggesting the loss of an oxygen atom. Finally, an exchangeable proton at δ 10.8 was seen.

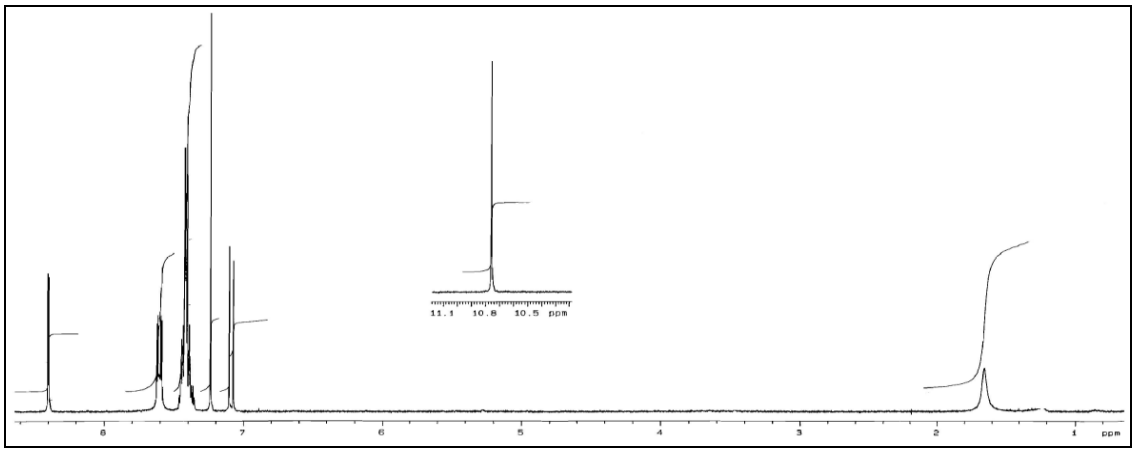


Figure 132: ^{1}H NMR spectrum (300 MHz) of aqabamycin C (**103c**) in $CDCl_3$

The ^{13}C NMR spectrum indicated 16 carbon signals including two carbonyls at δ 169.9 and 169.8, an oxygenated sp^2 carbon at δ 156.2, 6 quaternary sp^2 and eight sp^2 methines. The ESI MS spectrum gave the mass of 310 Dalton from the *quasi*-molecular ion at *m/z* 309.2 $[M-H]^-$. Compound **103c** has a molecular formula of $C_{16}H_{10}N_2O_5$, which differs from that of **103b** ($C_{16}H_{10}N_2O_6$) by one oxygen atom.

Table 19: ^{13}C NMR (125 MHz) data of compounds **103a-c**

Position	**103a**[a]	**103b**[b]	**103c**[c]
1-NH	-	-	-
2	173.6	173.3	169.9
3	133.5	133.1	134.1
4	138.3	138.5	133.9
5	173.5	173.1	169.8
1´	133.5	122.5	127.9
2´	128.8	132.7	127.4
3´	116.9	116. 6	137.8
4´	160.5	156.2	156.2
5´	121.1	116. 6	120.7
6´	135.9	132.7	138.6
1´´	121.0	120.8	120.9
2´´	130.1	128.1	129.8
3´´	130.1	136.1	129.3
4´´	130.9	160.8	130.9
5´´	128.8	121. 1	129.3
6´´	132.2	138. 8	129.8

a) MeOH-d_4 b) DMSO-d_6 c) $CDCl_3$

The HSQC spectrum allowed the assignment of each carbon to the corresponding proton.

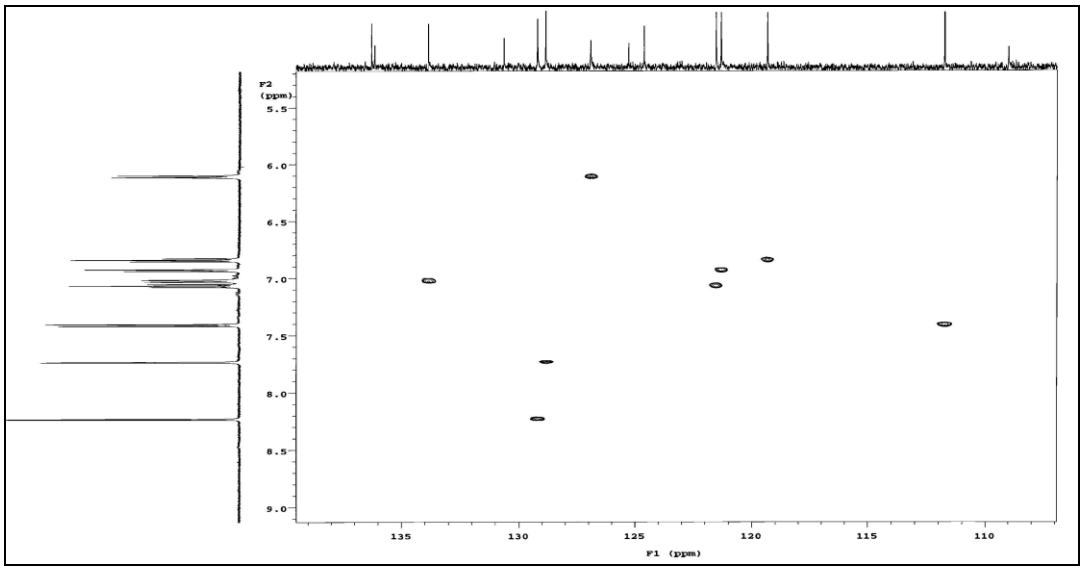


Figure 133: HSQC spectrum of compound aqabamycin C (**103c**) in $CDCl_3$

The structure of **103c** was deduced to be the new 3-(4-hydroxy-3-nitrophenyl)-4phenyl-pyrrol-2,5-dione, which is the 4'-deoxy derivative of **103b** and was named aqabamycin C.

6.9.4 Aqabamycin D

Compound **103d** was deduced as yellow powder similar to **103a -c**. The proton NMR spectrum was very simple and displayed only three proton signals in the aromatic region as two doublets at δ 8.32 (*J* = 2.2 Hz) and 7.12 (d, *J* = 8.8 Hz), and a doublet of doublet at δ 7.63 (dd, *J* = 8.8, 2.2 Hz) characteristic of a 1,2,4-trisubstituted aromatic ring as in **103b** and **103c.**

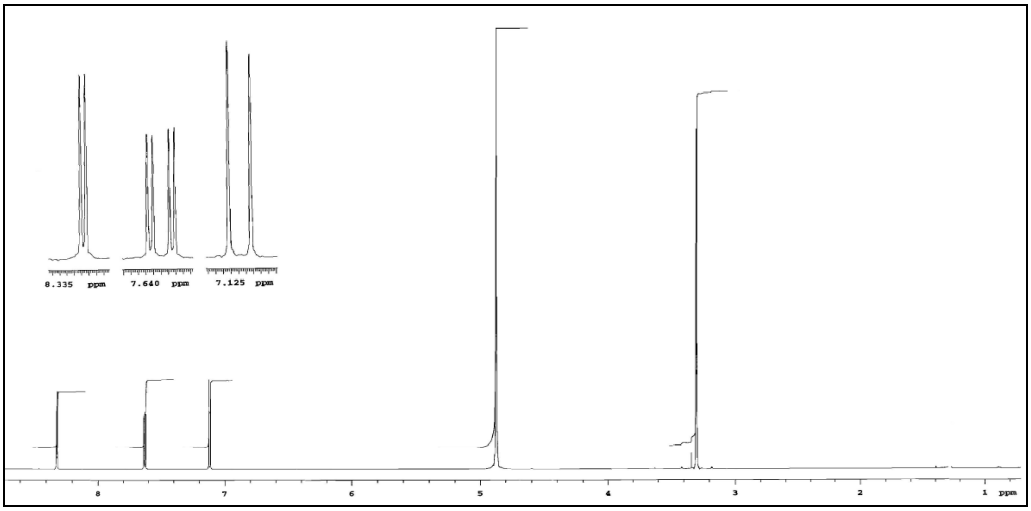


Figure 134: ^{1}H NMR spectrum (600 MHz) of aqabamycin D (**103d**) in MeOH-d_4

The molecular formula was found to be $C_{16}H_9N_3O_8$ containing 14 double bond equivalences. The fact that the ^{1}H NMR spectrum displayed only three sets of protons instead of nine as expected from the molecular formula suggested a symmetrical molecule. This symmetry was also proven by the ^{13}C NMR spectrum, which gave only 8 signals. By comparison of the data with those of **103a-c**, the structure of **103d** was deduced to be the new 3,4-bis-(4-hydroxy-3-nitrophenyl)pyrrol-2,5-dione, which was named aqabamycin D; it is a 3'-nitro derivative of aqabamycin B (**103b**).

6.9.5 Aqabamycin E

The yellow compound **103e** exhibited also similar physical properties as **103a-c**, although the proton NMR spectrum of **103e** showed a mixture of two similar compounds in the ratio 9:1. It was very similar to that of **103c** with the 1,2,4-trisubstituted aromatic system with two doublets at δ 8.25 (J = 2.2 Hz), 7.03 (J = 8.8 Hz) and a doublet of doublet at δ 7.51 (J = 8.7; 2.2 Hz). Finally, a signal due to a benzene ring was seen.

(+)-ESI MS indicated the *quasi*molecular ion at *m/z* 324 $[M+H]^-$, which lead to the molecular formula $C_{16}H_{11}N_3O_5$ indicating 13 double bond equivalences. The mass difference of Δm = 15 between **103c** and **103e** was attributed to an additional nitrogen and a proton in view of their molecular formula.

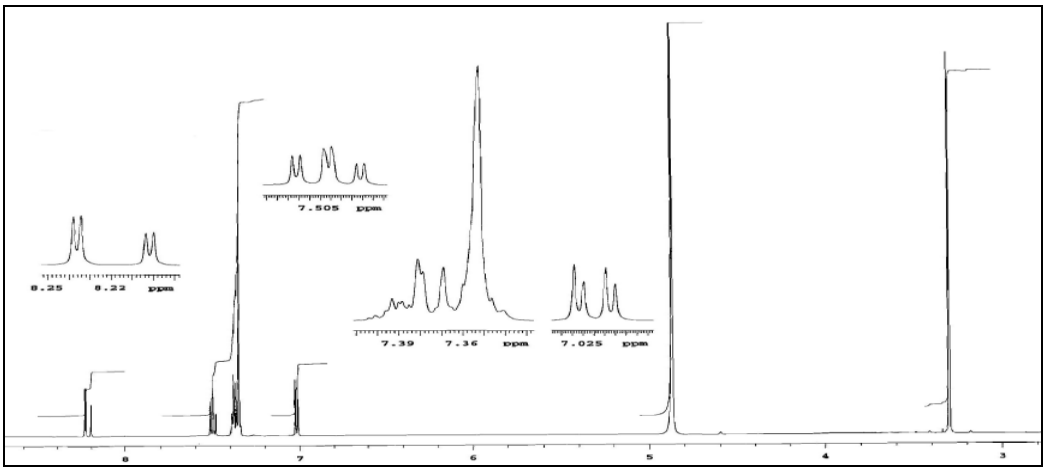


Figure 135: ^{1}H NMR spectrum (300 MHz) of aqabamycin E (**103e**) in MeOH-d_4

The ^{13}C NMR spectrum indicated also 16 carbon signals including one carbonyl group at δ 170.7 instead of two as in **103a -c**. Besides the absence of a carbonyl in **103e**, a quaternary carbon gave a signal at δ 149.7, which was not present in **103c.**

Both, **103c** and **103e** contained the same number of oxygen and carbon atoms as well as the same fragments and should have the same chromophore.

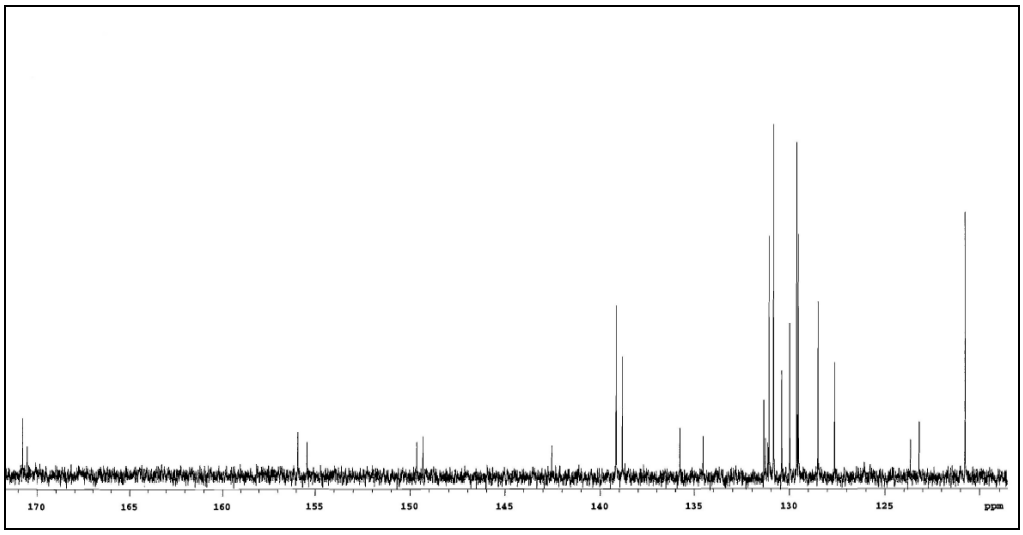


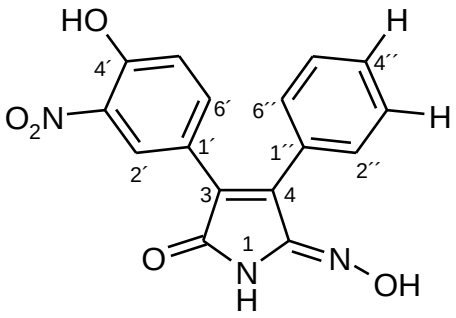
Figure 136: ^{13}C NMR spectrum (125 MHz) of aqabamycin E (**103e**) in MeOH-d_4

The upfield shift of the quaternary carbon to δ 149.7 in **103e** was interpreted as due to an oxime, and therefore the structure of **103c** was deduced as 3-(4-hydroxy-3-nitrophenyl)-4phenyl-pyrrol-2,5-dione-5-oxime (**103e-1**). The small satellite peaks in the ^{1}H NMR spectrum may be due to the isomeric 3-(4-hydroxy-3-nitrophenyl)-4phenyl-pyrrol-2,5-dione-2-oxime (**103e-2**) or to a *syn/anti* oxime mixture.

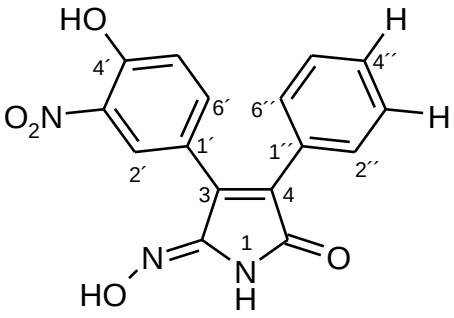
Table 20: ^{1}H NMR (600 MHz, *J* in Hz) data of compounds **103d-f**

Position	**103d** [a]	**103e**[a] **(**mixture of isomers)	**103f**
1-NH	-	-	11.73 (br s) [b]
2´	8.32 (d, 2.2)	8.22 (d, 2.2), 8.20 (d, 2.2)	8.27 (d , 2.2)
4´-OH	-	-	10.98 (s) [b]
5´	7.12 (d, 8.8)	2 x 7.02 (d, 8.8)	7.09 (d, 7.7)
6´	7.63 (dd, 8.8, 2.2)	2 x 7.52 (dd, 8.7, 2.2)	7.58 (dd, 8.7, 2.2)
2´´	7.63 (dd, 8.8, 2.2)	7.38 (m)	7.52 (dd, 8.7, 2.2)
3´´	7.12 (d, 8.8)	7.38 (m)	7.08 (d, 7.7)
4´´	-	7.38 (m)	-
5´´	-	7.38 (m)	-
6´´	8.32 (d, 2.2)	7.38 (m)	8.24 (d , 2.2)

a) in MeOH
b) in DMSO-d_6



103e-1



103e-2

6.9.6 Aqabamycin F

The yellow compound **103f** was obtained from fraction C. The proton NMR spectrum of **103f** in DMSO-d_6 indicated two pair of doublets and two pairs of doublets of doublets between δ 7.00- 8.20. After enlarging, two doublets of doublets at δ 7.54-7.58 and four doublets including two *ortho* and two *meta* coupling proton signals characteristic of two sets of 1,2,4-trisubstituted aromatic systems were discerned. In addition, two H/D exchangeable 1H singlets at δ 11.75 and 10.10 were visible, attributed to two chelated hydroxy groups. The only difference with respect to **103d** is the absence of the symmetry plane in **103f**.

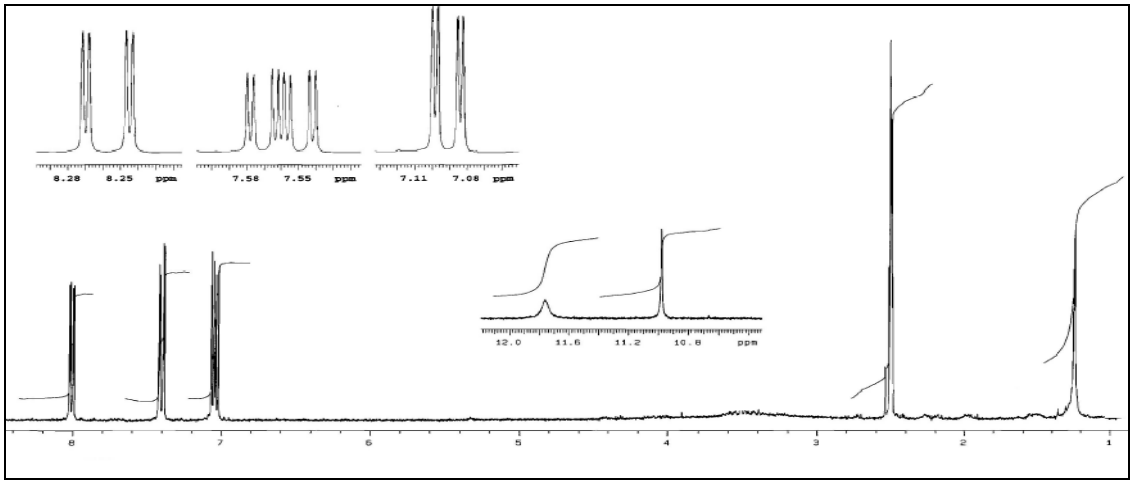


Figure 137: ^{1}H NMR spectrum (600 MHz) of aqabamycin F (**103f**) in DMSO-d_6

The ^{13}C NMR spectrum also displayed 16 carbons between δ 171 and 110 as in **103a-d** suggesting the same chromophore, but instead of two carbonyls as in **103a-d** only one at δ 170.2 was seen in compound **103f** and an upfield shift of a quaternary carbon at δ 149.7, which was interpreted as due to an oxime as in **103e**. ESI MS in-

dicated the *quasi*molecular ion at *m/z* 386. High resolution mass gave the molecular formula $C_{16}H_{10}N_4O_8$ containing one nitrogen and one proton more than **103d**.

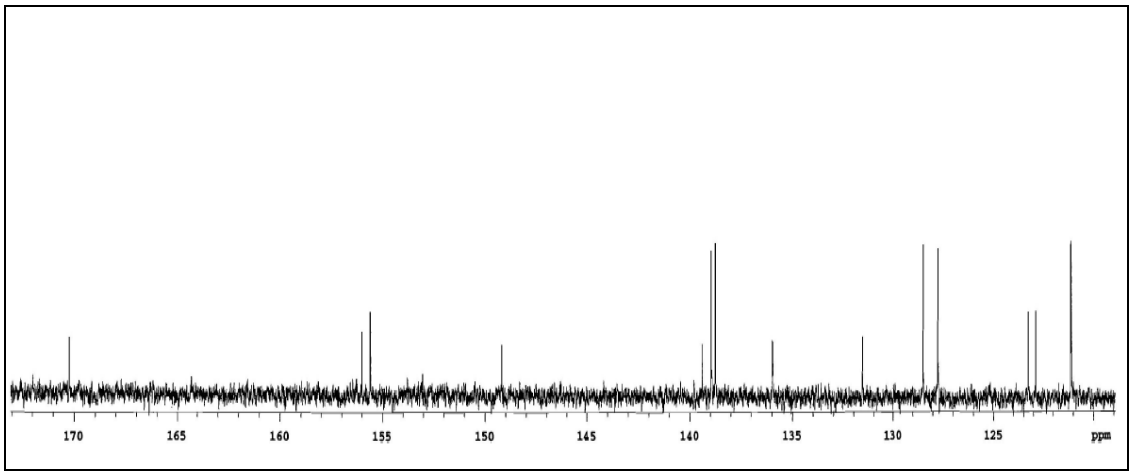


Figure 138: ^{13}C NMR spectrum (125 MHz) of aqabamycin F (**103f**) in MeOH-d_4

The HMBC and the H,H COSY spectra indicated the presence of two identical *o*-nitrophenole fragments. As the formula indicates a difference of NH with respect to **102d**, aqabamycin F is identified as compound **102f**.

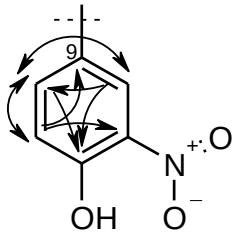


Figure 139: H,H COSY and HMBC correlations in aqabamycin F (**103f)**

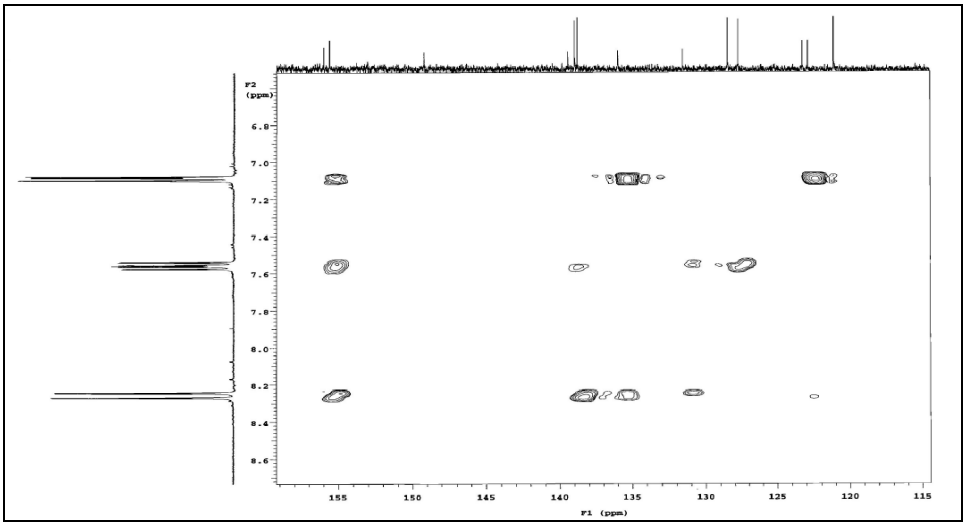


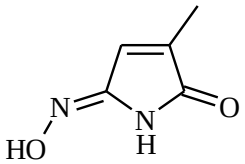
Figure 140: HMBC spectrum of aqabamycin F (**103f)** in MeOH-d_4

Table 21: ^{13}C NMR data (125 MHz) of **103d-f** in a) MeOH-d_4 and b) DMSO-d_6

Position	**103d**[a]	**103e-1**[a]	**103e-2**[a]	**103f**[b]
2	172.5	149.3	170.7	170.2
3	135.6	131.4	142.5	139.4
4	135.6	142.5	134.5	131.6
5	172.5	170.5	149.7	149.2
1´	121.6	123.7	123.2	123.3
2´	128.3	129.5	129.5	128.9
3´	136.3	134.5	135.8	136.0
4´	156.6	155.4	155.9	156.0
5´	121.5	120.8	120.8	121.0
6´	138.5	139.2	139.2	138.9
1´´	121.6	123.2	123.2	122.9
2´´	128.3	129.6	129.6	127.7
3´´	136.3	131.3	131.2	135.9
4´´	156.6	131.1	130.4	155.6
5´´	121.5	128.5	127.8	121.1
6´´	138.5	130.9	130.9	138.9

The (-)ESI MS/MS of aqabamycin F (**103f**) presented the fragments at *m/z* 368, 339 and 276, whose plausible fragments structures were proposed in the figure above (Figure 141) and corresponded to the lost of fragments ($[M\text{-}H\text{-}OH]^{2-}$, $[M\text{-}H\text{-}OH\text{-}NO]^{2-}$, $[M\text{-}H\text{-}NO_2]^{-}$, $[M\text{-}H\text{-}NO_2\text{-}HNO_3]^{-}$). The latter are typical in the ESI negative modus for aromatic nitro compounds.

Aqabamycin E (**103e**) and F (**103f**) are new natural products with the maleimide-5-oxime system, which is rare in natural products, in particular in bacteria. The 3-methyl-pyrrole-2,5-dione-5-oxime (**104**) was isolated in 2005 from the marine sponge *Pseudoceratina purpurea* and showed inhibitory activity on the growth of human cancer cells [MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS)].[185]



104

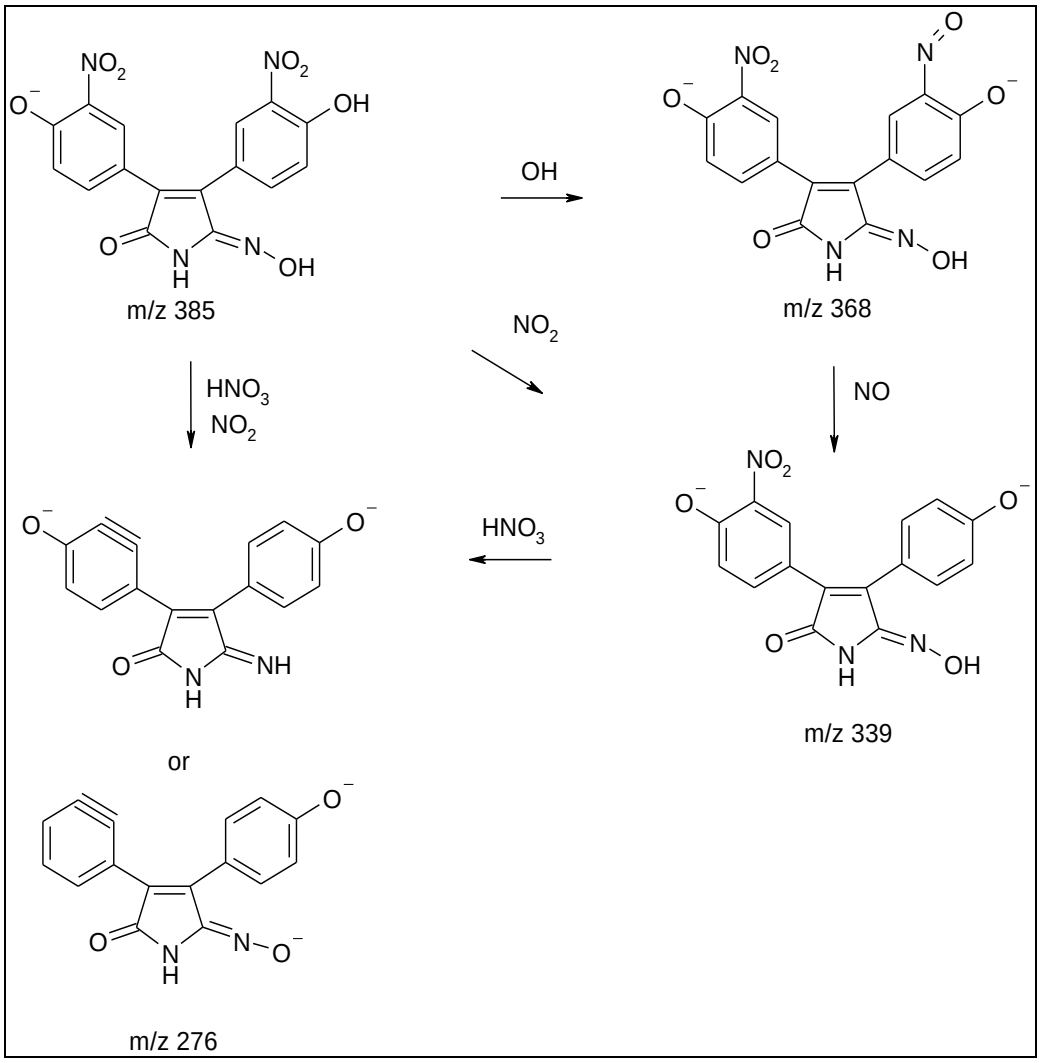


Figure 141: Proposal for MS/MS fragmentation of aqabamycin F (**103f)**

6.9.7 Aqabamycin G

Purification of fraction C gave the red solid **105**, which showed a brown colour with anisaldehyde/sulphuric acid. Compound **105** was fairly soluble in MeOH-d_4 and DMSO-d_6. The proton NMR spectrum in DMSO-d_6 displayed two broad singlets at δ 11.62 and 10.60 attributed to two acidic hydrogens; the aromatic region showed five doublets, two triplets at δ 7.02, 6.84 and a doublet of doublet at δ 7.01. With respect to the coupling constants, the signals were attributed to a 1,3,4- trisubstituted benzene moiety and an indol ring.

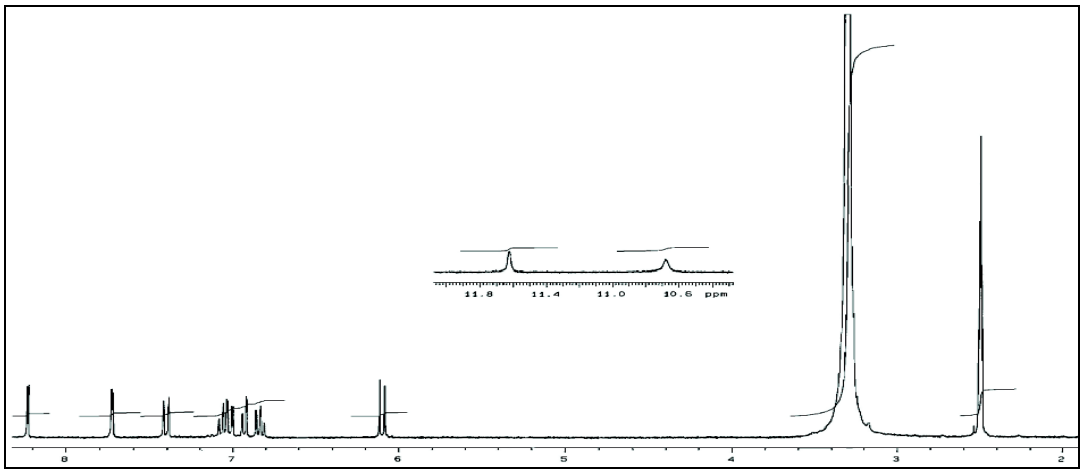


Figure 142: ^{1}H NMR spectrum (600 MHz) of aqabamycin G (**105**) in MeOH-d_4

The ^{13}C NMR spectrum indicated the presence of 18 carbon signals including two carbonyls at δ 173.1 and 172.6, eight sp^2 methines and eight quaternary sp^2 carbons.

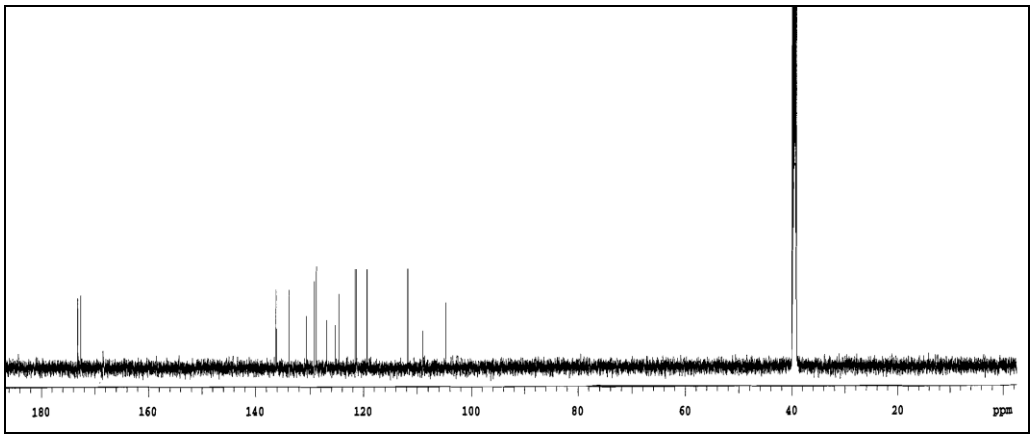
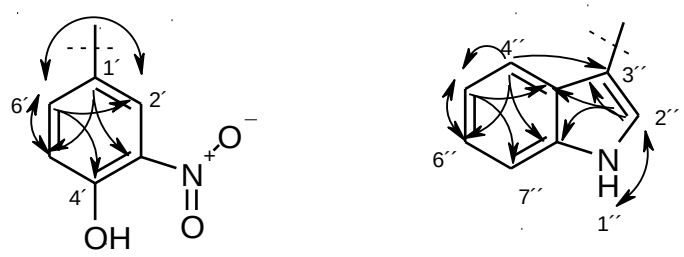


Figure 143: ^{13}C NMR spectrum (125 MHz) of aqabamycin G (**105**) in MeOH-d_4

From the molecular mass, the formula $C_{18}H_{11}N_3O_5$ was derived. The H,H COSY spectrum indicated couplings between the protons at δ 8.22 (2´-H), 6.10 (4´-H) and 7.01 (5´-H). In addition the two triplets and two doublets attributed to the indole ring coupled together allowing the construction of two fragments (I and II).



I II

Figure 144: H,H COSY and HMBC correlations in aqabamycin G (**105**)

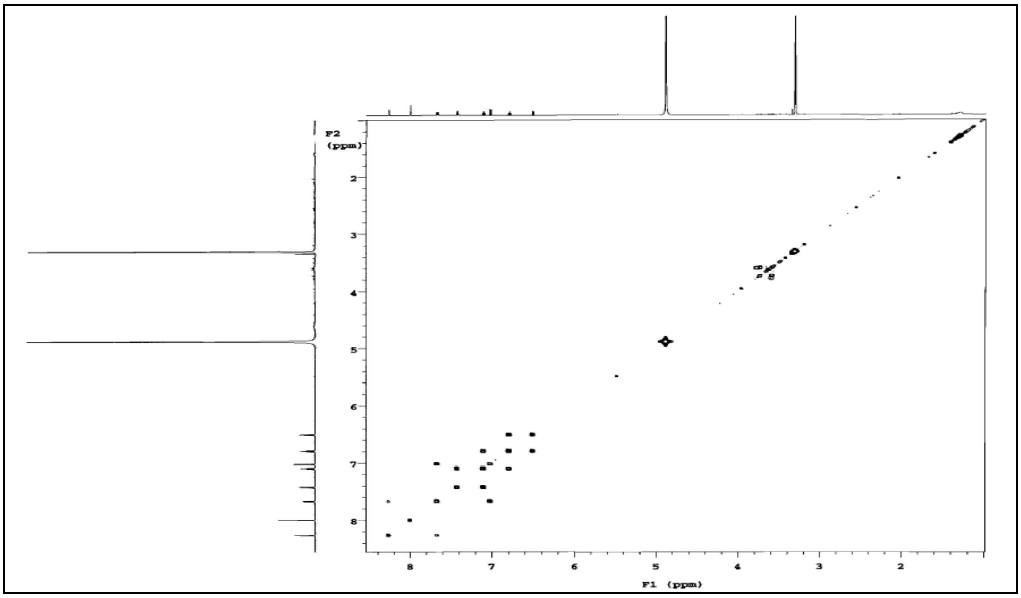


Figure 145: H,H COSY spectrum of aqabamycin G (**105**) in MeOH-d_4

The HMBC spectrum indicated correlations of the doublet at δ 7.73 (2´´-H) to the carbons at δ 136.3 (C-7´´a), 124.6 (C-3´´a), 104.0 (C-3´´) and confirmed the existence of the indole fragment.

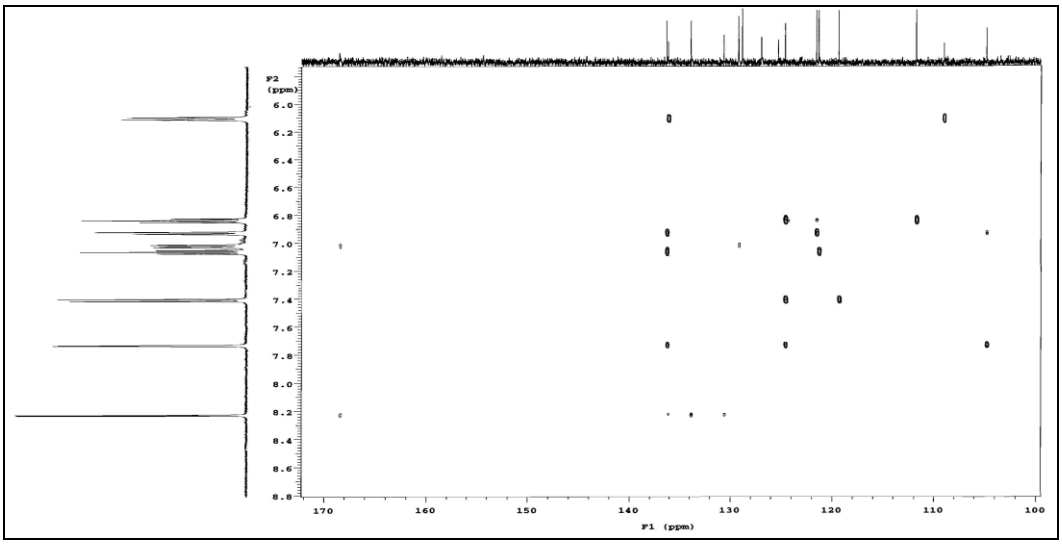
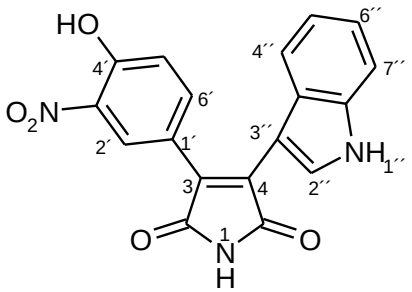


Figure 146: HMBC spectrum of aqabamycin G (**105**)

The protons at δ 7.01 (6´-H) and 8.22 (2´-H) showed HMBC correlations to the sp^2 carbon at 130.6 (C-3) and the proton at δ 7.73 (2´´-H) to the quaternary carbon at δ 125.3 (C-4). From the double bond equivalents (15) derived from the molecular formula $C_{18}H_{11}N_3O_5$, only 11 were assigned (nitro group, indol and benzene rings). The remaining fragment C_4HNO_2 contained the atoms C-3 and C-4 and two further carbonyl carbons (C-1 and C-5), which showed no correlation to other fragments. When these fragments were connected to a maleimide ring, 3-(4-hydroxy-3-nitrophenyl)-4-(1*H*-indol-3-yl)pyrrol-2,5-dione (**105**) resulted. This is also a novel natural product, which was named aqabamycin G.



105

Table 22: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) data of aqabamycin G (**105**) in DMSO-d_6

Position	$^{1}H^{b}$	$^{13}C^{b}$
1-NH	10.60 (br s)	-
2	-	173.2
3	-	130.6
4	-	125.3
5	-	172.6
1´	-	109.0
2´	8.22 (d, 2.5)	129.2
3´	-	136.1
4´	-	168.1
4´-OH	-	-
5´	6.10 (d, 9.1)	126.9
6´	7.01 (dd, 9.2, 2.2)	133.9
1´´-NH	11.62 (s)	-
2´´	7.73 (d, 2.5)	128.9
3´´	-	104.0
3´´a	-	124.6
4´´	6.93 (d, 8.1)	121.3
5´´	6.84 (t, 7.9)	119.3
6´´	7.05 (t, 8.1)	121.5
7´´	7.41 (d, 8.1)	111.8
7´´a	-	136.3

6.9.8 Aqabamycin H

The dark yellow compound **106** was separated from fraction C. It gave also an orange colour reaction with anisaldehyde/sulphuric acid. Compound **106** was fairly soluble in MeOH-d_4 and DMSO-d_6. The proton NMR spectrum in DMSO-d_6 displayed two broad singlets at δ 12.38 and 11.75 for two acidic protons. The aromatic region showed one singlet at δ 8.15, four multiplets at δ 8.14, 7.50, 7.30 and 7.30, in addition two triplets at δ 7.21, 7.14 and two doublets at δ 7.58 and 7.52 were also seen.

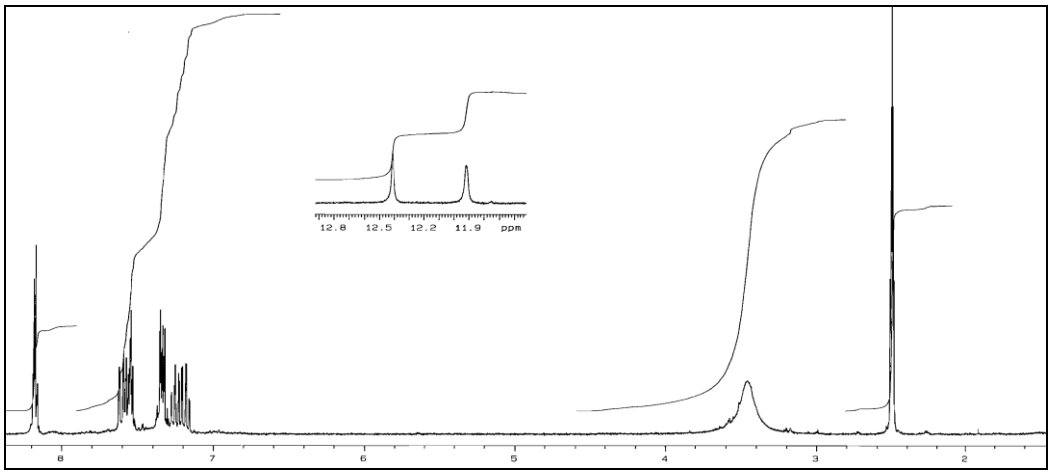


Figure 147: ^{1}H NMR spectrum (300 MHz) of aqabamycin H (**106**) in DMSO-d_6

The carbon NMR spectrum indicated 16 signals, among which nine were due to sp^2 methines and seven to quaternary carbons. HRESIMS gave $C_{16}H_{11}N_3O_2$ as molecular formula.

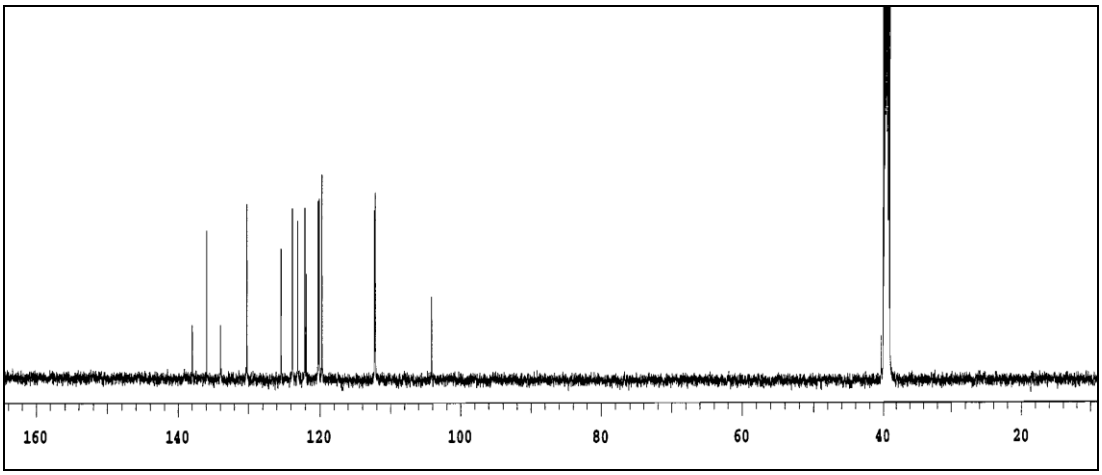
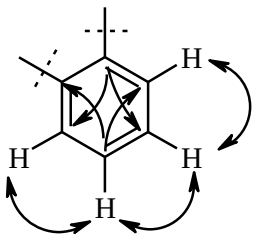
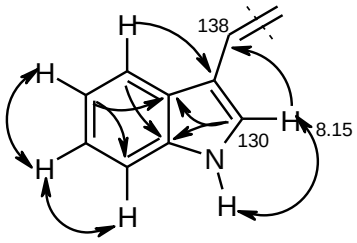


Figure 148: ^{13}C NMR spectrum (125 MHz) of aqabamycin H (**106**) in DMSO-d_6

From the H,H COSY and HMBC spectra, two fragments (I and II) could be easily identified. The connection of both fragments was deduced from the coupling between the proton at δ 8.15 to the quaternary carbon at δ 138 (see substructure II).

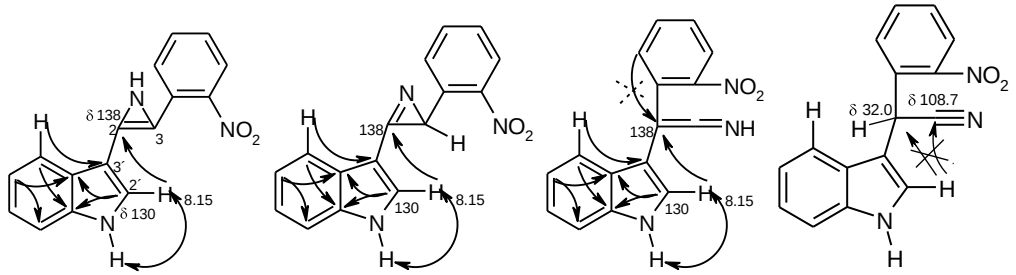


I



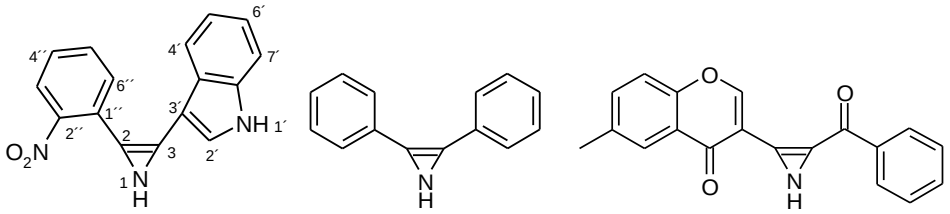
II

The search in AntiBase with the substructure and molecular formula gave no matches. The molecular formula gave 13 double bond equivalences. From both substructures, 10 double bond equivalences were accounted for. The remaining 3 double bond equivalents were arising from the nitro group, one double bond and one ring or an additional double bond. All these information suggested several isomeric structures, the most plausible are: the 1*H*-azirine (**106a**), the 2*H*-azirine (**106b**), a hetero cumulene (**106c**) and a nitrile derivative (**106d**)



106a **106b** **106c** **106d**

Structure **106b** possesses an sp^3 methine, which is not present in the proton spectrum. Additionally, the proton spectrum indicated two acidic protons, which appear only in structures **106a** and **c**. Therefore structure **106b** was excluded, although a rapid equilibrium between the two isomeric 2*H*-azirines could also explain the misisng sp^3-H signal. Considering the heterocumulene derivative (**106c**) we saw no correlation from the disubstituted benzene ring to the quaternary carbon at δ 138 (C-3). The carbon at position 2 should give a chemical shift between δ 180-200 and the heterocumulene should also be very instable. The nitrile derivative **106d** possesses like **106b** an sp^3 methine and reffering to the simulated chemical shifts for C-2 (δ 32.0) and C-3 (δ 108.7) and the absence of the nitrile band in its IR spectrum, structure **106d** was excluded. Structure **106a** showed two acidic protons (2 NH) and the proton at position 2´ in the indol ring showed a correlation to C-3. There are no correlations between the benzene ring and the quaternary sp^2 carbons C-2 and C-3, however, there was a weak HMBC correlation between 2'-H and C-3. As a result, structure **106a** was temptatively assigned and the name aqabamycin H was given.



106 **107** **108**

1*H*-Azirines are antiaromatic compounds and therefore not stable under usual conditions; cyclobutadiene is a well-known example. It is also known, however, that conjugation can stabilize these compounds, as it is e.g. found in biphenylidene (**107**). A similar azirine functionality has been described in the literature for the synthetic [1*H*]azirine-benzopyranone derivative **108** [186]. As compound **108** is stable, we may expected that aqabamycin H (**106a)** is also stabilized *via* tautomerism (Figure 149). It has to be further investigated if similar effects can also stabilize azirines, or if still other structure alternatives have to be searched for the metabolite from strain WMBA1-4.

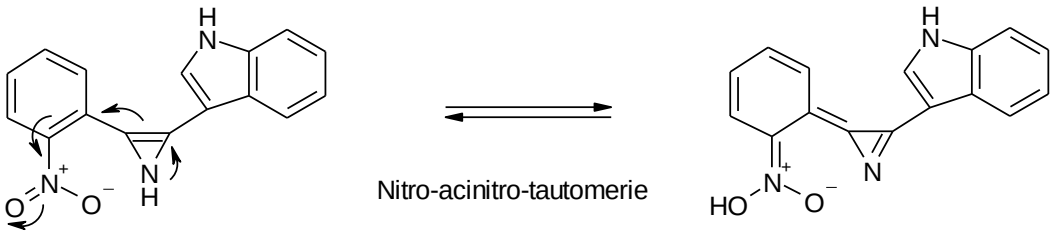


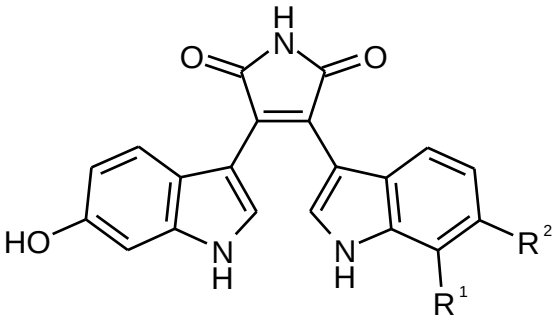
Figure 149: Nitro-acinitro tautomerie of compound **106a**

Nitro compounds like the novel 2,3-disubstituted maleimides **103a-f** and compound **105** are rare in nature. Since 1980, the bisindolylmaleimide derivatives arcyriarubin B (**109a**) and C (**109b**) in addition to arcyriaflavin B (**110a**) and C (**110b**) were isolated by Steglich *et al.* from the myxomycete *Arcyria denudata.*[187] Several maleimide derivatives are reported from marine basidiomacetes, e.g. himanimides A (**111a**) and C (**111**).[188]

Many of these maleimides are of special interest due to their important biological activities.[189] They are reported to cross the biological membrane due to the presence of the pyrrol-2,5-dione system.[190,191,192] Several related bis-indolylmaleimides were also synthesized, they act as protein kinase inhibitors and antitumor agents.[191]

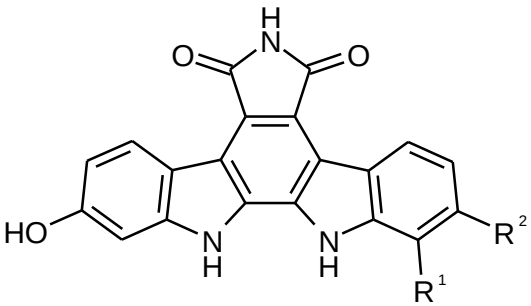
Table 23: ^{1}H and ^{13}C NMR data of aqabamycin H (**106**) in DMSO-d_6

Position	^{1}H	^{13}C
1-NH	12.38 (br s)	-
2	-	138.0
3	-	130.2
1´-NH	11.86 (s)	-
2´	8.15 (s)	130.2
3´	-	104.1
3a´	-	125.4
4´	7.58 (d)	120.1
5´	7.14 (t)	120.3
6´	7.21 (t)	122.1
7´	7.52 (d)	112.3
7a´	-	135.9
1´´	-	121.9
2´´	-	134.0
3´´	8.14 (m)	119.8
4´´	7.30 (m)	123.1
5´´	7.30 (m)	123.8
6´´	7.50 (m)	112.2



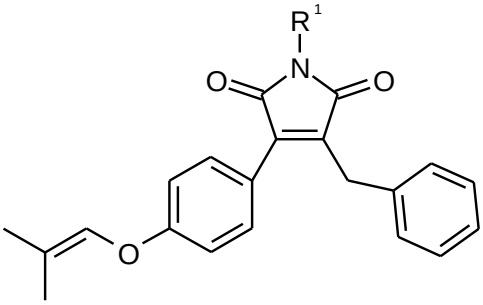
109a: $R^1 = R^2 = H$

109a: $R^1 = H$; $R^2 = OH$



110a: $R^1 = R^2 = H$

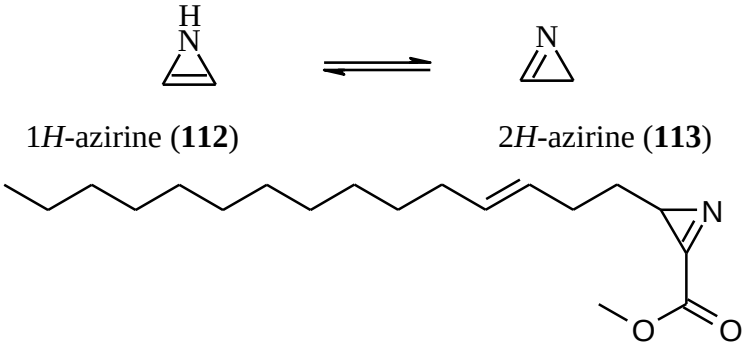
110b: $R^1 = OH$; $R^2 = H$



111a: R^1 = H

111b: R^1 = OH

Compound **106** belongs to the group of azirines. There are two isomeric azirines: the 1*H*- (**112**) and 2*H*-azirines (**113**). The 1*H* azirine is known only as a transition intermediate, and represents a cyclic conjugated system with four π-electrons. The few 2*H*-azirines, however, showed interesting chemical and biological behaviour:[193,194] The 2*H*-azacyclopropene dysidazirine (**113**) was isolated from the marine sponge *Dysidea fragilis* 1988.[195] Aqabamycin H (**106**) is the first 1*H*-azirine which is reported as natural product.



1*H*-azirine (**112**) 2*H*-azirine (**113**)

113

6.9.9 4-Hydroxy-3-nitrobenzaldehyde

The yellow compound **115** was isolated from fraction B. The very simple ^{1}H NMR spectrum exhibited a singlet at δ 9.88 attributed to an aldehyde proton and a pattern of three aromatic protons was characteristic of a 1,2,4-trisubstituted aromatic system, which was confirmed on the basis of the H,H COSY spectrum.

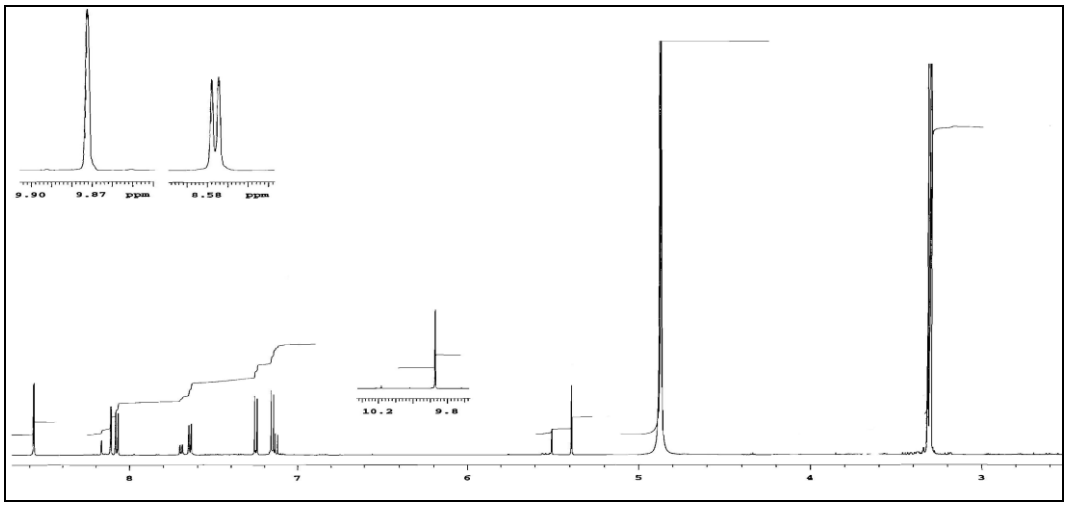
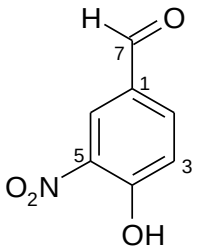


Figure 150: ^{1}H NMR spectrum (600 MHz) of 4-hydroxy-3-nitrobenzaldehyd (**115**) in MeOH-d_4

The (-)-ESI mass spectrum gave the *quasi*molecular mass of 166 ([M-H]$^-$) indicating a nitrogen containing compound. The ^{13}C NMR spectrum indicated 7 carbons including the signal of an aldehyde carbonyl at δ 191.0, an oxygenated sp^2 at δ 155.4, three sp^2 methines and two sp^2 quaternary carbons. A search in Chemical Abstracts delivered 4-hydroxy-3-nitro benzaldehyde (**115**), which previously had been synthesized by Yang et *a.l.*[196] Here it is reported from a natural source for the first time. The corresponding acid was also isolated previously from an undetermined bacterium in our group.[56]



115

4-Hydroxy-3-nitrobenzaldehyd (**115**) is reported to show nematicidal activity and inhibited the germination of the conidia of certain fungi and showed moderate antibacterial activity.[197]

6.9.10 (E)-3-(4-Hydroxy-3-nitro)-cinnamic acid

The yellow compound 116 was isolated from fraction C. The proton NMR spectrum of compound 116 was very similar to that of 115, with the 1,2,4-trisubstituted aromatic system.

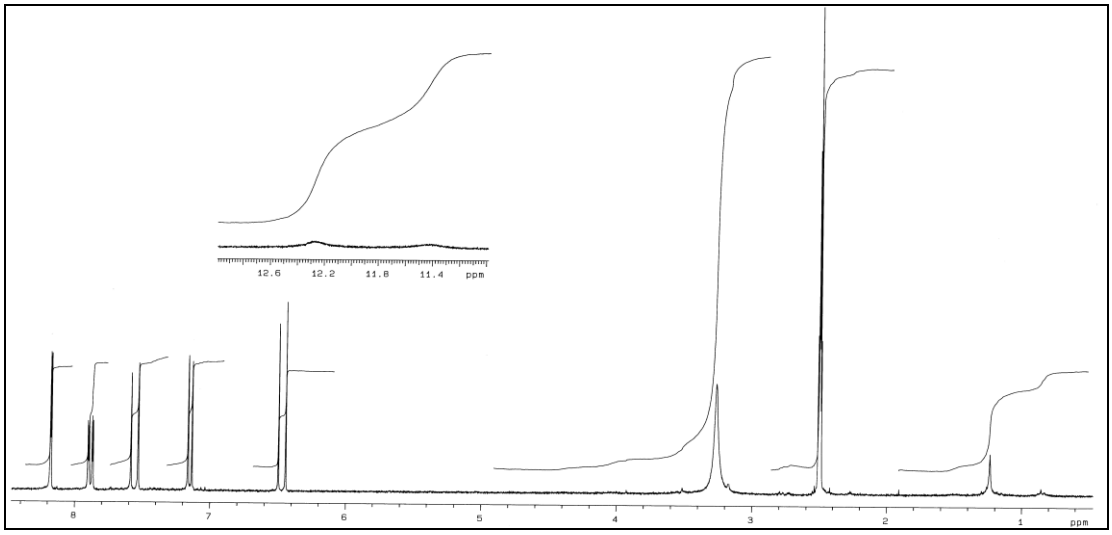
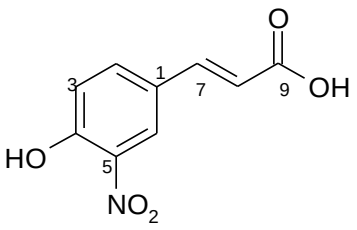


Figure 151: ^{1}H NMR spectrum (300 MHz) of **116** in DMSO-d_6

The difference was the absence of the aldehyde proton, which appeared at δ 9.88 in **115**. Instead two protons of a *trans* double bond at δ 7.58 and 6.48 and two broad singlets at δ 12.30 and 11.41 of exchangeable protons were seen.

By searching in Chemical Abstracts with the substructures from the ^{1}H NMR spectrum and the molecular mass (209 by EI and ESI MS), it arose that compound **116** was 4-hydroxy-3-nitrocinnamic acid, which was already synthesised by Kohlman *et al*.[198] and later by Yamasaki *et al*.[199] Nitrocinnamic acids are known to possess biological activities such as antibacterial, antiprotozoa or antioxidant properties.[200] Comparison of the proton NMR data with the literature[201] confirmed the structure.



116

6.9.11 3-Nitro-1*H*-indazole

Compound **117** was isolated as a pale yellow solid from fraction C. The ^{1}H NMR spectrum indicated four signals, particularly two doublets at δ 8.21 and 7.69 and two triplets at δ 7.57 and 7.48. This arrangement is typical for 1,2-disubstituted benzene ring.

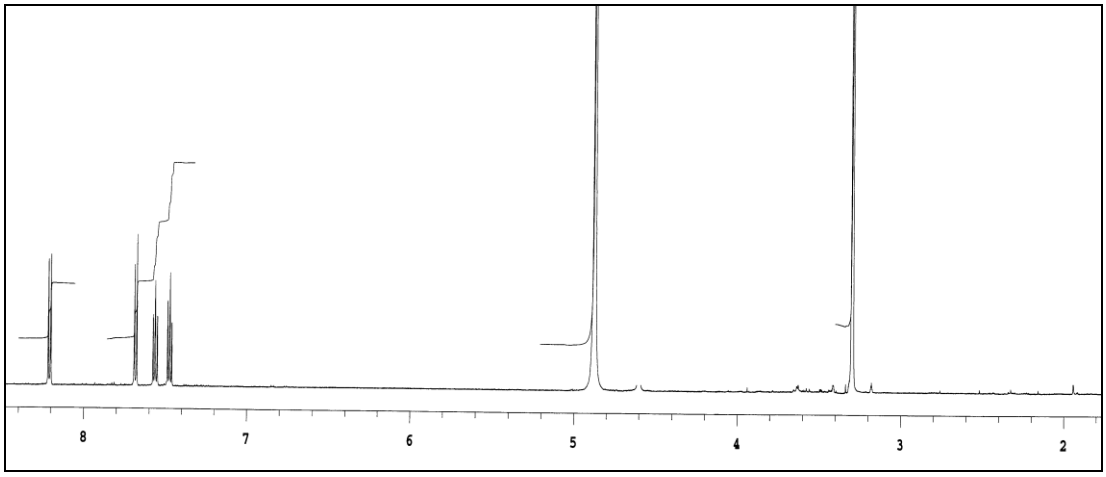
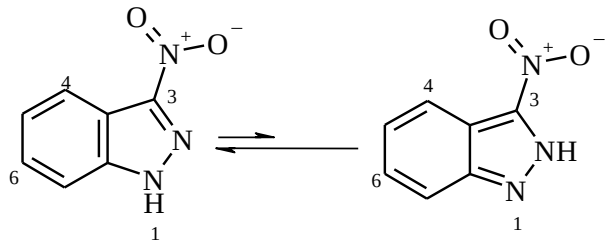
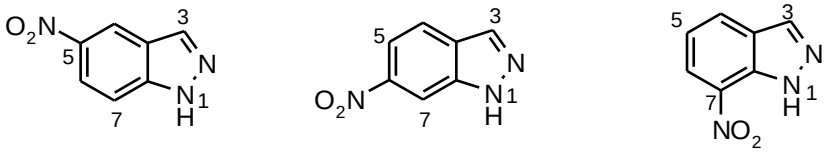


Figure 152: ^{1}H NMR spectrum (300 MHz) of 3-nitroindazole (**117a/b**) in MeOH-d_4

The ^{13}C NMR spectrum indicated 7 carbon signals. (-)-ESI MS gave the *quasi*-molecular ion at *m/z* 162, and (+)-HRESIMS gave the molecular formula $C_7H_5N_3O_2$. The search in Chemical Abstracts with the molecular formula and substructure gave 28 hits, which could be eliminated by close examination of the chemical shift of proton and carbon NMR data. The outcome of these comparisons revealed a closer similarity with indazoles, and especially the values of 3-nitroindazole, which was synthesized 1971 by Cohen-Fernandes,[202] showed a close similarity with the natural product, so that identity was assumed. 3-Nitroindazole shows annular tautomerism, [203] although the 1*H*-tautomer (**117a**) is more stable than 2*H*-tautomer (**117b**). The 5-, 6- and 7-nitroindazoles (**118a, b, c**) were examined in spinal column therapy as inhibitors for NG-nitro-L-arginine methyl ester (L-NAME), which is responsible for the elevation of blood pressure by spinal injury.[204] It is first time here that 3-nitroindazole was isolated from a natural source.



117a/b



118a **118b** **118c**

6.9.12 2-Hydroxy-indole-3-carbaldehyde

Compound **119** was isolated as white solid with a blue fluorescence at 366 nm. The ^{1}H NMR spectrum displayed 6 signals, one broad singlet at δ 14.12 of a chelated proton, an aldehyde singlet at δ 10.20, two doublets at δ 8.18 and 7.72 and two triplets at δ 7.54 and 7.38 characteristic of a 1,2-disubstituted aromatic ring.

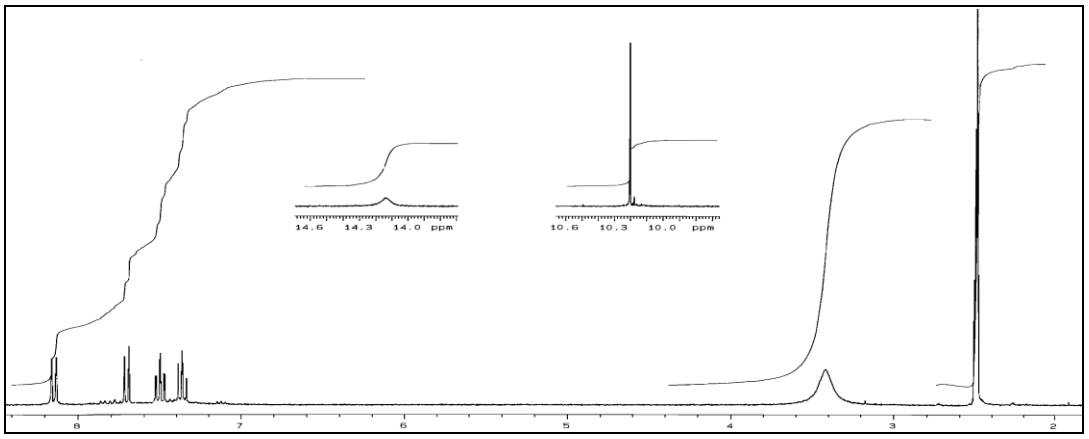
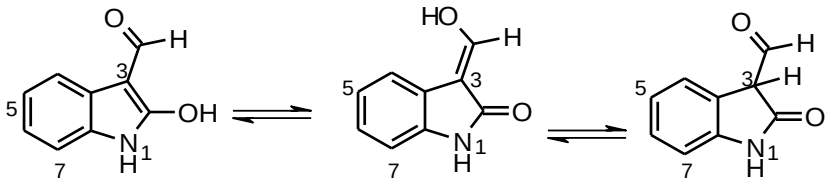


Figure 153:^{1}H NMR spectrum (300 MHz) of compound **119a** in DMSO-d_6

The EI MS spectrum gave the molecular ion at *m/z* 167 and (-)-ESI MS showed *quasi*molecular ions at 166 ([M-H]-) and 355 ([2M+Na-2H]-); (-)-HRESIMS gave the molecular formula $C_7H_4NO_4$. The search in Chemical abstracts delivered three plausible structures **119a**, **119b** and **119c**.



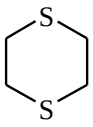
119a **119b** **119c**

Due to the presence of the aldehyde signal and the absence of an aliphatic methine proton, structure **119c** was excluded. Compound **119b** is the tautomer of 2-hydroxy-indole-3-carbaldehyde (**119a**), which was already reported as synthetic product [205] and is now described here as a new natural substance. 3-Hydroxy-2-carbaldehyd was excluded due to the comparison of the spectra with the literature.

2-Hydroxy-indole-3-carbaldehyde (**119a**) possesses antibacterial activity against Gram-positive bacteria as well as cytotoxic activity against mouse lymphocytic leukaemia and Jurkat-T-cell leukaemia (H. Anke, Kaiserlautern).

6.9.13 1,4-Dithiane

The proton NMR spectrum of the colourless oily **120** showed only one singlet at δ 2.18. EI MS gave a molecular ion at 120. The search in Chemical Abstracts with both information resulted in 1,4-dithiane as structure of compound **120**.



120

1,4-Dithiane is a groundwater contaminant associated with the production and the storage of ammunitions at certain military installations. Its oral LD_{50} in rats is reported as 3500 mg/kg of a range-finding study by the U.S. Army Medical Bioengineering Research and Development Laboratory.[206]

Additionally, benzoic acid and phenylacetic acid were isolated.

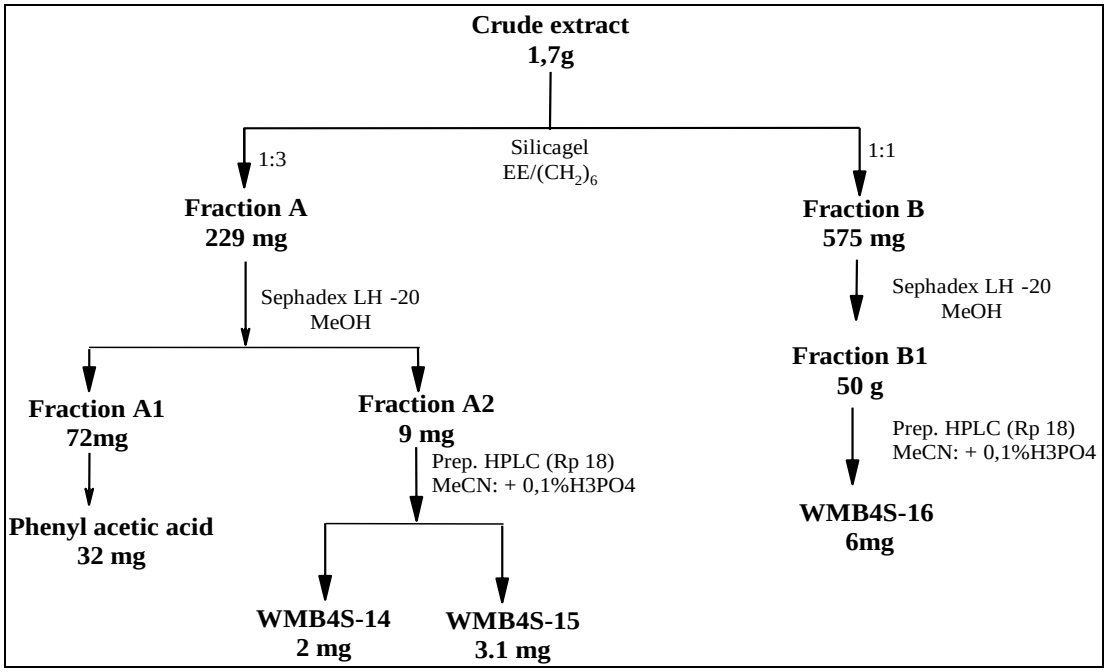


Figure 154: Work-up scheme 2 of the strain *Vibrio sp*. WMB4 in LB medium

6.9.14 Bis-indolylethane

Compound **121** was isolated from fraction B (LBm). The colourless oily compound gave a red colour with anisaldehyde/sulphuric acid was fairly soluble in MeOH-d_4 and DMSO-d_6. Its ^{1}H NMR spectrum displayed a broad H/D exchangeable 2H singlet at δ 10.68. The aromatic region showed four doublets at δ 7.48 and 7.35, two doublets at δ 7.12 and four triplets at δ 7.02 and 6.85. In the aliphatic region one methine quartet at δ 4.58 and one doublet at δ 1.78 attributed to a methyl group were also seen.

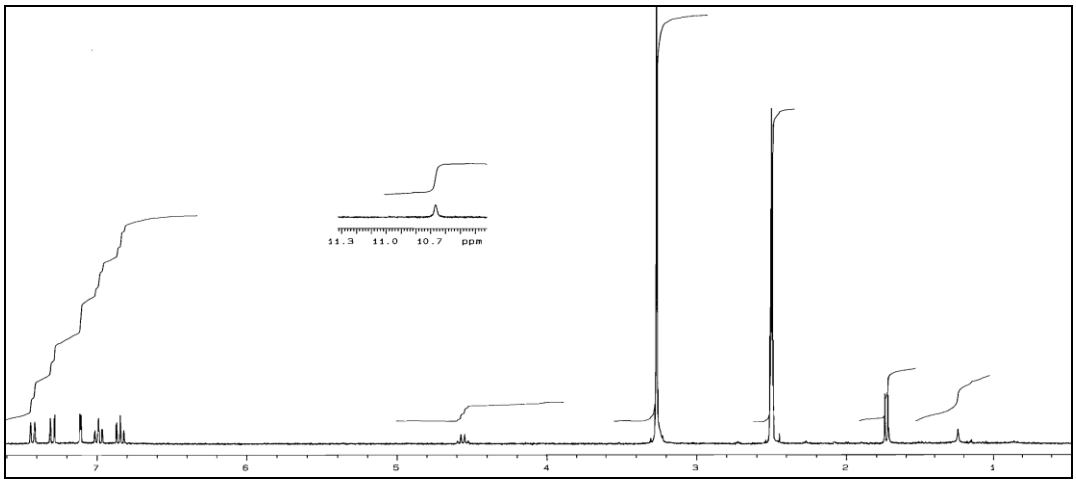
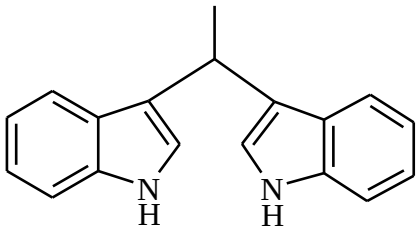


Figure 155: ^{1}H NMR spectrum (300 MHz) of vibrindole A (**121**) in DMSO-d_6

EI MS gave the molecular ion at *m/z* 260 and the (-)-HRESIMS of the *quasi*-molecular ion gave the molecular formula $C_{18}H_{15}N_2$. The search in AntiBase with the molecular weight and substructure delivered vibrindole A (**121**), which was first isolated from the marine strain *Vibrio parahaemolyticus* 1994 by Carmeli *et al.*[207]



121

6.9.15 3,3´phenyl-2-bis-indolylmethan and its cationic form Turbomycin B

Compound **122** was isolated as red oil from fraction B (Figure 154), which gave a red colour with anisaldehyde/sulphuric acid. The proton NMR spectrum indicated one broad singlet of intensity two at δ 10.90 and a singlet signal at δ 5.82. The sp^2 region indicated signals of an aromatic system at δ 7.33 (2H), 7.25 (2H) and 7.18 (1H) for a phenyl ring as well as doublets at δ 7.26 and 7.34 and triplets protons at δ 7.03 and 6.85 for four consecutives protons. In addition one singlet at δ 6.82 attributed to the proton in position 2-H of an indole ring was observed.

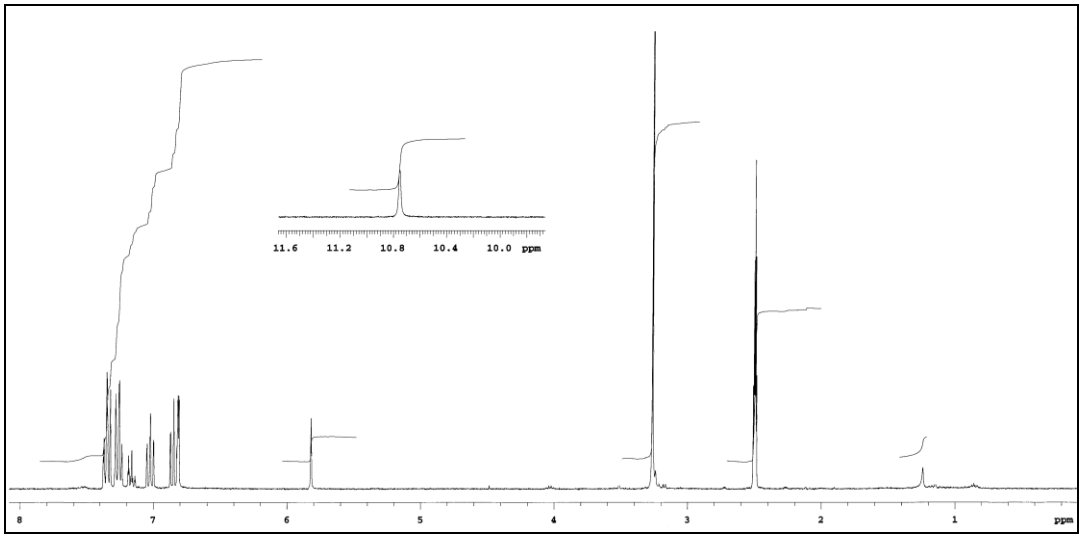


Figure 156:[1]H NMR spectrum (300 MHz) of phenyl-2-*bis*-indolylmethan (**122**) in DMSO-d_6

The ^{13}C NMR spectrum indicated 23 carbon signals, which were assigned by the HSQC spectrum as seven quaternary, fifteen sp^2 methines and one sp^3 methine.

From the H,H COSY and HMBC spectra, two fragments I and II can be easily suggested. EI MS indicated a molecular ion at *m/z* 322, however the (+)-ESI mass spectrum indicated a *quasi*molecular ion at *m/z* 321; the molecular formula $C_{23}H_{17}N_2$ was determined by (+)-HRESIMS.

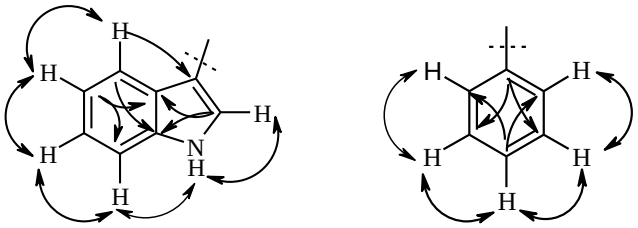
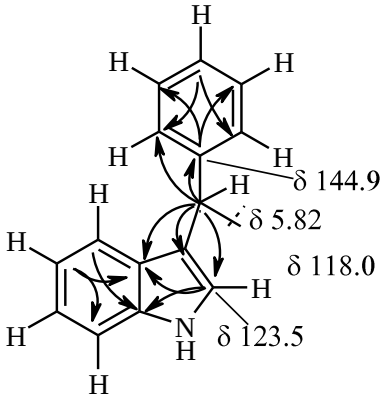
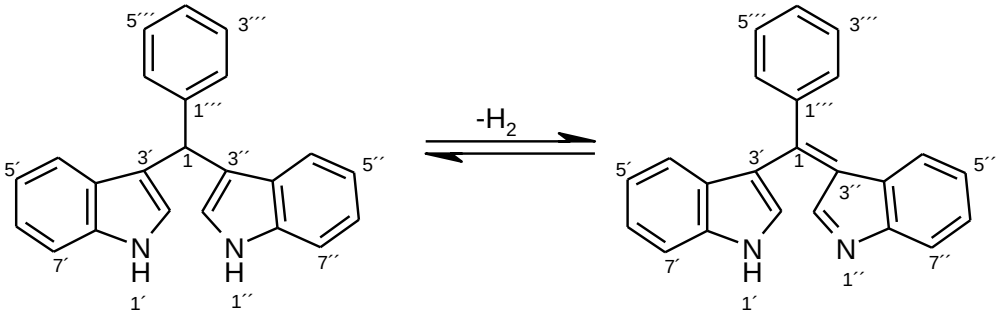


Figure 157: H,H COSY and HMBC correlations in fragments I and II

The careful inspection of the HMBC spectrum indicated an important coupling between the aliphatic methine proton at δ 5.82 (1-C*H*) to the quaternary carbons (C-1´´´, C-3´´, C-3´), indicating that both fragments were connected through that methine (see substructure III). The complete structure is then **122**. The search in Anti-Base gave compound **122** to be the phenyl-2-*bis*-indolylmethan. Compound **122** have in the EI MS a molecular weight 322, but the ESI MS spectrum gave the molecular weight 320, which can be explained by oxidation of the compound in the ion source.

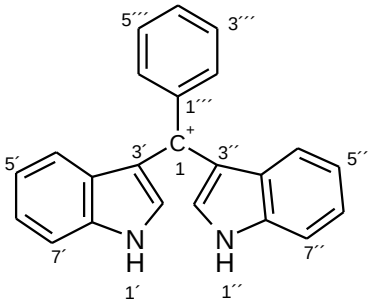


III



122a **122b**

To further support the structure, the red coloured turbomycin B (**123**) was synthesized according to the literature. The ^{1}H NMR spectrum of turbomycin B (**123**) was different from the one of compound **122,** with the absence of the sp^3 methine at δ 5.81 in turbomycin B**,** but the molecular mass was also 320 from (±)-ESI MS. So compound **122** may be in equilibrium between **122a** and **122b.** Structure **122b** is the free base of turbomycin B (**123**)



123

Bis (indolyl)methanes containing an sp^3 methine carbon are reported to be unstable and can spontaneously oxidize to form triarylmethane dyes such as **122b**. Bis (indolyl)-methane was reported as an chromogenic sensing molecule,[208] which is used to detect the CO_2 levels in blood.

Compound **122** showed cytotoxic activity due to an inhibition of the macromolecule biosynthesis in L1210 cells with IC_{50} values of 10-15 µg/ml. This cytotoxicity was previously unknown.

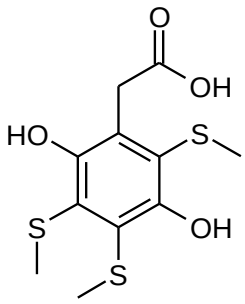
7 Summary

The battle to improve the quality of life by finding cures against diseases and extend the life expectancy of mankind is still one of the priorities in natural products chemistry. The war against the worldwide enemies cancer and AIDS is nowadays the number one priority in most research institutes. Although natural product chemistry has already made available a high number of biological active compounds, cancer cells as well as many vicious microbes continue to show an increasing resistance. Hence there is an urgent need for new medicines and new compounds with more efficiency. Exploring new sources, new techniques and new targets in collaboration with biological chemistry may help in meeting this challenge. The main goal of my work was to find new biological active metabolites from microorganisms, to overcome the problem a step ahead.

The dereplication through HPLC-UV-ESI MS/MS has proven to be a very efficient and powerful tool for the detection of interesting compounds in crude extracts, through the simultaneous interpretation of the fragmentation pattern and UV absorptions from different compounds present in the extracts. Twelve strains were selected in this work and examined mainly based on their biological (antibacterial, antifungal, antialgal, cytotoxic) activity as well as by the chemical screening (TLC, HPLC MS). Selected results are summarized below.

7.1 *Pseudoalteromonas* sp. T 268

The high antibacterially and antifungally active extract of marine *Pseudoalteromonas* sp. T 268 isolated from the intestine of the Antarctic krill *Euphausia superba*, gave in addition to homogentisate (**21**) and its methyl ester (**23**), 3-methylthiopropanoic acid (**20**), two new sulphur methyl derivatives of homogentisic acid named euphamycin A (**25**) and B (**27**): The latter two exhibited potent cytotoxic effects against suspension cell lines (IC_{50} = 10-15 µg/ml) and against HL-60 cells, which showed a cytotoxic effect with IC_{50} = 5 µg/ml; additionally, they showed antibiotic activity.



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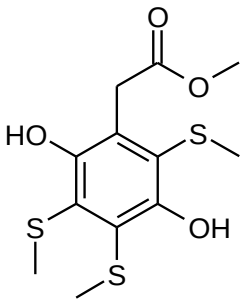
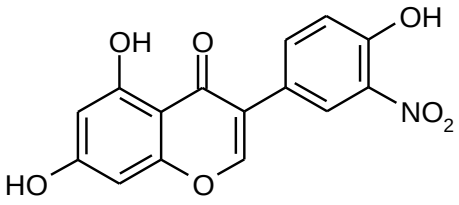


27

7.2 *Salegentibacter holothuriorum* T436

The bacterium *Salegentibacter holothuriorum* T436, which was isolated from the Eastern Weddell Sea, drew our attention by its high antibacterial and antifungal activity. Several compounds with various activities were isolated, and interestingly most of them belonged to the rare natural nitro products: 3´-mononitrogenistein (**43**) showed mild cytotoxic activity against L1210 and Jurkat cells, 4-hydroxy-3-dinitrophenyl-propionic acid (**40**) exhibited antibiotic activity against *Clostridium difficile*; further products were 2-hydroxy-3- (4-hydroxy-3-nitrophenyl)propionic acid methyl ester (**45**), 4-hydroxy-3-dinitrophenyl-propionic acid (**40)**, and the new nitro compounds 3- (4-hydroxy-3,5-dinitrophenyl)propionic acid methyl ester (**37c**), dinitro tyrosol (**38c**)**,** 4-hydroxy-3,5-dinitrophenyl-acetic acid (**39d**), genistin (**43**) and 2-hydroxy-3- (4-hydroxy-3-nitrophenyl)propionic acid methyl ester (**45**).



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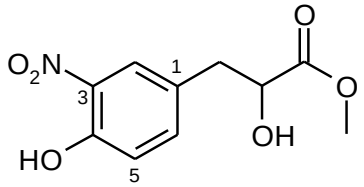

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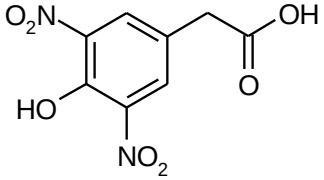
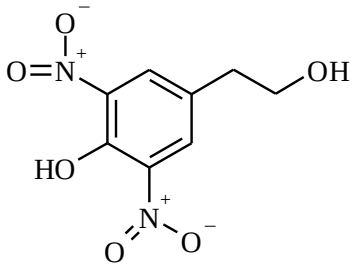
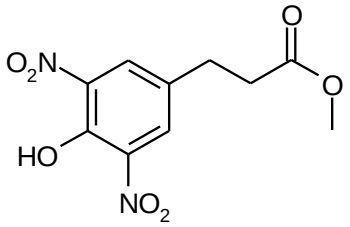


39d



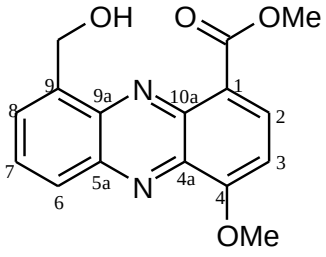
38c



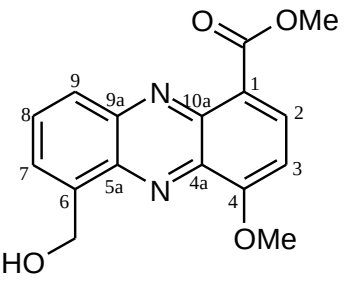
37c

7.3 Terrestrial *Streptomyces* sp. Ank 2

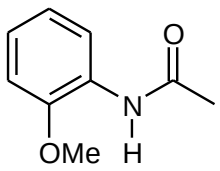
Besides its activity against *Bacillus subtilis*, *Mucor miehei*, *Escherichia coli*, *Streptomyces viridodiromogenes*, *Staphylococcus aureus*, *Chlorella vulgaris* and *Chlorella sorokiniana* and strong cytotoxic, the terrestrial *Streptomyces* strain Ank 2 produced trivial compounds such as indolcarboxylic acid, *p*-hydroxybenzoic acid, adenosine (**52**), deoxyadenosin (**53**), 4-hydroxyphenyl ethanol (**55**), the antitumor agent *N*-acetyl-tyramine (**56**), also aureothin (**50**) and pimprinethine (**51**) and series of new natural products such as *N*- (2-methoxyphenyl)-acetamide (**47**), 2,3-dimethoxybenzamide (**48**), 2-hydroxy-1- (4-hydroxy-3-methoxyphenyl)-ethanone (**49**) and finally a new phenazine derivate with its two structure possibilities (**46a,b**). Aureothin is reported to possess a variety of pharmacological properties such as anti-bacterial against *Helicobacter pylori* for the treatment of intestinal tract diseases.[209]



46a



46b



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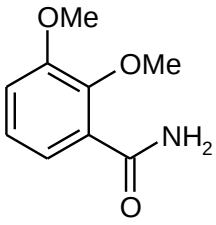

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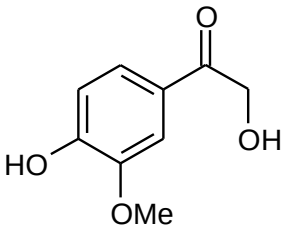
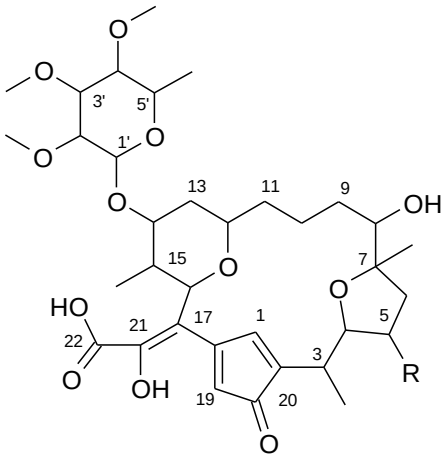
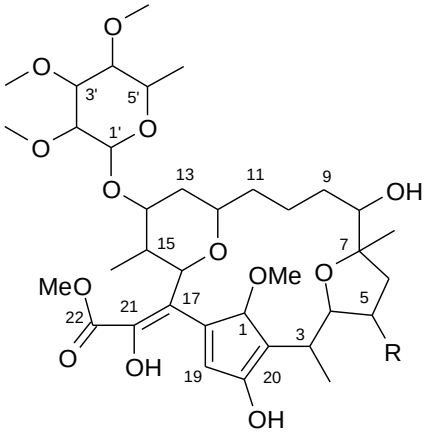


49

7.4 Terrestrial *Streptomyces* sp. GW 4723

The intensively black coloured terrestrial strain GW 4723 produced activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Mucor miehei* and *Candida albicans*. Its investigation delivered eight compounds, of which seven were new. The most predominant among them were the complex silamycins A and B (**62a,b**), which were elucidated *via* their methylation products **62c,d**.

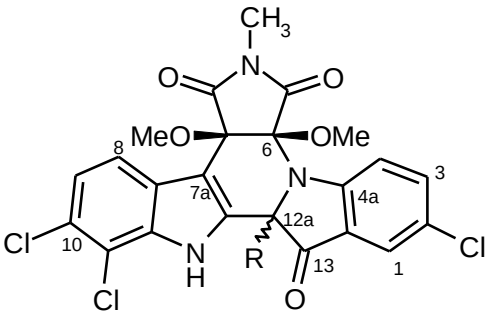




62a: R = Et **62c**: R = Et

62b: R = Me **62d**: R = Me

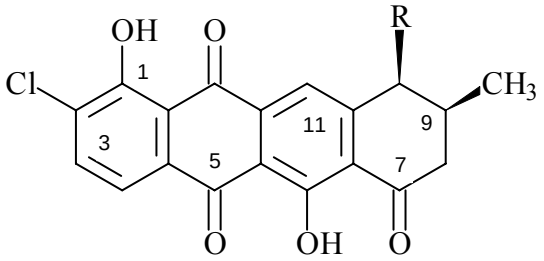
Silamycin A and B are the first compounds with a 19-membered carbocyclic ring possessing an unusual cyclopentendienone connected to an α-hydroxy carboxylic acid. The new bis-indoles moyopomycin A (**67a**) and B (**67b**) were also isolated. Silamycin A and B (**62a,b**) and moyopomycin A and B (**67a,b**) showed moderate activity in agar diffusion test against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*.



67a: R = OCH_3

67b: R = OH

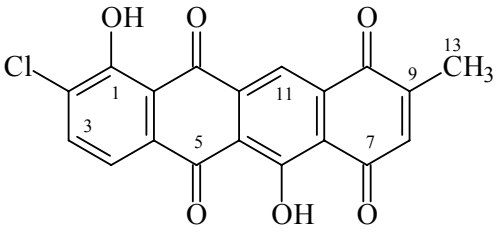
The highly active celastramycin B (**72a**) and the new metabolite deoxy-celastramycin B (**72b**) were also isolated from this strain.



72a: R = OH

72b: R = H

The new celastramycin D (**73**) and celastramycin E (**74**) are the first examples of tetracenquinone containing the thiomethyl group.



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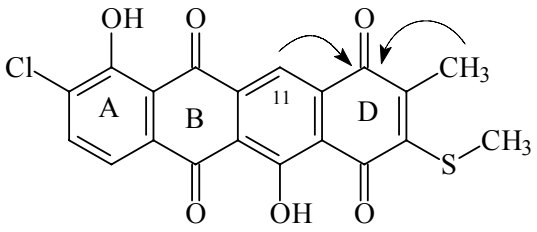
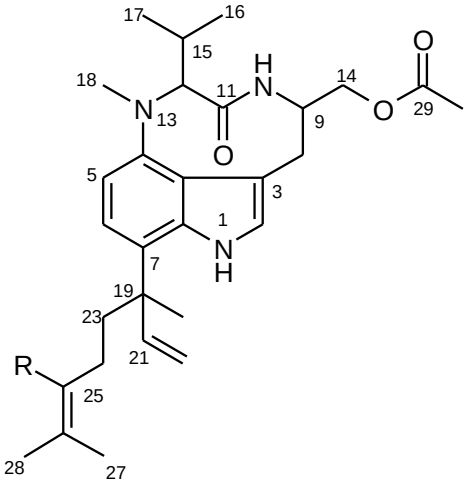


74

7.5 Terrestrial *Streptomyces* sp. AdM02

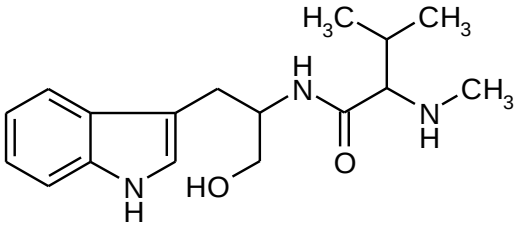
The terrestrial streptomycete AdM02 with activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* delivered 13 compounds including 5 known ones. Among them was the precursor of teleocidin, *N*-methyl-L-valtryptophanol (**81**), teleocidin A (**79**) and B (**80**), the antimicrobial antibiotic azomycin (**87**) and vanillic acid (**88**). The synthetically known lyngbyatoxin A acetate (**78**a), three cinnamic acid derivatives such as 2-hydroxy-6-methyl-cinnamic acid (**82a**), 2-methoxy-6-methyl-cinnamic acid (**82b**) and the antiinvasive and antimetastatic 2-methoxy-6-methyl cinnamic amide (**82c**), 3-hydroxy-5-hydroxyaminoisochroman-1-one (**85**) and 2-methoxy-*N*phenylbenzamide (**86**) were also isolated. A new lyngbyatoxin derivative was identified as 25-methyllyngbyatoxin A acetate (**78b**) and as well as one novel compound presenting an unprecedented skeleton heramide (**83**). The remarkable structure of the latter was supported by EI MS, ESI MS/MS

and HR MS/MS fragmentations and by careful interpretation of 1D and 2D NMR data.

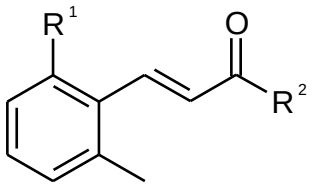


78a: R = H

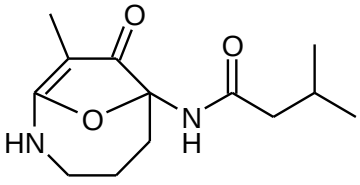
78b: R = CH_3



81



	R^1	R^2
82a	OH	OH
82b	OCH_3	OH
82c	OCH_3	NH_2



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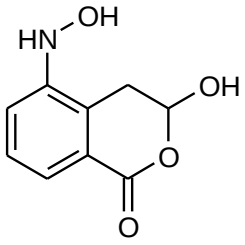

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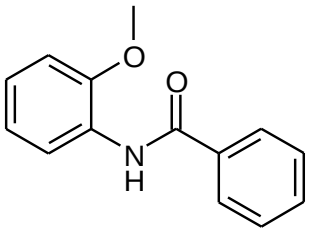
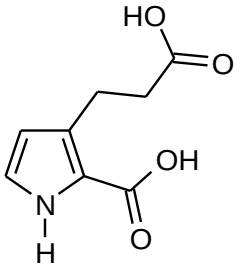


86

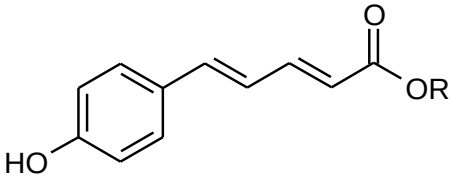
Although the new metabolites were found not to be responsible for the activity of this extract, samples have been submitted for additional biological tests. Results are not yet available.

7.6 Terrestrial *Streptomyces* sp. AdM19

The terrestrial streptomycete AdM19 was active against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Chlorella sorokiniana* and delivered eleven compounds from which three were known and identified as intomycin A (**94**), ferulic acid (**95**) and uracil. Eight were new from natural sources, 3-(carboxyethyl)-1*H*-pyrrole-2-carboxylic acid (**89**), avenalumic acid methyl ester and the new natural product avenalumic acid (**90a-b**), the new 5-(4-acetylaminophenyl)penta-2,4-dienamide, and the derivatives **91a-c** and the methylation products. The new 1phenyl-1*H*-pyrroles were named iguanen A (**92a**) and iguanen B (**93**).

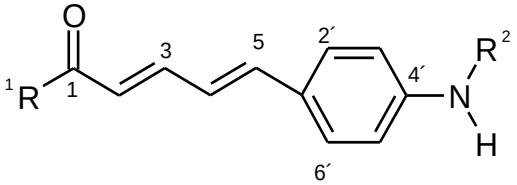


89

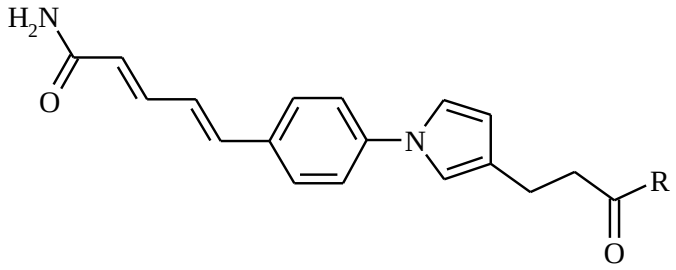


90a: R=CH_3

90b:R= H

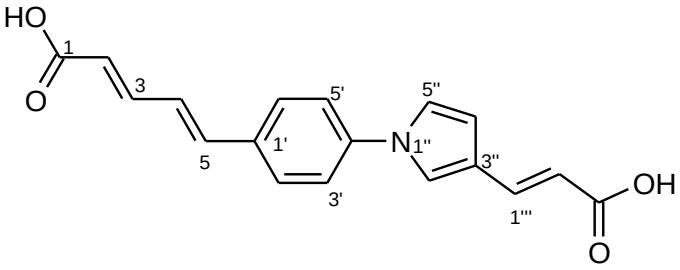


	R^1	R^2
91a	NH_2	$COCH_3$
91b	NH_2	H
91c	OH	$COCH_3$
91d	OCH_3	$COCH_3$



92a R= OH

92b R= OCH_3

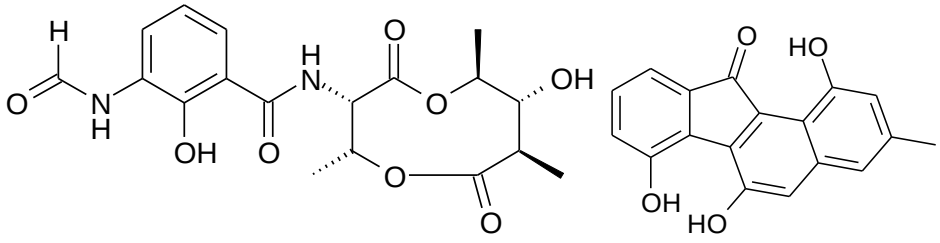


93

All these metabolites exhibited no antimicrobial activity in our screening tests; however, they have been submitted for immunsupression tests.

7.7 Terrestrial *Streptomyces* sp. AdM21

The antimicrobial terrestrial *Streptomyces* sp AdM 21 delivered a complex mixture of the antimycin A group (**97**) with masses between *m/z* 436 and 578, and the new urauchimycin D (**98**). Additionally, a new derivative of isoprekinamycin[162] identified as 1,6,7-trihydroxy-3-methylbenzo[a]fluoren-11-one (**99**) is reported here for the first time from a natural source.



98 **99**

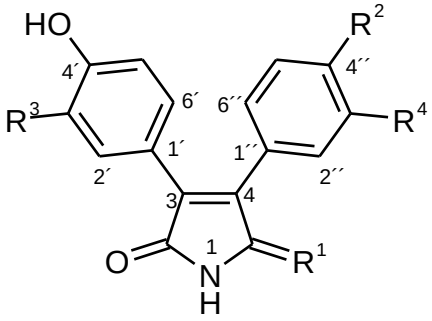
Prefluostatin belongs to the group of diazobenzo[a]fluorine. It showed weak cytotoxicity and moderate activity in the agar diffusion test against *Bacillus subtilis*,

Mucor miehei, Escherichia coli and *Staphylococcus aureus* at concentration of 20 μg per paper disk.

7.8 Marine *Vibrio* sp WMBA1-4

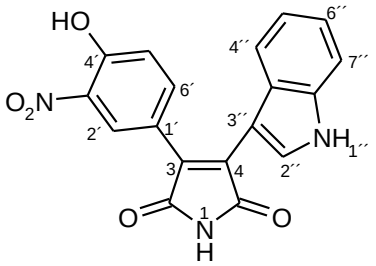
In cooperation with H. Anke from the Institute of Biotechnology and Drug Research (IBWF) in Kaiserslautern, a series of new nitro maleimides, including three new maleimide oximes and a new 1*H*-azirine derivate were isolated from the marine *Vibrio sp* WMBA1-4. Most of these compounds showed antibacterial activity against Gram positive bacteria like *Micrococcus luteus, Bacillus subtilis, and Bacillus brevis,* cytotoxic activity against breast cancer, colorectal cancer, mouse lymphocytes leukaemia and Jurkat-T cell leukaemia. The compounds were named as aqabamycins A (**103a**) - D (**103d**), the maleimide oximes aqabamycin E (**103e**) - F (**103f**), aqabamycin G (**105**), the 3-[3- (2-nitrophenyl)-1*H*-azirine-2-yl-1*H*-indole was called aqabamycin H (**106a**). Aqabamycin H belongs to the group of [1*H*]azirine: of the two possible isomeric azirines, the [2*H*]azirine is reported to be more stable. Despite the fact that [1*H*]azirines were observed only as transient intermediates, aqabamycin H (**106a**) is stable. The stability is explained through tautomerism (Figure 149).

The nematicidal 4-hydroxy-3-nitrobenzaldehyde (**115**) is reported here for the first time from a natural source. 4-hydroxy-3-nitrocinnamic acid (**116**), the 3-nitroindazole (**117**) with its annular tautomer and 2-hydroxy-1*H*-indole-3-carbaldehyde (**119**), the typical groundwater contaminant in military installation 1,4 dithiane (**120**), finally vibrindole A (**121**), the cytotoxic phenyl-2-indolylmethan (**122**) and its cationic form turbomycin were also isolated.

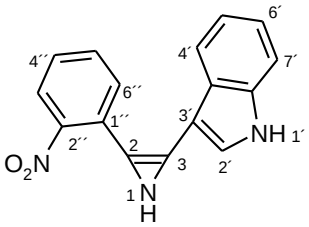


103

	R^1	R^2	R^3	R^4
103a	O	H	H	H
103b	O	OH	H	NO_2
103c	O	H	NO_2	H
103d	O	OH	NO_2	NO_2
103e	NOH	H	NO_2	H
103f	NOH	OH	NO_2	NO_2



105



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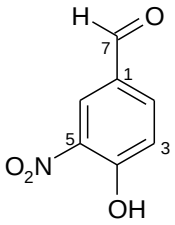
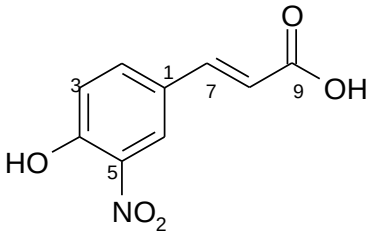


115



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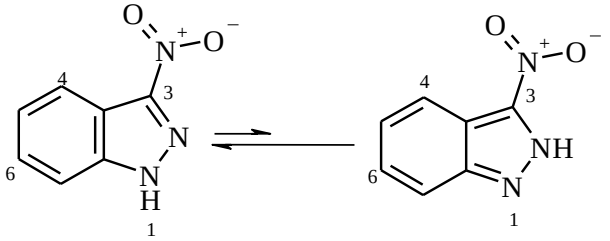
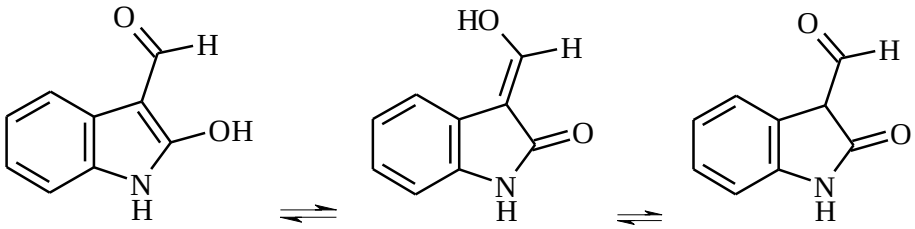
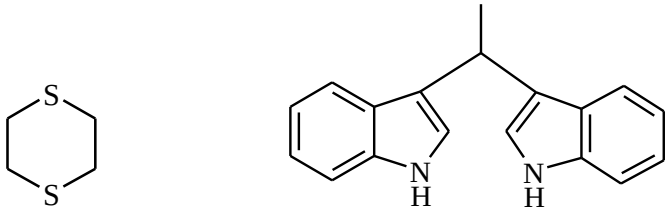


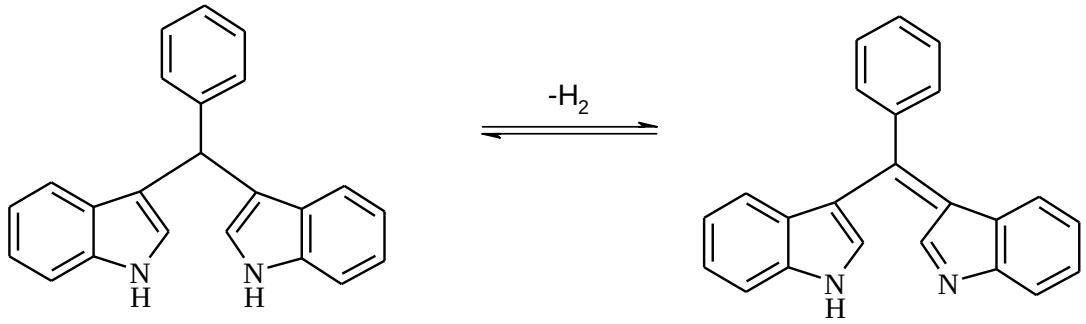
117



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



120 **121**



122

In total of the 12 studied strains, 96 compounds were isolated, including 49 new structures. In view of the structural diversity and different activities exhibited by various compounds it is right to say that the study of microorganisms as sources of new antibiotics is still very promising.

Strains	No of strains	No of isolated compounds	No of new compounds
Terrestrial *Streptomyces*	8	61	29
Marine *Pseudomonas*	1	5	3
Marine S*alegentibacter*	1	7	4
Marine sp undetermined	1	6	1
Marine *Vibrio* sp	1	17	12

8 Material 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 95 from Finnigan, High resolution EI MS with perfluorokerosine as standard. DCI MS: Finnigan MAT 95 (200 eV), gas reaction gas: NH_3. ESI MS with LCQ from Finnigan, ESI MS/MS with CID at10 to 35% (low energy), High resolution FT ICR MS with Apex-Q IV 7 Tesla from Bruker Daltonik with HP-Mix as standard. – **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 II**: Knauer HPLC equipment containing: spectral-digitalphotometer 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. - **HPLC MS (for Database)**: Mass spectrometer: Finnigan LCQ; UV/VIS-detector: Finnigan Surveyor PDA detector (Thermo Electron Corporation); HPLC-pump: Rheos 4000 (Flux Instrument); Deaerator: ERC-3415α (Flux Instruments); **Autosampler**: Jasco 851-AS Intelligent Sampler (Jasco); Control software HPLC: Janeiro (Flux Instruments); File system: Xcalibur (Finnigan); Databank software: MS-Manager (ACDLabs); **column**: EC 125/2 Nucleosil 100-5 C18 (Macherey-Nagel), Synergi 4µ MAX-RP 80A, 150×2.00 mm 4µ micron (Phenomenex); **Solvent**: Methanol LiChrosolv hypergrade for Liquid chromatography (Merck). **Programm**: start 10% Methanol to 100% Methanol in 20 min, 10 min 100 % Methanol, from 100 % Methanol to 10 % Methanol in 2 min.; flow rate: 300 µl/min. – **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/UV254 (Macherey-Nagel & Co.). **Glass plates for chemical screening**: Merck silica gel 60 F254, (10 x 20 cm). **Preparative thin layer chromatography (PTLC)**: 55 g Silica gel P/UV254 (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 x 20 cm) glass plates 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 (2,2dihydroxy indan-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,o'*-dianisidine reaction**: The reagent was prepared from 100 ml (0.032%) *o*-dianisidine in 1 N acetic acid, 1.5 g Na_2WO_4. 2 H_2O in 10 ml water, 115 ml acetone and 450 mg KI. The 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. **Tin(II)-chloride/hydrochloric acid/4-Dimethylaminobenzaldehyde: Spray reagent I:** 3 ml solution of tin (II)-chlorid (15 % water) mixed in 15 ml Hydrochlorid acid (37%) and 180 ml water, prepare in situ. **Spray reagent II:** 1 g 4-dimethylaminobenzaldehyde dissolved in 30 ml ethanol, 3 ml hydrochlorid acid (37%) and 180 ml 1 butanol**. Handling**: first spraying with reagent I, then drying on air and spraying again with reagent II.

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 Dar-

gatz 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, mannitol, yeast extract and malt extract were purchased from Merck, Darmstadt. **Antifoam solution:** Niax PPG 2025; Union Carbide Belgium N. V. (Zwiijndrecht). **Petridishes:** 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, Goettingen. **Laminar-Flow-Box:** Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1. **Brine shrimp eggs** (*Artemia salina*): SERA Artemia Salinenkrebseier, SERA Heinsberg. - Brine shrimp food: 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 seawater

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 sol.	20 mL
Stock sol.	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
$ZnSO_4.7\ H_2O$	0.056 g

$Al_2 (SO_4)_3.18\ H_2O$	0.056 g
$NiSO_4.6\ H_2O$	0.056 g
$CO (NO_3)_3.6\ H_2O$	0.056 g
TiO_2	0.056 g
$(NH_4)_6Mo_7O_{24}.4\ H_2O$	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 Nutrient compositions

M_{2+} medium (M_2 medium with seawater)

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Artificial seawater	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 without seawater

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

Luria-Bertani-Medium (LB) modified for North Sea strains: M_1-Medium

Trypton	5 g
Yeast extract	5 g
NaCl	10 g
Tap water	500 mL
Artificial seawater1	500 ml
pH	7.2±0.2

B-Medium: M_{11}

Corn starch	5g
A-Z Amine	2.5g
Beef extract (Powder)	3.8 g
Soya meal	1g
Yeast extract	2.5g
KNO_3	1.5g
Seaweed extract	2.5 ml
Marine salts mixture	33.3 g
pH	8.00 ± 0.2

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	1000mL

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 seawater	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 seawater	750 ml
Demineralised water	250 ml

8.5.2 Stock solutions and media for cultivation of algae

Fe-EDTA

0.7 g of $FeSO_4.7\ H_2O$ 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
$Na_2MoO_4.2H_2O$	13.0 mg
$Co\ (NO_3)_2.6H_2O$	10.0 mg

Salts are dissolved in 10 ml of demineralised water.

Solution B

$CuSO_4.5H_2O$	5.0 mg
H_3BO_3	10.0 mg
$ZnSO_4.7H_2O$	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
$MgSO_4.7\ H_2O$	0.075 g
NaCl	0.025 g
$CaCl_2.2\ H_2O$	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.

8.5.3 Storage of Strains

All bacteria strains were stored in liquid nitrogen. The strains were used to inoculate agar plates with the suitable media at room temperature.

8.5.4 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.

8.5.5 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.

8.5.6 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.

8.5.7 Brine shrimp microwell cytotoxicity assay

To a 500 ml separating funnel, filled with 400 ml of artificial seawater, 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 unhitched 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 shrimps larvae were transferred to a deep-well microtiter plates (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:

$$M = \left[\frac{(A - B - N)}{(G - N)} \right] \bullet 100 \text{ with}$$

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%.

8.5.8 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 and metabolite 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 and from bioLeads in Heidelberg. “Ank” and “ADM” are also terrestrial streptomycetes, which were collected by Prof. Laatsch and Prof Amin de Meijere and isolated by Prof. Anke in the Institute of Biotechnology and Drug Research (IBWF) in Kaiserslautern. The marine strain such as “T” (*Pseudoalteromonas* sp, and *Salegentibacter holothuriorum* sp) and “WMB” (*Vibrio* sp) were collected from the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven and identified by Prof. Anke at the Institute of Biotechnology and Drug Research (IBWF) in Kaiserslautern.

9.1 *Pseudoalteromonas* sp. T268

100-l of Strain *Pseudoalteromonas* sp T268 was fermented of M_1 Medium at 20°C with 120 rpm and aeration of 3-4 l/min for 67 hours at the Institute for Biotechnology and active agent research (Kaiserslautern).

9.1.1 Biological characterisation of the compounds

The compounds are tested against several fungi, gram positive and gram negative bacteria. Compounds **25** and **27** showed antibacterial activity grams positive and gram negative bacteria in the group of Prof. H. Anke using the agar diffusion test (Table 24) the minimal inhibitory concentration (MIC) assays (Table 25) and cytotoxic test (Table 26).

Table 24: Antimicrobial activity of the compounds from *Pseudoalteromonas* sp. T268 in agar diffusion test.

Compound	Inhibition zone (mm)50 µg /disc Bacteria	
	Bacillus brevis	*Enterobacter dissolvens*
25	16	-
27	12	-

- : no inhibition

Table 25: MIC of the metabolites from *Pseudomonas* sp. T 268 in the serial dilution assay

Organisms	MIC [µg/ml]			MIC [µg/ml]	
	27	**25**		**27**	**25**
Paecilomyces variotii	NT	50s	Bacteria:		
Penicillium notatum	NT	-	Gram-positive:		
Phytophthora infestans	NT	100c	*Bacillus brevis*	100c	50s
Mucor miehei	NT	-	*Bacillus subtilis*	100c	100c
Nematospora coryli	NT	-	*Micrococcus luteus*	NT	50s
Saccharomyces	NT	-	Gram-		

cerevisiae			negative:		
Ustilago nuda	NT	-	*Escherichia coli* K12	NT	50s

-: not active up to 100 µg/ml; nt: not tested; s: bacteriostatic/fungistatic; c: bactericidal/fungicidal

Table 26: Cytotoxic activities of the compounds from *Pseudoalteromonas* sp. T268.

Compound	L1210		Jurkat		MDA-MB-321		MCF-7		Colo-320	
	IC50	IC90	IC50	IC90	IC50	IC90	IC50	IC90	IC50	IC90
27	<20	20	<20	>100	100	>100	100	>100	35	50
25	10	20	10	50	15	20	30	50	10	50

IC_{50}: inhibition in proliferation of 50% of cells IC_{90}: inhibition in proliferation of 90% of cells

3-Methylthiopropanoic acid (20): $C_4H_8O_2S$, colourless oil. - R_f = 0.51 (CH_2Cl_2/MeOH 9:1). - IR (KBr): ν_{max} = 3380, 2945, 2833, 1450, 1030, 618 cm^{-1}. – ^1H NMR (DMSO-d_6, 300 MHz): δ = 10.15 (br, 1H, OH), 2.65 (t, *J* = 8.3, 14.4 Hz, 2H, 2-CH_2), 2.51 (t, *J* = 8.3, 15.1 Hz, 2H, 3-CH_2), 2.05 (s, 3H, SCH_3). – ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 172.9 (C_q, 1-CO), 34.0 (CH, 2-CH_2), 28.5 (CH, 3-CH_2), 14.6 (CH, S-CH_3).

Homogentisic acid (21): White substance. - R_f = 0.10 (CH_2Cl_2/MeOH 9:1). - UV (MeOH): λ_{max} (log ε) = 228 (3.22), 295 (3.08) nm. - IR (KBr): ν_{max} = 3394, 2925, 1712, 1521, 1458, 1393, 1213, 968, 819 cm^{-1}. - ^1H NMR (DMSO-d_6, 300 MHz): δ = 6.58 (d, *J* = 8.5 Hz, 1H, 3´-H), 6.53 (d, *J* = 2.9Hz, 1H, 6´-H), 6.46 (dd, *J* = 2.9, 8.5 Hz, 1H, 4´-H), 3.77 (s, 2H, 2-H_2). – EI MS (70 eV): *m/z* (%) = 168 ($[M]^{\bullet+}$, 42), 150 (50), 122 (100), 94 (42). – EI-HRMS: 168.04240 ($[M]^{\bullet+}$) (calcd. for $C_8H_8O_4$: 168.04172).

Homogentisic acid methyl ester (23): White substance. - R_f = 0.32 (CH_2Cl_2/MeOH 95:5). - UV (MeOH): λ_{max} (log ε) = 228 (3.22), 295 (3.08) nm. - IR (KBr): ν_{max} = 3405, 2951, 1724, 1510, 1459, 1211, 1024, 817 cm^{-1}. - EI MS (70 eV): *m/z* (%) = 182 ($[M]^{\bullet+}$, 58), 150 (85), 122 (100), 94 (30). – EI-HRMS: 182.05760 ($[M]^{\bullet+}$) (calcd. for $C_9H_{10}O_4$: 182.05737).

Euphamycin A (25): Light pale yellow substance. - R_f = 0.42 (CH_2Cl_2/MeOH 95:5). - R_t = 14.88 (LC MS). - UV (MeOH): λ_{max} (log ε) = 235 (3.83), 280 (3.30), 336 (3.63) nm. - IR (KBr): ν_{max} = 3379, 2923, 1587, 1392, 1171, 969, 855 cm^{-1}. - ^1H NMR (DMSO-d_6, 600 MHz): δ = 10.6 (s br, 1H, OH), 8.16 (s br, 1H, OH), 6.8 (s br, 1 H, OH), 3.86 (s, 2H, 2-H_2), 2.36 (s, 3H, 3´-SCH_3), 2.34 (s, 3H, 4´-SCH_3), 2.25 (s, 3H, 6´-SCH_3). – ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 172.2 (C_q, 1-CO), 151.8 (C_q, C-5'), 149.6 (C_q, C-2'), 127.8 (Cq, C-1'), 126.6 (C_q, C-4'), 124.6 (2 C_q, C-3',6'), 35.2 (CH_2, C-2), 18.8 (3´-S*C*H_3), 18.6 (CH, 4´-S*C*H_3), 17.6 (CH, 6´-S*C*H_3). – EI MS (70 eV): *m/z* (%) = 306 ($[M]^{\bullet+}$, 80), 288 (60), 260 (20), 245 (100), 217 (15), 199 (15). - (-)-ESI MS *m/z* (%) = 305 ($[M-H]^-$, 85), 633 ($[2M+Na-2H]^-$, 100), 961 ($[3M+2Na-3H]^-$, 62). - (-)-HRESIMS: 304.99840 $[M-H]^-$ (calcd. 304.99814 for $C_{11}H_{13}O_4S_3$).

Euphamycin B (27): Light pale brown substance. - R_f = 0.87 (CH_2Cl_2/MeOH 95:5). - R_t = 13.28 (LC MS). - UV (MeOH): λ_{max} (log ε) = 235 sh (3.83), 280 (3.30), 336 (3.63) nm. - IR (Film): ν_{max} = 3389, 2924, 1737, 1632, 1393, 1343, 1164 cm^{-1}. – ^1H NMR (DMSO-d_6, 600 MHz): δ = 3.96 (s, 2H, 2-CH_2), 3.61 (s, 3H, 1-OCH_3), 2.36 (s, 3H, 4´-SCH_3), 2.32 (s, 3H, 3´-SCH_3), 2.25 (s, 3H, 6´-SCH_3). – EI MS (70 eV): *m/z* (%) = 320 ($[M]^{\bullet+}$, 100), 288 (58), 260 (20), 245 (80), 217 (15), 199 (15). - (-) HRESIMS: 319.01391 $[M-H]^-$ (calcd. 319.01379 for $C_{12}H_{15}O_4S_3$).

9.2 Strain T48

The culture and harvest was doing by IBWF (Kaiserslautern). The fermentation resulted by 22°C in B medium in pH 8. The growth culture was filtrated and the mycelium was extracted with ethyl acetate and the pH of the filtrate after setup the pH to 4. The crude extract (1.5g) was separated by Sephadex with mobile phase (CH_2Cl_2/50%MeOH) with gave three fractions. Fraction three gave compound genistine (5 mg), fraction two was separated again on the Sephadex LH-20 column (CH_2Cl_2/40%MeOH) gave genistine, 4-(hydroxyphenyl)-acetic acid methyl ester, and sub fraction 2-2 after PTLC (CH_2Cl_2/10%MeOH) gave indolecarbaldehyde, indole-3-acetic acid and indole-3-acetic acid methyl ester. Fraction 1 gave after separation on silica gel 4-(hydroxylphenyl)-acetic acid (**29a**) and 4-(hydroxylphenyl)-acetic acid methyl ester (**29b**).

Genistine (28): White to light yellow powder. - R_f = 0.12 (CH_2Cl_2/10 % MeOH). - IR (KBr): ν_{max} = 3475, 2926, 2860, 2360, 2344, 1632, 1458, 1385, 1268, 840 cm^{-1}. - UV/VIS (MeOH): λ_{max} nm (log ε) = 432 (3.30), 268 (3.93), 231 (3.89). - ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 12.82 (1H, s, OH), 9.51 (s, 1H, OH), 8.41 (s, 1H, H-2), 7.41 (d, *J* = 8.8 Hz, 1H, 2´,6´-H), 6.82 (d, *J* = 8.7 Hz, 1H, 3´, 5´-H), 6.72 (d, *J* = 1.3 Hz, 1H, 8-H), 6.45 (d, *J* = 1.3Hz, 1H, 6-H), 5.40 (br s, 1H, OH), 5.10 (d, 1.8 Hz, 1´´-H), 4.59 (1H, br s, OH), 3.65 (m, 1H, 6´´-H_{2a}), 3.49 (m, 1H, -6´´-H_{2b}), 3.49 (m, 1H, 2´´-H), 3.32 (m, 1H, 3´´-H), 3.32 (m, 1H, 5´´-H). - ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 180.5 (C, C-5), 162.9 (C, C-6), 161.6 (C, C-7), 157.4 (C, C-4´), 157.2 (C, C-8a), 154.5 (CH, C-2), 130.1 (CH, C-2´), 130.1 (CH, C-6´), 122.5 (C, C-3), 120.9 (C, C-1´), 115 (CH, C-3´), 115 (CH, C-5´), 106.1 (C, C-4a), 99.9 (CH, C-1´´), 99.6 (C, C-6), 94.5 (CH, C-8), 77.2 (CH, C-2´´), 76.4 (CH, C-3´´), 73.1 (CH, C-5´´), 69.6 (CH, C-4´´), 60.6 (CH_2, C-6´´). - (-)-ESI MS: *m/z* (%) = 431 ($[M-H]^-$, 30), 477 ($[M+HCOO]^-$, 100), 863 ($[2M-H]^-$, 100), 909 ($[2M+HCOO]^-$, 90). - (+)-ESI MS: *m/z* (%) = 455 ($[M+Na]^+$, 5), 887 ([2M+Na], 100).

3-Indolylcarbaldehyde (30): Colourless solid. - R_f = 0.35 (CH_2Cl_2/10% MeOH). – ^{1}H NMR (MeOH-*d*$_4$, 300 MHz): δ = 9.89 (s, 1 H, CHO), 8.18 (d, *J* = 3 Hz, 1H, 4-H), 8.10 (s, 1H, H-2), 7.45 (d, *J* = 3 Hz, 1H, 7-H), 7.25 (m, 2 H, 5,6-H). - (-)-ESI MS: *m/z* (%) = 431.3 ($[M-H]^-$, 30). - (-)-ESI MS: *m/z* (%) = 144 ($[M-H]^-$, 100).

Indol-3-yl-acetic acid methyl ester (31b): Colourless solid, - R_f = 0.8 (CH_2Cl_2/5% MeOH). – ^{1}H NMR (MeOH-*d*$_4$, 300 MHz): δ = 7.52 (d, *J* = 7.6 Hz, 1H, 4-H), 7.35 (d, *J* = 7.6 Hz, 1H, 7-H), 7.15 (s, 1H, H-2), 7.10 (t, *J* = 7.5 Hz, 1 H, 5-H), 7.00 (t, *J* = 7.8 Hz, 1 H, 6-H), 3.76 (s, 2H, CH_2), 3.65 (s, 3H, OCH_3). - (-)-ESI MS: *m/z* (%) = 188.0 ($[M-H]^-$, 100); - (+)-ESI MS: *m/z* (%) = 212 ($[M+Na]^+$, 100), 401 ($[2M+Na]^+$, 20).

9.3 Strain T436

9.3.1 Fermentation and scale up

The fermentation was carried out by IBWF (Kaiserslautern). The optimum temperature for growth was between 21-27 °C in M1 medium without marine salt. The growth culture was filtrated and the mycelium was extracted with ethyl acetate. The

crude extract (1.5g) was separated by Silica gel with mobile phase (Ethyl acetate/Hexane) which gave two fractions A and B. Both fractions were separated by Sephadex LH-20 (MeOH) following by preparative HPLC (MeCN + 0.001% H_3PO_4) and gave **37c**, **38c, 39d**, **40**, **43** and **45.**

9.3.2 Biological activity

Pathogenic germ	Inhibition zone (mm)
*Bacillus brevis (*ATCC 9999)	17
*Bacillus subtilis (*ATCC 6633)	17
*Nematospora coryli (*ATCC 10647)	19
*Micrococcus luteus (*ATCC 381)	15
*Mucor miehei (*Tü 284)	0
Paecilomyces variotii (ETH 114646)	0

3´-Nitrogenistein (43): Yellow solid. - R_f = 0.55 (CH_2Cl_2/MeOH 9:1). - R_t = 12.49 min (LC MS). - UV (MeOH): λ_{max} (log ε) = 264 nm. – IR (KBr): ν_{max} = 3432, 2963, 2926, 1628, 1537, 1382, 1261, 1092, 1030, 803cm^{-1}. – ^{1}H NMR (MeOH-d_4, 600 MHz): δ = 8.20 (s, 1H, 2-H), 8.15 (d, *J* = 2.1 Hz, 1H, 6´-H), 7.80 (dd, *J* = 8.8, 1.9 Hz, 1H, 2'-H), 7.01 (d, *J* = 8.8 Hz, 1H, 3'-H), 6.28 (d, *J* = 2.1 Hz, 1H, 8-H), 6.18 (d, *J* = 2.1 Hz, 1H, 6-H). – (-)-ESI MS: *m/z* (%) = 314.3 [M-H]$^-$ (100). – (-)-ESI MS/MS (45 eV): *m/z* (%) = 314.2 [M-H]$^-$ (45), 297.1 (100). – (-)-ESI MS/MS (35 eV): *m/z* (%) = 297.1 (100), 280.2 (90), 267 (80). – (+)-APCI: *m/z* = 316 [M-H]$^+$. – (-)-APCI: *m/z* = 314 [M-H]$^-$.

4-Hydroxy-3-nitrophenyl-propionic acid (40): Yellow solid. - R_f = 0.55 (CH_2Cl_2/MeOH 9:1). - R_t = 7.49 min (LC MS). - UV (MeOH): λ_{max} nm (log ε) = 274 (1.78), 356 (2.32). – IR (KBr): ν_{max} = 3404, 2922, 1714, 1630, 1581, 1539, 1483, 1430, 1404, 1327, 1244. 1180, 1081, 907, 850, 762, 661, 600 cm^{-1}. - ^{1}H NMR (MeOH-d_4, 600 MHz): δ = 7.98 (d, *J* = 2.1 Hz, 1H, 2-H), 7.45 (dd, *J* = 8.4, 2.1 Hz, 1H, 6-H), 7.08 (d, *J* = 8.4 Hz, 1H, 5-H), 2.93 (t, *J* = 7.0 Hz, 2H, CH_2CH_2COO), 2.67 (t, *J* = 7.0 Hz, 2H, CH_2CH_2COO).

3,5-Dinitro-4-hydroxyphenyl-2-chloropropionic acid methyl ester (37b): Orange solid. – R_f = 0.62 (CH_2Cl_2/MeOH 9:1). - R_t = 10.34 min (LC MS). - UV

(MeOH): λ_{max} (log ε) = 347 (qual.) nm. – IR (KBr): ν_{max} = 3427, 2925, 2854, 1739, 1621, 1544, 1399, 1258, 1098, 701 cm^{-1}. – ^1H NMR (CD_3OD, 600 MHz): δ = 8.04 (s, 2H, 2´-H, 6´-H), 4. 68 (t, 1H, ^3J = 8.9 Hz, 2-H), 3.75 (s, 3H, 1-OCH_3), 3.18, 3.06 (ABX, ^2J = 15.1 Hz, ^3J = 8.9 Hz, 3-H). – (-)-APCI: *m/z* = 303 $[M-H]^-$.

2-Hydroxy-3-(4-hydroxy-3-nitrophenyl)propionic acid methyl ester (45): Yellow solid. – R_f = 0.59 (CH_2Cl_2/MeOH 9:1). - R_t = 9.14 min (LC MS). - UV (MeOH): λ_{max} nm (log ε) = 273 (2.27), 353 (2.93), 356. – IR (KBr): ν_{max} = 3433, 2925, 2853, 1740, 1630, 1538, 1489, 1383, 1328, 1252. 1181, 1098, 824, 765, 678 cm^{-1}. - ^1H NMR (MeOH-d_4, 600 MHz): δ = 7.98 (d, ^4J = 2.1 Hz, 1H, 2-H), 7.50 (dd, ^3J = 8.4 Hz, ^4J = 2.1 Hz, 1H, 6-H), 7.08 (d, ^3J = 8.4 Hz, 1H, 5-H), 4.28 (t, 1H, ^3J = 7.2 Hz, 2-H), 3.72 (s, 3H, 1-OCH_3), 3.10 (dd, ^3J = 4.2, 1.9 Hz, 1H, 3-H), 2.95 (dd, ^3J = 4.2, 1.9 Hz, 2H, 3-H). – (-)-APCI: *m/z* = 240 $[M-H]^-$.

3,5-Dinitro-4-hydroxyphenyl acetic acid (39d): Yellow solid. – R_f = 0.55 (CH_2Cl_2/MeOH 9:1). – R_t = 12.8 min (LC MS). – UV (MeOH): λ_{max} (log ε) = 349 (1.92) nm. – IR (KBr): ν_{max} = 3437, 1395, 1641, 1580, 1544, 1431, 1401, 1352, 1305, 1261, 1155, 910, 729, 606. cm^{-1}.– ^1H NMR (MeOH-d_4, 600 MHz): δ = 8.22 (s, 2H, 2´-H, 6´-H), 3.72 (s, 2H, 2-H). – EI MS (70 eV): *m/z* (%) = 242 ($[M]^{\bullet+}$, 65), 197 (100), 151 (20), 105 (16), 76 (16). – (-)-APCI: *m/z* = 241 $[M-H]^-$. – (-)-HRMS: 241.01027$[M-H]^-$ (calcd. 241.01022 for $C_8H_5N_2O_7$).

(3,5-Dinitro-4-hydroxyphenyl)propionic acid methyl ester (37b): Yellow solid. – R_f = 0.62 (CH_2Cl_2/MeOH 9:1). – EI MS (70 eV): *m/z* (%) = 270 ($[M]^{\bullet+}$, 20), 260 (45), 210 (100), 187 (10), 180 (10). – (-)-HRMS: 269.04160 $[M-H]^-$ (calcd. 269.04152 for $C_{10}H_9N_2O_7$).

Dinitrotyrosol (38c): Yellow solid. – R_f = 0.55 (CH_2Cl_2/MeOH 9:1). - R_t = 10.34 min (LC MS). - UV (MeOH): λ_{max} (lg ε) = 351 (c = 29µ/ml.) nm. – IR (KBr): ν_{max} = 3395, 2945, 2834, 1638, 2545, 1384, 1029, 618 cm^{-1}. – ^1H NMR (CD_3OD, 600 MHz): δ = 8.04 (s, 2H, 2´-H, 6´-H), 3.80 (t, 2H, ^3J = 6.8 Hz, 1-H), 2.82 (t, ^3J = 6.8 Hz, 2H, 2-H), 3.18, 3.06 (ABX, ^2J = 15.1 Hz, ^3J = 8.9 Hz, 3-H). – (-)-APCI: *m/z* = 227 $[M-H]^-$. – EI MS (70 eV): *m/z* (%) = 228 ($[M]^{\bullet+}$, 32), 197 (28), 180 (100), 151 (28), 105 (12). – (-)-HRMS: 227.03102 $[M-H]^-$ (calcd. 227.03095 for $C_8H_7N_2O_6$).

9.4 *Streptomyces* sp. Ank 2

The terrestrial strain Ank 2 was cultivated on M_2^+-Agar plate, during the four days incubation at 28°C. The plate was used to inoculate four Erlenmeyer flasks of 250 ml each in the same condition like these for the agar Plate. The resulting culture broth was extracted with ethyl acetate and used for pre-screening.

9.4.1 Biological activity of the crude extract

In the biological screening, the crude extract showed activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Candida albicans* and *Chlorella vulgaris* (Table 27)

Table 27: Biological activities of the crude extract Ank 2

Test-Organism	Diameter (mm)
Bacillus subtilis	21
Staphylococcus aureus	28
Streptomyces viridochromogenes (Tü 57)	21
Escherichia coli	22
Candida albicans	12
Mucor miehei	0
Chlorella vulgaris	11
Chlorella sorokiniana	0
Scenedesmus subspicatus	0

9.4.2 Fermentation and work-up

For the terrestrial streptomycete strain Ank 2, 100×1 l Erlenmeyer flasks each containing 250 ml of M_2 medium were inoculated from agar plates and grown for 5 days at 30 °C. The culture broth was mixed with *ca.* 1 kg Celite and separated by pressure filtration. The mycelial cake was extracted three times with ethyl acetate and acetone. Multiple separations of the combined extracts (5.1g) delivered 80 mg of antimycin A complex, and 100 mg of aliphatic fatty acids (mainly palmitic acid).

The water phase was extracted with XAD-16 (column 96 × 32 cm) and the resin washed with water and extracted with methanol. The methanol phase was concentrated and the aqueous residue extracted with ethyl acetate. All fractions are defatted with cyclohexane. The mycelium and the water extract were working up separely.

Chromatography of the water extract (1.0 g) on silica gel (CH_2Cl_2/MeOH gradient) and Sephadex LH-20 (CH_2Cl_2/MeOH 3:2) delivered 4 fractions, which were successively separated by PTLC (CH_2Cl_2/10%MeOH) and gave adenosine (10 mg), desoxyadenosine (10 mg), *p*-hydroxybenzoic acid (6 mg), *N*-acetyltyramine (**56**, 10 mg), 2,3-dimethoxybenzamide (**48**, 3 mg), 4-hydroxyphenyl ethanol (**55**, 4 mg). The mycelium extract was given on silica gel (CH_2Cl_2/MeOH gradient) and PTLC (CH_2Cl_2/10%MeOH) and delivered three fractions. RP18- HPLC (MeCN/H_2O gradient) of fraction 1 and yielded the major compound aureothin (**50**, 100 mg; R_t = 28.16 min, 88,0% azeotrope MeCN) and 6 mg of pimprinethine (**51**) (R_t = 22.52 min, 73.5% azeotrope MeCN). Fraction 2 gave 2.5 mg yellow phenazine derivate (**46**) (R_t = 20.32 min, 63.5% azeotrope MeCN).

9-Hydroxymethyl-4-methoxyphenazine-1-carboxylic acid methyl ester (**46**): Yellow solid. - R_f = 0.37 (CH_2Cl_2/5% MeOH). - UV (MeOH): λ_{max} = 252, 365 nm. - IR (neet): ν_{max} = 2925, 2854, 1727, 1600, 1535, 1463, 1440, 1285, 1236, 1205, 1105, 1037, 763 cm^{-1}. - ^1H NMR ($CDCl_3$, 300 MHz): δ 8.41 (d, *J* = 8.1 Hz, 1H, 2-H), 8.28 (br d, *J* = 8.9 Hz, 1H, 6-H), 7.83 (dd, *J* = 8.9, 6.6 Hz, 1H, 7-H), 7.74 (br d, *J* = 6.6 Hz, 1H, 8-H), 7.07 (d, *J* = 8.1 Hz, 1H, 3-H), 5.35 (s, 2H, 9-CH_2), 4.19 (s, 3H, 4-OCH_3), 4.08 (s, 3H, 1-COO*CH*$_3$). - ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 166.4 (C_q-CO), 158.2 (C_q-4), 144.0 (C_q-5a), 142.0 (C_q-10a), 140.6 (C_q-9a), 138.9 (C_q-9), 135.1 (CH-2), 134.1 (C_q-4a), 131.1 (CH-6), 129.6 (CH-7), 128.6 (CH-8), 122.0 (C_q-1), 105.2 (CH-3), 64.2 (CH_2OH), 56.6 (4-OCH_3), 52.3 (CO*OCH*$_3$). - EI MS *m/z* (%) = 298.2 (M^+, 72), 280.1 (50), 252.1 (44), 238.1 (40), 223.1 (20), 181.1 (28), 164.1 (32), 149.1 (56), 69.1 (100), 45 (56). - (+)-ESI MS *m/z* (%) = 321 ($[M+Na]^+$, 10), 619 ($[2M+Na]^+$, 100). – (+)-HRESIMS: 299.10265 $[M+H]^+$ (calcd. 299.10264 for $C_{16}H_{15}N_2O_4$).

***N*-(2-Methoxyphenyl)-acetamide** (**47**): colourless solid. - R_f = 0.57 (CH_2Cl_2/5% MeOH). - IR (neet): ν_{max} = 3251, 3138, 3063, 3023, 2966, 1659, 1597, 1544, 1495, 1462, 1434, 1369, 1323, 1291, 1271, 1252, 1221, 1180, 1118, 1047, 1025, 965, 924, 783, 751, 712, 656 cm^{-1}. - ^1H NMR ($CDCl_3$, 300 MHz): δ 8.36 (dd, *J* = 7.9, 1.7 Hz, 1H, 6-H), 7.78 (br s, 1H, NH), 7.06 (td, *J* = 7.7, 1.7 Hz, 1H, 4-H), 6.97 (td, *J* = 7.7, 1.4 Hz, 1H, 5-H), 6.85 (dd, *J* = 7.9, 1.5 Hz, 1H, 3-H), 3.88 (s, 3H, OCH_3), 2.20 (s, 3H, 2-OCH_3). - ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 168.1 (CO), 147.6 (C_q-2), 127.6

(C_q-1), 123.5 (CH-4), 121.0 (CH-5), 119.7 (CH-6), 109.8 (CH-3), 55.6 (OCH_3), 24.9 (CH_3). - EI MS *m/z* (%) = 165.1 (M^+, 90), 123.2 (100), 108.1 (53), 135.1 (45), 80.1 (18), 43.1 (12). - (+)-ESI MS *m/z* (%) = 166 ($[M+H]^+$, 62), 188 ($[M+Na]^+$, 100), 353 ($[2M+Na]^+$, 29).

2,3-Dimethoxybenzamide (**48**): Colourless solid. - R_f = 0.40 (CH_2Cl_2/5% MeOH). - UV (MeOH)[210]: λ_{max} (lg ε) = 248 (4.33), 307 (3.99) nm. - IR (neet): ν_{max} = 3449, 3337, 2939, 2842, 1661, 1574, 1518, 1476, 1427, 1385, 1267, 1225, 1203, 1172, 1093, 1056, 992, 879, 802, 759 cm^{-1}. - ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.91 (br s, 1H, NH), 7.72 (dd, *J* = 7.9, 1.7 Hz, 1H, 6-H), 7.18 (t, *J* = 8.1 Hz, 1H, 5-H), 7.09 (dd, *J* = 8.1, 1.7 Hz, 1H, 4-H), 5.96 (br s, 1H, NH), 3.94 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3). - ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 167.0 (CO), 152.6 (C_q), 147.9 (C_q), 125.9 (C_q), 124.4 (CH), 122.9 (CH), 115.8 (CH), 61.4 (OCH_3), 52.1 (OCH_3). - EI MS *m/z* (%) = 181 (M^+, 90), 164 (100), 149 (40), 135 (45), 122 (30), 106 (25), 91 (18), 77 (30).

2-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-ethanone (**49**): Colourless solid. - R_f = 0.31 (CH_2Cl_2/5% MeOH). - ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.52 (d, *J* = 1.9 1H, 2-H), 7.45 (dd, *J* = 8.3, 1.9 Hz, 1H, 6-H), 6.98 (d, *J* = 8.3Hz, 1H, 5-H), 4.83 (s, 2H, CH_2), 3.96 (s, 3H, 3-OCH_3). - ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 196.7 (CO), 151.1 (C_q-4), 146.9 (C_q-3), 126.3 (C_q-1), 122.8 (CH-6), 114.3 (CH-5), 109.5 (CH-2), 64.9 (CH_2), 56.1 (OCH_3). - (-)-ESI MS *m/z* (%) = 181 ($[M-H]^-$, 28), 385 ($[2M-2H+Na]^+$, 96).

Aureothin (50): Yellow solid. - R_f = 0.55 (*(*CH_2Cl_2/5% MeOH). - UV (MeOH): λ_{max} = 256, 350 nm. - IR (KBr): ν_{max} = 2924, 1665, 1590, 1540, 1511, 1469, 1416, 1376, 1337, 1257, 1167, 1109, 1050, 1013, 961, 890 cm^{-1}. - ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21 (d, *J* = 8.8 Hz, 1H, 3,5-H), 7.41 (d, *J* = 8.7 Hz, 1H, 2,6-H), 6.38 (s, 1H, 7-H), 6.23 (s, 1H, 9-H), 5.17 (t, *J* = 13.8, 6.9 Hz, 1H, 13-H), 4.87 (q, 2H, 12-CH_2), 3.99 (s, 3H, 20-OCH_3), 3.05 (q, 2H, 14-CH_2), 2.06 (s, 3H, 21-*CH_3*), 2.05 (s, 3H, 22-*CH_3*), 1.87 (s, 3H, 10-*CH_3*). - ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 181.2 (17-CO), 162.7 (C_q-19), 155.0 (C_q-15), 146.0 (C_q-1), 144.0 (C_q-4), 140.3 (C_q-8), 138.5 (C_q-11), 129.5 (CH-2,6), 128.2 (C_q-9), 126.0 (C_q-7), 124.1 (CH-3,5), 120.2 (C_q-16), 100.0 (C_q-18), (CH-6), 129.6 (CH-7), 73.6 (CH-13), 70.0 (CH-12), 57.8 (20-OCH_3), 38.0 (14-CH_3), 18.0 (10-CH_3), 9.99 (12-CH_3), 7.2 (21-CH_3). - (+)-ESI MS *m/z* (%) = 398

([M+H]$^+$, 10), 420 ([M+Na]$^+$, 10), 817 ([2M+Na]$^+$, 100). – (+)-HRESIMS: 398.15984 [M+H]$^+$ (calcd. 398.15982 for $C_{22}H_{24}NO_6$).

Pimprinethine (**51**): Colourless needles. - $R_{f\,=}$ 0.42 (CH_2Cl_2/5% MeOH). - UV (EtOH)[211]: λ_{max} (lg ε) = 225 (4.40), 268 (4.19), 284 (shoulder, 4. 12), 302 (shoulder, 4.02) nm. - ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.42 (t, 3H, *J* = 8Hz, -H), 2.85 (q, 2H, *J* = 8 Hz), 7. 18 -7.94 (m, 1H), 8.7 (br s, 1H). - ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 174.8, 150.6, 126.2 (C_q), 123.5 (C_q), 124.0 (CH), 121.9 (CH), 121.8 (CH), 119.5 (CH), 111.0 (CH), 111.9 (CH), 21.2 (CH_2), 10.5 (CH_3). - (+)-ESI MS *m/z* (%) = 213.1 ([M+H]$^+$, 100). - (-)-ESI MS *m/z* (%) = 211 ([M-H]$^-$, 45). – (+)-HRESIMS: 213.10213 [M+H] $^+$ (calcd. 213.10225 for $C_{13}H_{13}N_2O$).

***p*–Hydroxyphenyl-2-ethanol** (**55**): - R_f = 0.77 (CH_2Cl_2/ 10 %MeOH). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.08 (d, *J* = 8.8 Hz, 2H, 2',6'-H), 6.80 (d, *J* = 8.8 Hz, 2H, 3',5'-H), 3.82 (t, *J* = 7.3 Hz, 2H, 1-CH2), 2.80 (t, *J* = 7.3 Hz, 2H, 2-H_2). – 13C/APT NMR ($CDCl_3$, 125 MHz): δ = 154.2 (Cq-4'), 130.5 (Cq-1'), 130.2 (CH-2',6'), 115.4 (CH-3',5'), 63.8 (CH_2-1), 38.2 (CH_2-2). – CI MS (NH_3): *m/z (*%) = 294 ([M + NH_4], 2), 173 ([M + NH_4 + NH_3], 51), 156 ([M + NH_4], 100). – EI MS (70 eV.): *m/z (*%) = 138 ([M]$^+$,32), 107 ([M- (CH2-OH)]$^+$., 100), 86 (26), 84 (38).

***N*-Acetyl-tyramine** (**56**): White powder. - R_f = 0.37 (CH_2Cl_2 / MeOH10%). – ^{1}H NMR (MeOH-d_4, 300 MHz): δ = 7.02 (d, *J* = 8.8 Hz, 2H, 2,6-H), 6.75 (d, *J* = 8.9 Hz, 2H, 3,5-H), 3.38 (t, *J* = 6.2 Hz, 2 H, 2'-CH2), 2.68 (t, *J* = 6.1 Hz, 2 H, 1'-CH_2), 1.92 (s, 3H, 5'-CH3). – EI MS (70 eV) (%): *m/z (*%) = 179 ([M]$^+$, 5), 120 ([M- (NH_2-C = O-CH3)], 100), 107 (35).– (+)-ESI MS: *m/z (*%) = 180 ([M + H]$^+$,100)

9.5 *Streptomyces* sp AdM5

The terrestrial *Streptomyces* sp. AdM5 formed on agar red colonies. For screening, the strain was cultivated on a shaker with 95 rpm for five 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 35 mg of a red crude extract. TLC (CH_2Cl_2/10% MeOH) revealed a unpolar red zone (R_f = 0.65), which turned violet with anisaldehyde/sulphuric acid. In the biological screening, the extract shows activity again *Streptomyces viridochromogenes* (Tü 57), *Mucor mie-*

hei, Chlorella sorokiniana, Escherichia coli, Staphylococcus aureus, Candida albicans, Scenedesmus subspicatus.

9.5.1 Scale up and isolation

For the scale up, the strain AdM 5 was cultivated on a 25 L scale under the same conditions as for the screening. The well grown pink coloured culture broth was mixed with diatomaceous earth (1 kg) and filtered through a pressure filter. The filtrate was gived through a XAD-column and the mycelium was extracted separately with ethyl acetate. The water residue was chromatographed on silica gel using a CH_2Cl_2 inclusively MeOH gradient, followed by PTLC (20x20cm, CH_2Cl_2/5% MeOH) and Sephadex LH-20 (MeOH), with gived 6 fractions: The purification of Fractions I to IV delivered respectively indol-3-carboxylic acid, phenyl acetic acid (**57**) and phenyl acetamide (**58**), palmitic acid and indole-3-ethanol. The mycelium extract (1.5g) was first purified through Sephadex LH-20 (DCM/50%MeOH) but the obtained fractions showed no separation. The extract was chromatographed on CH_3COONa-PTLC (DCM/MeOH, 97:3) followed by separation on aluminium oxide column with DCM acidifying with 2N HCl, the obtained fractions were subjected again to normal PTLC (DCM/5%MeOH) and give rot-orange undecylprodigiosine, rot-purple butylcycloheptylprodigiosine (**61**, 50 mg) and a violet compound with a molecular weight of 393 Dalton (60 mg).

Phenylacetamide (**58**): White crystals. – No colour reaction with anisaldehyde/sulphuric acid. – R_f = 0.60 (CH_2Cl_2/MeOH 9:1). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.08 (brs, 1H, OH), 7.15 (m, 5H, Ar-H), 3.66 (s, 2H, 2-H). – EI MS (70 eV): *m/z* (%) = 136 ($[M]^{\bullet+}$, 38), 92 (18), 91 (100), 65 (15).

Undecylprodigiosine (**59**): Orange crystals. – Pink colour reaction with anisaldehyde/sulphuric acid. - UV (MeOH) λ_{max} = 526, 499, 370 nm. - IR (KBr) ν_{max} = 2924, 2852, 1618, 1579, 1408, 1385, 1042 cm^{-1}. – $R_{f\,=}$ 0.72 (CH_2Cl_2/MeOH 5%). – R_t = 18.60 min (LC MS). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 15.4 (m, 1H) 13.85 (brs), 9.06 (brs), 7.34 (m, 1H, 13-H), 6.98 (s, 1H, 6H), 6.95 (dd, *J* = 3.8, 1.1 Hz, 1H, 15-H), 6.81 (d, *J* = 3.8 Hz, 1H, 3-H), 6.38 (dd, *J* = 3.8, 2.7 Hz, 1H, 14-H), 6.18 (d, *J* = 3.8 Hz, 1H, 2-H), 6.10 (s, 1H, 9-H), 4.02 (s, 3H, 8-OCH3), 2.79 (m, 2H, 1'-H), 1.70 (m, 2H, 10'-H), 1.42-1.20 (m, 16 H), 0.92 (t, *J* = 6.6 Hz, 3H, 11'-H). – EI MS (70 eV):

m/z (%) = 393 ($[M]^{\bullet+}$, 100), 378, 348, 337, 229, 252, 91. – (+)-ESI MS: *m/z* (%) = 394 ($[M+H]^+$, 100), 783 ($[2M+H]^+$, 10).

Butylcycloheptylprodigiosine (61): Red crystals. – Pink colour reaction with anisaldehyde/sulphuric acid. – UV (MeOH) λ_{max} = 534, 499, 362 nm. – IR (KBr) ν_{max} = 2927, 2856, 1616, 1385, 1035 cm^{-1}. – R_f = 0.67 (CH_2Cl_2/MeOH 5%). – R_t = 17.27 min (LC MS). – ^1H NMR ($CDCl_3$, 300 MHz): δ = 15.44 (br), 13.85 (brs), 9.06 (brs), 7.29 (dd, *J* = 3.5, 1.3 Hz, 1H, 13-H), 7.10 (s, 1H, 6-H), 6.90 (d, *J* = 3.8 Hz, 1H, 15-H), 6.49 (s, 1H, 2-H), 6.34 (dd, *J* = 3.8, 1.3 Hz, 1H, 14-H), 6.09 (s, 1H, 9-H), 4.02 (s, 3H, 8-OCH3), 3.10 (m, 1H, 1'-H_a), 2.58 (m, 1H, 1'-H_b), 2.00-160 (s, 10H, 2´, 3´, 4´, 5´, 9´´-H), 1.85 (m, 2H), 1.70 (m, 1H), 1.58 (m, 1H), 1.39 (m, 1H), 1.20 (m, 2H,), 0.92 (t, 3H, 11'-H). – EI MS (70 eV): *m/z* (%) = 393 ($[M]^{\bullet+}$, 100), 378, 348, 311, 299, 252. – (+)-ESI MS: *m/z* (%) = 392 ($[M+H]^+$, 100), 787 ($[2M+H]^+$, 5).

9.6 *Streptomyces* sp GW4723

9.6.1 Fermentation and work up

Well grown agar subcultures of terrestrial *Streptomyces* sp. were used to inoculate 100 1-l Erlenmeyer flaks containing each 250 ml of M_2 medium at standard conditions. The flaks were placed on a linear shaker at 28°C for 4-5 days. The dark culture broth was worked up followed the scheme (Figure 47), and the obtained crude extract was subjected to flash chromatography on silica gel using CD_2Cl_2/MeOH gradient and resulted in four fractions. The TLC of the crude extract obtained from the extraction of the water phase exhibited no yellow bands as from the ethyl acetate extract, instead colourless bands, which became blue on spraying with anisaldehyde/sulphuric acid. The purification of this crude extract on Sephadex-LH20 (CH_2Cl_2/50%MeOH) and finally on preparative HPLC (MeCN/30% H_2O) delivered silamycin A (**62a,** 40 mg) and B **(62b**, 25 mg). The mycelial cake was extracted with ethyl acetate, the water phase was passed through XAD-16 and latter eluted with methanol. The ethyl acetate of the mycelium and methanol extracts were evaporated to dryness and worked separately on the view of their TLC. The ethyl acetate fraction was subjected to Sephadex LH-20 using (CD_2Cl_2/50% MeOH) and four fractions were obtained. The PTLC (CD_2Cl_2/5% MeOH) of the fraction II delivered celastramycin B (7 mg), furan 2,4-dicarboxylic acid methyl ester and deoxycelastramycin B

(2 mg). Fraction III was chromatographed on Sephadex LH-20 (CD_2Cl_2/40% MeOH) resulting in two sub-fractions IIIa and IIIb, the sub-fraction IIIa indicated on TLC (CD_2Cl_2/7% MeOH) only one major compound and it was identified as celastramycin D (2 mg). A PTLC (CD_2Cl_2/5% MeOH) of the sub-fraction IIIb followed by Sephadex LH-20 (MeOH) delivered celastramycin E (**72**, 5 mg), The preparative HPLC of fraction IV using MeCN-H_2O delivered moyopomycin A (**67a**, 8 mg) and B (**67b**, 3 mg) with retention time 37 min and 36 min respectively.

9.6.2 Biological Activity

Antibacterial and antifungal activities were semi quantitatively determined using the agar diffusion method with 9 mm paper disc with 40 μg by silamycin A (**62a**) and B (**62b**) /disk. moyopomycin A (**67a**) and B (**67b**) at the concentration of 20 μg/plate and celastramycin B (**72a**), 10-deoxycelastraymcin B (**72b**), celastramycin D (**73**) and E (**74**) with 20 and 40 μg per paper disc showed moderate activity against, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* and no activity at the mentioned concentration against the growth of *Streptomyces viridochromogenes* (Tü 57), *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*.

Table 28: Biological activities of compound

	Conc./paper disk μg/ paper disc	EC	SA	BS	SV	MM	CV	CS	SS
62a	40	15	16	15	-	-	-	-	-
62b	40	11	13	12	-	-	-	-	-
67a	20	11	12	13	-	-	-	-	-
67b	20	10	10	12	-	-	-	-	-
72a	40	14	30	20	19	14	14	14	14
72b	20	13	17	13	-	-	-	-	-
73	40	11	13	11	13	-	-	-	-
74	20	11	12	10	-	-	-	-	-

EC: *Escherichia coli*, SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, SV: *Streptomyces viridochromogenes*, M: *Mucor miehei*, CV: *Chlorella vulgaris*, CS: *Chlorella sorokiniana*, SS: *Scenedesmus subspicatus*

Silamycin A (**62a**): – Slight yellow solid. - R_f (CH_2Cl_2% MeOH): 0.33. - IR (KBr) ν_{max} = 3276, 2963, 2933, 2925, 1616, 1458, 1384, 1202, 1116, 1084,1036, 973 cm-1. - UV (MeOH) λ_{max} (log ε): 310 (7.00), 203 (7.31); NMR data see Table 3 and Table 4. – EI MS *m/z* (%) 678 (M^+, 2), 472 (12), 454 (10), 189 (40), 101 (100), 88 (86), 45 (30). – (-)-ESI MS *m/z* (%) 677 ($[M-H]^-$, 100), 1355 ($[2M-H]^-$, 40). - (+)-HRESIMS *m/z* 679.368205 $[M+H]^+$ (calcd. 679.36881 for $C_{36}H_{55}O_{12}$).

Silamycin B (**62b**): Slight yellow solid. - R_f (CH_2Cl_2% MeOH): 0.38. - IR (KBr) ν_{max} =3297, 2964, 2931, 1624, 1559, 1458, 1385, 1202, 1116, 1085, 1036 cm^{-1}. - UV (MeOH) λ_{max} (log ε): 308 (6.65). NMR data see Table 3 and Table 4. - (+)-ESI MS *m/z* (%) 665 ($[M+H]^-$, 20), 687 ($[M+Na]^+$, 100), 1351 ($[2M+Na]^-$, 70). - (-)-ESI MS *m/z* (%) 663 ($[M-H]^-$ 100), 1327 ($[2M-H]^-$, 90). - (+)-HRESIMS *m/z* 665.35243 $[M+H]^+$ (calcd. 665.35316 for $C_{35}H_{53}O_{12}$).

1-Methoxysilamycin A methyl ester (**64a**): $C_{38}H_{60}O_{13}$, colourless solid. - UV/VIS (MeOH) λ_{max} (log ε) : 361 (2.95), 311 (3.53), 221 (3.71). - ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.69 (s, 1H, OH), 6.48 (s, 1H, OH), (6.31 (brs, 1H, 19-H), 5.20 (brs, 1H, 1-H), 4.87 (brs, 1H, 1'-H), 4.50 (d, 1H, *J* = 9.6 Hz, 4-H), 4.47 (d, *J* = 10.8 Hz, 1H, 16-H), 3.91 (brd, *J* = 2.3 Hz, 1H, 14-H), 3.87 (brt, *J* = 11.5 Hz, 1H, 12-H), 3.74 (s, 3H, 22-OMe), 3.67 (m, 1H, 5'-H), 3.51 (brt, *J* = 2.1Hz, 1H, 2'-H), 3.47 (s, 3H, 1-OMe), 3.39 (m, 1H, 3'-H), 3.39 (s, 3H, 4'-OMe), 3.36 (s, 3H, 2'-OMe), 3.31 (s, 3H, 3'-OMe), 3.12 (d, *J* = 8.8 Hz, 1H, 7-H), 3.61 (t, *J* = 9.4 Hz, 1H, 4'-H), 2.92 (m, 1H, 15-H), 2.56 (m, 1H, 5-H), 2.05 (m, 2H), 1.95 (m, 1H, 3-H), 1.90, 1.50 (m, 2H), 1.90, 1.40 (m, 2H), 1.70, 1.10 (m, 2H), 1.60 (m, 2H), 1.15 (d, *J* = 6.2 Hz, 3H, 6'-H), 1.02 (s, 3H, 7-Me), 0.93 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 0.73 (d, *J* = 6.8 Hz, 3H, 15-Me), 0.51 (d, *J* = 7.1 Hz, 3H, 3-Me). - ^{13}C NMR (DMSO-d_6, 150.8 MHz): δ 150.7 (C_q, C-20), 149.6 (C_q, C-22), 138.4 (C_q, C-21), 122.4 (C_q, C-18), 119.5 (C_q, C-17), 111.7 (C_q, C-2), 98.1 (CH, C-1'), 92.8 (CH, C-19), 84.0 (C_q, C-7), 81.4 (CH, C-4'), 80.7 (CH, C-3'), 79.1 (CH, C-8), 78.8 (CH, C-4), 78.5 (CH, C-14), 76.3 (CH, C-16), 76.9 (CH, C-2'), 77.1 (CH, C-1), 69.6 (CH, C-12), 67.7 (CH, C-5'), 59.8 (4'-OCH_3), 58.5 (1-OCH_3), 58.0 (2'-OCH_3), 56.5 (3'-OCH_3), 55.9 (22-OCH_3), 46.6 (CH_2, C-6), 42.7 (CH, C-3), 40.0 (CH, C-3), 38.4 (CH_2, C-11), 36.0 (CH, C-15), 33.8 (CH_2, C-13), 29.1 (CH_2, C-9), 29.0 (CH_2, C-10), 22.6 (5-CH_2CH_3), 19.4 (7-CH_3), 17.5 (CH_3, C-6'), 10.0 (3-CH_3), 13.36 (15-CH_3), 13.35 (5-CH_2CH_3);

Moyopomycin A (**67**a): Yellow compound. - R_f = 0.83 (CH_2Cl_2/5 % MeOH). - IR (KBr): ν_{max} = 3524, 2926, 2351, 2284, 1725, 1634, 1464, 1436, 1385, 1304, 1179, 1113, 1079, 1020, 806, 792 cm^{-1}. – UV (MeOH): λ_{max} (log ε) = 376 (3.44), 302 (3.84), 233 (4.5) nm. – ^{1}HNMR (600 MHz, $CDCl_3$): see Table 7. – ^{13}C NMR (150 MHz, $CDCl_3$): see Table 6. – (-)-ESI MS: *m/z* (%) = 548 ($[M-H]^-$, 26), 550 ($[2M-H]^-$, 26), 1118 ($[2M-2H+Na]^-$, 50); 1121 ($[2M-2H+Na]^-$, 50). – EI MS: *m/z* (%) = 549 (M^+, 16), 551 (15), 553 (3), 554 (5), 520 (98), 518 (100). – HRESIMS: 548.018982 $[M-H]^-$ (calcd. 548.01883 for $C_{24}H_{17}N_3O_6Cl_3$).

Moyopomycin B (**67b**): Yellow compound. R_f = 0.52 (CH_2Cl_2/5 % MeOH). - IR (KBr): ν_{max} = 3535, 2932, 1723, 1635, 1465, 1385, 1115, 1022, 876, 792 cm^{-1}. - UV (MeOH): λ_{max} (log ε) = 372 (3.12), 300 (3.67), 236 (4.43) nm. – ^{1}HNMR (600 MHz, $CDCl_3$): see Table 7. – ^{13}C NMR (150 MHz, $CDCl_3$): see Table 6. - (-)- ESI MS: *m/z* (%) = 534 ($[M-H]^-$, 96), 536 ($[M_2-H]^-$, 100), 1093 ($[2M-2H+Na]^-$, 88). - HRESIMS: 534.00327 $[M-H]^-$ (calcd. 534.00318 for $C_{23}H_{15}N_3O_6Cl_3$).

10-Deoxycelastramycin B (**72b**): Yellow solid. - R_f = 0.90 (CH_2Cl_2/5 % MeOH); IR (KBr): ν_{max} = 3475, 2926, 2860, 2360, 2344, 1632, 1458, 1385, 1268, 840 cm^{-1}; UV (MeOH): λ_{max} nm (log ε) = 432 (3.30), 268 (3.93), 231 (3.89); ^{1}HNMR (600 MHz, $CDCl_3$): δ 12.25 (s, 1H, 1-OH), 12.10 (s, 1H, 6-OH), 7.75 (d, ^3J = 8.1 Hz, 1H, H-3), 7.62 (d, J = 8.1 Hz, 1H, H-4), 6.98 (br s, 1H, H-11), 2.95 (ddd, J = 1.3, 5.3, 16.0 Hz, 1H, H_{2a}-8), 2.91 (ddd, J = 1.2, 3.6, 16.0 Hz, 1H, H_{2a}-10), 2.59 (ddd, J = 1.1, 10.0, 16.4 Hz, 1H, H_{2b}-10), 2.47 (dd, J = 10.9, 15.9 Hz, 1H, H_{2b}-8) 2.40 (m, 1H, H-9), 1.16 (d, J = 6.5 Hz, 3H, 9-CH_3); ^{13}C NMR (151 MHz, $CDCl_3$): δ 198.4 (C, C-7), 194.4 (C, C-4), 180.1 (C, C-5), 163.5 (C, C-6), 157.5 (C, C-1), 153.8 (C, C-10a), 137.5 (C, C-3), 137.2 (CH, C-11a), 130.3 (C, C-6a), 129.0 (C, C-6a), 133.5 (C, C-4a), 121.4 (CH, C-11), 120.1 (CH, C-4), 116.4 (C, C-5a), 115.8 (C, C-1a), 47.5 (CH_2, C-8), 36.7 (CH_2, C-10), 30.2 (CH_2, C-9), 21.4 (CH_3); - (-)-ESI MS: *m/z* (%) = 355 ($[M-H]^-$, 100), 357 ($[M-H]^-$, 40), 733 ($[2M-2H+Na]^-$, 30); EI MS: *m/z* (%) = 356 (M^+, 30), 358 (12), 328 (52), 314 (100); HRESIMS: 357.05258 $[M+H]^+$ (calcd. 357.05244 for $C_{19}H_{14}O_5Cl$).

Celastramycin D (**73**): Yellow solid. - R_f = 0.90 (CH_2Cl_2/5 % MeOH); IR (KBr): ν_{max} = 3475, 2926, 2857, 2363, 1734, 1717, 1700, 1685, 1653, 1636, 1559,

1540, 1507, 1457, 1385, 1265, 807, 746 cm^{-1}; UV/VIS (MeOH): λ_{max} nm (log ε) = 444 (2.80), 270 (3.50), 226 (3.57) ; ^1H NMR (600 MHz, $CDCl_3$): δ 12.29 (s, 1 H, 1-OH), 12.05 (s, 1 H, OH-6), 7.86 (1H, s, 11-H), 7.75 (d, *J* = 8.1 Hz, 1H, 3-H), 7.65 (d, *J* = 8.1 Hz, 1H, 4-H), 6.98 (d, *J* = 1.5 Hz, 1H, 8-H), 1.41 (d, *J* = 1.41 Hz, 3H, 13-H_3); (-)-ESI MS: *m/z* (%) = 367 ($[M-H]^-$, 100), 369 (44); EI MS: *m/z* (%) = 368 (M^+, 100), 370 (40), 340 (44), 312 (25), 300 (21); - (-)-HR ESIMS: 367.001402 $[M-H]^-$ (calcd. 367.00148 for $C_{19}H_8O_6Cl$).

Celastramycin E (**74**): Orange solid, yellow in solution. - R_f = 0.87 (CH_2Cl_2/5 % MeOH); IR (KBr): ν_{max} = 3475, 2928, 2362, 2339, 1734, 1700, 1635, 1653, 1559, 1540,1521, 1457, 1384, 1281, 1204, 1105, 754, 688, 668 cm^{-1}; UV/VIS (MeOH): λ_{max} (log ε) = 433 (3.64), 270 (4.17), 231 (4.23) nm; ^1H NMR (600 MHz, $CDCl_3$): δ 12.26 (s, 1H, 6-OH), 12.21 (s, 1H, 1-OH), 7.85 (s, 1H, 11-H), 7.81 (d, *J* = 8.09 Hz, 1H, 3-H), 7.68 (d, *J* = 8.1 Hz, 1H, 4-H), 2.18 (s, 3H, 13-H_3), 2.76 (s, 3H, SCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 192.5 (C, C-12), 181.1 (C, C-7), 180.7 (C_q, C-5), 178.3 (C_q, C-10), 164.5 (C, C-6), 158.0 (C, C-1), 155.6 (C, C-8), 140.1 (C, C-10a), 139.6 (C, C-9), 138.0 (CH, C-3), 136.3 (C, C-11a), 133.0 (C, C-4a), 130.4 (C, C-6a), 129.8 (C, C-2), 120.5 (CH, C-11), 120.4 (C, C-4), 118.8 (C, C-5a), 115.9 (C, C-1a), 16.4 (CH_3), 13.5 (CH_3, C-13); (-)-ESIMS: *m/z* (%) 413 ($[M-H]^-$, 100), 415.0 ($[M-H]^-$, 30); EI MS: *m/z* (%) 414 (M^+, 100), 416 (32), 381 (78), 339 (10), 300 (44), 302 (12), 244 (14); HRESIMS: 412.989391 $[M-H]^-$ (calcd. 412.98920 for $C_{20}H_{10}O_6ClS$), 415.0050210 $[M+H]^+$ (calcd. 415.00377 for $C_{20}H_{12}O_6ClS$).

9.7 *Streptomyces* sp. GW 14/1869

The terrestrial *Streptomyces* sp. GW 14/1869 formed on agar a white aerial mycelium with a red colouration. 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 40 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 did not inhibit the growth of *Streptomyces viridochromogenes* (Tü 57), *Mucor miehei, Chlorella sorokiniana, Escherichia coli, Staphylococcus aureus, Candida albicans, and Scenedesmus subspicatus*.

9.7.1 Scale up and isolation

For the scale up, the strain GW 14/1869 was cultivated on a 25 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 was given through a XAD-column and the mycelium was extracted separately with ethyl acetate and the combined solutions were concentrated under vacuum. The residue was chromatographed on silica gel using a CH_2Cl_2/MeOH gradient, followed by PTLC (20 x 20 cm, CH_2Cl_2/5% MeOH) with gave 4 fractions: Fractions I and II are respectively phthalate ester and indol-3-carboxylic acid, the purification of fraction III on Sephadex LH-20 (MeOH) gave celastramycin B (2.3 mg). Fraction IV was purified by chromatography on PTLC (CH_2Cl_2/5% MeOH) and delivered julimycin Q3,3 (**77**, 40 mg).

Julimycin Q3,3 (**77**): Yellow orange solid, pink with sodium hydroxide. - R_f = ($CHCl_3$/MeOH). – ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.41 (d, ^3J = 8.3 Hz, 1H, 7-H), 7.21 (d, ^3J = 8.2 Hz, 1H, 6-H), 5.92 (s, 1H, 10-H), 5.60 (m, ^2J = 4.2-6.4 Hz, 1H, 12-H), 5.51 (s, 1H, 10-OH), 5.20 (s, 1H, 3-OH), 3.01 (d, ^3J = 4,2 Hz1H,), 2.45 (AB-system, ^3J = 18.3 Hz, 2H, 4-H), 1.65 (s, 3H, 14-H), 1.40 (d, ^3J = 7.1Hz, 11-H), 1.39 (s, 3H, 15-H). ─ ^{13}C- NMR (DMSO-d_6, 75 MHz): δ = 199.4 (C_q-1), 189.9 (C_q-5), 169.6 (C_q-13), 161.3 (C_q-9), 134.5 (C_q-8), 129.8 (CH-7), 128.7 (C_q-9a), 126.5 (C_q-5a), 113.2 (CH-6), 68.4 (CH-3), 67.1 (CH-12), 62.5 (C_q-10a), 62.4 (C_q-4a), 56.9 (CH-10), 49.5 (CH_2-2) 45.6 (CH-4), 31.4 (CH_3-11), 22.6 (CH_3-15), 21.2 (CH_3-14). ─ (+)-ESI MS: *m/z* (%) = 1522.7 ($[2M+Na]^+$, 20), 773.1 ($[M+Na]^+$, 100). ─ (-)-ESI MS: *m/z* (%) = 1497 ($[2M-H]^-$, 10), 749 ($[M-H]^-$, 100). - (+)-HRESIMS: *m/z* = 773.20504 $[M+Na]^+$ (calcd. 773.20522 for $C_{38}H_{38}O_{16}Na$), 768.24976 $[M+NH_4]^+$ (calcd. 768.25390 for $C_{38}H_{42}NO_{16}$).

Bis(2-ethylhexyl)phthalat (**77**): Pale oil. – R_f: 0.58. EI MS (70 eV): m/z (%) = 390 (1) $[M]^+$, 279 (40), 167 (40), 149 (100). - (+)-ESI MS: *m/z* = 390 $[M+Na]^+$, 403 $[M+Na]^+$. - ^{1}H NMR (300 MHz, CD_3OD): δ = 7.70 (m, CH, 2H), 7.51 (m, CH, 2H), 4.20 (m, CH2, 4H), 1.66 (m, CH, 2H); 1.30 (m, CH_2, 16H), 0.95 (m, CH_3, 12H).

9.8 *Streptomyces* sp AdM02

For the terrestrial streptomycete strain AdM02, 20×1 l Erlenmeyer flasks each containing 250 ml of M_2 medium were inoculated from agar plates and grown for 3 days at 30 °C. The culture broth was mixed with *ca.* 1 kg Celite and separated by pressure filtration. The mycelial cake was extracted three times with ethyl acetate and acetone. Multiple separations of the combined extracts (2.2 g) delivered 80 mg of teleocidin family complex (**79a-c)** and 200 mg of azomycin (**87**).

The water phase was extracted with XAD-16 (column 96 × 32 cm) and the resin washed with water and extracted with methanol. The methanol phase was concentrated and the aqueous residue extracted with ethyl acetate. Chromatography of this extract (2.2 g) on Silica gel (DCM and DCM/MeOH gradient) delivered eight fractions, which were successively separated by Sephadex LH-20 (CH_2Cl_2/MeOH 6:4), PTLC ($CHCl_3$/10% MeOH). RP HPLC (MeCN/H_2O gradient). The Sephadex LH-20 (MeOH) purification of fraction 2 delivered two sub-fractions 2A and 2B. The preparative HPLC of fraction 2A using MeCN/50%H_2O as gradient yielded lyngbyatoxin acetate (**78a**) and its derivate **78b**. The preparative HPLC of fraction 2B using MeCN/80%H_2O gradient gave two new cinnamic acid derivates **82b and c**. The PTLC Fraction 4 (CH_2Cl_2/8%MeOH) gave 27 mg of the yellow new compound **83**. Purification fraction 5 on Sephadex LH-20 (CH_2Cl_2/50%MeOH) followed by PTLC (CH_2Cl_2/8%MeOH) yielded compound (**82a**, 5 mg). The polar fraction 8 was purified on PTLC using (CH2Cl2/15%MeOH) and delivered 18.9 mg of *N*- Methyl-L-val-tryptophanol (**81**). Fraction 3 gave 3-Hydrox-5-hydroxyamino-isochroman-1-one (**85**), *N*-(2-Methoxyphenyl) benzamide (**86**) and vanillic acid.

9.8.1 Biological Activity of the crude extract AdM21

Test-Organism	Diameter (mm)
Bacillus subtilis	14
Staphylococcus aureus	18
Escherichia coli	15
Brine shrimp	100%

***N*- Methyl-L-val-tryptophanol** (**81**): Colourless crystals. - ^{1}H NMR (300 MHz; MeOH-d_4): δ = 7.62 (d, *J* = 8.1 Hz, 1H, 4-H), 7.29 (d, *J* = 8.1 Hz, 1H, 7-H), 7.10 (s,

1H, 2-H), 7.07 (t, *J* = 7.98 Hz, 1H, 6-H), 6.98 (t, *J* = 7.98 Hz , 1H, 5-H), 4.40 (m, 1H, 9-H), 3.80 (br t, 1H, 12-H), 3.62 (2H, m, 14-H), 3.10 (br. dd, *J* = 14.7, 9.3 Hz, 1H, 8-H), 2.90 (dd, *J* = 14.6, 9.3 Hz, 1H, 8-H), 2.40 (m, 1H, 15-H), 2.01 (br s, 3H, 18-CH_3), 0.98 (m, 6H, 16,17-CH_3).

Lygnbyatoxin A acetate (**78a**): Colourless oil. - UV (MeOH) λ max (log ε)[130]: 229 (4261), 297 (1270). - IR (film) ν_{max} 3422, 1654 cm^{-1}. - ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 9. - (+)-ESI MS: *m/z* (%) = 480.1 ($[M+H]^+$, 15), 502.3 ($[M+Na]^+$, 50), 981.1 ($[2M+Na]^+$, 100). - (-)-ESI MS: *m/z* (%) = 478 ($[M-H]^-$, 100). - (+)-HRESIMS: *m/z* = 480.32216 $[M+H]^+$ (calcd. 480.32208 for $C_{29}H_{42}N_3O_3$).

Lygnbyatoxin A acetate homologue (**78b**): Colourless oil. - UV (MeOH) λ_{max} (log ε) : 229 (4261), 297 (1270); IR (film) ν_{max} 3422, 1654 cm^{-1}. - ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 9. - (+)-ESI MS: *m/z* (%) = 516.4 ($[M+Na]^+$, 100), 1009.2 ($[2M+Na]^+$, 85). - (-)-ESI MS: *m/z* (%) = 478 ($[M-H]^-$, 100). - (+)-HRESIMS: *m/z* = 494.33812 $[M+H]^+$ (calcd. 494.33773 for $C_{30}H_{44}N_3O_3$).

2-Hydroxy-6-methyl-cinnamic acid (**82a**): White solid, blue fluorescence at 366 nm, with anisaldehyde/sulphuric acid its gave a orange colour. - R_f = 0.42 (CH_2Cl_2/8 % MeOH), - UV (MeOH): λ_{max} (log ε) = 204 (4.15), 232 (3.88), 282 (4.02), 327 (3.61) nm. - IR (Film): ν_{max} = 3252, 2549, 2364, 1684, 1600, 1578, 1464, 1440, 1348, 1301, 1263, 1191, 1035, 981, 878, 778, 755, 670 cm^{-1}. - ^{1}H, ^{13}C NMR see Table 10. - EI MS: *m/z* (%) = 178 (78) [M^+], 160 (76) [M^+-18], 132 (100) [M^+-46]. - (-)-HRESIMS: *m/z* = 177.05519 $[M-H]^-$ (calcd. 177.05571 for $C_{10}H_9O_3$).

2-Methoxy-6-methylcinnamic acid (**82b**): White solid. - R_f = 0.57 (CH_2Cl_2/8 % MeOH). - UV/vis (MeOH): λ_{max} (log ε) = 202 (3.99), 277 (3.25) nm. - IR (neat): ν_{max} = 2929, 2857, 2369, 1691, 1644, 1596, 1469, 1437, 1267, 1202, 1144, 1082, 990, 800, 777, 723 cm^{-1}. - ^{1}H, ^{13}C NMR see Table 10. – EIMS: *m/z* (%) = 192 ([M^+, 100]), 175 ([M^+-18, 17]), 161 ([M^+-31, 78]), 146 ([M^+-46, 40]). - HREI MS: *m/z* = 192.07830 [M^+] (calcd. 192.07810 for $C_{11}H_{12}O_3$).

2-Methoxy-6-methylcinnamic amid (**82c**): White solid. – R_f = 0.48 (CH_2Cl_2/8% MeOH). - UV/vis (MeOH): λ_{max} (log ε) = 205 (3.60), 277 (3.07) nm. - IR (neat): ν_{max} = 3344, 3204, 2930, 2857, 2364, 1668, 1595, 1470, 1434, 1265, 1204, 1137, 1081, 998, 777, 722 cm^{-1}. - ^{1}H, ^{13}C NMR see Table 10. - EI MS: *m/z* (%) =

191.1 (18) [M^+], 160.1 (100) [M-31], 146.1 (18) [M-46]. - (+)-ESI MS: *m/z* (%) = 192 ([M+H]$^+$, 100), 214 ([M+Na]$^+$, 60), 409 ([2M+Na]$^+$, 14). - HREIMS: *m/z* = 191.09420 [M^+] (calcd. 191.09409 for $C_{11}H_{13}NO_2$).

3-Methyl-N-(8-methyl-7-oxo-9-oxa-2-aza-bicyclo[4.2.1]non-1(8)-en-6-yl)-butyramide (**Heramide**, **83**): Yellow solid. - R_f = 0.31 (CH_2Cl_2/8 % MeOH), blue fluorescent at 366 nm; no colour with anisaldehyde/sulphuric acid. - UV/vis (MeOH): λ_{max} (log ε) = 203 (3.89), 232 (3.88), 305 (3.56), 340 (3.74) nm. - IR (Film): ν_{max} = 3241, 2960, 2874, 2362, 1667, 1573, 1451, 1374, 1292, 1245, 1198, 1163, 1100, 1024, 992, 924, 760, 734 cm^{-1}. – ^{1}H, ^{13}C NMR data see Table 11. - (+)-ESI MS: *m/z* (%) = 275 ([M+Na]$^+$, 100). - (-)-ESI MS: *m/z* (%) = 251 ([M-H]$^-$, 100). - (+)-HRESIMS: *m/z* = 253.14887 [M+H]$^+$ (calcd. 253.15468 for $C_{13}H_{21}N_2O_3$).

3-Hydrox-5-hydroxyamino-isochroman-1-one (**85**): Colourless oil, blue fluorescent at 254 and 366 nm. - It gave a pink colour with anisaldehyd/sulphuric acid. - R_f = 0.35 (CH_2Cl_2/8 % MeOH). – UV/vis (MeOH): λ_{max} (log ε) = 217 (3.58), 256 (sh), 341 (2.85) nm. - IR (Film): ν_{max} = 3373, 3266, 2924, 2857, 1678, 1641, 1605, 1496, 1465, 1380, 1236, 1149, 1094, 1031, 800, 756 cm^{-1}. - ^{1}H, ^{13}C NMR see Table 12. - CI MS: *m/z* (%) = 213 ([M+NH_4]$^+$, 41), 196 ([M+H]$^+$, 100). - HREI MS: *m/z* = 195.0529 [M]$^+$ (calcd. 195.05262 for $C_9H_9NO_4$).

***N*-(2-Methoxyphenyl)benzamide** (**86**): Colourless crystals. – IR (film)[143]: ν_{max} 3426 (NH), 2838 (OCH3), 1671 (C=O), 1601 (C = C, conj.), 1530 (NC=O), 1254 (C = N), 750 and 709 (o-subst. and monosubst.), 1290 and 1026 (ArC-O-C), 1450 and 1431 (CH_3) cm^{-1}. - ^{1}H NMR (300 MHz; MeOH-d_4): δ = 8.08 (1H, d, *J* = 7.9 Hz, 6-H), 7.98 (2H, d, *J* = 6.8 Hz, 9,13-H), 7.60 (d, *J* = 7.1 Hz, 1H, 12-H), 7.58 (t, *J* = 8.1 Hz, 1H, 11-H), 7.54 (t, *J* = 8.1 Hz, 1H, 10-H), 7.20 (t, *J* = 8.2 Hz, 1H, 4-H), 7.10 (d, *J* = 8.2 Hz, 1H, 3-H), 6.98 (t, *J* = 7.9 Hz, 1H, 5-H), 3.90 (s, 3H, OC*H*$_3$). - ^{13}C NMR (75 MHz; CD_3OD) 152.2 (C_q, C-2) 136.1 (C_q, C-8), 133.0 (CH, C-11), 129.8 (CH, C-10, 12), 128.4 (CH, C-9,13), 128.0 (C_q, C-1), 126.8 (CH, C-4), 123.9 (CH, C-6), 121.6 (CH, C-5), 111.9 (CH, C-3), 56.4 (OCH_3). - (+)-ESI MS: *m/z* (%) = 228 ([M+H]$^+$,15), 250 ([M+Na]$^+$, 100), 477 ([2M+Na]$^+$, 60). - (+)-HRESIMS: *m/z* = 228.10198 [M+H]$^+$ (calcd. 228.10191 for $C_{14}H_{14}NO_2$).

Azomycin (**87**): White crystals. - UV/vis (MeOH): λ_{max} (log ε) = 217 (3.58), 256 (sh), 341 (2.85) nm. - IR (Film): ν_{max} = 3373, 3266, 2924, 2857, 1678, 1641, 1605, 1496, 1465, 1380, 1236, 1149, 1094, 1031, 800, 756 cm^{-1}. - ^1H NMR (300 MHz, DMSO-d_6): δ = 7.20 (s, 2H). - CI MS: *m/z* (%) = 114.0 ($[M+H]^+$), 131.0 ($[M+NH_4]^+$, 100), 148.1 ($[M+NH_3+NH_4]^+$, 70), 244.1 ($[2M+NH_4]^+$, 15). - EI MS: *m/z* (%) = 113 ([M^+, 100]), 97 ([M^+-16, 12]), 83 ([M^+-30, 55]), 67 ([M^+-46, 20]), 56 ([M^+-57, 30]).

9.9 *Streptomyces* sp. AdM19

9.9.1 Fermentation procedure and work-up

The terrestrial *Streptomyces* sp AdM19 was cultivated using M_2 medium for five days on a linear shaker culture. The brown culture broth was filtrated under filter press followed by the extraction of the mycelium cake 4 times with ethyl acetate and finally with acetone. The water phase was chromatographed on XAD-16 and eluted with methanol. The methanolic phase was evaporated under dryness. The mycelium and water extracts was mixed together on the view of their TLC comparison and delivered 8g of crude extract.

The 8g was first chromatographed on Sephadex LH-20 using (CH_2Cl_2/50%MeOH) as eluent and delivered four fractions. The oily fraction 1 was discarded. The chromatography of fraction 3 on silica gel, followed by PTLC delivered intomycin A. Triturating of fraction 4 with methanol delivered 1.3 g of iguanen A (**92 a**). the filtrate was chromatographed on Sephadex LH-20 (CH_2Cl_2/50%MeOH) and delivered sub-fractions A (not further studied), B and C. Sub-fraction C was chromatographed on sephadex LH-20 using MeOH as eluent followed by PTLC to give uracil and 3- (carboxy-ethyl)pyrrole-2-carboxylic acid (**89**, 8 mg). Silica gel chromatography of fraction B delivered in addition to a (10 mg), 5- (4-acetylaminophenyl)penta-2,4-dienamide (**91**, 15 mg) and a complex mixture which gave yellow colouration on spraying with anisaldehyde/sulphuric acid, the latter was methylated with diazomethane followed by separation on PTLC and delivered avenalumic acid methyl ester (**90**, 6 mg). Purification of fraction 2 by PTLC (CH_2Cl_2/8%MeOH) delivered ferulic acid and iguanen B (**92b**, 5 mg).

9.9.2 Biological activities

Compounds **89, 90, 91, 92a** and **93** were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57) and *Escherichia coli*, the fungi *Mucor miehei* and *Candida albicans*, and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana and Scenedesmus subspicatus* in the agar diffusion test at a concentration of 50 μg/paper and exhibited small activities against *M. miehei* (10 mm) and *Candida albicans* (11 mm).

3-(Carboxy-ethyl)pyrrole-2-carboxylic acid (**89**): White powder. - R_f = 0.27 (CH_2Cl_2/10%MeOH). - UV (MeOH): λ_{max} (lg ε) = 263 (3.80) nm. - IR (Film): ν_{max} = 1692, 1631, 1548, 1485, 1427, 1325, 1299, 1260, 1219, 1201, 1125, 1090, 1029, 897, 790 cm^{-1}. - ^1H NMR ($CDCl_3$, 300 MHz): δ = 11.32 (br s, 1H, 1-H), 6.80 (t, *J* = 2.8 Hz, 1H, 5-H), 6.02 (t, *J* = 2.5 Hz, 1H, 4-H), 2.93 (t, *J* = 7.4 Hz, 1H, 1'-H_2), 2.46 (t, *J* = 8.20 Hz, 1H, 2'-H_2). - ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 173.9 (C, C-3'), 162.0 (2-*CO*OH), 130.0 (C, C-3), 121.8 (CH, C-5), 118.5 (C, C-2), 110.0 (CH, C-4), 34.7 (CH_2, C-2'), 22.0 (CH_2, C-1'). - (+)-ESI MS *m/z* (%) = 206.0 ($[M+Na]^+$, 76), 410.9 ($[2M+2Na]^+$, 100). - (-)-ESI MS *m/z* (%) = 182.0 ($[M-H]^-$, 100), 364.7 ($[2M-H]^+$, 50). - EI MS *m/z* (%) = 183 (M^+, 16), 139 (60), 137 (40), 120 (8), 119 (20), 106 (28), 94 (36), 80 (100), 44 (16). - HREIMS: 183.05249 $[M]^+$ (calcd. 183.05262 for $C_8H_9NO_4$).

Avenalumic acid methyl ester (**90a**): Yellow powder. - R_f = 0.34 (CH_2Cl_2/10%MeOH). - UV (MeOH): λ_{max} (log ε) = 332 (3.26), 318 (3.22), 211 (3.47) nm. - ^1H NMR ($CDCl_3$, 300 MHz): δ = 9.78 (br s, 1H, OH), 7.40 (d, *J* = 8.7 Hz, 2H, 2',6'-H), 7.37 (dd, *J* = 15.2, 10.5 Hz, 1H, 3-H), 7.00 (d, *J* = 15.1Hz, 1H, 5-H), 6.90 (dd, *J* = 15.4, 10.5 Hz, 1H, 4-H), 6.78 (d, *J* = 8.7 Hz, 2H, 3', 5'-H), 3.76 (s, 3H, OCH_3). - ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 166.7 (C, C-1), 158.6 (C, C-4), 145.5 (CH, C-3) 140.9 (CH, C-5), 128.9 (CH, C-2', 6'), 126.9 (C, C-1') 123.0 (CH, C-4), 118.5 (CH, C-2), 115.7 (CH C-3', 5'), 51.0 (O*CH*$_3$). - EI MS *m/z* (%) = 204 (M^+, 88), 178 (20), 173 (28), 147 (28), 144 (52), 145 (100), 127 (44), 115 (32), 84 (20), 65 (28). - HR EI MS: 204.07850 $[M]^+$ (calcd. 204.07864 for $C_{12}H_{12}O_3$).

5-(4-Acetylaminophenyl)penta-2,4-dienamide (**91a**): Yellow powder. - R_f = (CH_2Cl_2 /3%MeOH). - UV (MeOH): λ_{max} (log ε) = 329 (3.84), 317 (3.84), 248 (sh),

207 (3.92) nm. - IR (Film): ν_{max} = 3324, 1666, 1593, 1532, 1513, 1413, 1320, 1267, 1180, 1121, 999, 848 cm^{-1}. - ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 10.00 (1H, s), 7.58 (d, *J* = 8.7 Hz, 1H, 2',6'-H), 7.48 (d, *J* = 8.7 Hz, 1H, 3',5'-H), 7.19 (dd, *J* = 15.0, 9.6 Hz, 3'-H), 6.94 (dd, *J* = 15.6, 9.6 Hz, 4'-H), 6.08 (d, *J* = 15.1 Hz, 2-H), 6.84 (d, *J* = 15.6 Hz, 5-H), 2.05 (s, 1H, CH_3). - ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 168.9 (*CO*CH_3), 167.5 (C, C_1), 140.4 (CH, C-3), 140.2 (C, C-4'), 138.2 (CH, C-5), 131.6 (C, C-1'), 128.0 (CH, C-2', 6'), 126.0 (CH, C-4), 125.3 (CH, C-2), 119.5 (CH, C-3', 5'), 24.6 (CH_3). - (+)-ESI MS *m/z* (%) = 253 ($[M+Na]^+$, 38), 483 ($[2M+Na]^+$, 100), 713 ($[3M+Na]^+$, 45). - (+)-HRESIMS: 231.11237 $[M+H]^+$ (calcd. 231.11281 for $C_{13}H_{15}N_2O_2$), 253.09435 $[M+Na]^+$ (calcd. 253.09476 for $C_{13}H_{14}N_2O_2Na$).

(2E,4E)-5-(4-Acetylaminophenyl)penta-2,4-dienoic acid (**91c**): Yellow powder. - R_f = 0.30 (CH_2Cl_2 /10%MeOH). - UV (MeOH): λ_{max} (log ε) = 330 (4.12), 317 (sh), 286 (4.17), 247 (3.96) nm. - IR (Film): ν_{max} = 3315, 1672, 1596, 1535, 1513, 1411, 1373, 1321, 1264, 1179, 1019, 968, 852 cm^{-1}. - ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.33 (br s, 1H, OH), 10.01 (s, 1H, NH), 7.60 (d, *J* = 8.7 Hz, 2H, 2'-, 6'-H), 7.49 (d, *J* = 8.7 Hz, 2H, 3'-, 5'-H), 7.33 (ddd, *J* = 15.1, 10.3, 4.9 Hz, 1H, 3-H), 5.96 (d, *J* = 15.1 Hz, 1H, 2-H), 6.98 (m, 4, 2H, 5-H), 2.05 (s, 1H, CH_3). - ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* = 168.3 (N*CO*CH_3), 167.5 (C, C-1), 144.5 (CH, C-3), 140.0 (C, C-4'), 139.5 (CH, C-5), 130.7 (C, C-1'), 127.7 (CH, C-2', 6'), 124.9 (CH, C-4), 121.2 (CH, C-2), 118.9 (CH, C-3', 5'), 24.0 (CH_3). - (-)-ESI MS *m/z* (%) = 230 ($[M-H]^-$, 100), 461 ($[2M-H]^-$, 36), 483 ($[2M-2H+Na]^-$, 60). - (+)-HRESIMS: 232.09638 $[M+H]^+$) (calcd. 232.09683 for $C_{13}H_{14}NO_4$).

(2E,4E)-5-(4-Acetylaminophenyl)penta-2,4-dienoic acid methyl ester (91d): Yellow powder. - R_f = 0.46 (CH_2Cl_2 /8%MeOH). - ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.58 (d, *J* = 8.7 Hz, 2H, 2', 6'-H), 7.49 (d, *J* = 8.7 Hz, 2H, 3',5'-H), 7.33 (m, 1H, 3-H), 6.00 (d, *J* = 15.1 Hz, 1H, 2-H), 6.98 (m, 2H, 4-, 5-H), 3.76 (s, 3H, OCH_3), 2.08 (s, 1H, CH_3). - EI MS *m/z* (%) = 245.2 (76), 203.1 (8), 186.1 (40), 144.1 (100), 143.1 (34), 43.0 (16).

Iguanen A (**92a**): Yellow powder. - R_f = 0.47 (CH_2Cl_2 /10%MeOH). - UV (MeOH): λ_{max} (log ε) = 336 (4.16), 318 (4.10), 247 (sh) nm. - IR (Film): ν_{max} = 3402, 2256, 2129, 1666, 1524, 1383, 1051, 1026, 1005, 825, 746 cm^{-1}. - ^{1}H NMR and ^{13}C NMR data see Table 14. - (+)-ESI MS *m/z* (%) = 643.0 ($[2M+Na]^+$, 100), 664.9

([2M+2Na-H]$^+$, 58), 952.9 ([3M+Na]$^+$, 30), 975.0 ([3M+2Na-H]$^+$, 42). - (-)-ESI MS *m/z* (%) = 309 ([M-H]$^-$, 10), 619 ([2M-H]$^-$, 44), 641 ([2M-2H+ Na]$^-$, 44), 929 ([3M-H]$^-$, 40), 973 ([3M-2H+Na]$^-$, 100). - (+)-HRESIMS: 311.13864 [M+H]$^+$ (calcd. 311.13903 for $C_{18}H_{19}N_2O_3$).

Iguanen methyl ether (**92b**): Yellow powder. - R_f = 0.76 (CH_2Cl_2 /10%MeOH). - IR (Film): ν_{max} = 3356, 3196, 2952, 2926, 2855, 1667, 1602, 1523, 1488, 1436, 1411, 1378, 1353, 1319, 1254, 1198, 1172, 1138, 1046, 998, 934, 850, 828 cm^{-1}. - ^{1}H NMR and ^{13}C NMR data see Table 14. - (+)-ESI MS *m/z* (%) = 347.1 ([M+Na]$^+$, 8), 671.0 ([2M+Na]$^+$, 100), 994.8 ([3M+Na-H]$^+$, 48). - EI MS *m/z* (%) = 324 (M$^+$, 76), 265 (22), 251 (100), 206 (52), 44 (27). - (+)-HRESIMS: 325.15398 [M+H]$^+$ (calcd 325.15468 for $C_{19}H_{21}N_2O_3$).

Iguanen B (**93**): Yellow powder. - R_f = 0.32 (CH_2Cl_2 /8%MeOH). - UV (MeOH): λ_{max} (log ε) = 339 (4.02), 318 (4.00), 233 (sh), 206 (4.02) nm. - IR (Film): ν_{max} = 3354, 2948, 2915, 2854, 1669, 1604, 1528, 1490, 1437, 1419, 1380, 1351, 1323, 1254, 1198, 1170, 1046, 998, 934, 850cm^{-1}. - ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.87 (brt, *J* = 1.5Hz, 2''-H), 7.68 (d, *J* = 9.2 Hz, 2H, 2'-, 6'-H), 7.63 (d, *J* = 9.0 Hz, 2H, 3'-, 5'-H), 7.51 (d, *J* = 15.6 Hz, 1H, 1'''-H), 7.50 (brt, *J* = 2.4 Hz, 1H, 5''-H), 7.33 (dd, *J* = 15.2, 9.2 Hz, 1H, 3-H), 7.12 (dd, *J* = 15.5, 9.7 Hz, 1H), 7.10 (d, *J* = 15.3 Hz, 1H), 6.70 (brt, *J* = 1.6 Hz, 1H 2''-H), 6. 16 (d, *J* = 15.8 Hz, 1H, 2'''-H), 6.02 (d, 1H, *J* = 15.1Hz, 1-H). - (-)-ESI MS *m/z* (%) = 308 ([M-H]$^-$, 100), 639 ([2M-H]$^-$, 40). - (+)-HRESIMS: 310.10700 [M+H]$^+$ (calcd. 310.10739 for $C_{18}H_{16}NO_4$).

Inthomycin A (**94**): White powder. - R_f = 0.29 (CH_2Cl_2/MeOH (9:1). - IR (KBr): ν_{max} = 3422cm-', 2928, 1655, 1391,1059, 645. - ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.20 (s, 1H, 13-H), 7.10 (br.s, 1H, NH), 6.90 (s, 1H, 12-H), 6.78 (dd, *J* = 15, 1H, 11 Hz, 8-H), 6.38 (m, 2H, 5-H, 6-H), 5.92 (t, *J* = 11 Hz, 1H, 7-H), 5.78 (m, 1H, 9-H), 5.20 (d, *J* = 5.6 Hz, 1H, 3-OH), 4.60 (d, *J* = 5.6 Hz, 1H, 3-H), 3.48 (d, *J* = 8Hz, 2H, 10-H), 1.78 (s, 3H, 4- CH_3), 1.18 (s, 3H, 2- CH_3), 0.98 (s, 3H, 2-CH_3). - ^{13}C NMR and APT (DMSO-d_6, 125 MHz): δ = 179.0 (Cq, 1-CO), 151.9 (Cq, 11-C), 151.1 (Cq, 13-C), 140.0 (Cq, 4-C), 128.8 (CH, 9-C), 127.9 (CH, 8-C), 127.0 (CH, 7-C), 124.4 (CH, 6-C), 123.3 (CH, 5-C), 121.9 (CH, 12-C), 73.1 (CH, 3-C), 45.4 (Cq, 2-C), 18.2 (CH_2, 10-C), 24.8 (CH_3, 2-C), 21.4 (CH_3, 2-C), 19.8 (CH_3, 4-C). (+)-ESI

MS *m/z* (%) = 313 ($[M+Na]^+$, 100), 603 ($[2M+Na]^+$, 10). - (+) HRESIMS: 313.15227 $[M+Na]^+$ (calcd. 313.15227 for $C_{16}H_{22}N_2O_3Na$).

Ferulic acid (95): Pale yellow Crystals. - R_f = 0.42 (CH_2Cl_2/MeOH 9:1. - ^1H NMR (300 MHz, DMSO-d_6): δ = 9.80 (br, s), 7.50 (d, *J* = 15.9 Hz, 1H, 2-H), 7.25 (d, *J* = 1.5 Hz, 1H, 5-H*)*, 7.10 (dd, *J* = 8.6 Hz, 1H, 9-H), 6.80 (d, *J* = 8.6 Hz, 1H, 8-H), 6.38 (d, *J* = 15.9 Hz, 1H, 3-H), 3.80 (s, 3H, OMe).

9.10 *Streptomyces* sp AdM21

The terrestrial strain AdM21 was cultivated on M_2^+-agar. After four days at 28°C, a white aerial mycelium was formed. The plates were used to inoculate four Erlenmeyer flasks of 250 ml each in the same condition like these for the agar plates. The resulting culture light yellow was extracted with ethyl acetate and used for pre-screening.

9.10.1 Primary screening

The chemical screening showed the presence of some colourless bands which turns violet then black with anisaldehyde/sulphuric acid (R_f = 0.33, CH_2Cl_2/MeOH = 9:1). In pharmacological screening the strain shows an inhibition in the proliferation of cells tumour MCF 7.

9.10.2 Biological Activity of the crude extract AdM21

Test-Organism	Diameter (mm)
Bacillus subtilis	12
Staphylococcus aureus	22
Streptomyces viridochromogenes (Tü 57)	11
Escherichia coli	12
Candida albicans	22
Mucor miehei	23

9.10.3 Fermentation and work-up of strain ADM21

For the terrestrial *Streptomyces* sp. AdM21, 100×1 l Erlenmeyer flasks each containing 250 ml of M_2 medium were inoculated from agar plates and grown for 3 days at 30 °C. The culture broth was mixed with *ca.* 1 kg celite and separated by

pressure filtration. The mycelial cake was extracted three times with ethyl acetate and acetone. Multiple separations of the combined extracts (5.1g) delivered 80 mg of antimycin A complex, and 100 mg of aliphatic fatty acids (mainly palmitic acid).

The water phase was extracted with XAD-16 (column 96 × 32 cm) and the resin washed with water and extracted with methanol. The methanol phase was concentrated and the aqueous residue extracted with ethyl acetate. Chromatography of this extract (1.5 g) on Sephadex LH-20 (CH_2Cl_2/MeOH 6:4) delivered fractions I-IV, which were successively separated by PTLC ($CHCl_3$/10% MeOH). RP HPLC (MeCN/H_2O gradient) of fraction II yielded urauchimycin D (**98**, $t_{R\,=}$ 18.20 min, 45,7% azeotrope MeCN, 2.5 mg), and 5 mg of *trans*-cyclo (Pro,Val) (**96**, $t_{R\,=}$ 17.30 min, 60% azeotrope MeCN, 5 mg). Fraction III gave 5 mg of the violet fluorenone **99** ($R_{f\,=}$ 0.51, CH_2Cl_2/5% MeOH). Fraction IV gived after PTLC 3 subfractions: the subfractions III was separated through preparative RP HPLC (MeCN/H_2O gradient) and gived antimycin A mixture and 40 mg of PHB.

***Trans*-cyclo(Pro-Val)** (**96**): Colourless solid. – R_f = 0.66 (CH_2Cl2 /10 %MeOH).– ^{1}H NMR (CDCl3, 300 MHz): δ = 5.92 (br s, 1H, 4-NH), δ = 4.10 (t, *J* = 6.2 Hz, 1 H, 6-H), δ = 4.00 (dd, *J* = 9.7, 3.7 Hz, 1H, 3-H), 3.60-3.50 (m, 2 H, 9-CH_2), 2.40-1.80 (m, 4 H, 7,8-H), 1.50 (ddd, *J* = 7.1 Hz, 1 H, 10-CH_3), 0.96-092 (d, *J* = 6.5 Hz, 6 H, 11,12-CH_3). – ESI MS: *m/z (*%) = 219 ($[M + Na]^+$, 100).

Antimycin A-mixture (**97**): Oil. - R_f = 0.36 (CH_2Cl_2/2 % MeOH). - ^{1}H NMR ($CDCl_3$, 300 MHz,): δ = 12.64 (s, 1H, 2'-OH), 8.56 (d, *J* = 8.2 Hz, 1H, 4'-H), 8.52 (d, ^3J = 1.2 Hz, 1H, C*H*O), 8.10 (*br*s, 1H, 3'-NH), 7.24 (d, *J* = 8.3 Hz, 1H, 6'-H), 7.17 (d, *J* = 7.6 Hz, 1H, 3-NH), 6.90 (t, *J* = 8.2 Hz, 1H, 5'-H), 5.78 (q, *J* = 7.2 Hz, 1H, 4-H), 5.38 (t, *J* = 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 rest R).

Urauchimycin D (**98**): Yellow solid. – R_f = 0.81 (CH_2Cl_2/5 % MeOH). – $[\alpha]^{20}_D$ = +53° (*c* 0.1, acetone). – UV/vis (MeOH): λ_{max} (lg ε) = 201 (4.02), 221 (3.77), 348 (2.99) nm. – IR (KBr): ν_{max} = 3790, 3662, 3420, 2926, 2855, 2242, 1663, 1583, 1414, 1385, 1206, 1139, 657 cm^{-1}. – ^{1}H-, ^{13}C NMR see

Table **16**. – (+)-ESI MS: *m/z* (%) = 417 ($[M+Na]^+$, 100), 810.8 (28) $[2M+Na]^+$. – (-)-ESI MS: *m/z* (%) = 393 (100) $[M-H]^-$, 809 (98) $[2M-2H+Na]^-$. - (+)-HRESIMS:

m/z = 395.14492 ($[M+H]^+$) (calcd. 395.14490 for $C_{18}H_{23}N_2O_8$), 417.12688 $[M+Na]^+$ (calcd. 417.12685 for $C_{18}H_{22}N_2O_8Na$).

1,6,7-Trihydroxy-3-methylbenzo[a]fluoren-11-one; prefluostatin (**99**): Purple solid. - R_f: 0.47 (5% MeOH/CH_2Cl_2). – UV/vis (MeOH): λ_{max} (lg ε) = 225 (4.54), 258 (4.52), 301 (4.23), 477 (3.75) nm. – IR (KBr): ν_{max} = 3225, 2955, 2918, 2850, 2350, 1711, 1667, 1614, 1585, 1464, 1390, 1366, 1263, 1216, 1160, 1096, 761, 669 cm^{-1}. – ^1H and ^{13}C NMR data see Table 17. – (+)-ESI MS: *m/z* (%) = 293 (10) $[M+H]^+$, 606.8 (4) $[2M+Na]^+$. – (-)-ESI MS: *m/z* (%) = 291 ($[M-H]^-$, 100), 583 (80) $[2M-2H]^-$. - (+)-HRESIMS: *m/z* = 293.08096 $[M+H]^+$ (calcd. 293.08084 for $C_{18}H_{13}O_4$).

9.11 *Streptomyces* sp. Ank 5

The terrestrial strain Ank 5 was cultivated on M_2^+-Agar plates, during the four days incubation at 28°C, agar plates of the growth strain were obtained with a white mycelia. The plates were used to inoculate four Erlenmeyer flasks of 250 ml each in the same condition like these for the agar plates. The resulting culture light yellow was extracted with ethyl acetate and used for pre-screening. The chemical screening showed the presence of some colourless bands, which turned violet, green, blue with anisaldehyde/sulphuric acid.

9.11.1 Biological Activity

The crude extract showed remarkable antifungal activity against *Candida albicans* and a moderate activity against *Streptomyces viridochromogenes* (Tü 57) and *Staphylococcus aureus.* Those activities are as mentioned in the literature, [212] since the butenolides did not exhibited antibacterial activities they were attributed to antimycins, which were isolated in high amounts from this strain.

Table 29: Biological activities of the crude extract Ank 5

Test organism	Diameter (mm)
Staphylococcus aureus	12
Streptomyces viridochromogenes (Tü 57)	11
Candida albicans	32
Brine shrimps	100%

9.11.2 Fermentation and work up

For the terrestrial *Streptomyces* sp Ank 5 100 ×1 l Erlenmeyer flasks each containing 250 ml of M_2 medium were inoculated from agar plates and grown for 3 days at 30 °C with 95 rpm. The culture broth was mixed with *ca.* 1 kg celite and separated by pressure filtration. The mycelial cake was extracted three times with ethyl acetate and acetone. The water phase was extracted with XAD-16 (column 96 × 32 cm) and the resin washed with water and extracted with methanol, which was concentrated and the aqueous residue extracted with ethyl acetate. Multiple separations of the combined extracts (1.5g) on Sephadex LH-20 (column 3 × 70 cm, CH_2Cl_2/MeOH 1:1) delivered 100 mg of antimycin A-complex (**97**) and butenolide (**102**, 20 mg).

(4*S*)-4-Hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**102**): $C_{13}H_{20}O_3$, Oil. - R_f = 0.37 (CH_2Cl_2/5 % MeOH). - UV/vis (MeOH)[212]: λ_{max} (lg ε) = 206 nm (2.31). - ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.48 (dd, *J* = 5.6, 1.5 Hz, 1H, 3-H), 6.16 (dd, *J* = 5.6, 2.0 Hz, 1H, 4-H), 5.03 (m, 1H, 4-H), 2.54 (m, 1H, 10-H), 2.18 (s, 3H, 12-CH_3), 1.70 (m, 1H), 1.60 (m, 2H), 1.50-1.20 (m, 10H), 1.10 (d, *J* = 7.0 Hz, 3H, 13-CH_3). – (+)-ESI MS *m/z* (%) = 247 ($[M+Na]^+$, 20), 471 ($[2M+Na]^+$, 100).

9.12 *Vibrio* sp. WMB4

9.12.1 Fermentation of *Vibrio* sp. WMBA1-4

Vibrio sp. WMBA1-4 was fermented in M11 media with different marine salts concentrations and at different temperatures to select the optimum condition for the bacterial growth and the production of antimicrobial compounds. The optimum temperature was 22-27 °C with 33.5–75 g/l of marine salts mixture in M11.

Five fermentations (100 litres) were carried out with *Vibrio* sp. in M11 at 25 °C with 150 rpm, by pH 4 and aeration of 3-4 l/min. The duration of fermentation (Biostat U20) varied between 46-72 hours. The fermentation process was ended when the oxygen partial pressure started to increase again. 18 g of an oily crude extract were obtained and exhibited UV absorbing spots on TLC, which developed a characteristic yellow to orange colour reaction upon spraying with anisaldehyde/sulphuric acid.

The strain was fermented in a small scale (5x 2L of M1). The chromatogram of the crude extract of WMBA1-4 showed, in the non-polar range, a large number of

peaks that have indole-like UV spectra in the analytical HPLC. After various methods of separation such as silica gel column chromatography, filtration and reversed phase HPLC bis-indol derivates were isolated. (Figure 154)

9.12.2 Biological activities

Compound **103a**, **103b**, **103c**, **103d**, **103e**, **103f**, **105**, **106**, **115**, **116**, **117** , **119** were tested against fungi, gram positive and gram negative bacteria in the group of prof. H. Anke using the agar diffusion test (Table 30), the minimal inhibitory concentration (MIC) assays (Table 31) and cytotoxic test (Table 32).

Table 30: Antimicrobial activities of compounds from *Vibrio* sp. WMBA1-4 in agar diffusion test

Compound	Inhibition zone (mm) 50 µg /disc		
	Bacteria	Fungi	
	B. brevis	*N. coryli*	*M. miehei*
103a	10	12i	
103c	16		
103d	15		
103e	21		
103f	20		
105	20		
106	10		
115	18		13
116	12	30d	
117		12	
119	11		

Table 31: MIC of the metabolites from *Vibrio* sp. WMBA1-4 in the serial dilution assay

Organisms	MIC [µg/ml]									
	103a	**103c**	**103d**	**103e**	**103f**	**105**	**106**	**115**	**116**	**119**
Paecilomyces variotii	50s	50s	-	50s	-	-	-	100s	-	-
Penicillium notatum	-	-	-	-	-	-	100s	50s	-	-
Phytophthora infestans	-	100s	nt	100s	-	-	50s	50s	nt	
Mucor miehei	-	50s	-	50s	-	100s	-	50s	-	-
Nematospora coryli	6-7s	50s	100s	50c	100s	50c	25c	100c	50c	50c
Saccharomyces cerevisiae	-	-	100c	-	-	-	-	100c	-	-
Ustilago nuda	-	100s	-	-	-	-	50c	50c	-	50c
Bacteria:										
Gram-positive:										
Bacillus brevis	100c	25s	100s	6-7s	12.s	25s	50s	50s	-	-
Bacillus subtilis	100s	25c	50s	6-7s	12.5s	25s	12.5s	50s	-	100 s
Micrococcus luteus	100s	25s	100s	12.5s	12.5s	50s	12.5s	50c	-	-
Gram-negative:										
Escherichia coli K12	100c	25c	50s	6-7s	12.5s	25s	12.5s	50s	100s	-
Enterobacter dissolvens	-	-	-	-	-	-	-	-	-	-

- : not active up to 100 µg/ml; nt: not tested s: bacteriostatic/fungistatic; c: bactericidal/fungicidal

Table 32: Cytotoxic activities [µg/ml] of compounds from *Vibrio* sp. WMBA1-4.

Com-pound	L1210		Jurkat		MDA-MB-321		MCF-7		Colo-320	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
103a	30	>100	>100	>100	>100	>100	>100	>100	40	>100
103b	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
103c	50	>100	>100	>100	>100	>100	>100	>100	>100	>100
103d	15	>100	60	>100	>100	>100	>100	>100	>100	>100
103e	15	>100	20	>100	25	>100	20	>100	50	>100
103f	15	>100	25	>100	>100	>100	>100	>100	>100	>100
105	15	>100	50	>100	>100	>100	>100	>100	>100	>100
106	20	100	20	>100	25	90	>100	>100	30	100
115	50	>100	>100	>100	>100	>100	>100	>100	>100	>100
117	50	80	>100	>100	>100	>100	>100	>100	>100	50
119	40	>100	20	>100	100	>100	>100	>100	>100	V

Aqabamycin A (**103a**): Yellow solid. – R_f = 0.41 (CH_2Cl_2/MeOH 9:1). - R_t = 14.30 min (LC MS). - UV (MeOH): λ_{max} (log ε) = 232 nm (4.21), 384 nm (3.81). – IR (KBr): ν_{max} = 2295, 1799, 1709, 1607, 1514, 1351, 1276, 1175, 1020 cm^{-1}. – ^1H NMR (MeOH-d_4, 300 MHz) and ^{13}C NMR (125 MHz, MeOH-d_4): see Table 18 and Table 19. - (-)-ESI MS *m/z* (%) = 264 ($[M-H]^-$, 100), 528 ($[2M-H]^-$, 25). - (-)-HRESIMS: 264.06654 $[M-H]^-$ (calcd. 264.06661 for $C_{16}H_{10}NO_3$).

Aqabamycin B (103b): Yellow solid. – R_f = 0.56 (CH_2Cl_2/MeOH 9:1). - R_t = 13.82 min (LC MS). – UV (MeOH): λ_{max} (log ε) = 232 nm (4.33), 253 nm (4.30), 386 nm (3.95). – IR (KBr): ν_{max} = 2352, 1766, 1715, 1625, 1607, 1536, 1513, 1422, 1346, 1320, 1279, 1250, 1175, 1024, 995 cm^{-1}. – ^1H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR (125 MHz, DMSO-d_6): see Table 18 and Table 19. - EI MS (70 eV): *m/z* (%) = 326.1 ($[M]^{\bullet+}$, 80), 255 (20). - (-)-ESI MS *m/z* (%) = 325 ($[M-H]^-$, 100), 528 ($[2M-H]^-$, 25); - (-)-HRESIMS: 325.04653$[M-H]^-$ (calcd. 325.04660 for $C_{16}H_9N_2O_6$).

Aqabamycin C (**103c**): Yellow solid. – R_f = 0.62 (CH_2Cl_2/MeOH 9:1). - R_t = 15.99 min (LC MS). – UV (MeOH): λ_{max} (log ε) = 228 nm (4.43), 265 nm (4.24), 364 nm (3.96). – IR (KBr): ν_{max} = 2849, 2159, 1771, 1717, 1625, 1537, 1537, 1485, 1445, 1421, 1344, 1322, 1255, 1183, 1126, 1079, 1020 cm^{-1}. – ^1H NMR ($CDCl_3$, 300

MHz): see and ^{13}C NMR (125 MHz, $CDCl_3$): see Table 18 and Table 19. - EI MS (70 eV): *m/z* (%) = 310 ($[M]^{\bullet+}$, 100), 277 (18), 263 (10), 239 (32). - (-)-ESI MS *m/z* (%) = 309 ($[M-H]^-$, 45), 641 ($[2M+Na-2H]^-$, 30). - (-)-HRESIMS: 309.05172 $[M-H]^-$ (calcd. 309.05169 for $C_{16}H_9N_2O_5$).

Aqabamycin D (**103d**): Yellow solid. – R_f = 0.60 (CH_2Cl_2/MeOH 9:1). R_t = 15.02 min (LC MS). – UV (MeOH): λ_{max} (log ε) 232 nm (4.49), 265 nm (4.37), 362 nm (4.05) nm. – IR (KBr): ν_{max} = 2219, 2308, 1724, 1690, 1613, 1564, 1514, 1481, 1415, 1341, 1250, 1151, 1102, 1078, 1012, 974. – ^1H NMR (MeOH-d_4, 600 MHz): see Table 20. - ^{13}C NMR (125 MHz, MeOH-d_4): see Table 21. – EIMS (70 eV): *m/z* (%) = 371 ($[M]^{\bullet+}$, 100), 300 (20), 254 (2). - (-)-ESI MS *m/z* (%) = 370 ($[M-H]^-$, 100), 762 ($[2M+Na-2H]^-$, 70). - (-)-HRESIMS: 370.03155 $[M-H]^-$ (calcd. 370.03168 for $C_{16}H_8N_3O_8$).

Aqabamycin E (103e): Yellow solid. – R_f = 0.58 (CH_2Cl_2/MeOH 9:1). - R_t = 15.58 min (LC MS). – UV (MeOH): λ_{max} (log ε) = 312 (4.23) nm. – IR (KBr): ν_{max} = 3267, 2924, 2288, 1704, 1627 cm^{-1} (oxime), 1535, 1484, 1420, 1342, 1320, 1251, 1177, 1135, 1079, 1026, 1008, 960 cm^{-1}. – ^1H NMR (MeOH-d_4, 600 MHz): see Table 20. - ^{13}C NMR (125 MHz, MeOH-d_4): see Table 21. – EI MS (70 eV): *m/z* (%) = 325 ($[M]^{\bullet+}$, 100), 308 (15), 278 (10), 262 (12). - (-)-ESI MS *m/z* (%) = 324 ($[M-H]^-$, 40), 671 ($[2M+Na-2H]^-$, 30). - (-)-HRESIMS: 324.06254 $[M-H]^-$ (calcd. 324.06259 for $C_{16}H_{10}N_3O_5$).

Aqabamycin F (**103f**): Yellow solid. - R_f = 0.44 (CH_2Cl_2/MeOH 9:1). - R_t = 14.79 min (LC MS). – UV (MeOH): λ_{max} (log ε): 321 (3.99) nm. – IR (KBr): ν_{max} = 1712, 1626 (oxime), 1529, 1484, 1422, 1342, 1320, 1252, 1178, 1135, 1082, 1023, 964 cm^{-1}. – ^1H NMR (MeOH-d_4, 600 MHz): see Table 20. - ^{13}C NMR (125 MHz, MeOH-d_4): see Table 21. – EI MS (70 eV): *m/z* (%) = 386 ($[M]^{\bullet+}$, 25), 370 (5), 328 (5). - (-)-ESI MS *m/z* (%) = 385 ($[M-H]^-$, 100), 792 ($[2M+Na-2H]^-$, 100). - (-)-HRESIMS: 385.04244$[M-H]^-$ (calcd. 385.04258 for $C_{16}H_9N_4O_8$).

Aqabamycin G (**105**): Yellow solid. - R_f = 0.50 (CH_2Cl_2/MeOH 9:1), R_t = 16.02 min (LC MS). – IR (KBr): ν_{max} = 3380, 1707, 1608, 1535, 1423, 1346, 1246, 1165, 1024 cm^{-1}. – ^1H NMR (MeOH-d_4, 600 MHz) and ^{13}C NMR (125 MHz, DMSO-d_6) see Table 22. – (-)-ESI MS: *m/z* (%) = 348 ($[M-H]^-$, 95), 719 ([2M+Na-

2H]$^-$, 100), 1090 ([3M+2Na-3H]$^-$, 60). - (-)-HRESIMS: 348.06250 [M-H]$^-$ (calcd. 348.06259 for $C_{18}H_{10}N_3O_5$).

Aqabamycin H (**106**): Dark yellow solid. - R_f = 0.43 (CH_2Cl_2/MeOH 9:1). R_t = 16.55 min (LC MS). – UV (MeOH): λ_{max} (lg ε): 350 (3.28), 406 (3.31) nm. – IR (KBr): ν_{max} = 3398, 2117, 1636, 1457, 1353, 1207 cm^{-1}. – ^{1}H NMR (MeOH-d_4, 600 MHz) and ^{13}C NMR (125 MHz, DMSO-d_6) see Table 23. – EI MS (70 eV): *m/z* (%) = 277 ([M]$^{\bullet+}$, 100), 260 (20), 247 (12), 218 (20), 204 (15). - (-)-ESI MS *m/z* (%) = 276 ([M-H]$^-$, 95), 552 ([2M-H]$^-$, 40). - (-)-HRESIMS: 276.07801 [M-H]$^-$ (calcd. 276.07784 for $C_{16}H_{10}N_3O_2$).

3-Nitro-4-hydroxybenzaldehyde (**115**): Yellow substance. - $R_{f\,=}$ 0.79 (CH_2Cl_2 /8%MeOH). - UV (MeOH): λ_{max} (log ε) = 264 (4.13), 346 (3.65) nm. - IR (Film): ν_{max} = 1686 cm^{-1}. - ^{1}H NMR (300 MHz, MeOH-d_4): δ = 9.88 (s, 1H, 7-H), 8.12 (d, *J* = 2.0 Hz, 1H, 6-H), 7.62 (dd, *J* = 8.63, 2.0 Hz, 1H, 2-H) 7.25 (d, *J* = 8.7 Hz, 1H, 3-H). - ^{13}C NMR (125 MHz, MeOH-d_4): δ = 191.0 (COH-7), 155.4 (C-4), 136.2 (CNO_2-5), 136.2 (CH-2), 124.4 (CH-6), 120.9 (CH-3), 120.9 (C-1). - (-)-ESI MS *m/z* (%) = 166 ([M-H]$^-$, 35), 355 ([2M+Na-2H]$^-$, 100), 543 ([3M+2Na-3H]$^-$, 40). - (-)-HRESIMS: 166.01448 [M-H]$^-$ (calcd. 166.01457 for $C_7H_4NO_4$).

4-Hydroxy-3-nitrocinnamic acid (**116**): Yellow substance. - R_f = 0.50 (CH_2Cl_2/MeOH 9:1). – UV (MeOH): λ_{max} (log ε) = 360 (3.4), 278 (4.31) nm. – IR (KBr): ν_{max} = 1679, 1620, 1530, 1484, 1286, 1161 cm^{-1}. – ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 12,30 (brs, 1H, 9-OH), 11.41 (brs, 1H, 4-OH), 8.20 (d, *J* = 2.1 Hz, 1H, 6-H), 7.92 (dd, *J* = 8.4 Hz, *J* = 2.1 Hz, 1H, 2-H), 7.58 (d, *J* = 16.1 Hz, 1H, 7-H), 7.18 (d, *J* = 8.4 Hz, 1H, 3-H), 6.45 (d, *J* = 16.2 Hz, 1H, 8-H). - ^{13}C NMR (125 MHz, MeOH-d_4)[201]: δ = 171.2 (9-C), 156.6 (4-C), 142.3 (C-7) 136.2 (5-CNO_2), 136.2 (2-CH), 128.4 (Cq-1), 126.2 (CH-6), 121.7 (Cq-8), 121.4 (CH-3). - EI MS (70 eV): *m/z* (%) = 209 ([M]$^{\bullet+}$, 100), 192 (12). - (-)-HRESIMS: 208.02515 [M-H]$^-$ (calcd. 208.02514 for $C_9H_6NO_5$).

3-Nitro-1*H*-indazole (**117**): Pale yellow solid. - R_f = 0.56 (CH_2Cl_2/MeOH 9:1). – UV (MeOH): λ_{max} (log ε) = 326 (3.5), 234 (3.6) nm. – IR (KBr): ν_{max} = 1679, 1620, 1530, 1484, 1286, 1161 cm^{-1}. – ^{1}H NMR (MeOH-d_4, 300 MHz): δ = 8.21 (brs, 1H, 9-OH), 11.41 (brs, 1H, 4-OH), 8.20 (d, *J* = 8.5 Hz, 1H, 7-H), 7.69 (d, *J* = 8.5 Hz, 1H, 4-H), 7.57 (t, *J* = 8.5 Hz, 1H, 5-H), 7.48 (t, *J* = 8.5 Hz, 1H, 6-H). - ^{13}C NMR (125

MHz, MeOH-d_4): δ = 149.0 (Cq-3), 143.4 (Cq-7a), 129.5 (CH-4) 126.4 (CH-6), 121.6 (Cq-3a), 116.9 (CH-5), 112.6 (CH-7). - (-)-ESI MS *m/z* (%) = 162 ([M-H]$^-$, 18), 346 ([2M+Na-2H]$^-$, 8), 532 ([3M+2Na-3H]$^-$, 20), 727 ([4M+3Na-4H]$^-$, 60), 913 ([5M+4Na-5H]$^-$, 100). - (-)-HRESIMS: 162.03801 [M-H]$^-$ (calcd. 162.03089 for $C_7H_4N_3O_2$).

2-Hydroxy-1*H*-indole-3-carbaldehyde (**119**): Colourless oil. - R_f = 0.44 (CH_2Cl_2/MeOH 9:1). – UV (MeOH): λ_{max} (log ε) = 298 (4.07), 236 (4.02), 242 (4.02) nm. – IR (KBr): ν_{max} = 3192, 2854, 1697, 1670, 1465, 1257, 1138, 1088, 920, 801, 740 cm^{-1}. – ^{1}H NMR (MeOH-d_4, 300 MHz): δ = 14.12 (br, 1H, 1-NH or 2-OH), 10.20 (s, 1H, CHO), 8.18 (d, *J* = 8.0 Hz, 1H, 7-H), 7.72 (d, *J* = 8.0 Hz, 1H, 4-H), 7.54 (t, *J* = 8.5 Hz, 1H, 5-H), 7.38 (t, *J* = 8.5 Hz, 1H, 6-H). – EI MS (70 eV): *m/z* (%) = 161 ([M]$^{\bullet+}$, 2), 146 (100), 118 (30), 91 (18), 63 (10).

1, 4-Dithiane (**120**): Colourless oil. - R_f = 0.51 (CH_2Cl_2/MeOH 9:1). – IR (KBr): ν_{max} = 3430, 2923, 1634, 1399, 1057, 616 cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.18 (s). – EI MS (70 eV): *m/z* (%) = 120 ([M]$^{\bullet+}$, 100), 74 (30), 61 (90).

Vibrindole A (**121**): Colourless oil. - R_f = 0.40 (CH_2Cl_2/MeOH 9:1). – UV (MeOH): λ_{max} (log ε) = 223 (4.55), 282 (4.90) nm; – IR (neat): ν_{max} = 3416, 2958, 2936, 1612, 1456, 743 cm^{-1}. – ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 10.68 (br, 2H, 2-NH), 7.48 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 1.2 Hz, 2H), 7.02 (t, *J* = 8.0 Hz, 2H), 6.85 (t, *J* = 8.0 Hz, 2H), 4.58 (q, *J* = 7.1 Hz, 1H), 1.78 (d, *J* = 7.1 Hz, 3H). – EI MS (70 eV): *m/z* (%) = 260 ([M]$^{\bullet+}$, 40), 245 (100), 217 (10), 144 (10), 122 (15). - (-)-HRESIMS: 259.12434 [M-H]$^-$ (calcd. 259.12406 for $C_{18}H_{15}N_2$).

Phenyl-2-bis-indolylmethan and its cationic form Turbomycin B (122): Red solid. - R_f = 0.57 (CH_2Cl_2/MeOH 9:1). – UV (MeOH): λ_{max} (log ε) = 223 (4.82), 281 (4.00), 290 (3.96) nm. – IR (KBr): ν_{max} = 3412, 2944, 2833, 1619, 1455, 1417, 1338, 1217, 1095, 1227, 744, 701 cm^{-1}. – ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 10.90 (br, 2H, 2-NH), 7.34 (d, *J* = 7.1 Hz, 2H), 7.33 (t, *J* = 7.2 Hz, 2H), 7.26 (d, *J* = 7.1 Hz, 2H), 7.25 (d, *J* = 7.6 Hz, 2H), 7.18 (t, *J* = 7.2 Hz, 1H), 7.03 (t, *J* = 8.1 Hz, 2H), 6.85 (t, *J* = 8.0 Hz, 2H), 6.82 (d, *J* = 2.5 Hz, 2H), 5.82 (s, 1H). - ^{13}C NMR (125 MHz, DMSO-d_6): δ = 144.9 (Cq), 136.5 (2Cq) 128.3 (2CH), 128 (2CH), 126.6 (2Cq), 125.7 (CH), 123.5 (2CH), 120.8 (2CH), 119.1 (2CH), 118.1 (2CH), 118.0 (2Cq), 111.4 (2CH), 40.0 (CH). - (-)-ESI MS *m/z* (%) = 319 ([M-H]$^-$, 100), 683

([2M+HCOO$^-$], 40). - (+)-ESI MS *m/z* (%) = 321 ([M+H]$^+$, 10), 663 ([2M+Na]$^+$, 10). (+)-APCI MS *m/z* (%) = 322 ([M+H]$^+$, 10). (-)-APCI MS *m/z* (%) = 319 ([M+H]$^+$, 100). – EI MS (70 eV): *m/z* (%) = 322 ([M]$^{\bullet+}$, 100), 245 (55), 206 (55), 144 (10), 122 (10). - (-)-HRESIMS: 321.13866 [M-H]$^-$ (calcd. 321.13970 for $C_{23}H_{17}N_2$).

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Lebenslauf

Am 07.09.1973 wurde ich als fünftes Kind der Eheleute Nana Colette und Yao David in Jaunde, Kamerun geboren.

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Seit Oktober 2004 arbeite ich unter Anleitung von Prof. Dr. H. Laatsch an der vorliegenden Dissertation mit dem Titel „Aqabamycins, Rare Nitro Maleimides and other Novel Metabolites from Microorganisms; Generation and Application of an HPLC-UV-ESI MS/MS Database“

Ich besitze die kamerunische Staatsbürgerschaft.

Göttingen, den 09.12.2007

Fotso-Fondja Yao Clarisse Blandine

Publications List

1. **Clarisse B. Fondja Yao**, *M. Schiebel, E. Helmke, H. Anke, and H. Laatsch*: Marine Bacteria, XXXIII: Prefluostatin and New Urauchimycin Derivates Produced by *Streptomycete* Isolates. *Z. Naturforsch*. 61B (**2006**) 320-325

2. I. Sajid, **Clarisse B. Fotso-Fondja Yao**, K.A Shaaban, S Hasnain and H Laatsch, Antifungal and antibacterial activities of indigenous S*treptomyces* isolates from saline farmlands: pre-screening, ribotyping and metabolite diversity. *World J. Microb. Biot*. (**2009**) 601-610

3. Serge Fotso, **Clarisse B. Fotso-Fondja Yao** and Hartmut Laatsch. Iguanen A and B: New Secondary metabolites from terrestrial *Streptomyces* sp AdM 19. *Eur. J. Org. Chem.* (to be submitted)

4. **Clarisse B. Fotso Fondja Yao**, Wael Zereini, S. Fotso, H. Anke and H. Laatsch, Euphamycin A and B from the Marine *Pseudoalteromonas* sp. T 268. *J. Antibiot*. (to be submitted)

5. **Clarisse B. Fotso Fondja Yao,** Wael Zereini, S. Fotso, H. Anke and H. Laatsch. Aqabamycin A-G: Novel Nitro Maleimides from a Marine *Vibrio* species. II. Structure Elucidation. *J. Antibiot*. (to be submitted)

6. Wael Zereini, **Clarisse B. Fotso-Fondja Yao**, Hartmut Laatsch, Heidrun.Anke. Aqabamycins A-G: Novel Nitro Maleimides from a Marine *Vibrio* species. I. Taxonomy, Fermentation, Isolation and Biological Activities, *J. Antibiot*. (to be submitted)

7. Serge Fotso, **Clarisse B**. **Fotso-Fondja Yao** and H. Laatsch. Silamycin A and B: First members of an unusual 15-membered cyclic antibiotic Isolation, Structure Elucidation and Biological Activities. *Eur J. Org. Chem.* (to be submitted)

8. Serge Fotso, **Clarisse B. Fotso-Fondja Yao** and H. Laatsch. Moyopomycin A and B: Two unusual metabolites from the terrestrial *Streptomyces sp*. GW 4723. *J. Antibiot*. (to be submitted)

9. Serge Fotso, **Clarisse B. Fondja-Fondja Yao** and H. Laatsch, Rare naphthacenequinones from the terrestrial *Streptomyces* sp. GW 4723. *J. Nat. Prod*. (to be submitted)

10. **Clarisse B. Fotso-Fondja Yao**, Serge Fotso, and Hartmut Laatsch A rare bicyclo ring system and cinnamic derivatives from *Streptomyces* sp. AdM 02, *Eur J. Org. Chem.* (in preparation)

11. Serge Fotso, **Clarisse B. Fotso-Fondja Yao**, Iris Grün-Wollny, and Hartmut Laatsch Isolation, Structure elucidation of 2-hydroxy-Luisol A novel tetraol from a Marine *Streptomyces* sp. (Actinomycetes) and reduction products of Luisol A. *Z. Naturforsch*. (in preparation)

12. **Clarisse B. Fotso-Fondja Yao**, Serge Fotso, and Hartmut Laatsch New secondary metabolites from the terrestrial *Streptomyces* Anke 2. *Z. Naturforsch*. (in preparation)

13. **Clarisse B. Fotso Fondja Yao,** Wael Zereini, S. Fotso, H. Anke and H. Laatsch. Aqabamycin H, a Novel Azirin Derivative from a Marine *Vibrio* species from a Marine *Vibrio* species. *Angew. Chem* (in preparation)

POSTER

1. **Clarisse B. Fotso-Fondja Yao**, W. Zereini, S. Fotso, H. Anke and H. Laatsch. 1. Göttinger Chemie-Forum, **2007** „Aqabamycin A-H: Rare Nitro Maleimides and a New Azirin from a *Vibrio* sp. WMB4“

2. **Clarisse B. Fotso-Fondja Yao**, S. Fotso, Grün Wollny, H. Anke and H. Laatsch „Begabt, … genial, … GW 4723, ein einzigartiger Produzent ungewöhnlicher Strukturen „ Poster awarded "Best Poster" and „Best complexe structure" of the 20-Irseer Naturstofftage der DECHEMA e.V. See Newsletter No. 9, der Fakultät für Chemie, October **2008**, page 6 (http://www.uni-goettingen.de/de/28481.html)

COMMUNICATIONS

1. **Fotso-Fondja Yao C. B**. and Laatsch, H.: Kinamycin the never-ending story, Goslar, Germany 18-20 September **2005**

2. **Fotso-Fondja Yao C. B**. and Laatsch, Myxomycete compounds from Bacteria. Do slime molds have also symbionts? Tübingen, Germany 18-20 September **2006**

3. **Fotso-Fondja Yao C. B**. and Laatsch H.: A potent skin tumor promoter Lyngbyatoxin A acetate derivative and a new untypical chromophor from *Streptomyces* sp. AdM 02 Tübingen, Germany 19-21 September **2007**