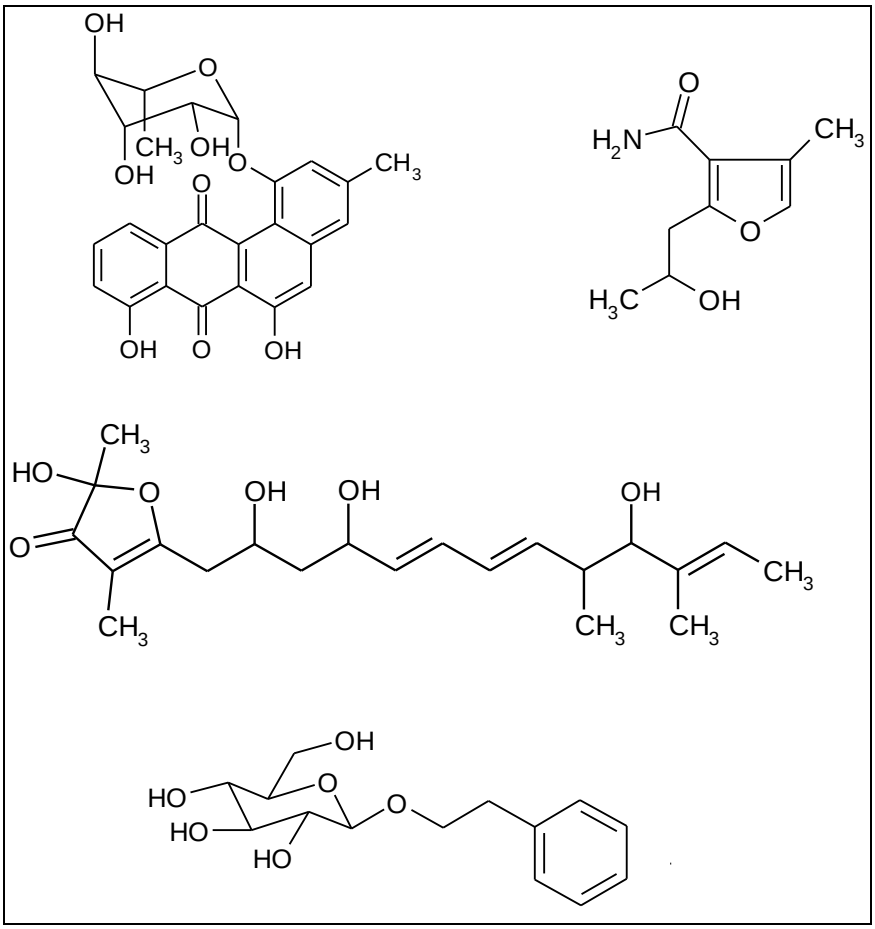
Humaira Naureen

Dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside, Actinofuranone C and Further New Bioactive Secondary Metabolites from Terrestrial *Streptomyces* spp.



Dissertation

Dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside, Actinofuranone C and Further New Bioactive Secondary Metabolites from Terrestrial *Streptomyces* spp.

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultäten

der Georg-August-Universität zu Göttingen

vorgelegt von

Humaira Naureen

aus

Chakwal (Pakistan)

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D7

Referent: Prof. Dr. H. Laatsch

Korreferent: Prof. Dr. U. Diederichsen

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

For my parents

and my husband

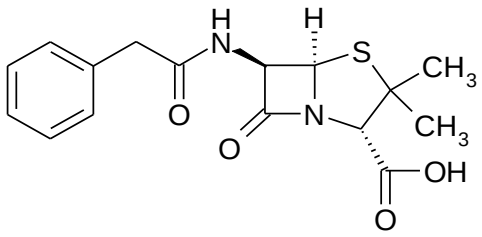
Table of Contents

1 Introduction

1.1 Importance of natural products in drug discovery

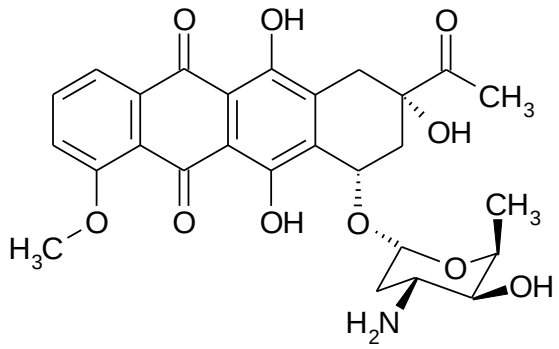
The term *Secondary metabolites* can be defined as "chemical substances which are made by living organisms and do not actively participate in primary metabolism. These chemicals that are usually found in few families or species and don't seem to serve a purpose in minute-to-minute activities of cells."[1, 2]

The World Health Organization estimates that approximately 80 percent of the world's population relies on traditional medicines for their primary health care.[3] History of medicines starts with the onset of human civilization when man started to use specific medicinal plant parts for treating their illnesses. Until the discovery of penicillin (**1**) by Fleming in 1928, most of the natural products were obtained from plants. In the early 1940s, the success of penicillin in treating infections during World War II opened up a new era of drug discovery from microorganisms. Microorganisms remain a fruitful source of structurally diverse bioactive natural products including penicillins, cephalosporins, aminoglycosides, tetracyclines and polyketides.[2]



1

In addition to a number of antibacterial and antifungal agents isolated from microorganisms, current therapeutic applications of microbial metabolites have expanded to anticancer agents (daunorubicin, **2**),[4] cholesterol-lowering agents (lovastatin, **3**),[5] antihelmintic agents (avermectin B1a, **4**),[6] immunosuppressive agents (rapamycin, **5**)[7] and antidiabetic agents (acarbose, **6**)[8], etc.[9]



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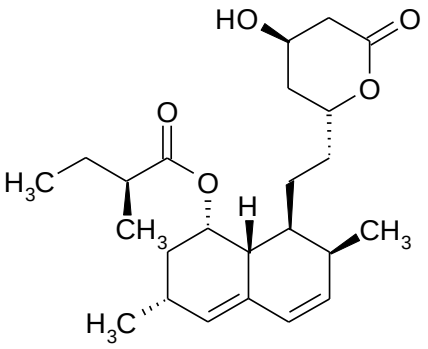

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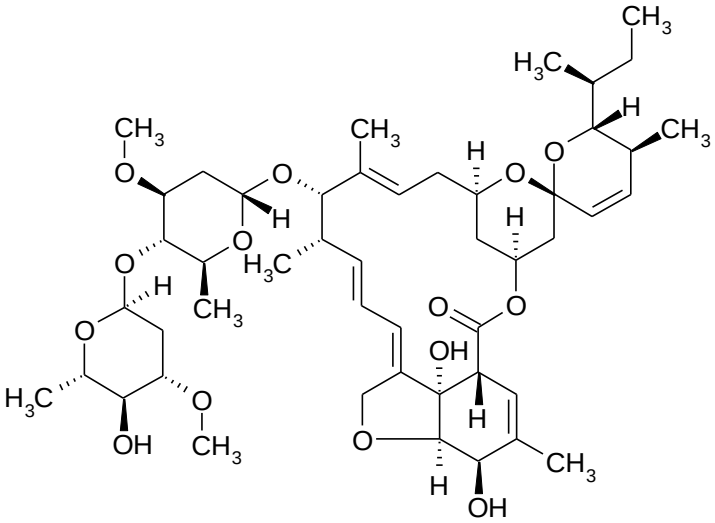

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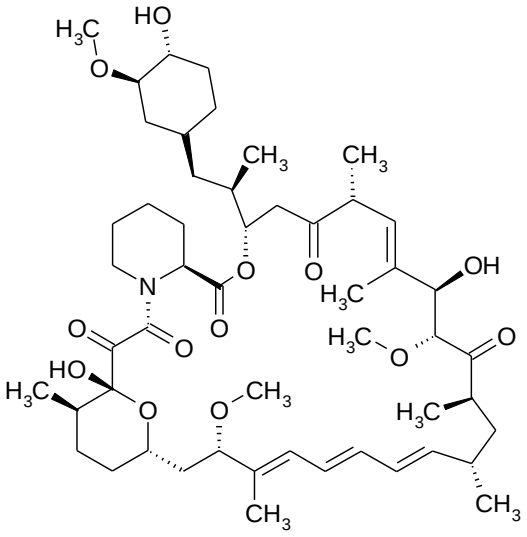

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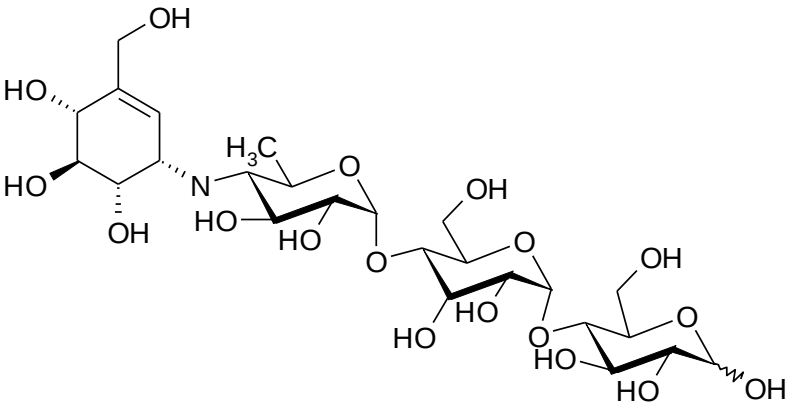

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However, due to the rise of resistance in infectious microorganisms and emergence of new diseases like AIDS, hepatitis, Swine flu, and a number of cancer types; the development of new drugs is needed. Over the last decade, a variety of advanced techniques such as combinatorial chemistry, high-throughput screening (HTS) and rational drug design have provided significant achievement in natural product drug discovery research.[10]

A search in the literature revealed that out of the total number of approved drugs in United States from 1981-2007, 10-20% are natural products.[11] In a recent review, Newman *et al*. reported an update of their previously published chart (1981-2006)[12] where they showed a data of new drugs in the market from 1981-2008 which indicated an increase of new natural compounds from 28% to 33% in two years.[13]

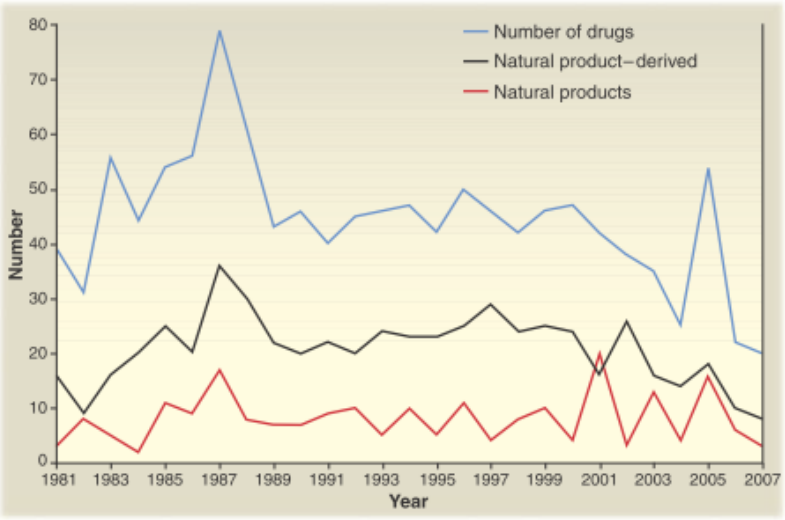
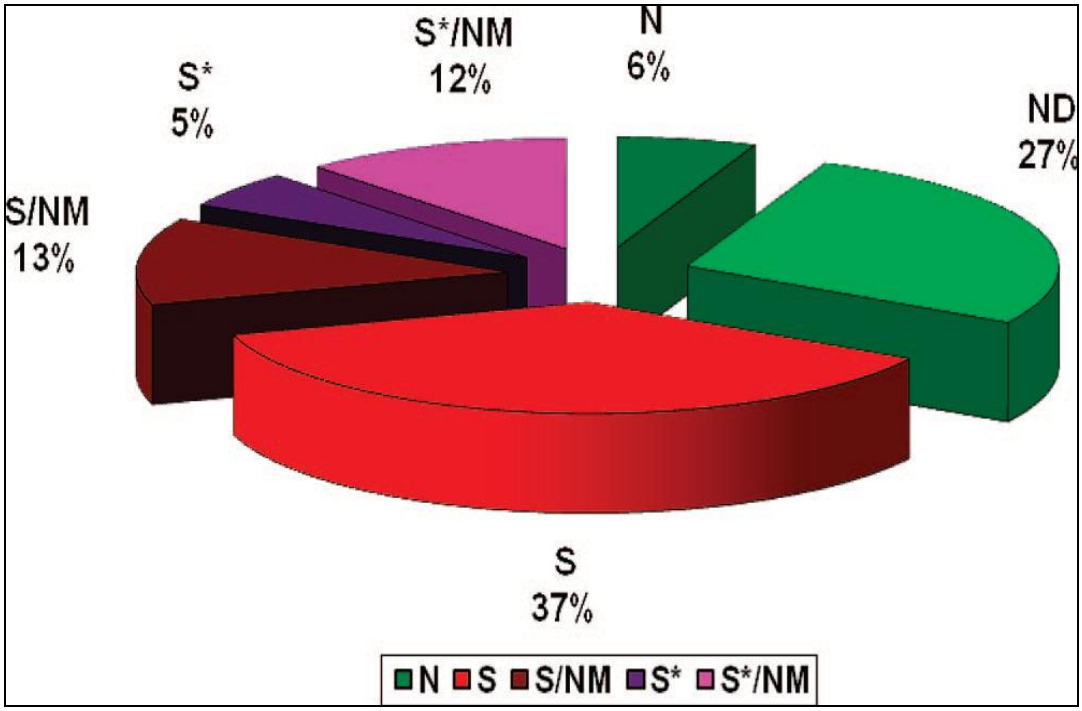


Figure 1: Number of drugs approved in the United States from 1981-2007. [11]



N	Unmodified natural product
ND	Derived from a natural product and is usually a semi synthetic modification.
S	Synthetic compound with no natural product conception
S/NM	Synthetic compound showing competitive inhibition of the natural product substrate
S*	Made by total synthesis, but the pharmacophore came from natural product.
S*/NM	Synthetic compound with natural product pharmacophore showing competitive inhibition of natural product substrate

Figure 2: New chemical entities from natural sources, 1/1981-10/2008 [13]

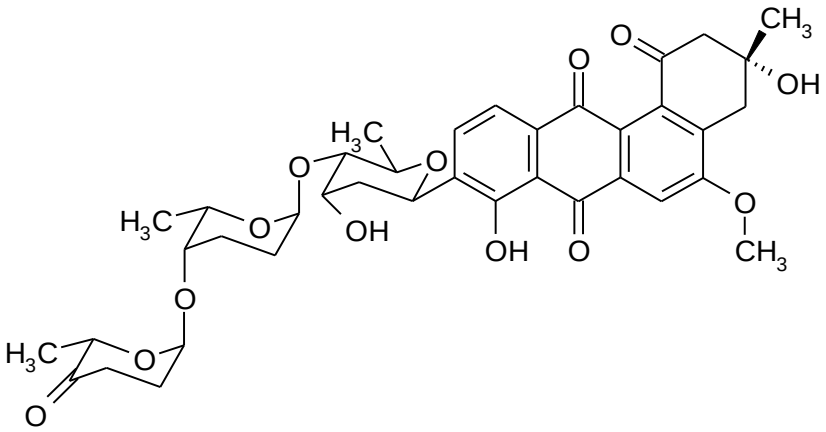
1.2 Recently isolated metabolites from *Streptomyces* spp.

Streptomycetes are Gram-positive, sporulating bacteria with high GC content, predominantly distributed in soil. [14] More than 500 species of these fascinating bacteria have been identified.[15] They are characterized by a complex secondary metabolism producing antibiotics and other metabolites with medicinal activities. The genus *Streptomyces* is an important group of family Streptomycetaceae, belonging to the order Actinomycetales and the phylum Actinobacteria. Among the total biologically

active compounds that have been obtained so far from microbes, 45% are produced by actinomycetes.[16]

Among the many metabolites isolated from *Streptomyces*, main groups can be classified into β-lactam antibiotics, ansamycins, aminoglycosides, tetracyclines, macrolides and anthracyclines etc.[17]

Angucyclines are natural microbial quinones related to tetracyclines and anthracyclines which possess a characteristic structural feature, a benz[a]anthraquinone ring system assembled in an angular fashion.[18] They show a broad spectrum of biological activities including antibacterial,[18, 19] antifungal,[20] antitumor,[18,19] antiviral,[21] enzyme inhibitory[22, 23] and platelet aggregation inhibition properties.[24, 25] Angucyclines are mostly isolated from the genus *Streptomyces*. Three novel angucyclines NO5WA963A, B and C (**7**-**9**) with antiproliferative activity against cancer cells were recently isolated from a *Streptomyces* strain.[26]



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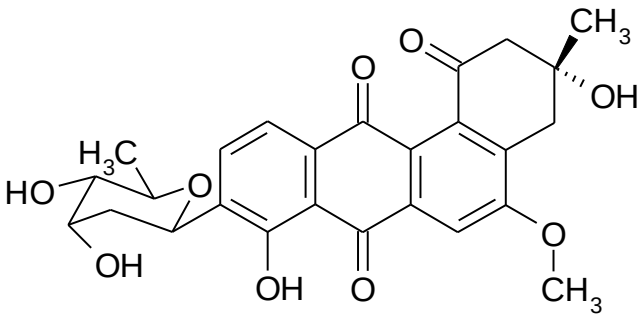

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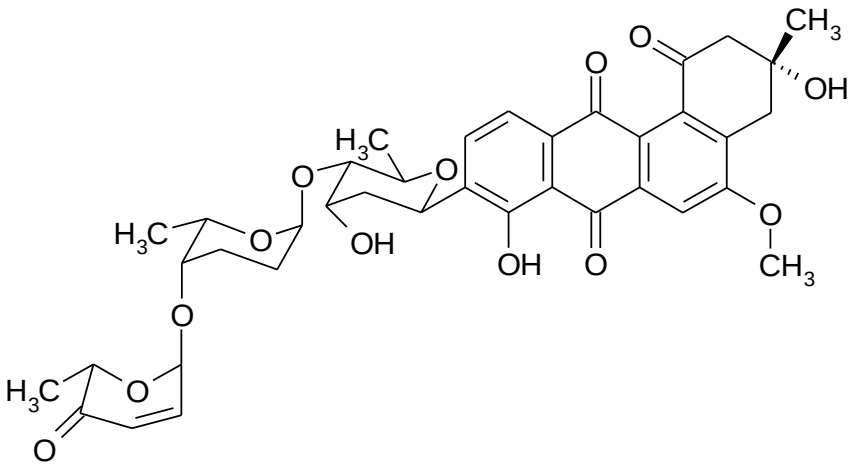
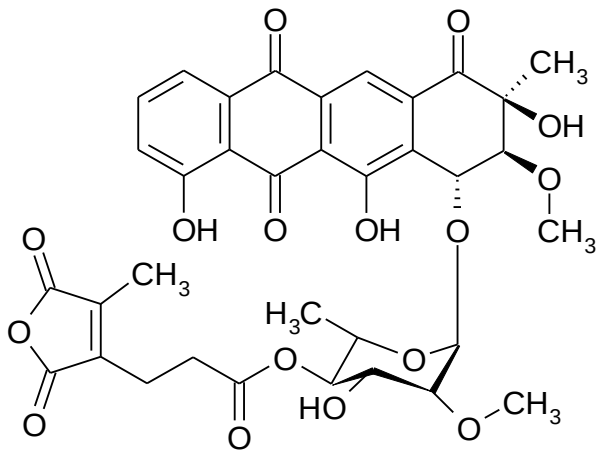


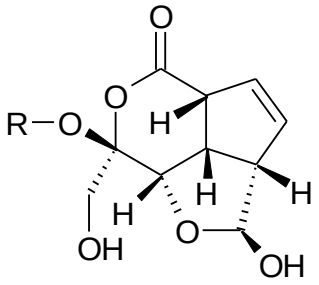
9

More than 70 natural angucyclines and their aglycones have been published so far. The related anthracyclines are the most effective chemotherapeutic agents in cancer treatment produced from *Streptomyces* sp. e.g. daunorubicin, adriamycin etc. A new anthracycline antibiotic, aranciamycin anhydride (**10**) was isolated from *Streptomyces* sp. Tü 6384 which showed growth inhibitory activity against selected human tumor cell lines.[27]

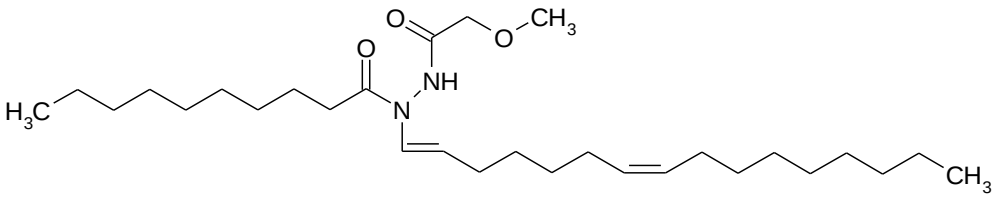


10

Novel and diverse natural products can provide new ways and ideas to synthetic chemists for development and improvement in drug discovery research. Lucknolides A (**11**) and B (**12**) with unprecedented, highly functionalised tricyclic ketal-lactone ring were isolated recently in our group from terrestrial *Streptomyces* sp. Ank 289.[28] Moreover, two novel alkylhydrazines; elaiomycin B (**13**) and C (**14**) were isolated from *Streptomyces* strain BK 190.[29]



11: R = H
12: R = CH_3



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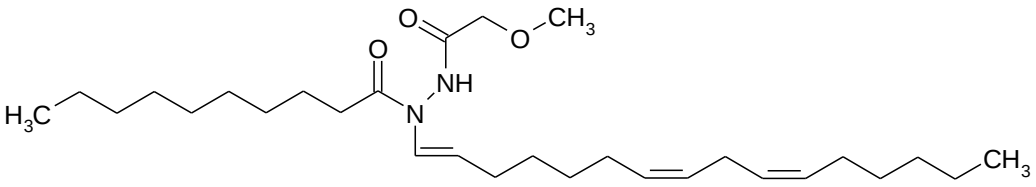
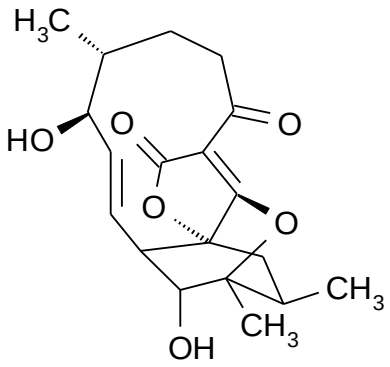


14

Abyssomicins are bioactive complex spirotetronate metabolites discovered by Fiedler and Süssmuth in Tübingen (Germany) in 2004.[30] Abyssomicin I (**15**), a new member of this class was recently isolated from a soil-derived *Streptomyces* strain and exhibited inhibitory effects on tumour cell invasion with IC_{50} value of 11μM.[31] Additionally, in our group, two new derivatives; *ent*-homoabyssomicin A (**16**) and B (**17**) with opposite stereochemistry were isolated from terrestrial *Streptomyces* sp. Ank 210.[32]



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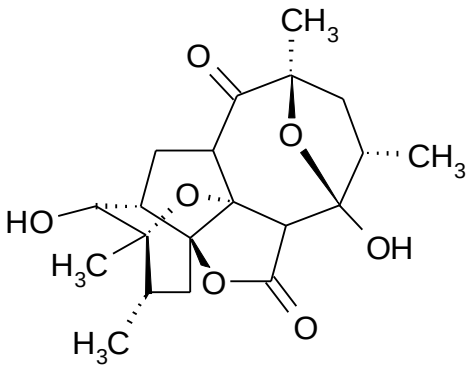

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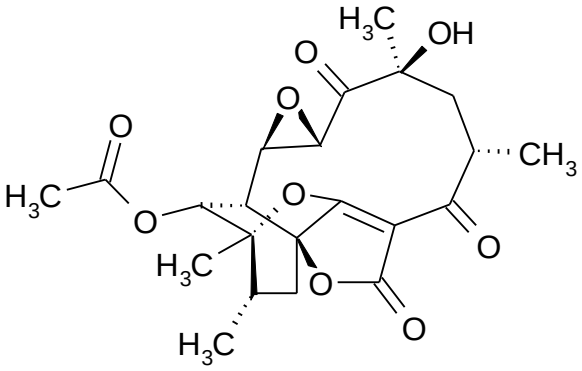
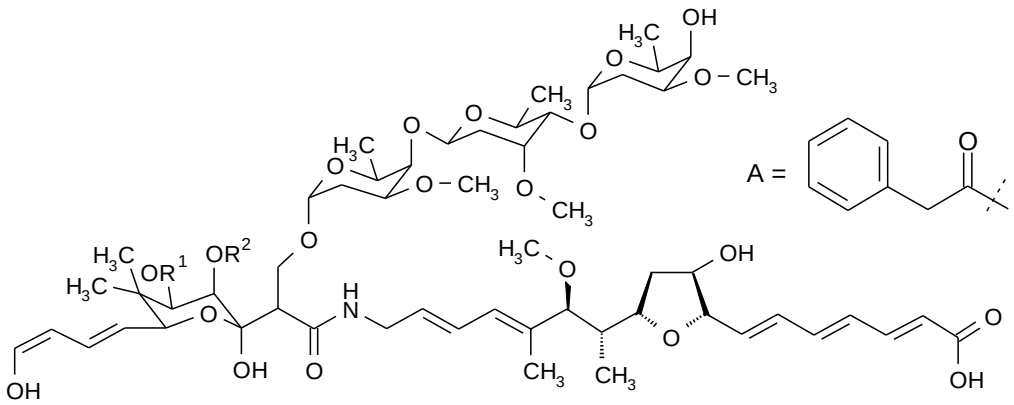


17

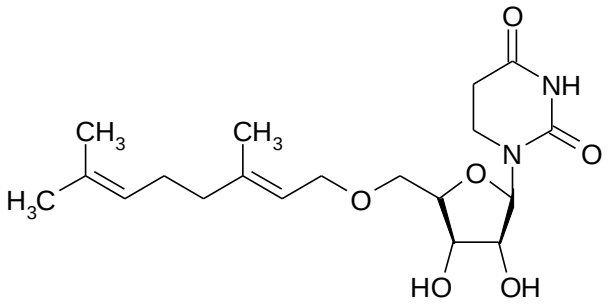
Fiedler and co-workers also isolated two new derivatives of elphamycin-type antibiotics, namely phenelphamycin G (**18**) and H (**19**) from *Streptomyces albospinus* Acta 3619. The compounds **18** and **19** showed a narrow antibacterial spectrum with a profound inhibitory activity against *Propionibacterium acnes*. Moreover, both compounds also showed weak inhibition of acetylcholine esterase (ACE).[33]



18: R^1 = H, R^2 = A
19: R^1 = A, R^2 = H

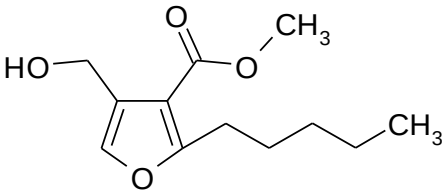
The influenza virus is a major pathogen, which causes catastrophic mortality among human and animals. The recent occurrence of the Avian influenza in Asia and

subsequently worldwide emergence of swine-origin influenza virus A (H1N1) highlighted the strong need of safe and effective treatments. Recently, a novel compound; JBIR-68 (**20**) with anti-influenza virus activity was discovered from *Streptomyces* sp. RI18.[34]



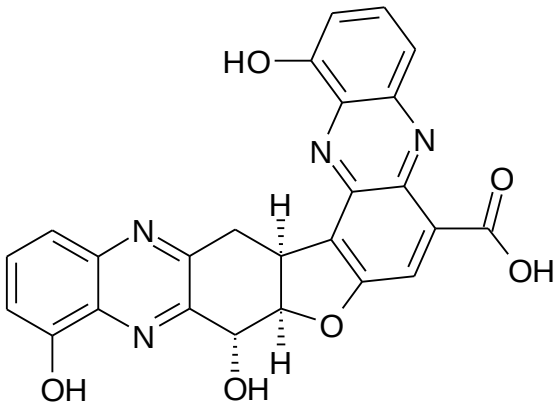
20

Highly-substituted furan compounds play an important role not only as synthetic intermediates and useful building blocks in organic chemistry but also as pharmaceuticals, fragrances and flavours.[35] A cytotoxic metabolite containing 2,3,4-trisubstituted furan ring namely HS071 (**21**) was isolated from *Streptomyces* sp. HS-HY-071 which revealed *in vitro* activity against HCT-116 cancer cells with an IC_{50} of 18.2 μg/ml.[36]



21

Furthermore, three new phenazine derivatives, named izumiphenazines A-C (**22**-**24**) were isolated from soil-derived *Streptomyces* sp. IFM 11204. Compounds B and C in combination with TRIAL (TNF-related apoptosis-inducing ligand) showed synergistic activity in sensitising TRIAL-resistant AGS cells, thereby suggesting their possible use against human gastric adenocarcinoma.[37]



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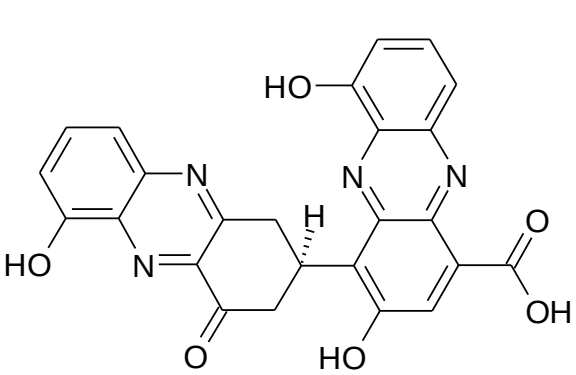

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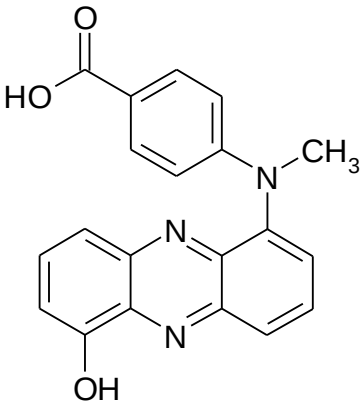

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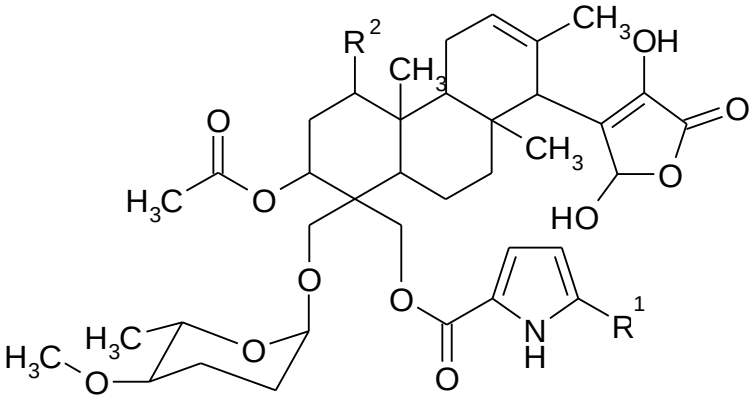
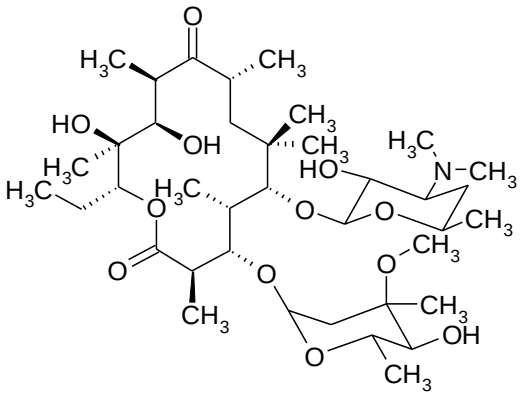
Phenalenolactones A-D (**25**-**28**) are new terpenoglycoside antibiotics obtained from *Streptomyces* sp. Tü 6071. These compounds showed an inhibitory activity against Gram-positive bacteria.[38]



25: $R^1 = CH_3$, $R^2 = OH$
26: $R^1 = H$, $R^2 = OH$
27: $R^1 = CH_2OCH_3$, $R^2 = OH$
28: $R^1 = CH_3$, $R^2 = H$

Macrolides are a further important group of secondary metabolites with interesting biological activities. Until now, nearly one hundred 14-membered macrolides and some other compounds with different ring sizes up to a 60-membered ring including polyene macrolides, macrodiolides, macrotetrolides and macrolide lactams were produced from actinomycetes.[39] The well known antibacterial antibiotic, erythromycin (**29**), a 14-membered ring macrolide, used for people allergic to penicillins was also isolated from *Streptomyces erythreus*.[40] A new cytotoxic 16-membered tetraene macrolide JBIR-100 (**31**) was recently isolated from a newly identified *Streptomyces* strain.[41] Additionally, another polyene macrocyclic lactam, sceliphrolactam (**30**) is reported from a wasp-associated *Streptomyces* sp. The compound **30** displayed antifungal activity against amphotericin B-resistant *Candida albicans*.[42]



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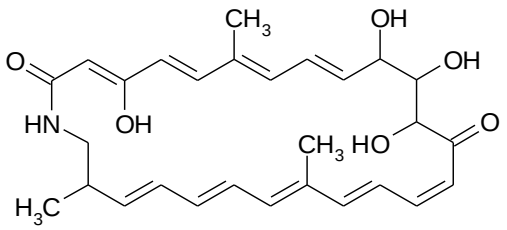

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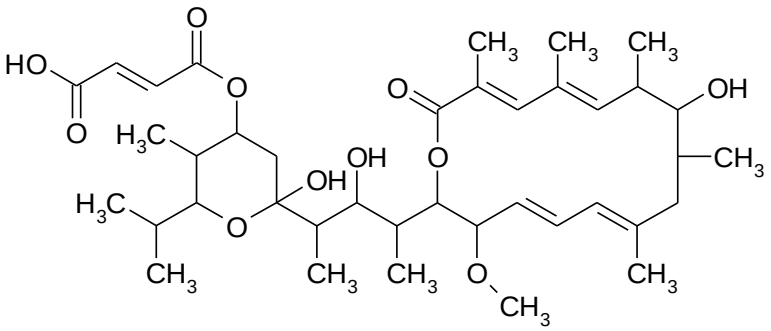
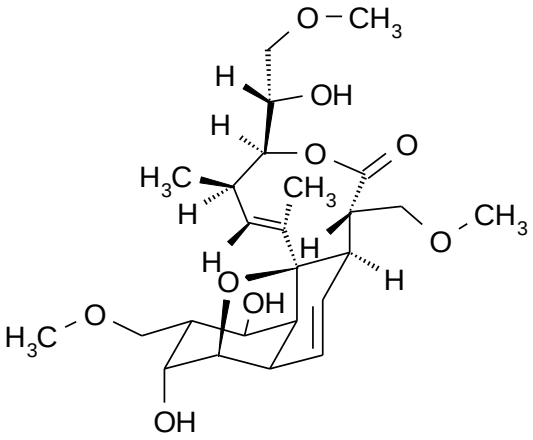


31

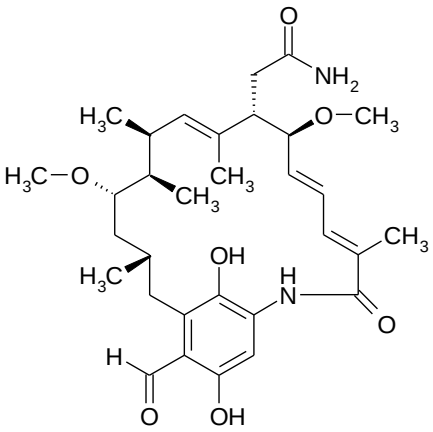
Branimycin (**32**), the most complex member of nargenicin antibiotics, was isolated in our research group form terrestrial *Streptomyces* strain GW 60/1571.[43] It exhibited profound antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and particularly against *Streptomyces viridochromogenes*

(Tü 57). Due to the interesting structure and high biological activities, it is also recently synthesized by Mulzer *et al*.[44]



32

The ansamycins are a growing class of complex macrolactam antibiotics from streptomycetes. They contain a characteristic cyclic structure in which an aliphatic ansa chain forms a bridge between two non-adjacent positions of a cyclic π-system. Most of them exhibit antibacterial, antifungal and antitumor activities. Rifamycins, streptovaricins and geldanamycins are important members of this class. A new geldanamycin analogue; 11-methoxy-17-formyl-17-demothoxy-18-O-21-O-dihydrogeldanamycin (**33**) was isolated from *Streptomyces hygroscopicus*. The compound **33** showed considerable cytotoxicity against human cancer cell lines (breast cancer, MCF-7, skin melanoma SK-MEL-2 and lung carcinoma COR-L23).[45] In addition, two new derivatives of a non-quinone geldanamycin DHQ3 (**34**), DHQ7 (**35**) and DHQ8 (**36**) were reported from a genetically engineered strain of *Streptomyces hygroscopicus*. These compounds showed improved inhibitory activity against heat shock protein (Hsp90) as compared to geldanamycin.[46]



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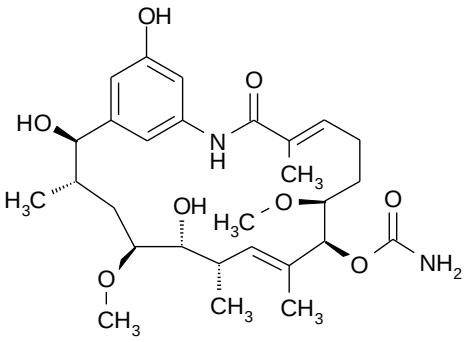

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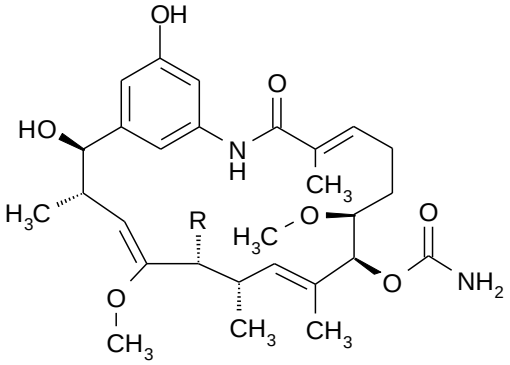
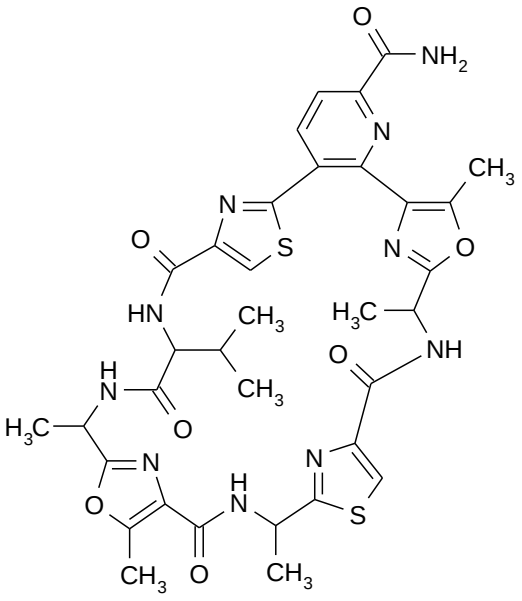


35: R = OH

36: R = $OCONH_2$

Thiopeptide antibiotics containing numerous thiazole and oxazole rings and other specified residues such as dehydroamino acids, are a large group of macrocyclic peptides produced by bacteria. Many thiopeptides possess a broad spectrum of biological activity, most prominently high potency against Gram-positive bacteria including multidrug-resistant *Staphylococcus aureus* (MRSA). JBIR-83 (**37**) and JBIR-84 (**38**) are the recent examples of this group isolated from *Streptomyces* sp. RI19.[47] These compounds are derivatives of promothiocin A (**39**) which was also isolated from a *Streptomyces* strain in 1994.[48] The compound **37** showed weak free radical-scavenging activity.[47]



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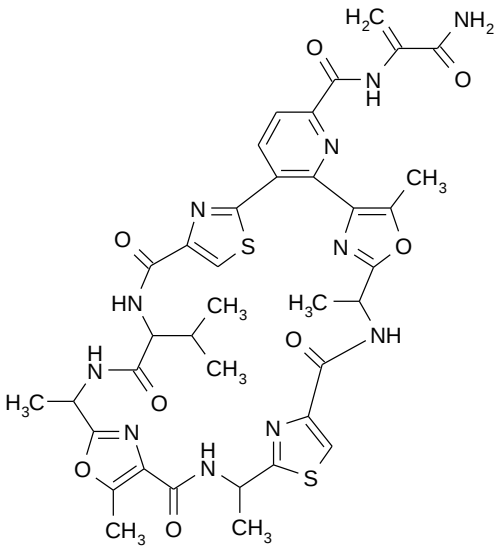

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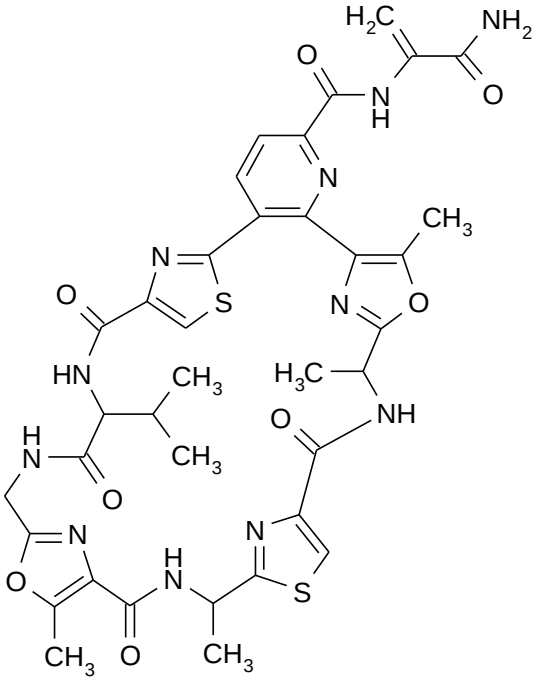
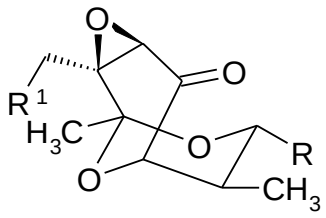


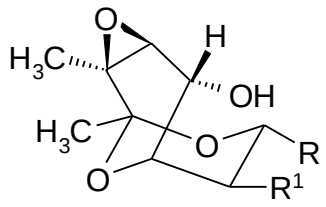
39

Lymphatic filariasis is a parasitic disease caused by two nematodes *Burgia malayi* and *Wucheria bancrofti*. Recently three new tirandamycins, TAM E (**42**), F (**43**), G (**44**) along with already known TAM A (**40**) and B (**41**) were discovered from *Streptomyces* sp. 17944. Among them, TAM B (**41**) selectively inhibited asparagine tRNA synthetase of *B. malayi* and killed the adult *B. malayi* parasite.[49]



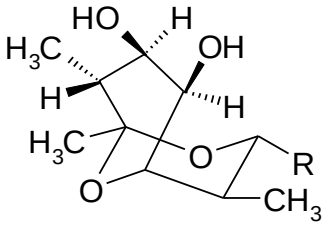
40: $R^1 = H$

41: $R^1 = OH$



42: $R^1 = CH_3$

43: $R^1 = H$



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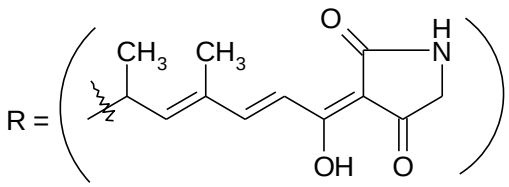


2 Objectives of the present research work

Natural products have not only played a crucial role in medicine but also contributed to agrochemicals, pesticides and in cosmetics. However, the new chemical entities as commercial therapeutic agents from nature have been declined after the so called "golden period of natural product drug discovery". New and innovative approaches such as advances in genomics, searching for new secondary metabolites from unexplored environments and application of new and automotive screening technologies are required for more successful natural product drug discovery research.

- The main objectives of this work are isolation, purification and structure elucidation of new and preferably bioactive secondary metabolites from streptomycetes obtained from different ecological sources. For this purpose, biological and chemical (TLC/HPLC-MS) screening for selection of suitable strains is required to be performed in a sequence outlined in the following steps;

- "Talented strains" need to be identified by the biological activity tests against different pathogenic bacteria, fungi, algae as well as brine shrimp cytotoxicity assay. They should also be selected on the basis of TLC spot patterns such as polarity, interesting colour reactions with anisaldehyde/sulphuric acid or Ehrlich's reagent.

- After screening and adaptation of the culture conditions (pH, duration of the fermentation, medium type) of the selected strains, the fermentation on a large scale should be carried out for isolation of different metabolic constituents.

- The crude extracts obtained from large-scale fermentation must be separated by various chromatographic techniques (silica gel, Sephadex LH-20, RP-18 column chromatography, HPLC, PTLC etc.) into pure metabolites.

- *Dereplication*: The pure compounds will be identified as new or known metabolites by various spectroscopic methods (NMR, MS, IR, UV, X-ray crystal analysis if possible) and through the search in different databases such as AntiBase, the Dictionary of Natural Products and the Chemical Abstracts.

Finally, the isolated new and pure metabolites will be submitted to different bio-assays (antimicrobial tests, brine shrimp assay). It should be stated that also the known compounds might exhibit new bioactivities, when tested against new targets.

3 General techniques

3.1 Collection of strains

The bacterial strains for this research project were obtained from different collaborations with microbiological groups summarized as follows:

- The terrestrial *Streptomyces* spp. (code beginning with Ank) were obtained from Prof. Dr. H. Anke, Institute for Biotechnology and Drug Research, Kaiserslautern, Germany.

- The terrestrial *Streptomyces* spp. (code beginning with GW) were obtained from the laboratory of Dr. Iris Grün-Wollny, Giessen, Germany.

- The terrestrial *Streptomyces* spp. (code beginning with WO) was obtained from Prof. Dr. Wolf (for searching of compounds with activity against plant-pathogenic fungi).

3.2 Working up procedure of selected bacterial strains

A well-defined strategic procedure is required for investigation of large number of bacterial strains in order to select the most appropriate strain. To achieve this purpose, biological and chemical screening techniques must be combined. Then the cultivation of interesting strain is scaled up and isolation procedure will be employed to isolate the pure metabolites, structure elucidation is then performed followed by the activity tests for the isolated compounds were carried out. The general procedure for evaluating bacterial strains to be investigated is summarized in Figure 3.

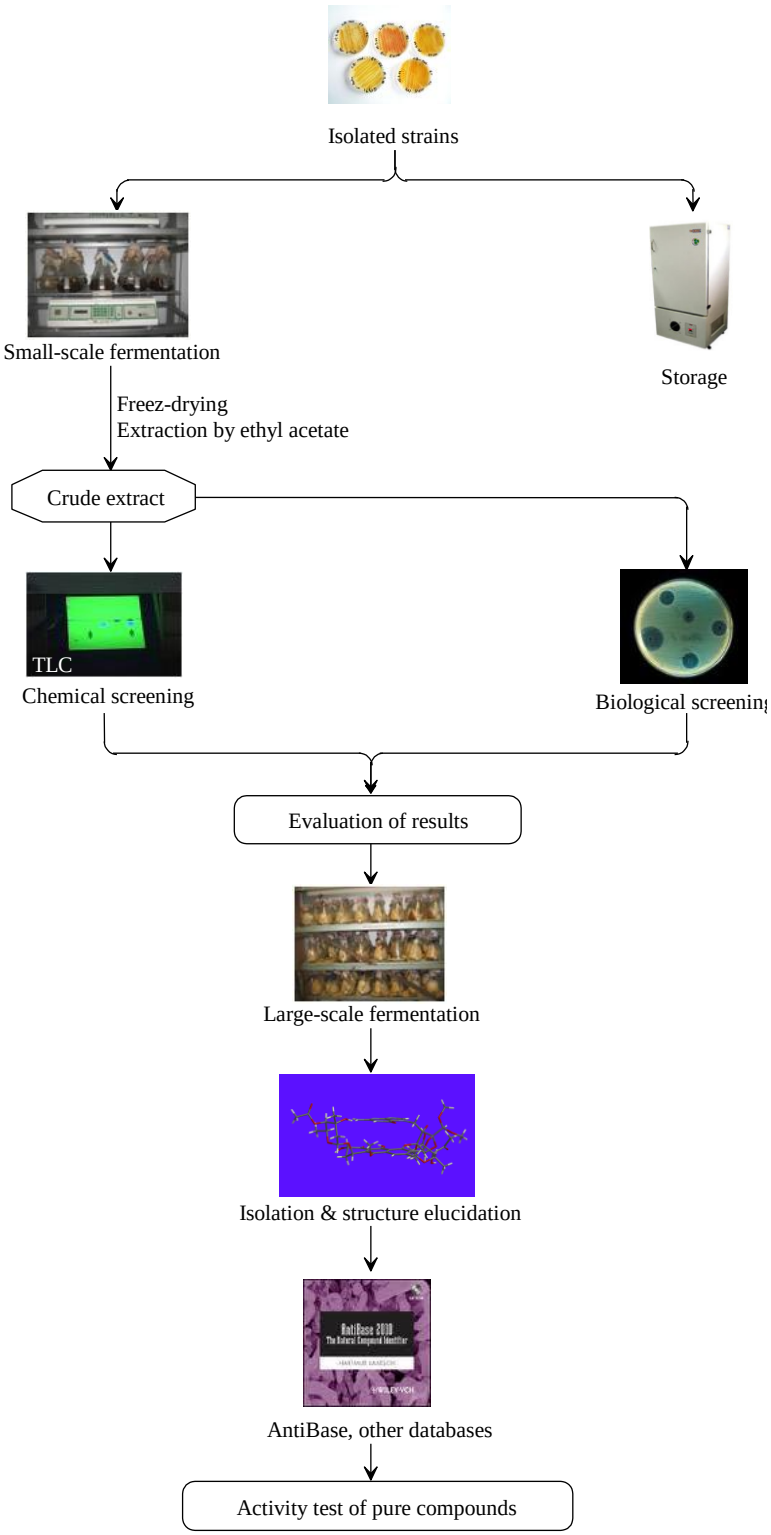


Figure 3: General screening of the selected strains.

3.3 Pre-screening

About 30% of the total received strains were able to produced biologically active metabolites or further interesting properties. Two different techniques i.e. the biological and chemical screening methods were applied to select the promising strains. In this respect, the so-called pre-screening is an essential and important process for isolating finally bioactive and/or chemically interesting compounds.

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

3.4 Chemical screening

The isolation of pure bioactive metabolites from bacteria is a multi-task, expensive and time-consuming process. For this reason, it is wise to avoid the unnecessary work such as re-isolation of known metabolites from the crude extracts or from partially purified fractions. To achieve this purpose, chemical screening is one of the most economical methods at an early stage of separation. TLC (thin layer chromatography) is the simplest and cheapest method for detection of bacterial constituents in the crude extract. It is easy to perform, quick and requires only simple equipment for sufficiently reproducible results in comparison with other methods like HPLC or LC MS. A spot of the crude extract is developed on a TLC with a CH_2Cl_2/MeOH solvent system. The developed zones on TLC plate are visualized under UV light and interesting zones are further localized by exposure to spray reagents. Many spray reagents are available for the detection, some of them are specific and other are universal. The following spray reagents are routinely used in our group:

- Anisaldehyde/sulphuric acid gives different colour reactions with many structural elements.
- Ehrlich's reagent is a specific reagent used to determine indoles and some other nitrogen containing compounds; indoles turn pink, blue or violet, pyrroles and furans become brown, anthranilic acid derivatives change to yellow.

- Concentrated sulphuric acid is especially used for polyenes. Short conjugated chains are showing a brown or black colour, carotenoids develop a blue or green colour.

- NaOH is used for the detection of *peri*-hydroxy-quinones, which turn red, blue or violet. The deep red prodigiosins are showing the colour of the yellow free base.

- Chlorine/*o,o′*-dianisidin is used as universal reagent for the detection of peptides.

3.5 Biological and pharmacological screening

In order to screen a crude extract for bioactive substances, an appropriate test is required. Many screening programs have been applied in natural product chemistry, which are usually divided into two categories: general screening bioassays and specialized screening. These screening programs will be different in the pharmaceutical industry or at university research groups. In any case, all bioassays should have high capacity, low cost, sensitivity and must give rapid answers. There are mainly two types of screening: the *vertical screening*, mostly used for industry shows high selectivity and narrow results (1:10,000-1:20,000).[50] Here, there is a focus on activity against specific enzymes or receptors, studying more in detail the biological activity of compounds, their target receptors and site of action. Examples of bioassays done in industry are tests in order to study specifically anticancer mechanisms by *in vitro* methods, the search for new antibiotics, inhibitors of platelet aggregation and many other enzymatic systems by high-throughput screening. However, due to lack of facilities, time and work force, this type of bioassays are seldom used at university level. Instead, the *horizontal screening* is used in university research groups. It exhibits low selectivity, but provides broad results and a quick overview about existing activities in a sample.

In our group, the crude extract is screened using the agar diffusion test with five bacteria spp. (Gram-positive, Gram-negative), fungi, microalgae as representatives of plants and brine shrimps (*Artemia salina*) as synonym for higher organisms. The crude extracts are tested against different microorganisms as mentioned in

Figure **4**. The brine shrimp toxicity has a strong correlation with cellular toxicity and is a good indicator of 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 for antimicrobial compounds. In the case of positive response, the samples are forwarded to industrial partners for a more detailed vertical screening. The cytotoxicity tests in our group are carried out at Oncotest GmbH (Freiburg), applications in agriculture are tested by BASF AG (Ludwigshafen), etc.

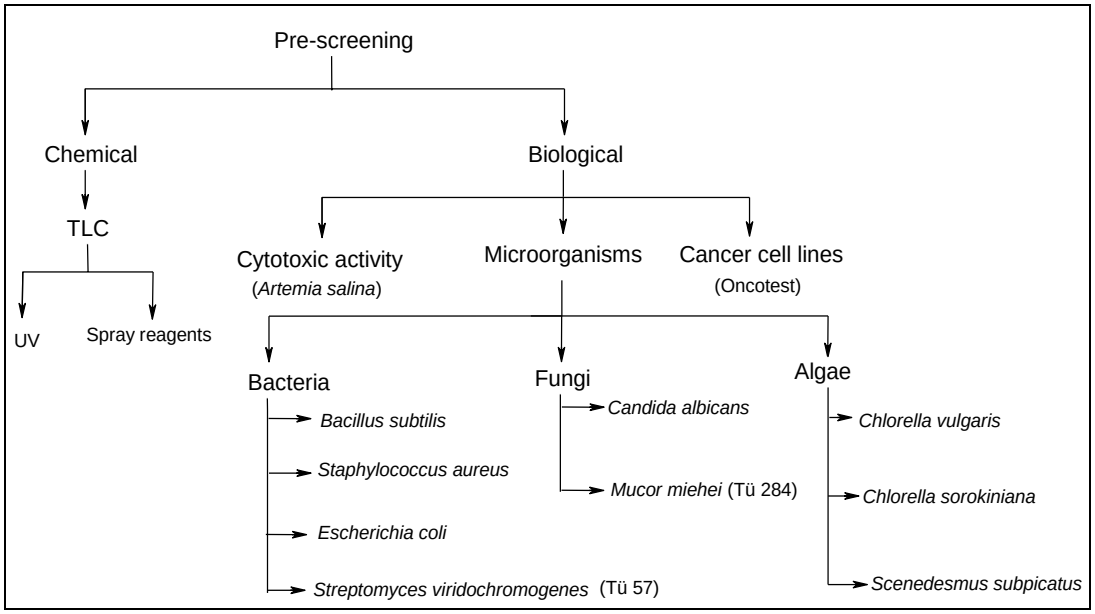


Figure 4: General strategy for pre-screening

3.6 Cultivation and scale up

Based on the pre-screening results, the cultivation and scale up of the selected strains are carried out for further investigation. In some cases, the optimisation of the culture conditions are needed to improve the microbial yield and particularly of the interesting secondary metabolites.

For large-scale cultivation, well-developed agar cultures are used to inoculate 100 of 1L Erlenmeyer flasks each containing 250-300 ml medium (pH 7.8) and cultivated on a linear shaker at 28 °C. The well-grown bacterial culture is harvested after 5-7 days, mixed with Celite (diatomaceous earth as filter aid) and filtered under pressure using a filter press to separate the water phase and mycelium. The filtrate is adsorbed on XAD-16 resin, washed with water and extracted with methanol. The mycelium is also exhaustively extracted with ethyl acetate and acetone. The organic phase is evaporated under vacuum and the remaining crude extracts are used for subsequent chromatographic work.

3.7 Isolation methods

The isolation methods mainly depend upon the amount of crude extracts and properties of the compounds of interest e.g. polarity etc. Firstly, the crude extract is defatted with cyclohexane and then subjected to silica gel chromatography using stepwise gradients of various solvent systems (CH_2Cl_2/MeOH, cyclohexane/ethyl acetate etc.). After fractionation by first column chromatography, the separated fractions are further subjected to size exclusion chromatography using Sephadex LH-20 column. Size-exclusion chromatography (Sephadex LH-20) offers advantage of a high recovery rate and minimizes the destruction of compounds. Further isolation techniques may include PTLC, RP-18, silica gel chromatography or again Sephadex LH-20. All steps are guided by monitoring by TLC.

3.8 Dereplication

Since the "Golden Age of Antibiotics" in the 1950s, natural product chemists have to face the steadily increasing problem of how to minimize the re-isolation of known compounds and to optimise the discovery of new compounds. Till to date, approximately 170,000 natural compounds have been isolated with an average of 700 new microbial metabolites published annually. Several techniques have been developed to identify known compounds at an early stage of purification; these complementary processes for rapid recognition of known compounds or the elucidation of a partial structure of an unknown compound to prioritise or conclude an isolation have come to be termed *dereplication*.[51] For this reason, a number of techniques have been adopted. The comparison of UV as well as mass data in conjunction with HPLC retention time of compounds from our own database is an efficient method. The advantage of this method is that it requires a tiny amount of sample and can even be directly applied to crude extracts. The disadvantage is that an authentic sample must be available for comparison that in most cases is not given. Moreover, the identification of new compounds can be managed by comparison of the molecular weight, the fragmentation pattern and the chromophore of the respective compound. Recently, a HPLC-UV-ESI-MS/MS database with more than 600 compounds has been established in our group for dereplication of a large number of crude extracts. This method is based upon retention time, UV data and molecular weight information like the mass and MS/MS fragmentations obtained by LC/MS analysis. The identification of given component could be accomplished by comparison of these data with reference values as well as the related structures with same chromophore or aglycone.

We have access to important leading databases such as The Dictionary of Natural Products (Chapman and Hall),[52] AntiBase (Wiley-VCH)[53] and the Chemical Abstracts. The Dictionary of Natural Products (DNP) allows the dereplication of nearly all natural products including plant metabolites. However, lower search capabilities as well as limited spectroscopic information are the disadvantages of this database. In contrast, AntiBase is more developed, efficient and practical method for dereplication of microbial natural products. It includes more than 39,000 metabolites from microbial sources. A wide range of sub-structure search capabilities and identification of known compounds by mass or high-resolution mass spectra as well as by comparison of ^{1}H NMR data in many cases are advantageous features. It also offers an access to the ^{13}C NMR data for almost all known compounds and thousands of original 1D and 2D spectra are available for comparison. The most comprehensive worldwide database, however, for the final confirmation of the novelty of compounds are the Chemical Abstracts.

4 Investigation of the selected bacterial strains

4.1 Terrestrial *Streptomyces* sp. GW54/453

During the primary screening, the crude extract of terrestrial *Streptomyces* sp. GW 54/453 displayed on TLC several UV absorbing zones, which showed interesting colours from violet to blue after spraying with anisaldehyde/sulphuric acid. The antimicrobial assay of the extract showed moderate activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. It also exhibited 100% activity against *Artemia salina*.

For further investigation, the strain was cultivated on M_2 medium as a 25L shaker culture for 7 days. After harvesting, it was filtered through filter press using Celite. The water phase was passed through Amberlite XAD-16 column and eluted with methanol. The mycelium was extracted with ethyl acetate and acetone and brought to dryness under vacuum.

On the basis of similar TL chromatograms, both extracts were combined and applied to low pressure silica gel column chromatography using a dichloromethane/methanol gradient. The fractions were further purified using different chromatographic techniques to get pure metabolites (see workup scheme).

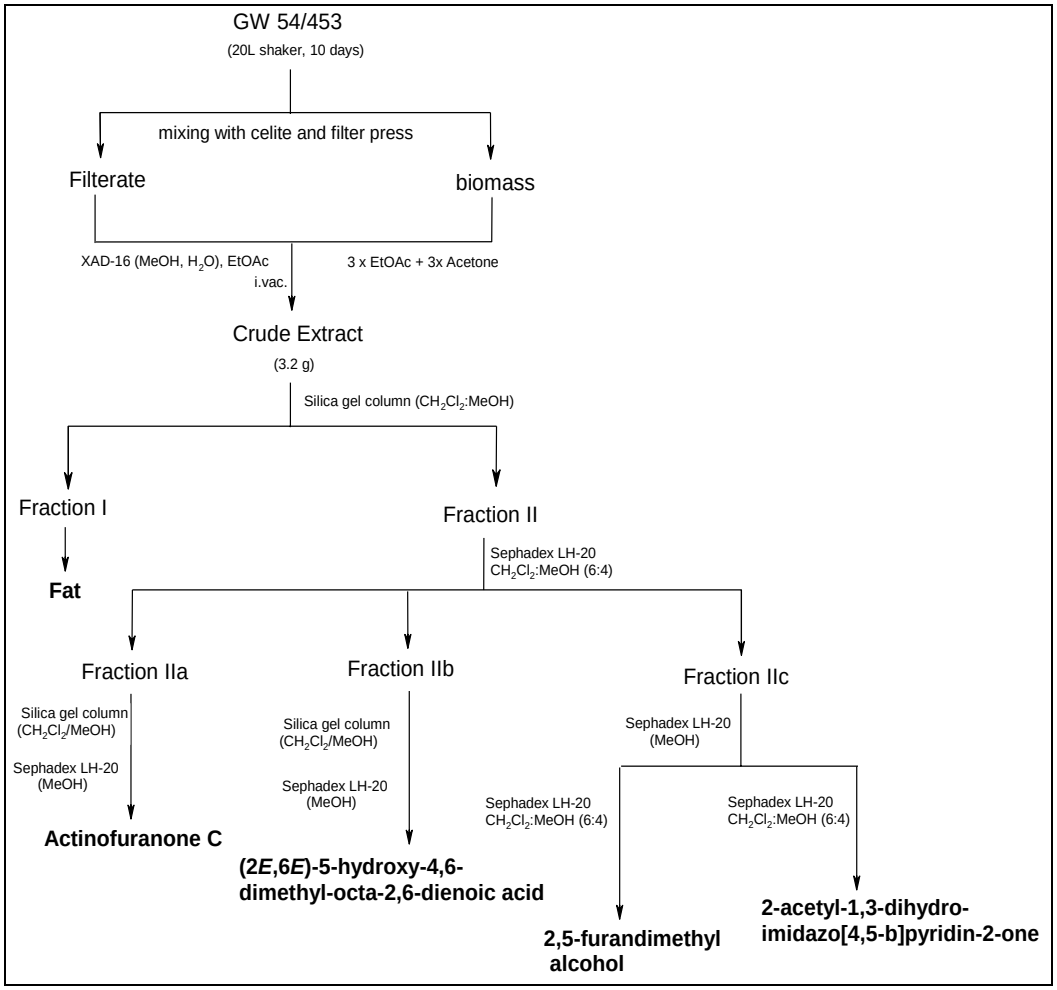


Figure 5: Workup scheme of terrestrial *Streptomyces* sp. GW 54/453.

4.1.1 (2*E*,6*E*)-5-Hydroxy-4,6-dimethyl-octa-2,6-dienoic acid

Compound **45a** was isolated as colourless oil from fraction IIa after purification with silica gel using CH_2Cl_2/MeOH. It was not UV absorbing at both 254 and 366 nm and turned to dark blue and later to green after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR and HSQC spectra of **45a** displayed signals of three olefinic protons, two among them at δ 7.09 (δ_C 153.9) and 5.91 (δ_C 121.1) were belonging to a *trans* double bond (J = 15.7); the third one gave a multiplet at δ 5.52 (δ_C 123.4). An oxygenated methine doublet (δ_H 3.81, δ_C 81.5) together with one sp^2 methine (δ_H 2.54, δ_C 40.4) were also visible. Finally, three methyl signals were visible, two of them at δ 1.63 (d, J= 7.9; δ_C 13.2) and 1.62 (s; δ_C 10.8) were attached to an olefinic carbon, while the third one was doublet at δ 0.93 (J = 6.8 Hz; δ_C 16.3), indicating a

$CHCH_3$ fragment. In the ^{13}C NMR spectra, two additional quaternary carbons were detected for a carbonyl (δ 171.0) of ester/ or acid, and an olefinic residue (δ 135.5).

The molecular weight of **45a** was established by DCI MS as 184 Dalton. On EI MS, the molecule showed a loss of 18 amu (*m/z* 166) due to the elimination of H_2O. By HR (+)-ESIMS, the molecular formula was determined as $C_{10}H_{16}O_3$.

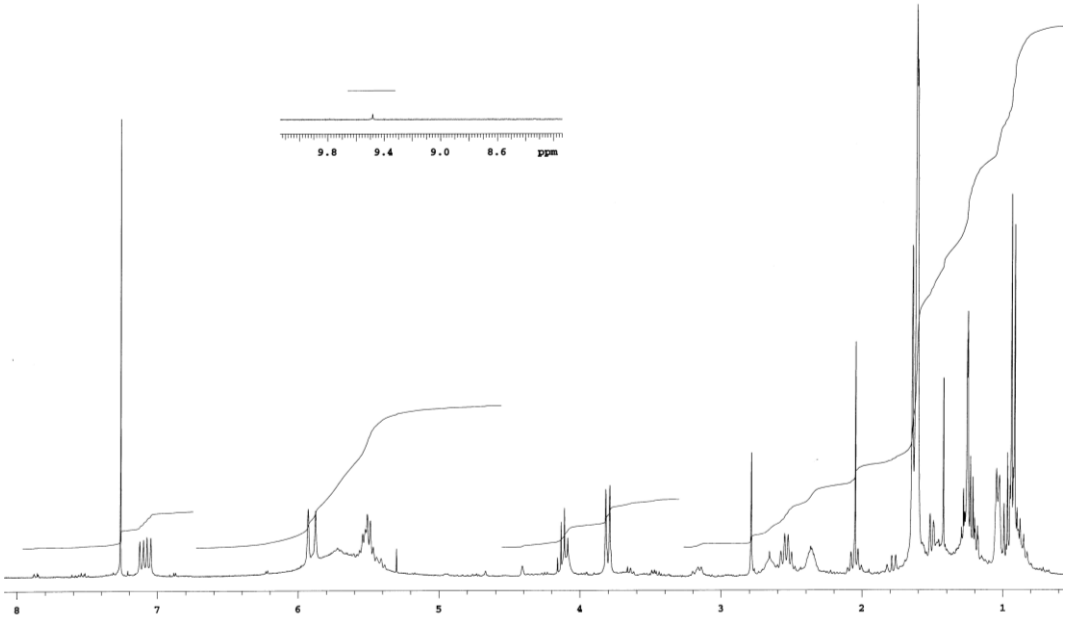


Figure 6: ^1H NMR spectrum ($CDCl_3$, 300 MHz) of (2*E*,6*E*)-5-hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (**45a**).

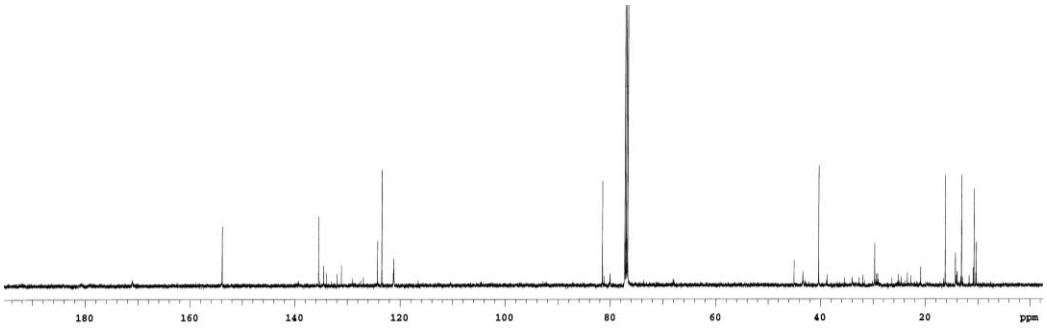


Figure 7: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of (2*E*,6*E*)-5-hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (**45a**).

The final structure of compound **45a** was derived from 2D NMR measurements (H,H COSY and HMBC). Based on the HMBC correlations, an α,β-enone system

was established as the β-olefinic proton (H-3, 7.09) displayed a ^{3}J coupling towards the carbonyl atom (C-1, 171.0); this was confirmed by a ^{2}J coupling from the α-proton (H-2, 5.91) *versus* the same carbonyl. Moreover, the α-olefinic proton directed obvious correlations with the methyl doublet of C-9 (δ 16.3) which is directly attached to the methine carbon C-4 (δ 40.4) affording the partial structure **A**. On the other hand, a terminal isobutene system was recognized. In accordance, the methyl doublet C-8 (13.2) is directly attached to the olefinic methine as shown by H, H COSY between H-7 (δ 5.52) and CH_3-8 (δ 1.63). The quaternary olefinic carbon; C-6 (δ 135.5) showed a correlation from its directly attached methyl singlet CH_3-10 (1.62, 10.8). The latter showed ^{3}J coupling to the hydroxy-methine C-5 (δ 81.5). Alternatively, a direct attachment between CH(OH)-5 and the terminal isobutene system was established. The *trans* configuration of the Δ^{6} double bond is indicated by an Overhauser coupling between H-5 and H-7, thus constructing the complemented partial structure **B** of (*E*)-2-methyl-but-2-en-1-ol.

Both partial structures **A** and **B** were combined by an essential H,H COSY coupling (^{3}J) between H-4 and H-5 beside to their inter-correlated (^{2}J) HMBC connectivities. Finally, the remaining OH of the molecule was included in the terminal carboxylic acid of **45a**, assigning its final structure as (2*E*,6*E*)-5-hydroxy-4,6-dimethyl-octa-2,6-dienoic acid. The free carboxylic group of **45a** was further deduced by methylation (**45b**), at where the methyl ester singlet (δ 3.73) was proved by the ^{1}H NMR spectrum, beside to the delivered corresponding molecular formula $C_{11}H_{18}O_3$ by HR-ESIMS. A search in the different databases (AntiBase, Dictionary of Natural Products and the Chemical Abstracts) proved the novelty of **45a**.

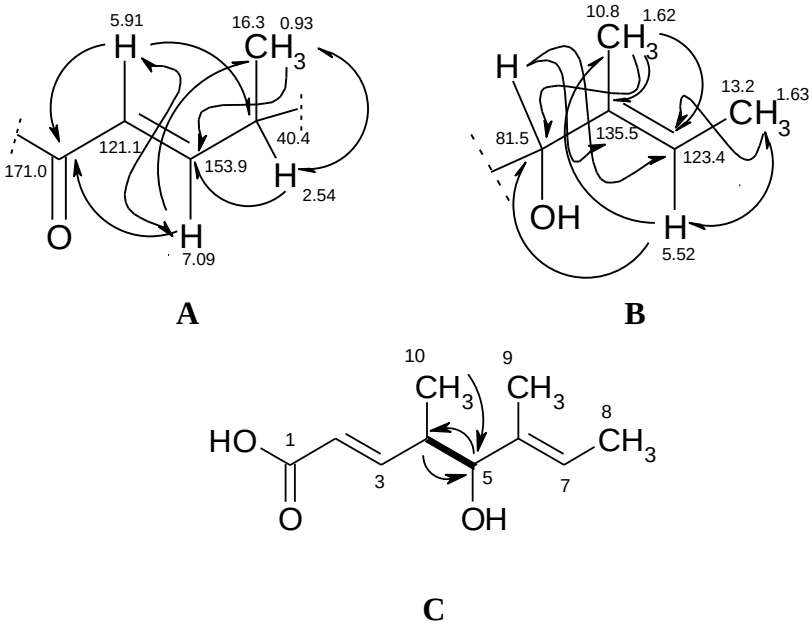
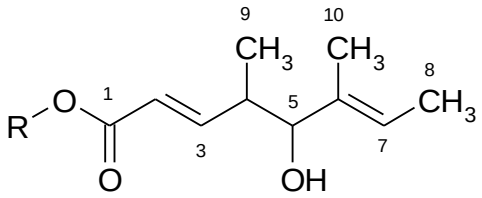


Figure 8: HMBC (→), and H, H COSY (↔,▬) connectivities of partial structures A, B, C of (2*E*,6*E*)-5-hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (**45a**).



45a: R = H
45b: R = CH_3

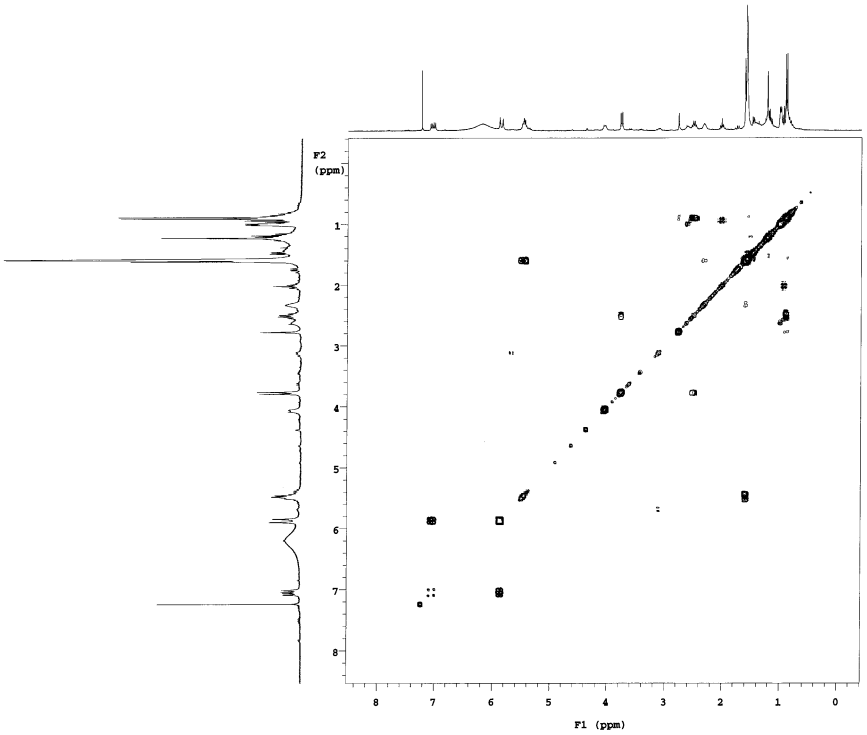


Figure 9: $^{1}H,^{1}H$ COSY spectrum ($CDCl_3$, 300 MHz) of (2*E*,6*E*)-5-hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (**45a**)

Table 1: ^{13}C and ^{1}H NMR ($CDCl_3$, 125 and 300 MHz) assignments of (2*E*,6*E*)-5-hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (**45a**) and its methyl ester derivative (**45b**)

Position	**45a**		**45b**
	δ_C	δ_H (mult.; *J* in Hz)	δ_H (mult.; *J* in Hz)
1	171.0	-	-
1-OCH_3	-	-	3.73 (s)
2	121.1	5.91 (d, 15.7)	5.91 (d, 15.7)
3	153.9	7.09 (dd, 15.7,8.1)	6.99 (dd, 15.7,8.1)
4	40.4	2.54 (m)	2.54 (m)
5	81.5	3.81 (d, 8.5)	3.79 (d, 8.5)
6	135.5	-	-
7	123.4	5.48 (q, 6.8)*	5.52 (m)
8	13.2	1.63 (d, 7.9)	1.63 (d, 7.9)
9	16.3	0.93 (d, 6.8)	0.93 (d, 6.8)
10	10.8	1.62 (s)	1.62 (s)

* value from 600 MHz

4.1.2 Actinofuranone C

The compound **46** was isolated as a light yellow solid from subfraction IIb after purification on silica gel. It showed UV absorbance at 254 nm and stained firstly to violet with anisaldehyde/sulphuric acid and few minutes later turned to green.

The ^{1}H NMR/HMQC spectra of **46** displayed five olefinic signals, located between δ 6.23~5.42 (δ 137.8~122.8), among them two *trans* protons (dd, J =15.2, 10.4) at δ 6.23 (134.3) and 6.08 (132.6) were displayed. Three oxygenated methines were visible in the region of δ 4.29~3.64 (83.3-68.0) together with a non-oxygenated one at δ 2.34 (δ 41.6). Two methylene multiplets were shown, the first of them was sp^2-linked (δ_H 2.71; δ_C 38.4), while the other was sp^3-attached (δ_H 1.80 and 1.66; δ_C 45.2). Ultimately, five methyl signals were observed, three of them were olefinic (δ_H 1.66~1.58; δ_C 13.2-5.8), while the remaining two methyls at δ 1.42 (δ 22.2) and 0.85 (δ 18.5) were sp^3 linked.

The molecular weight was determined by both positive and negative ESIMS modes as 380 Dalton. HR (+)-ESIMS established the corresponding molecular formula as $C_{21}H_{32}O_6$.

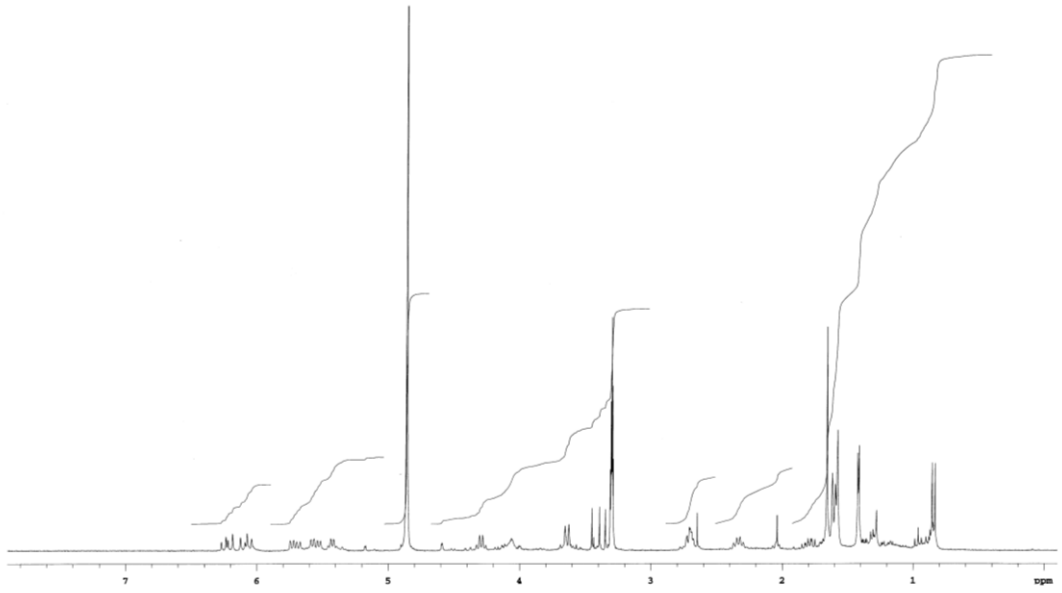


Figure 10: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of actinofuranone C (**46**).

Alternatively, the ^{13}C NMR spectrum displayed five quaternary carbons: the first two were at δ 205.1 and 186.4 for a ketone carbonyl and a β-olefinic carbon attached to oxygen, respectively. The remaining three were at δ 138.9, 104.1 and

110.1. In accordance, compound **46** was constructed from five methyls, two methylenes, three oxy-methines, five olefinic methines, an sp^2 methine, and five quaternary carbons, including one ketone carbonyl.

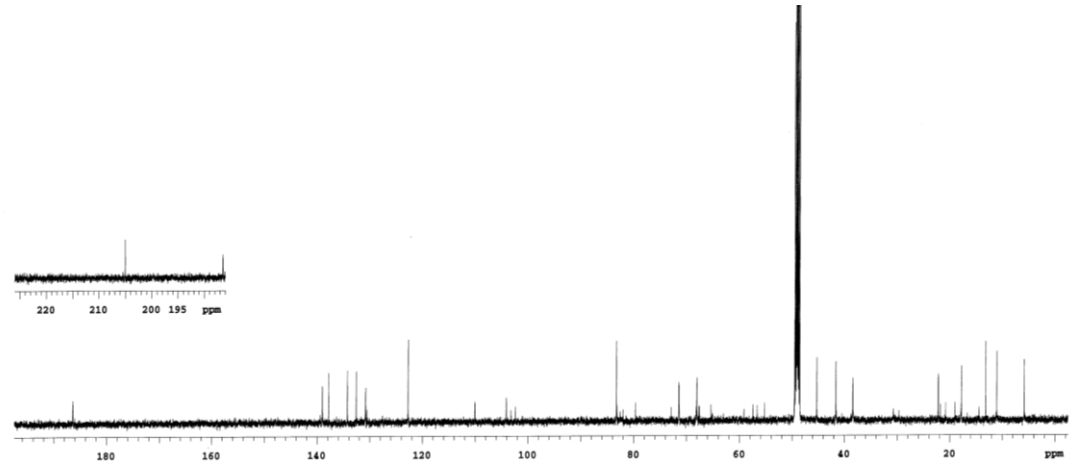


Figure 11: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of actinofuranone C (**46**).

A search with the above spectroscopic data in different databases (AntiBase, DNP and the Chemical Abstracts) indicated that the compound was a new natural product. To find out the final structure, it was subject to intensive 2D experiments (H,H COSY, HMBC, NOE). As in **45a**, compound **46** showed a terminal isobutene moiety, of which the methyl doublet of CH_3-18 (δ 1.61) showed a COSY cross signal with CH-17 (δ 5.42). The C_q-16 (δ_C 138.9) and its attached methyl CH_3-21 (δ_C 11.1) were recognized by H→C correlations. A further analysis of the 2D experiments showed clearly that the terminal nine carbon atoms of compound **46** (part. struct. **A**) were identical with structure **45a**. The *trans* configuration of the Δ^{16} double bond is indicated by an Overhauser coupling between H-15 and H-17.

The olefinic carbons CH-11 (δ 6.23) and CH-10 (δ 5.55) exhibited H,H COSY couplings towards each other and with the sp^2 oxymethine CH-9 (δ 4.29) confirming their connection with each other. The latter oxy-methine (H-9) showed in turn a further H,H COSY coupling with the methylene group CH_2-8 (δ 1.80, 1.66). This conclusion was proven by the HMBC correlations, constructing the partial structure **B**. In accordance, the two partial structures **A** and **B** were combined through C-11 and C-12, as they correlated by an H,H COSY signal between H-11 (δ 6.23) and H-12 (δ 6.08) and by HMBC couplings from H-11 to C-13 (δ 139.0) and *vice versa*. Therefore, the partial structure **C** was created.

The remaining partial structure ($C_8H_{11}O_4$) bears 3 double bond equivalents. Alternatively, the sp^3-linked singlet methyl CH_3-1 (δ 1.42) directed two essential HMBC correlations at the ketone carbonyl C-3 (δ 205.1, ^3J) and to C-2 (δ 104.1, ^2J) while the olefinic-bonded methyl CH_3-19 (δ 1.66) showed three connectivities at C-3 (^3J), and the joined α-β olefinic carbons; C-4 (δ 100.1, ^2J) and C-5 (186.4, ^3J). Alternatively, the sp^2-attached methylene CH_2-6 (δ 2.71) displayed three correlations at C-5 (^2J), C-4 (^3J) and the hydroxy-methine C-7 (δ 68.0) beside to an H,H COSY connectivity with the latter (H-7, δ 4.07). This recognized the partial structure **D**, as furanone moiety, which in turn has an acetal carbon C-2 (δ 104.1) and a disubstituted enone system (Figure 12). Finally, the two partial structures **C** and **D** were joined with the help of a clear connectivity between CH_2-8 and CH-7; hence the final structure was fixed as **46** for actinofuranone C.

Compound **46** was obtained as a mixture of epimers at C-2: the carbon signal of atom C-2 appeared as two singlets. This indicates that the configuration at C-2 is not stable and is equilibrated, however a similar behaviour was not reported for the closely related compound actinofuranone A [54] (**47**).

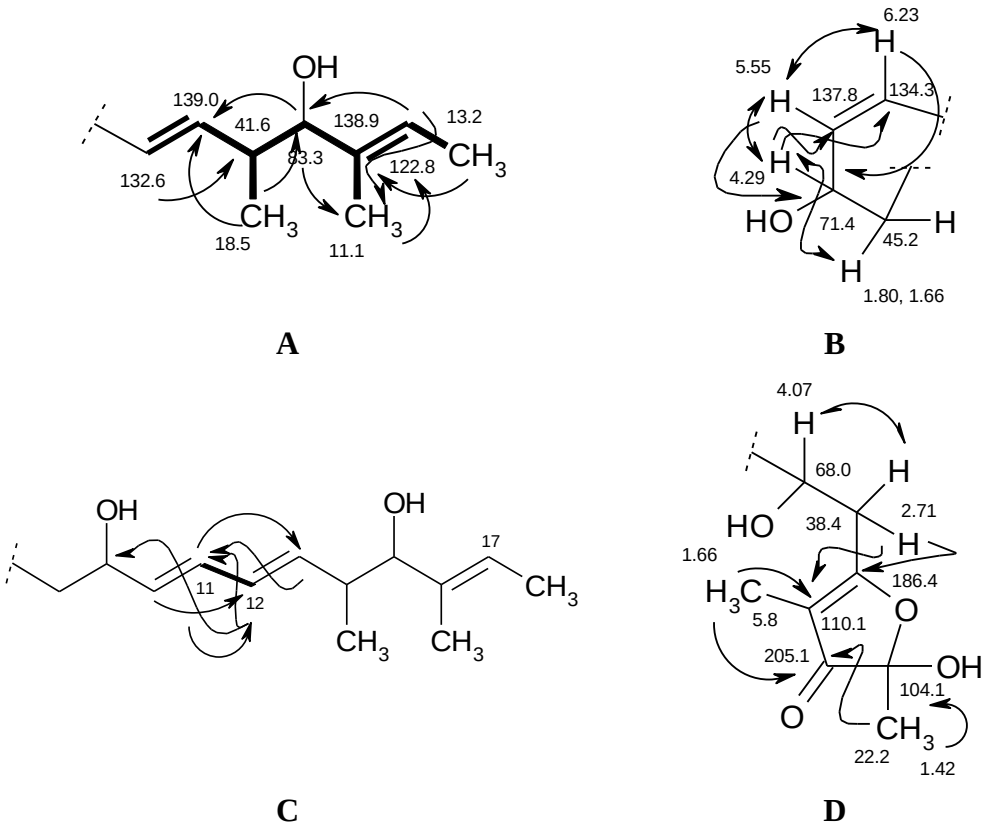
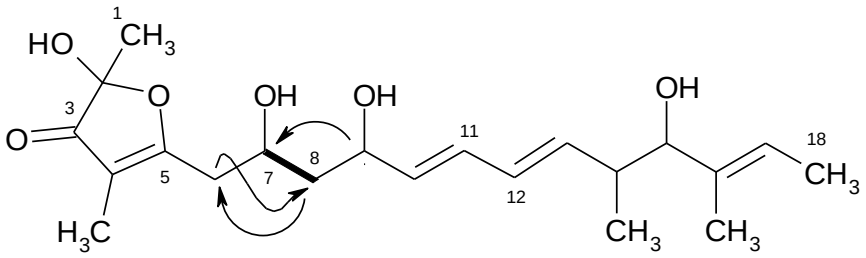
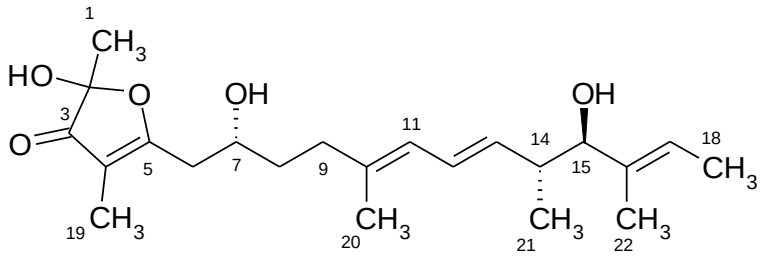


Figure 12: HMBC (→), and H,H COSY (↔, ▬) connectivities in the partial structures A, B, C, D of actinofuranone C (**46**)

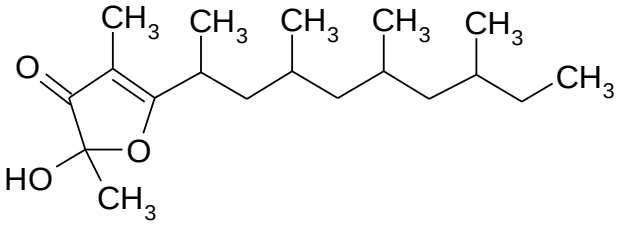


46

Actinofuranone C (**46**) is highly related to the recently reported Actinofuranones A (**47**) and B, produced by *Streptomyces* sp.[54] The sole difference between actinofuranone A (**47**) and our actinofuranone C (**46**) is a replacement of the CH_2-9 and CH_3-20 in actinofuranone A (**47**) by CH(OH)-9 and hydrogen in **46**, respectively. Actinofuranones A and B were found to possess weak *in vitro* cytotoxicity against mouse splenocyte T-cells and macrophages with IC_{50} values of 20 μg/ml. Another closely related compound AS-183 (**48**) isolated from a fungus *Scedosporium* spp. was reported as a potent inhibitor of cholesterol ester formation by inhibiting Acyl-CoA: cholesterol acyltransferase (ACAT) in human cells.[55]



47



48

Table 2: NMR (CD_3OD, *J* in Hz) assignments of actinofuranone C (**46**)

Position	δ_C [a]	δ_H [b]	Position	δ_C [a]	δ_H [b]
1	22.2	1.42, 1.43 (2 s)	12	132.6	6.08 (dd, 15.1, 10.4)
2	104.1	-	13	139.0	5.71(ddd,14.7,8.0,6.8)
3	205.1	-	14	41.6	2.34 (m)
4	110.1	-	15	83.3	3.64 (d, 8.3)
5	186.4	-	16	138.9	-
6	38.4	2.71 (m)	17	122.8	5.42 (q, 6.9)
7	68.0	4.07 (m)	18	13.2	1.61 (d, 6.7)
8	45.2	1.80 (m), 1.66 (m)	19	5.8	1.66 (s)
9	71.4	4.29 (q, 6.8)	20	18.5	0.85 (d, 6.9)
10	137.8	5.55 (ddd, 14.4, 10.4, 3.4)	21	11.1	1.58 (s)
11	134.3	6.23 (dd, 15.2, 10.4)			

[a]125 MHz; [b]300 MHz

Although more than 200 structurally related compounds are known from nature, the actinofuranones belong to a relatively rare polyketide group with a 3-furanone ring system with a C2-hemiketal and a C-5 unsaturated alkyl chain. The polyketide side chain of actinofuranones is probably assembled by a bacterial modular polyketide synthase (PKS). Müller *et al*.[56] described a hypothetical biosynthesis of the furanone moiety in aurafurones[57] which might involve several reaction steps, catalysed by post-PKS monooxygenase enzymes. The polyketide chain after release from the PKS cyclizes to a six-membered lactone moiety and is hydroxylated at the α-carbon. By Baeyer-Villiger oxidation, oxygen is incorporated into the C-C bond to form a carbonic acid ester derivative, which after cleavage by losing carbon dioxide resulted in a triketone moiety. After intramolecular nucleophilic attack of enolic hydroxyl group on the carbonyl carbon of the triketone moiety, the final furanone ring is formed. At the final steps, hydroxylation might occur at C-7 and C-9 to form Actinofuranone C (**46**).

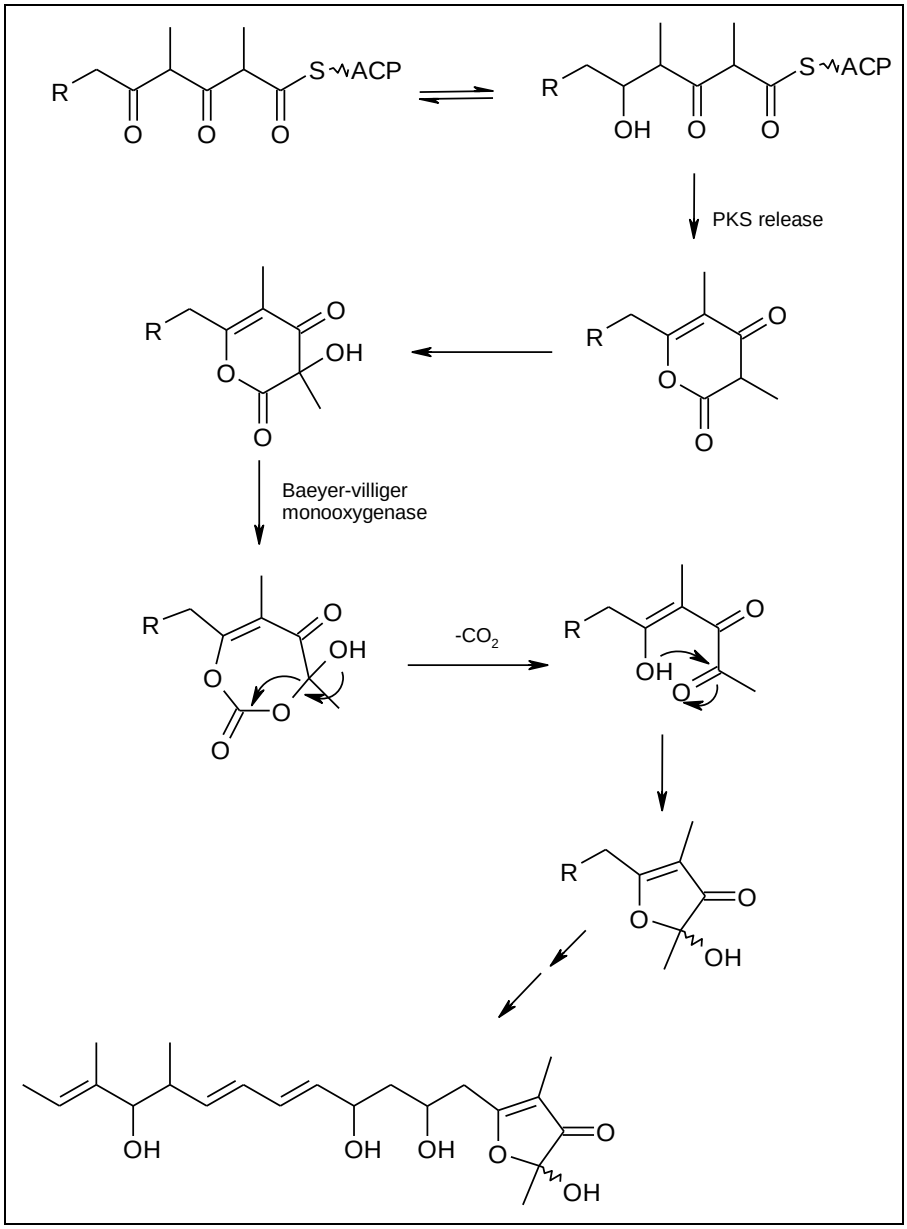


Figure 13: Hypothetical biosynthetic pathway[56] of the furanone ring in actinofuranone C (**46**).

4.1.3 4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one

4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**49**) was isolated as yellow solid by PTLC followed by Sephadex LH-20 (CH_2Cl_2/40%MeOH) from sub-fraction IIc. It was highly UV absorbing at 254 nm and showed an intense yellow colour after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum revealed two aromatic protons at δ 8.22 (d, J = 5.0), δ 7.19 (d, J = 5.0), a methyl singlet at δ 2.61 probably attached to a carbonyl moiety (C=O) or an aromatic ring and a broad singlet at δ 11.05 showing a replaceable proton -NH or -OH. The molecular weight of the compound was determined by EIMS as 177 Dalton and HREIMS gave the molecular formula as C_8 H_7 N_3 O_2.

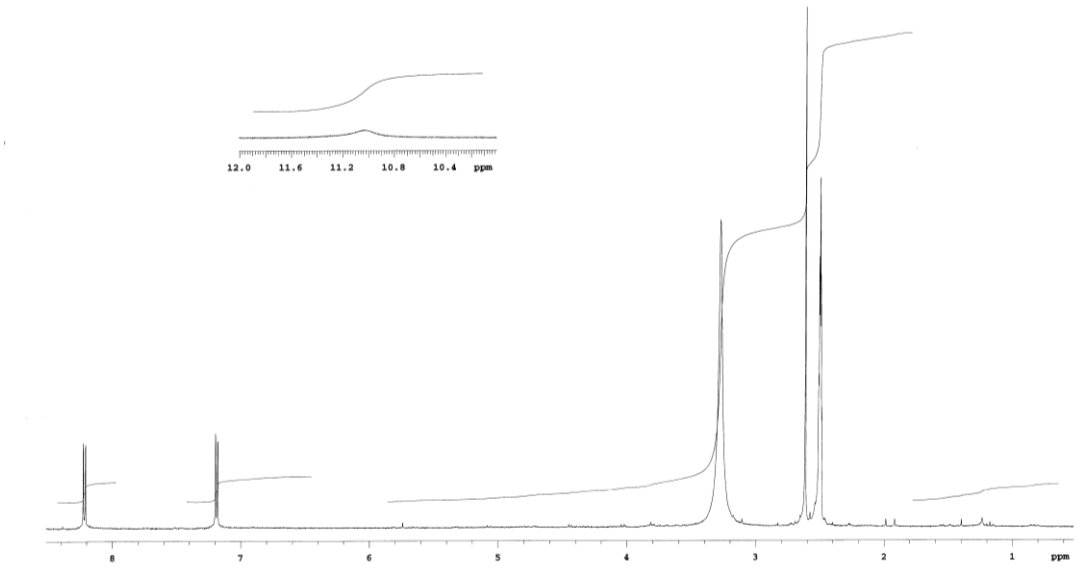


Figure 14: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**49**).

The ^{13}C spectrum showed two aromatic methine signals at δ 140.8 and 107.1, five quaternary sp^2 carbon signals and a methyl at δ 25.7.

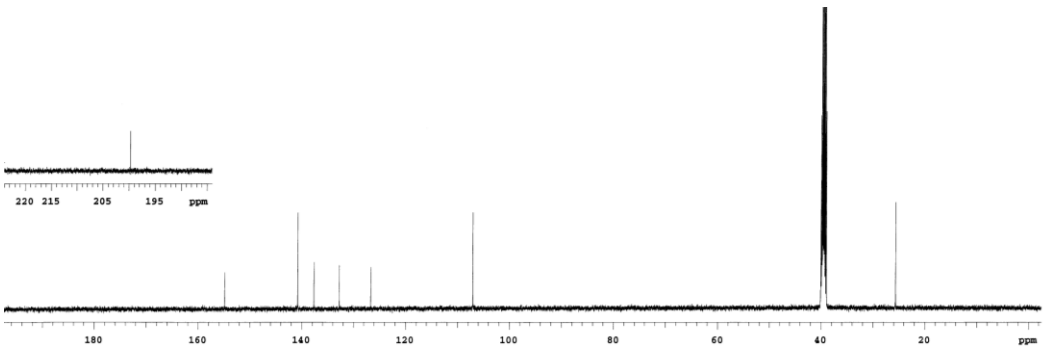
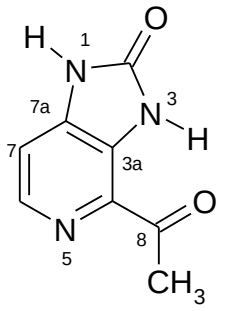


Figure 15: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**49**).

The search in AntiBase using NMR data as well as the molecular formula provided the hit as 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**49**) which was recently isolated in our group and reported in 2009.[58]

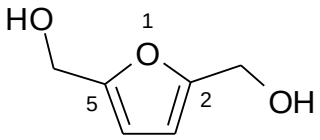


49

Dihydroimidazo-pyridine derivatives have diverse biological and pharmaceutical applications as protein-kinase inhibitors, anti-tumor, antiviral and anti-tuberculosis agents.

4.1.4 2,5-Furan-dimethanol

2,5-Furan-dimethanol (**50**) was purified from fraction IIc as a colourless oil, which showed a brown colour with anisaldehyde/sulphuric acid. In the ^{1}H NMR spectrum, two singlets at δ 6.22 (1H) and 4.60 (2H) and a broad signal at δ 2.45 appeared. The molecular weight was determined by EIMS as 128 Dalton. The search in AntiBase by using these spectroscopic data resulted in 2,5-furan-dimethanol (**50**), which was further confirmed by comparing the data with an authentic spectrum. 2,5-Furan-dimethanol was reported to possess a moderate antimicrobial activity against the fungus *Nematospora coryli* and the yeast *Saccharomyces cerevisiae*.[53]



50

4.2 Terrestrial *Streptomyces* sp. Ank 245

Despite of lacking antimicrobial activity, the terrestrial *Streptomyces* sp. Ank 245 was selected during the primary screening of the extract on basis of several col-

oured zones ranging from violet to blue and orange on TLC after spraying with anisaldehyde/sulphuric acid.

Well-grown agar culture plates were used to inoculate 100 of 1L Erlenmeyer flasks each containing 300 mL of M_2 medium (pH, 7.8) and incubated at on 28 °C for 7 days on a linear shaker. After harvesting, the dark brown culture broth was filtered by means of a filter press to separate mycelium and water phase. The water phase was adsorbed on Amberlite XAD-16 adsorber resin and eluted with methanol. The eluate was evaporated and the aqueous residue was extracted with ethyl acetate. The mycelium was exhaustively extracted with ethyl acetate and acetone. On the basis of similar chromatograms, both crude extracts were combined for further chemical investigation. For this purpose, the crude extract was applied to silica gel column chromatography using a CH_2Cl_2/MeOH gradient. From the fractions obtained thereof, purified metabolites were obtained using different chromatographic techniques (see work-up scheme).

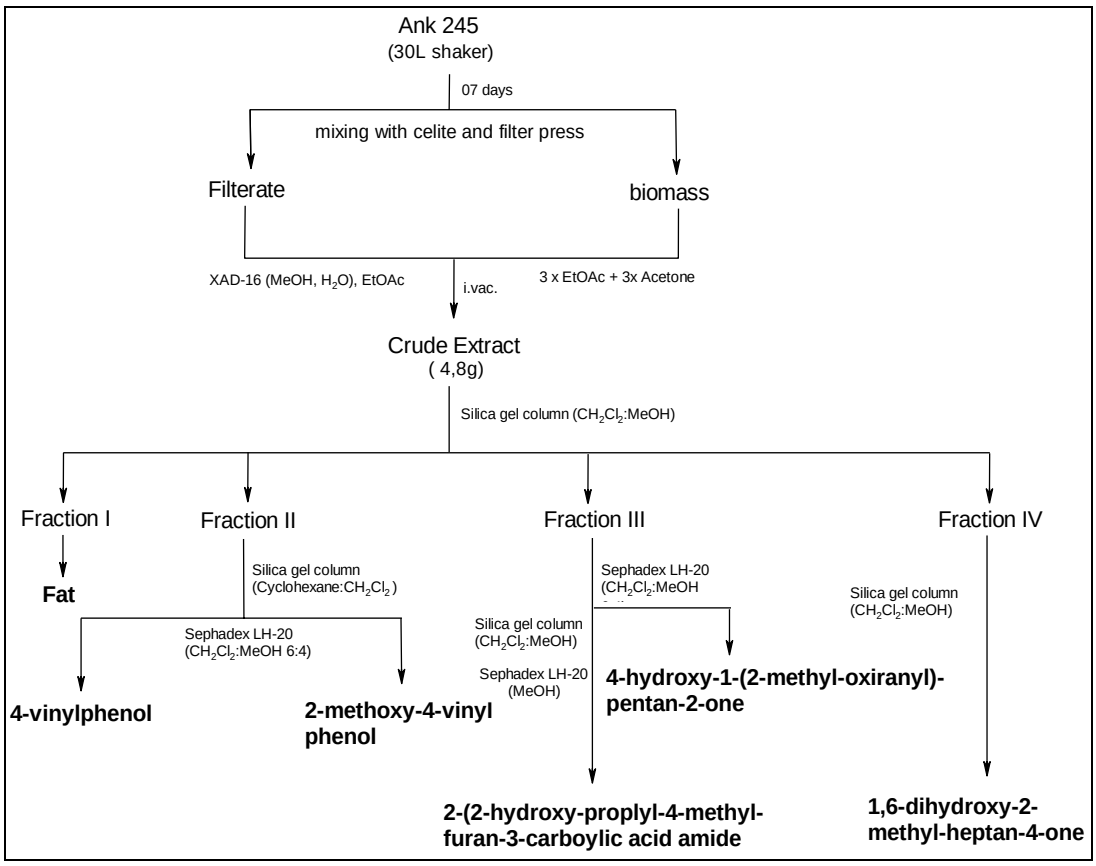


Figure 16: Work up scheme of terrestrial *Streptomyces* sp. Ank 245.

4.2.1 1,6-Dihydroxy-2-methyl-heptan-4-one

Compound **51** was obtained as middle polar colourless oil, which gave on TLC with anisaldehyde/sulphuric acid a dark green and later dark blue zone. It was not UV absorbing or fluorescent, i.e. **51** was neither aromatic nor unsaturated. The molecular weight of **51** was established by CIMS and (+)-ESIMS as 160 Dalton, and the corresponding molecular formula was recognized as $C_8H_{16}O_3$ by HRESIMS.

The ^{1}H NMR spectrum of **51** revealed the existence of two methyl doublets at δ 1.14 (J = 6.3 Hz) and 0.87 (J = 6.7 Hz), one of them being connected with an oxygenated methine (δ 4.18). Moreover, the multiplets of an oxygenated methylene group were visible at δ 3.44 and 3.27. Finally, a 5H multiplet, corresponding to two methylenes and one methine proton, was located in the region of δ 2.50~2.19. Based on the H,H COSY correlations (Figure 20), two partial structures were deduced, the first of them being a hydroxyl-isopropyl moiety (CH_2-CH[OH]-CH_3), while the other one was corresponding to an isobutyl-oxy pattern (HO-CH_2-CH(CH_3)-CH_2).

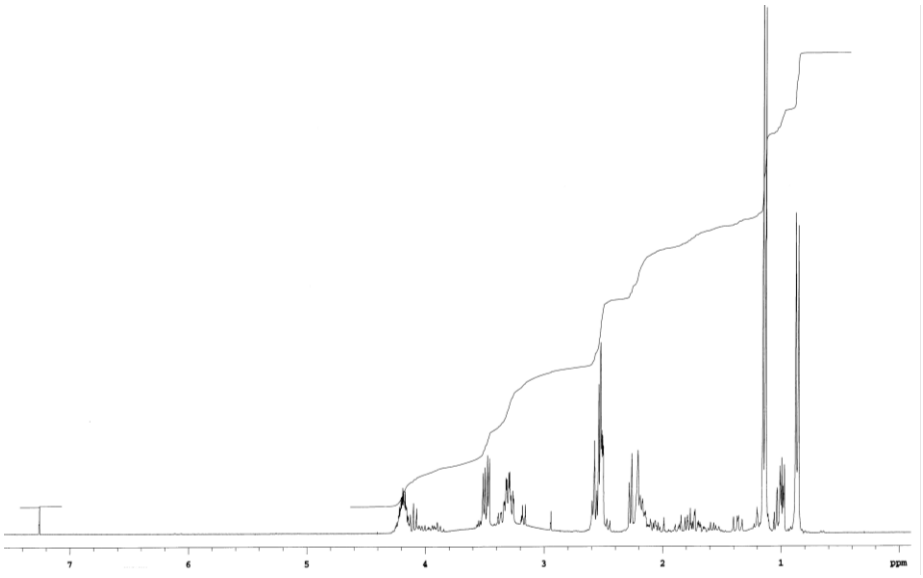


Figure 17: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 1,6-dihydroxy-2-methyl-heptan-4-one (**51**).

The ^{13}C/HMQC spectra indicated the presence of eight carbon signals, which were classified into the following categories: each one ketone carbonyl (δ 211.9), oxy-methine (δ 63.9), oxy-methylene (δ 67.3) and a methine (δ 31.9), two methylenes (δ 51.6, 47.6), and the already discussed two methyls (δ 22.5, 16.9). Based on the above partial structures and molecular formula, a search in AntiBase, the Dic-

tionary of Natural Products (DNP) and the Chemical Abstracts did not reveal a corresponding structure, pointing to a new compound.

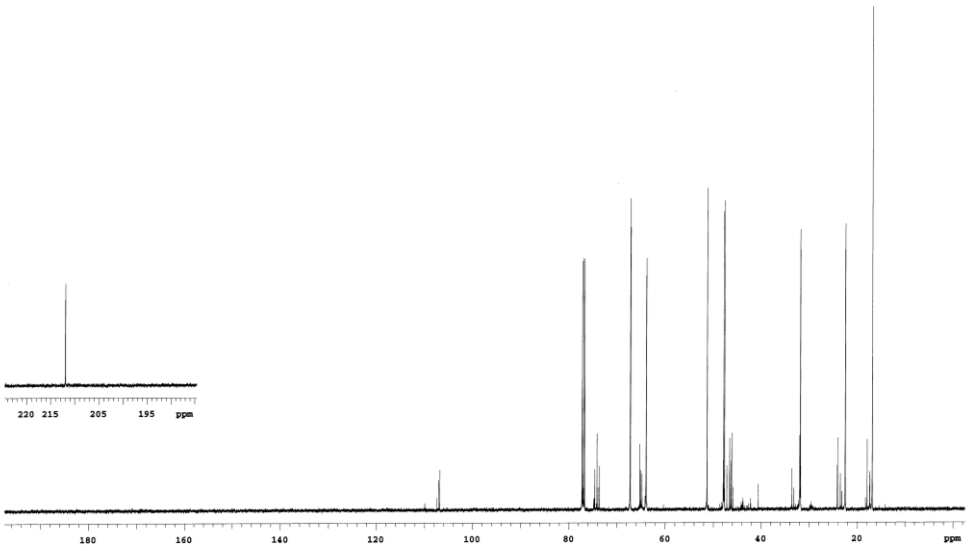


Figure 18: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 1,6-dihydroxy-2-methyl-heptan-4-one (**51**).

According to the HMBC spectrum, the two partial structures (isopropyl and isobutyl) were connected to each other *via* a ketone carbonyl (δ 211.9) on the basis of the ^3J correlation directed from the oxymethine proton H-6 (δ 4.18) of the isopropyl group towards the carbonyl. On the other hand, protons of the two sp^2-linked methylenes H_2-3 (δ 2.50 and 2.25) and H_2-5 (δ 2.50) showed ^2J couplings to the same carbonyl C-4 (δ 211.9), in addition to ^3J connectivities between each other. Accordingly, structure of **51** was fully deduced as 1,6-dihydroxy-2-methyl-heptan-4-one (**51**).

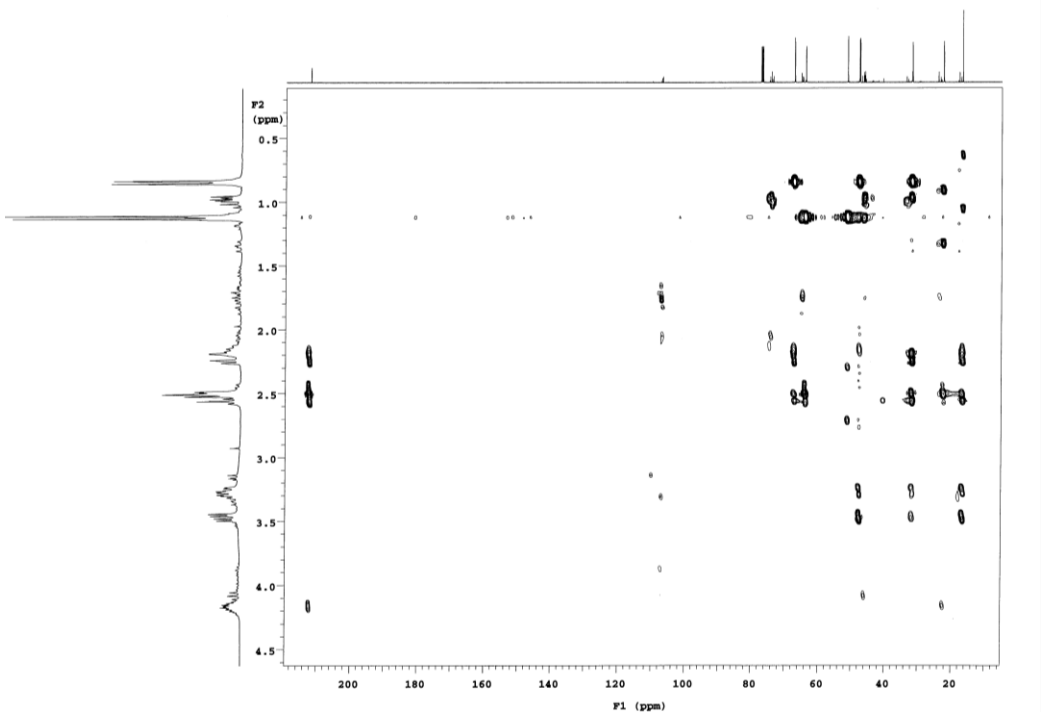


Figure 19: HMBC spectrum ($CDCl_3$, 600 MHz) of 1,6-dihydroxy-2-methyl-heptan-4-one (**51**).

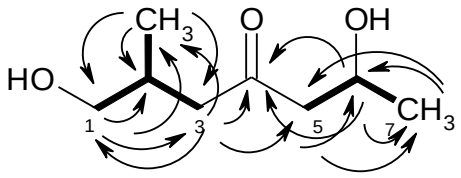
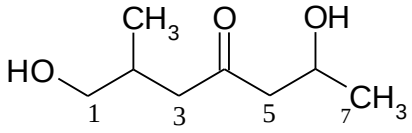


Figure 20: HMBC (→), and H,H COSY (▬) connectivities of 1,6-dihydroxy-2-methyl-heptan-4-one (**51**)



51

4.2.2 4-Hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one

As compound closely related to **51**, the derivative **52** was obtained as yellow oil with higher polarity, giving an orange colouration on spraying with anisaldehyde/sulphuric acid and heating, which turned later to violet. The compound showed

as well no UV activity, as indicative of a neither aromatic nor unsaturated system. Its molecular weight was obtained as 158 Dalton by CI MS and (+)-ESIMS. HRESIMS of **52** deduced its molecular formula as $C_8H_{14}O_3$, with 2 amu lesser than **51**, containing, however, one double bond equivalents more.

The ^{1}H and HMQC spectra of **52** revealed a high similarity in its pattern to those of **51**. Consequently, the same hydroxy-isopropyl group was confirmed (H,H COSY, Figure 23) where the oxy-methine multiplet (δ 4.25), its related methyl doublet (δ 1.19, 6.2 Hz) and the associated methylene (δ 2.62) were observed. On the other hand, the isobutyl fragment in **51** was altered here into a methyl singlet appearing at δ 1.20, along with one 2H singlet corresponding to an oxygenated methylene (δ 3.44). The remaining methylene protons (H_2-3) appeared at δ 2.80 (dd, J = 16.0, 9.2 Hz) and δ 2.54 (dd, J = 9.6, 4.5 Hz), respectively. The ABX-splitting of CH_2-3 seems to be due to a W-coupling with CH_2-1, but not with 2-CH_3.In accordance, the sole difference between compounds **52** and **51** was attributed to the expulsion of 2 H during a ring closure between C-1 and C-2 *via* oxygen in **51** to give an oxirane ring in **52**. This conclusion was confirmed by the fact that the methine carbon C-2 in **51** (δ 31.9) had changed into a quaternary oxygenated one appearing at δ 72.6. Moreover, the methyl doublet of 2-CH_3 in **51** (δ 0.87) appeared here as singlet (δ_H 1.20, δ_C 24.3) with a downfield shift, assuming its position at the oxygenated C-2.

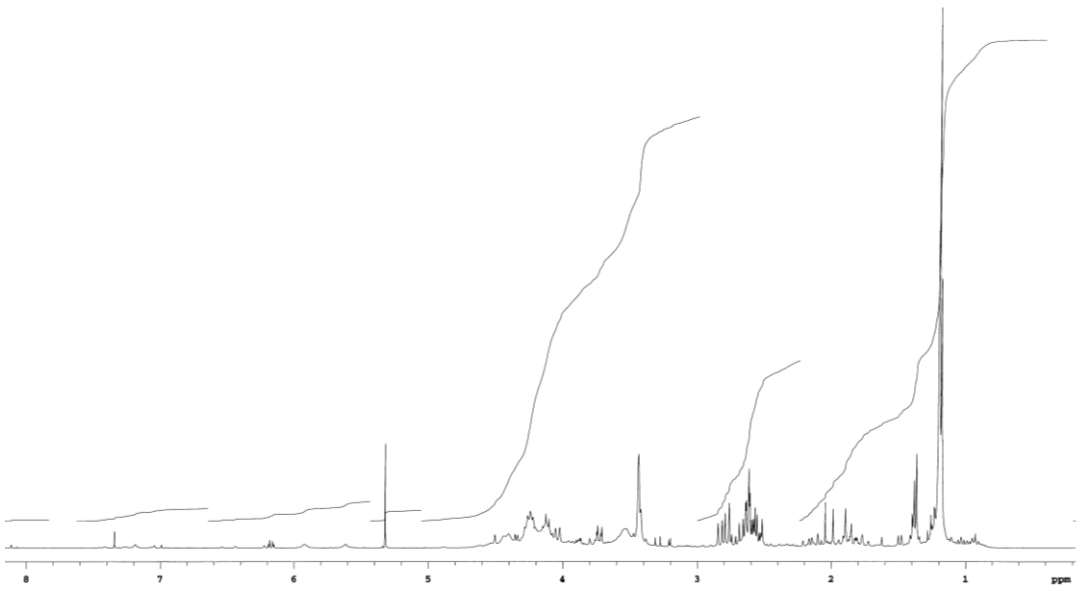


Figure 21: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**52**).

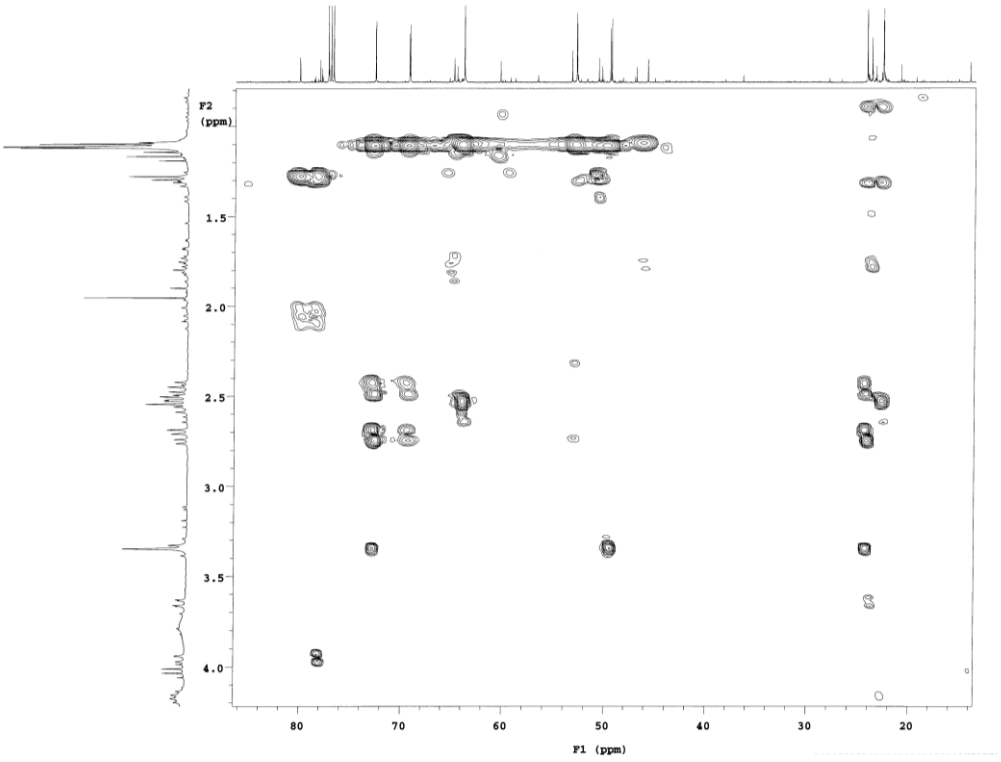


Figure 22: HMBC spectrum ($CDCl_3$, 600 MHz) of 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**52**).

The structure of **52** was finally deduced by HMBC correlations (Figure 23), showing the same correlation with the centred ketone carbonyl (δ 212.6) from both sides as in compound **51**. So, the structure of **52** was elucidated as 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one, as additional new structure.

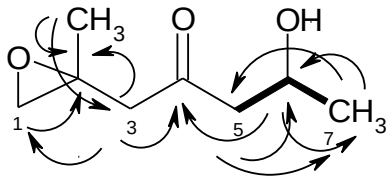


Figure 23: Selected HMBC (→), and H,H COSY (▬) connectivities of 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**52**)

4.2.3 2-(2-Hydroxy-propyl-4-methyl-furan-3-carboxylic acid amide

Compound **53** was obtained as colourless solid of middle polarity, which was detected as UV-absorbing zone, which turned pink after spraying with anisaldehyde/sulphuric acid. The molecular weight of 183 Dalton was determined by ESIMS,

as indicative of the existence of an odd number of nitrogen atoms in the structure. In the EI mass spectrum, the compound lost 44 amu, affording a base ion peak at *m/z* 139, as indicative of the loss of terminal $CHOCH_3$ group from the parent molecule and confirmed by HREIMS of the respective peak. The molecular formula was recognized by HRESIMS as $C_9H_{13}NO_3$.

The ^{1}H NMR of **53** displayed one quartet at δ 7.09 (J = 1.1 Hz), pointing to its allylic position to a neighbouring methyl at δ 2.13 (J =1.2 Hz) attached to an sp^2 carbon. Two broad signals of amide protons were visible at δ 7.24 and 6.19. In the aliphatic region, an oxymethine multiplet (δ 4.14), a methylene showing ABX splitting (δ 2.94, J = 14.7, 11.5, 4.0) and a methyl doublet (δ 1.26) were visible. According to the H,H COSY correlations (Figure 27), the latter three aliphatic signals constituted a hydroxy-isopropyl moiety [CH_2-CH(OH)-CH_3], as in the above two compounds, **51** and **52**.

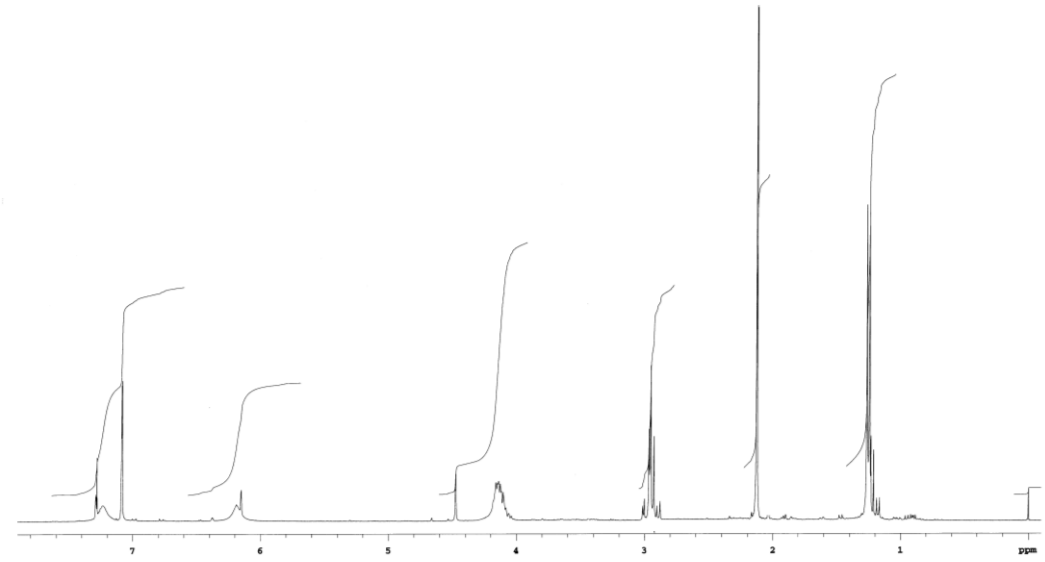


Figure 24: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 2-(2-hydroxy-propyl-4-methyl-furan-3-carboxamide (**53**).

The ^{13}C/HMQC spectra displayed the presence of nine carbon signals, among them one at δ 167.2 for a carboxamide ($CONH_2$), and two oxygenated sp^2 carbons (δ 155.9, 138.4), of which the last one was a methine (δ_H 7.09). Two signals of quaternary sp^2 carbons were visible at δ 119.6 and δ 118.6. In the aliphatic region, four carbon signals were detected, one of them a methyl (δ 9.5), while the remaining three carbons (CH, δ 67.2; CH_2, δ 36.6, CH_3, δ 23.6) are corresponding to a 2-hydroxy-propyl moiety. In accordance, structure **53** bears a carboxyl amide group and a 2-

hydroxy-propyl system. After subtraction these two groups from the empirical formula, C_5H_4O remained with three double bond equivalents, representing two olefinic double bonds, one of them being a propenyl group (CH_3-C=CH-). The two olefinic bonds must be therefore included in a ring system i.e. a furan, connected with the three substituted groups Me, $CONH_2$ and the 2-hydroxy-propyl unit. Using these partial structures along with the molecular formula, a search in different databases afforded no hit, pointing to a new compound.

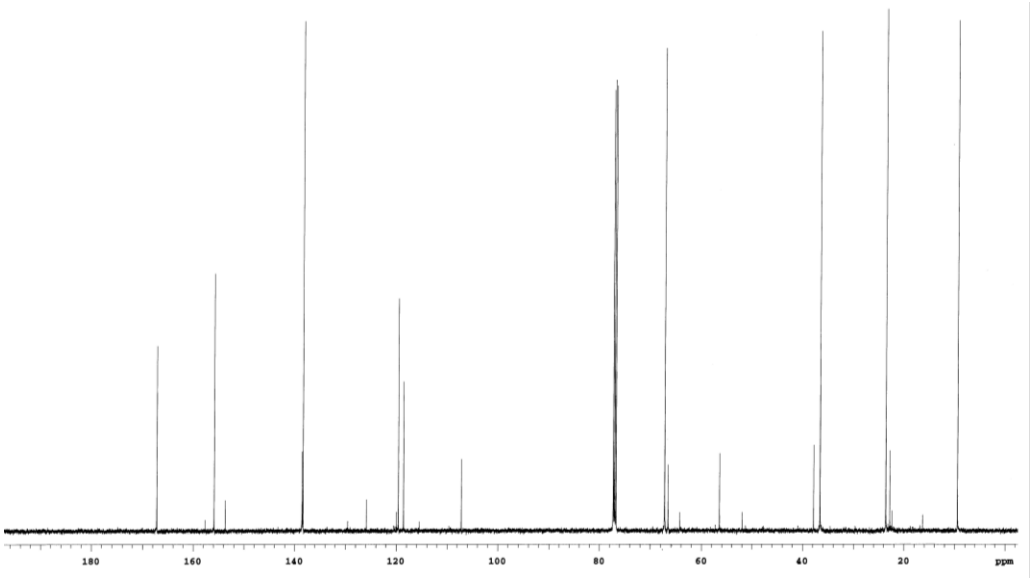


Figure 25: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 2-(2-hydroxy-propyl-4-methyl-furan-3-carboamide (**53**).

According to the HMBC correlations (Figure 27), the 2-hydroxy-propyl group in **53** was connected at C-2 of the furan ring. This was derived from ^3J and ^2J correlations from the hydroxy-methine H-7 (δ 4.14) and the methylene H_2-6 (δ 2.95) group, respectively, with C-2 (δ 155.9) of the furan ring. Moreover, CH_2-6 showed a ^3J coupling towards the quaternary atom C-3 (δ 118.6) in the furan ring. On the other hand, the oxymethine carbon in the furan ring was fixed at C-5 (δ 138.4), as ^3J couplings from H-5 to C-2 (155.9) indicated. Hence, the sp^2-linked methyl group (4-CH_3) is located at C-4 (δ 119.6), where it displayed three obvious correlations with C-5 (δ 138.4, ^3J), C-4 (δ 119.6, ^2J) and C-3 (δ 118.6, ^3J). Finally, the carboxamide group is bound at C-3. Respectively, compound **53** was confirmed as 2-(2-hydroxy-proplyl-4-methyl-furan-3-carboylic acid amide. The isomeric Pyrrolecarboxylic acid was excluded because of their strongly deviating shifts for C-2 and C-5 (calculated by ACD), while **53** was fitting nearly perfectly.

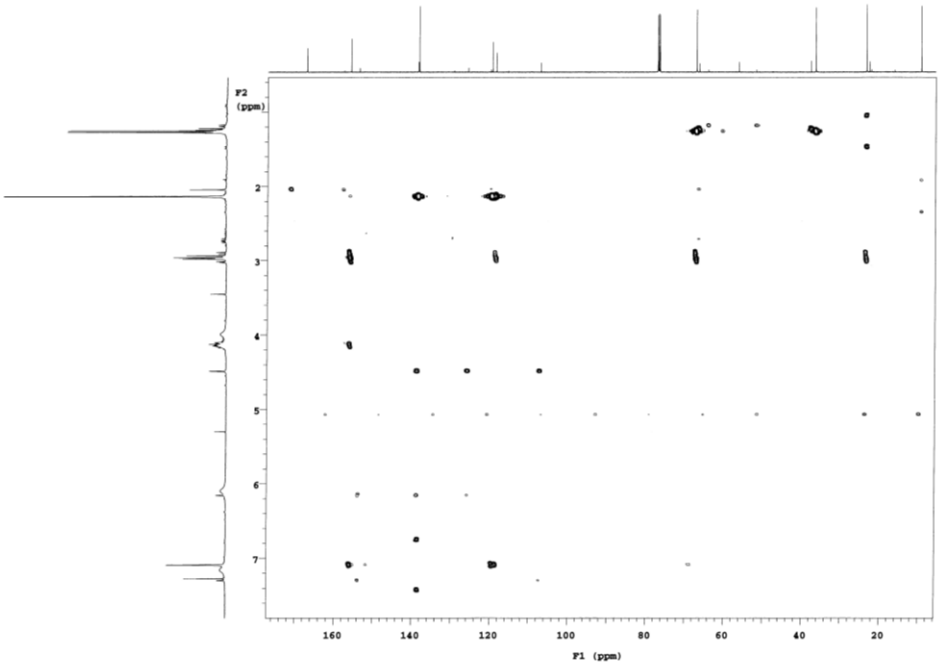


Figure 26: HMBC spectrum ($CDCl_3$, 600 MHz) of 2-(2-hydroxy-propyl-4-methyl-furan-3-carboxamide (**53**).

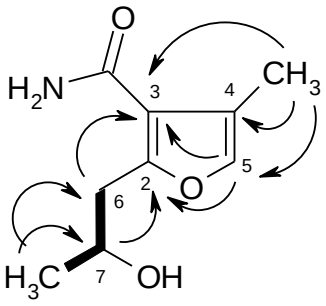


Figure 27: Selected HMBC (→), and H,H COSY (▬) connectivities of 2-(2-hydroxy-propyl-4-methyl-furan-3-carboxamide (**53**).

Table 3: NMR ($CDCl_3$) assignments of compounds **51-53** (*J* in Hz).

Position	**51**		**52**		**53**	
	δ_C [a]	δ_H [b]	δ_C [a]	δ_H [b]	δ_C [a]	δ_H [b]
1	67.3	3.44 (dd), 3.27 (m)	69.3	3.44 (s)	-	-
2	31.9	2.19 (m)	72.6	-	155.9	-
2-CH_3	16.9	0.87 (d, 6.7)	24.3	1.20 (s)	-	-
3	47.6	2.50 (m), 2.25 (m)	49.6	2.80 (dd, 16.0, 9.2), 2.54 (dd, 9.6,4.5)	118.6	-
3-$\underline{C}ONH_2$	-	-	-	-	167.2	-
3-$CO\underline{NH_2}$					-	6.19, 7.24 (s br, NH_2)
4	211.9	-	212.6	-	119.6	-
4-CH_3	-	-	-	-	9.5	2.13 (d, 1.2)
5	51.6	2.50 (m)	52.9	2.62 (m)	138.4	7.09 (q, 1.1)
6	63.9	4.18 (m)	63.9	4.25 (m)	36.6	2.94 (ABX, 14.7, 11.5, 4.0)
7	22.5	1.14 (d, 6.3)	22.7	1.19 (d, 6.2)	67.2	4.14 (m)
8	-	-	-	-	23.6	1.26 (d, 6.2)

[a]125 MHz; [b]300 MHz

Feeding experiments to elucidate the biosynthesis of **51-53** have not been performed so far, so that the following considerations are highly speculative. The first two compounds (**51**, **52**) are considered as building blocks of the furan carboxamide **53**. In the first step, the triketide unit might be combined with dihydroxy-acetone phosphate followed by decarboxylation or dehydration, yielding compounds **51** and **52**, respectively, or cyclisation and dehydrogenation might afford the corresponding furancarboxamide **53** (**Figure** 28).

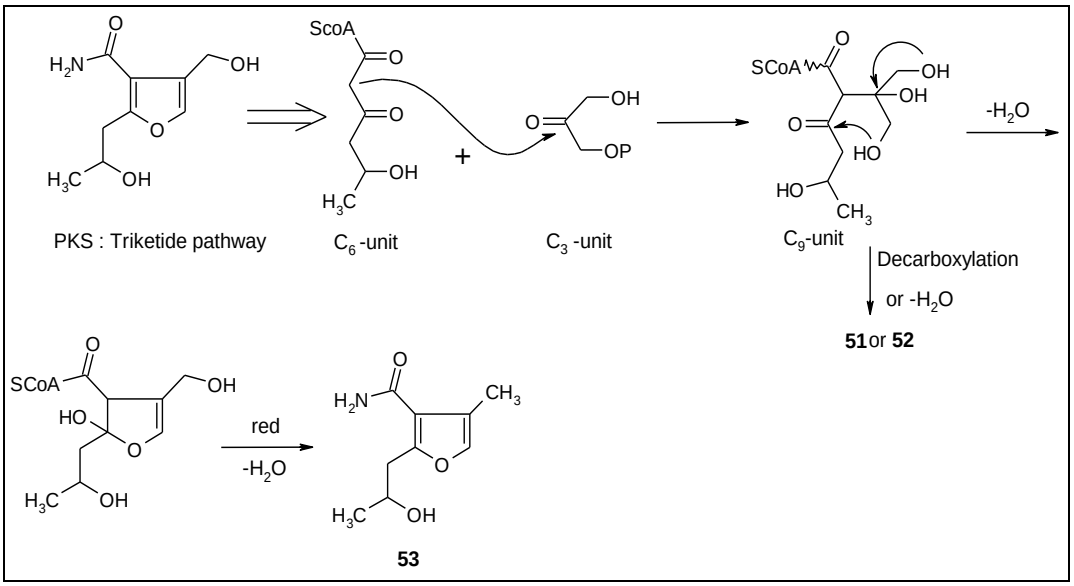


Figure 28: Proposed biosynthesis of compounds **51-53**.

4.2.3.1 Biological activity

Diverse antimicrobial activities were carried out for the extract in comparison with the isolated compounds. This was performed on the basis of agar diffusion method (40 µg/disc), and examined against eleven microbial tests with *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Rhizoctonia solani* and *Pythium ultimum*. In spite of the high chemical interest of the strain extract, it showed, among with its delivered components, no activity against the whole types of microbial tests. On the other hand, examination of the newly isolated compounds (**51-53**) exhibited cytotoxic activities, ranging between moderate and weak, against brine shrimps (*Artemia salina*) (Table 4).

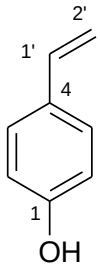
Table 4: Cytotoxic activities of compounds (**51-53**) against brine shrimps (*Artemia salina*).

Compounds	Brine shrimp (10 μg/ml)
51	8.3 %
52	1.5 %
53	3.0%

4.2.4 4-Vinylphenol

The fraction II was subjected to silica gel column chromatography (CH_2Cl_2/MeOH) to deliver UV- absorbing colourless oil, which had a strong fragrance. It stained to dark pink on heating after spraying with anisaldehyde/sulphuric acid reagent. In the ^{1}H NMR spectrum, two 2H doublets at δ 6.79 and δ 7.31 with the same coupling constant clearly indicated a 1,4-disubstituted aromatic pattern containing an electron-donating group. In addition, two olefinic protons (δ 5.13 and 5.61) with large coupling constant (>12 Hz) and a proton at δ 6.65 representing a vinyl system were also visible. The search in AntiBase with the above spectroscopic data resulted in *p*-vinylphenol (**54**).

4-Vinylphenol (**54**) is a common metabolite and flavouring component of fruits, flowers, fungi and bacteria. It is reported to have activity against fungi causing serious decay to pine trees.[59]



54

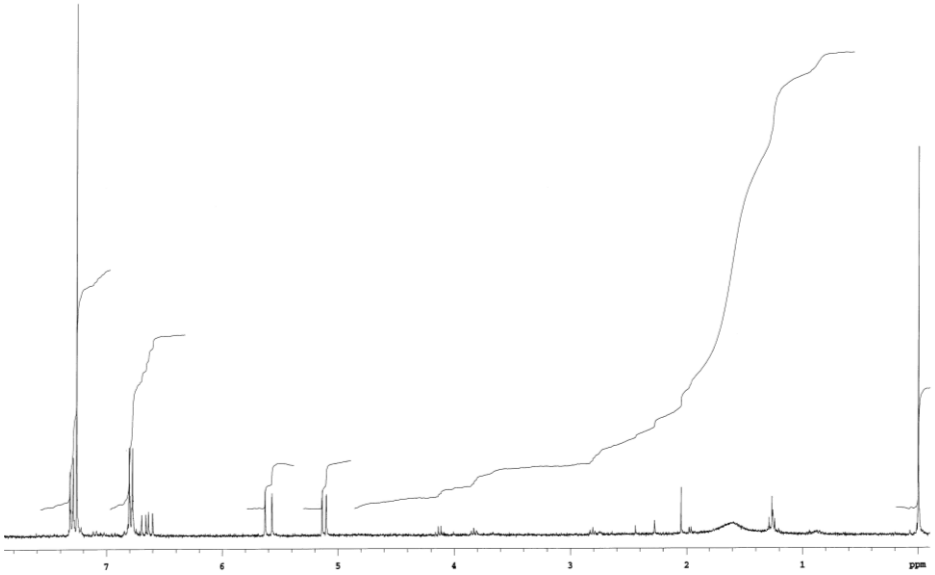


Figure 29: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 4-vinylphenol (**54**).

4.2.5 2-Methoxy-4-vinylphenol

Compound **55** was isolated from fraction II as colourless oil as well. It was UV absorbing at 254 nm and stained to violet after spraying with anisaldehyde/sulphuric acid reagent. The ^{1}H NMR spectrum revealed a 1,2,4-trisubstituted aromatic pattern showing two doublets and 1 doublet of doublet between δ 6.80 and 7.00. Moreover, three olefinic protons were also visible as in compound **54** representing again a vinyl system. Additionally, a methoxy signal at δ 3.92 was present. The mass of the compound was determined by GC-MS and EIMS as 150 Dalton. The search in AntiBase and comparing the spectrum with ACD program identified the compound as 2-methoxy-4-vinylphenol, also known as 4-vinylguaiacol.

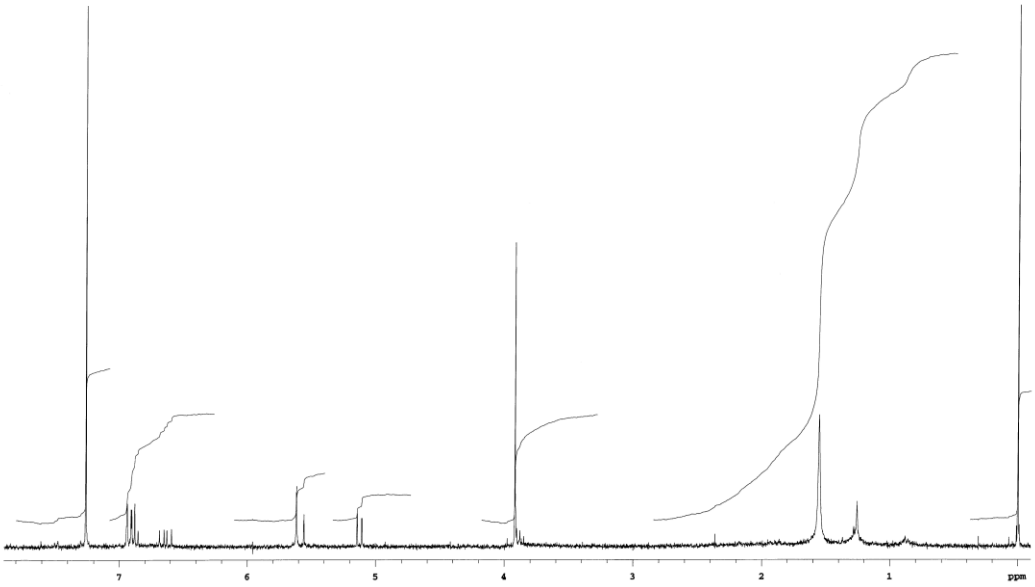
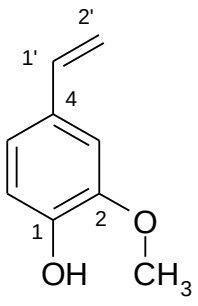


Figure 30: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 2-methoxy-4-vinylphenol (**55**).



55

4.3 Terrestrial *Streptomyces* sp. Ank 250

The crude extract of the terrestrial *Streptomyces* sp. Ank 250 showed high activity against Gram-positive bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis*, moderate activity against *Escherichia coli* and *Streptomyces viridochromogenes* (Tü 57). It also exhibited high cytotoxicity against brine shrimps (*Artemia salina*).

After usual workup and fermentation on large scale, the crude extract was subjected to silica gel column for fractionation and further purified by different chromatographic techniques.

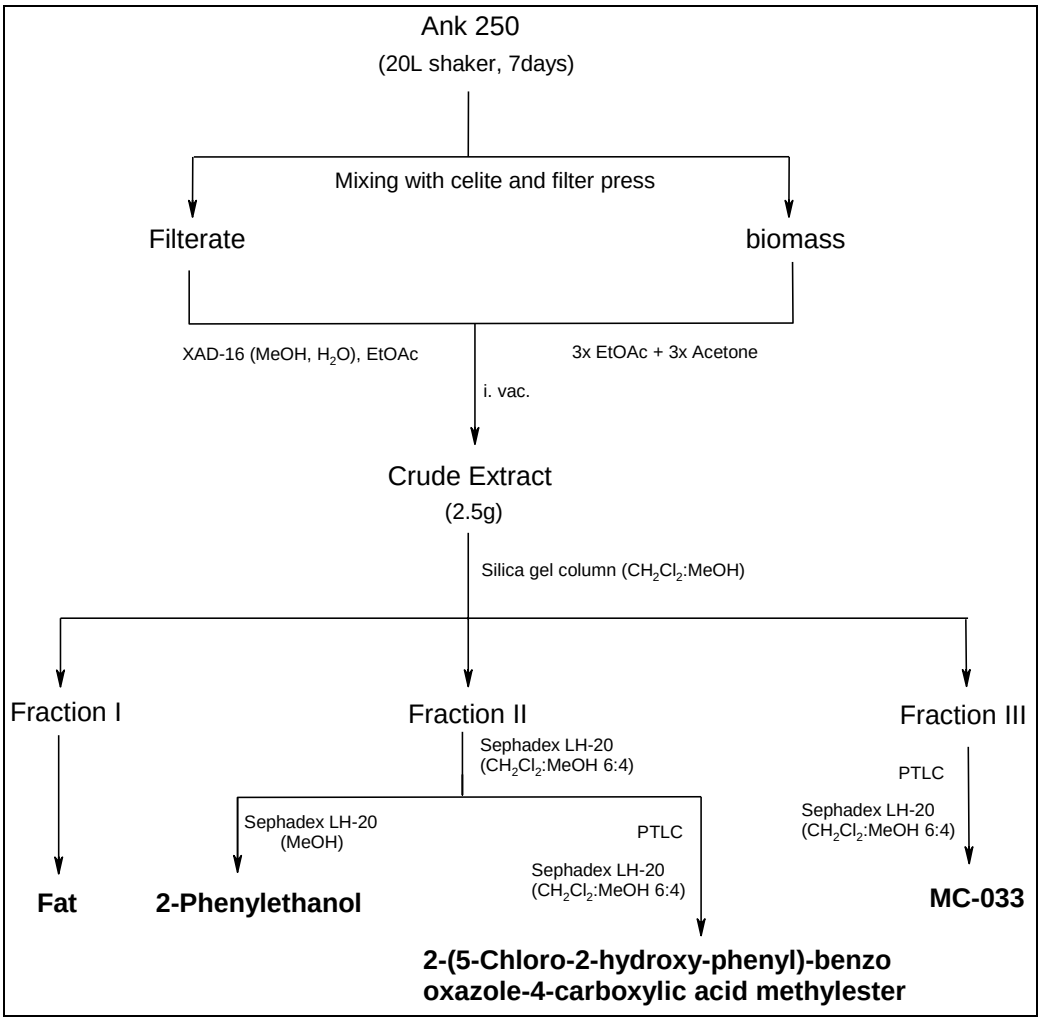


Figure 31: Work up scheme of terrestrial *Streptomyces* sp. Ank 250

4.3.1 2-(5-Chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester

The compound **56** was isolated from fraction II after purification by PTLC and Sephadex LH-20 (CH_2Cl_2/40%MeOH) as a highly green fluorescent, yellow solid. In the ^1H NMR spectrum, six protons in the aromatic region, a broad singlet (1H, δ 11.78) of an acidic proton (OH or NH) and a methoxy signal at δ 4.03 were visible. The ^{13}C NMR spectrum revealed 15 well-resolved carbon signals, among them two carbonyl signals at δ 165.2 and 163.0 as indication of an ester, amide or acid. In addition, six quaternary carbons between ~ δ 160.0-111.0, six aromatic methine carbons and a methoxy signal at δ 52.5 were also visible.

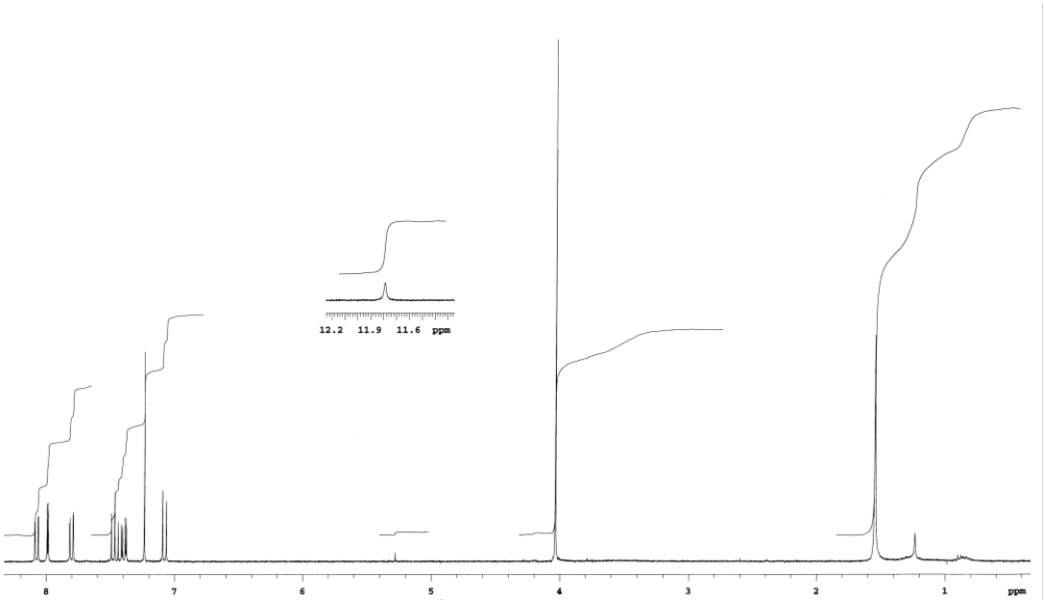


Figure 32: ^1H NMR spectrum ($CDCl_3$, 300 MHz) of 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**).

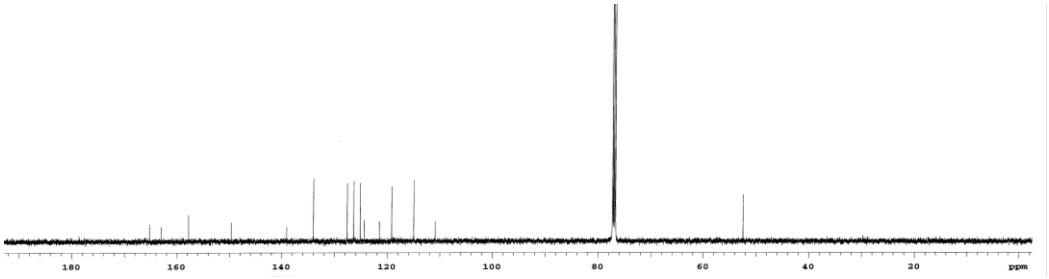
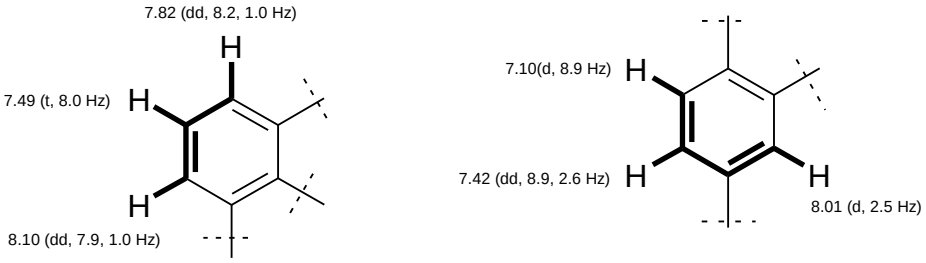


Figure 33: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**).

The (+)-ESIMS spectrum of the compound provided a *pseudom*olecular ion peak at *m/z* 326 $[M+Na]^+$ and an isotopic peak at *m/z* 328, which was 1/3rd of the molecular ion peak as an indication for the presence of chlorine. The molecular formula was determined by HRESIMS as $C_{15}H_{10}ClNO_4$.

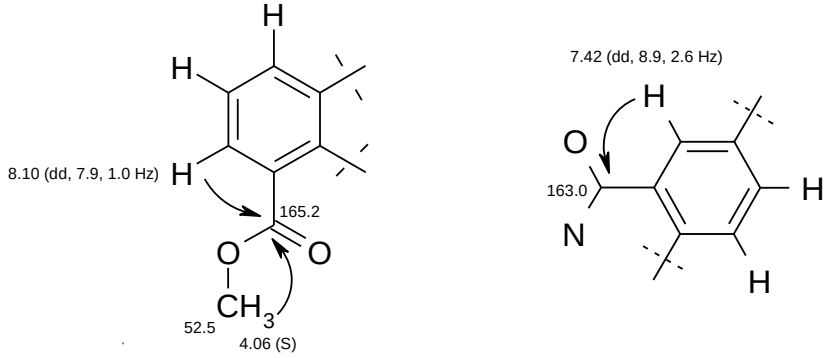
From the H,H COSY spectrum, two sub-structures **A** and **B** were constructed, one of which was 1,2,3 trisubstituted aromatic ring, and the other was a benzene ring displaying an ABX system.



A **B**

Figure 34: H,H COSY connectivities (**—**) of 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**).

The search in AntiBase with the sub-structures and the molecular formula gave no exact hit indicating the compound as a new natural product. The complete structure was elucidated with the help of HMBC spectrum where the proton at δ 8.10 and methoxy protons (δ 4.03, s) showed a ^3J correlation with carbonyl at δ 165.2 indicating the attachment of acetyl group with the aromatic ring (substructure **C**). The proton at δ 8.01 also exhibited ^3J correlation with the carbon at δ 163.0, which in turn could be attached with oxygen on one side and nitrogen on the other side (substructure **D**).



C **D**

Finally, the structure was fully elucidated as the new 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**) with the help of the remaining HMBC correlations.

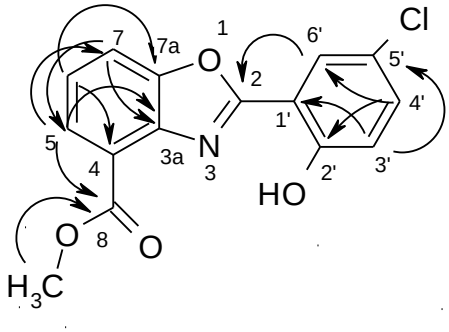
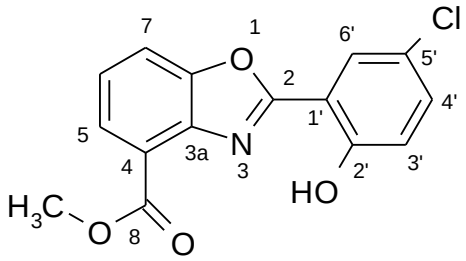


Figure 35: HMBC (→) correlations of 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**).



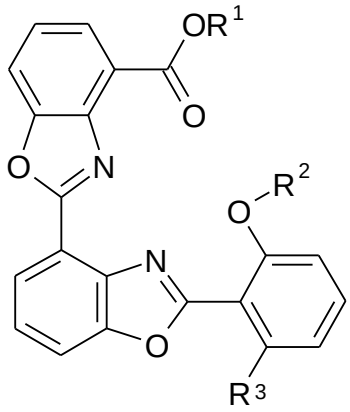
56

Table 5: NMR ($CDCl_3$, *J* in Hz) assignments of 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**)

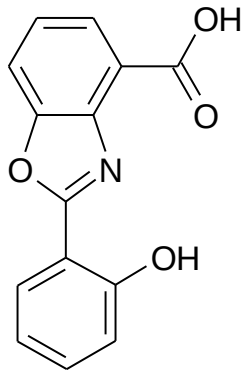
No.	2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**)	
	δ_C [a]	δ_H [b] (*J* in [Hz])
1	-	-
2	163.0	-
3	-	-
3a	139.1	-
4	121.5	-
5	127.6	8.10 (dd, 7.9, 1.0)
6	125.1	7.49 (t, 8.0)
7	114.5	7.82 (dd, 8.2, 1.0)
7a	149.7	-
8	165.2	-
8-OCH_3	52.5	4.06 (s)
1'	110.9	-
2'	157.8	-
2'-OH	-	11.80 (br s)
3'	119.2	7.10 (d,8.9)
4'	134.0	7.42 (dd, 8.9, 2.6)
5'	124.4	-
6'	126.4	8.01 (d, 2.5)

[a]125 MHz; [b]300 MHz

So far, few benzoxazole derivatives are known from *Streptomyces* sp. including cytotoxic UK-1[60] (**57**), AJI-9561[61] (**58**) and nataxazole[62] (**59**) etc. UK-1 (**57**) selectively inhibits human topoisomerase II as it has ability to form complexes with divalent and trivalent metal ions and due to this ability, it also forms complexes with double-stranded DNA in the presence of Mg^{2+} ions.[63] Recently, a new antibiotic caboxamycin (**60**) was isolated, which is structurally highly similar to compound **56**. It was obtained from a deep sea *Streptomyces* strain, which showed inhibitory activity against Gram-positive bacteria, selected human tumor cell lines and the enzyme phosphodiesterase.[64] However, compound **56** did not show antimicrobial or cytotoxic activity in our test strains.



57: $R^1 = CH_3$, $R^2 = R^3 = H$
58: $R^1 = R^2 = H$, $R^3 = CH_3$
59: $R^1 = CH_3$, $R^2 = H$, $R^3 = CH_3$



60

4.3.2 MC-033

The compound MC-033 (**62**) was obtained as a colourless amorphous powder during the purification by PTLC of fraction III. It was strongly UV-absorbing at 254 nm and showed a green colour after spraying with anisaldehyde/sulphuric acid.

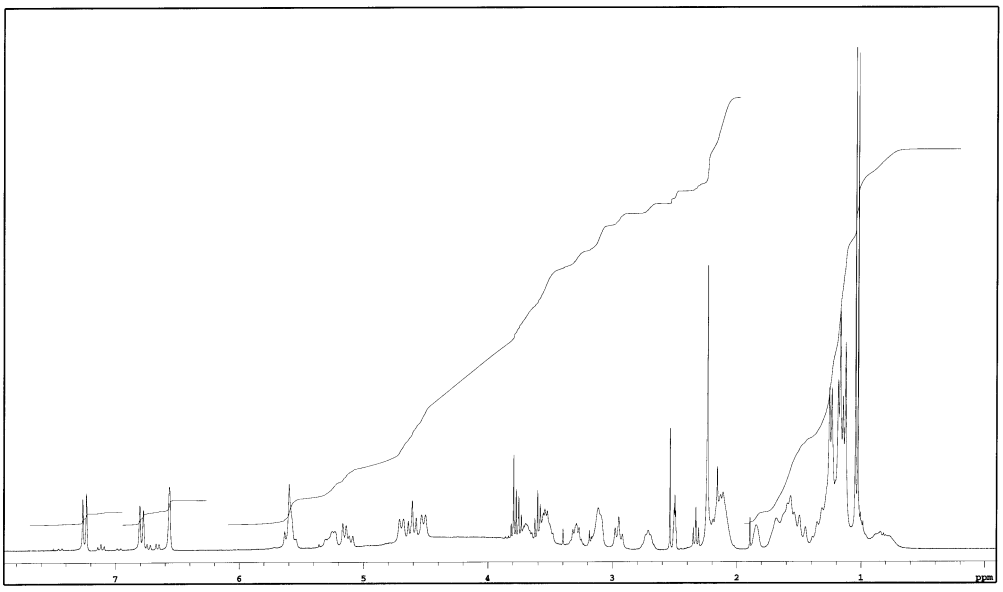


Figure 36: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of MC-033 (**62**).

The ^{1}H NMR spectrum showed two *ortho*-coupled protons at δ 7.25 and 6.79 (J = 8.7) and a singlet (1H, δ 6.57) in the aromatic region. There were also many signals in the olefinic and sugar region probably containing one or more sugars.

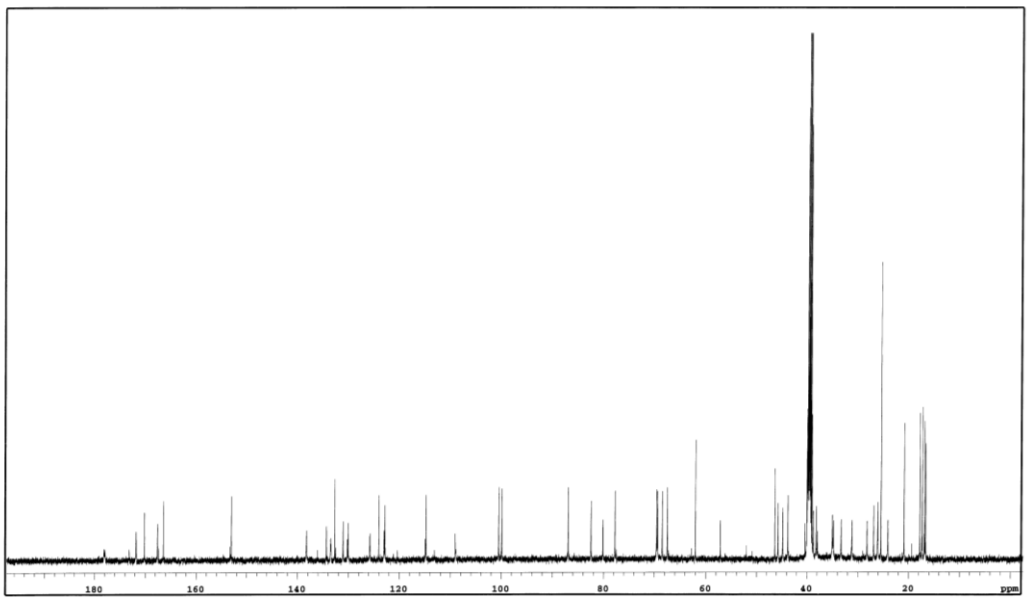
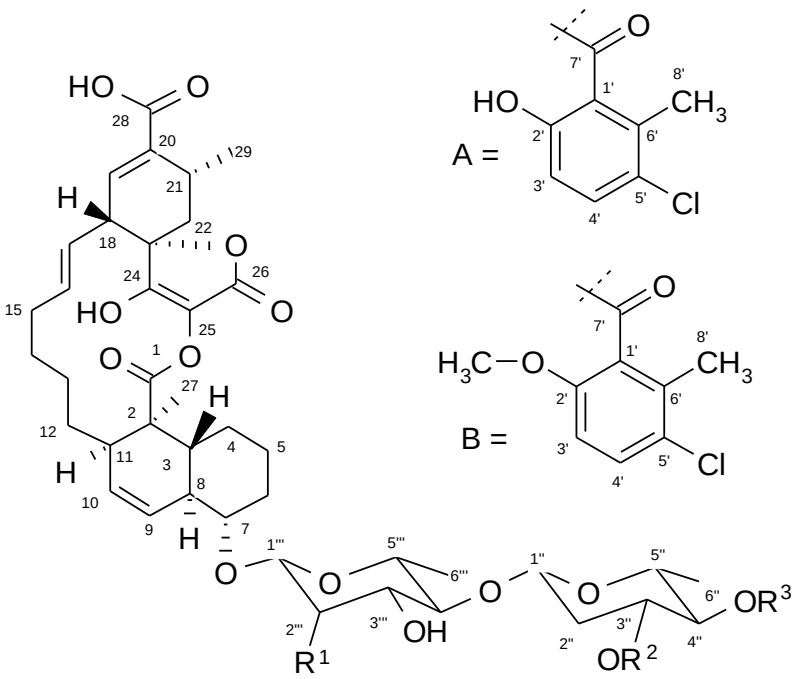


Figure 37: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of MC-033 (**62**).

The complex ^{13}C NMR spectrum displayed thirty-nine carbon signals including four carbonyl signals δ (~160-170) representing ester or acid, an aromatic oxymethine at δ 153.0 and several signals in the aromatic region between δ ~140-110. The two anomeric signals at δ 100.3 and 99.9 clearly indicated the presence of two sugar moieties. It also revealed several methyl and methylene carbons in the aliphatic region.

The ESI mass spectrum of the compound revealed an $[M-H]^-$ ion peak at *m/z* 939 having the isotopic peak [M+2] of chlorine. The HRESIMS provided the molecular formula as $C_{49}H_{61}O_{16}Cl$. The search with the help of molecular formula and the spectroscopic data in the AntiBase provided 2 hits i.e. MC-031 (**61**) and MC-033 (**62**).

MC-031 (**61**) and MC-033 (**62**) belong to the chlorothricin (**63**) type of antibiotics, isolated from a *Streptomyces* sp. as cholesterol biosynthesis inhibitors, [65] which only differ in the position of group A on the sugar moiety. To confirm the structure, the compound was subjected to 2D NMR measurements. In the HMBC experiment, the 4"-H signal (δ 4.62, t, J = 9.2 Hz) showed ^3J correlation with the carbonyl (C-7') at δ 170.9. The complete NMR assignments of compound **62** are given in Table 6 in comparison with the literature.



61: R^1 = H, R^2 = A, R^3 = H

62: R^1 = H, R^2 = H, R^3 = A

63: R^1 = H, R^2 = B, R^3 = H

Table 6: NMR ($CDCl_3$, *J* in Hz) assignments of MC-033 (**62**) in comparison with literature.[65]

Position	Experimental		Literature	
	δ_C	δ_H	δ_C	δ_H
1	177.9	-	177.3	
2	46.5	-	47.5	
3	38.2	1.53 (m)	37.9	
4	26.1	1.85 (m)	26.5	
5	24.1	1.72 (m), 1.26 (m)	24.3	
6	35.0	1.21 (m)	36.1	
7	82.3	3.16 (m)	83.0	
8	43.7	1.68 (m)	43.7	
9	122.8	5.23 (d)	123.2	
10	131.1	5.24	130.0	
11	45.7	1.84 (m)	46.6	
12	33.3	1.22 (m)	33.1	
13	26.9	0.79 (m)	28.0	
14	28.2	1.32 (m)	28.4	

15	31.2	2.45 (m)	32.3	
16	138.3	5.32 (m)	138.1	
17	124.0	5.02 (t)	124.5	
18	44.8	3.26 (m)	46.0	
19	134.3	6.60, (d)	138.4	
20	132.7	-	133.6	
21	25.4	2.85 (m)	26.9	
22	34.8	1.22 (m)	34.9	
23	80.2	-	80.4	
24	166.5	-	159.4	
25	114.8	-	115.5	
26	170.1	-	165.0	
27	16.7	1.25 (d, 6.0)	16.7	
28	171.8	-	170.1	
29	20.9	1.03 (d, 6.1)	20.7	
1'	122.8	-	122.8	
2'	153.1	-	154.4	
3'	109.1	6.79 (d, 8.6)	109.9	
4'	130.1	7.25 (d, 8.7)	130.7	
5'	125.7	-	126.8	
6'	133.5	-	134.0	
7'	167.6	-	166.7	
8'	16.9	2.24 (s)	16.8	
1"	99.9	4.69 (d, 8.9)	99.9	4.69 (br d, 9.5)
2"	38.7	1.56 (m)	38.7	1.55 (m), 2.17 (m)
3"	67.4	3.68 (m)	67.4	3.68 (m)
4"	77.8	4.60 (t, 9.2)	77.8	4.60 8 (t, 9.5)
5"	69.4	3.56 (m)	69.3	3.55 (m)
6"	17.3	1.24 (br d, 6.0)	17.2	1.24 (d, 6.0)
1"	100.5	4.51 (br d, 9.7)	100.6	4.52 (br d, 9.5)
2"'	40.0	2.24 (m), 1.56 (m)	39.9	1.31 (m), 2.12 (m)
3"'	68.5	3.28 (m)	68.4	3.51 (m)
4"'	86.9	2.96 (t, 8.6)	87.0	2.93 (t, 9.5)
5"'	69.6	3.56 (m)	69.5	3.29 (m)
6"'	17.8	1.03 (d, 6.1)	17.8	1.16 (d, 6.0)

MC-033 (**62**) was firstly isolated in 1992 along with other three new cholesterol biosynthesis inhibitors from a *Streptomyces* sp.[65] They belong to the group of spiro-tetronate antibiotics that exhibit broad biological activities including antibacterial,

antitumor, antimalarial and cholesterol biosynthesis inhibition. They possess an unusual aglycone that contains a characteristic tetronic acid (spiro-linked to a cyclohexane ring) conjugated with a *trans*-decalin system by a carboxylic ester as in chlorothricin (**63**).[66] The aglycone moiety of MC-033 (**62**) could be biosynthesised from condensation of oxaloacetate and malonyl-CoA. The resulting compound would be hydroxylated at C-3 of the lactone ring, or may be acetylated in that position from the carboxy-terminus of the polyketide chain, followed by a Baeyer-Villiger oxidation. The other possibility is that the main polyketide chain does not terminate with propionate at C-1, but rather with the acetate unit, which give rise to C-25/C-26.[67] (Figure 38).

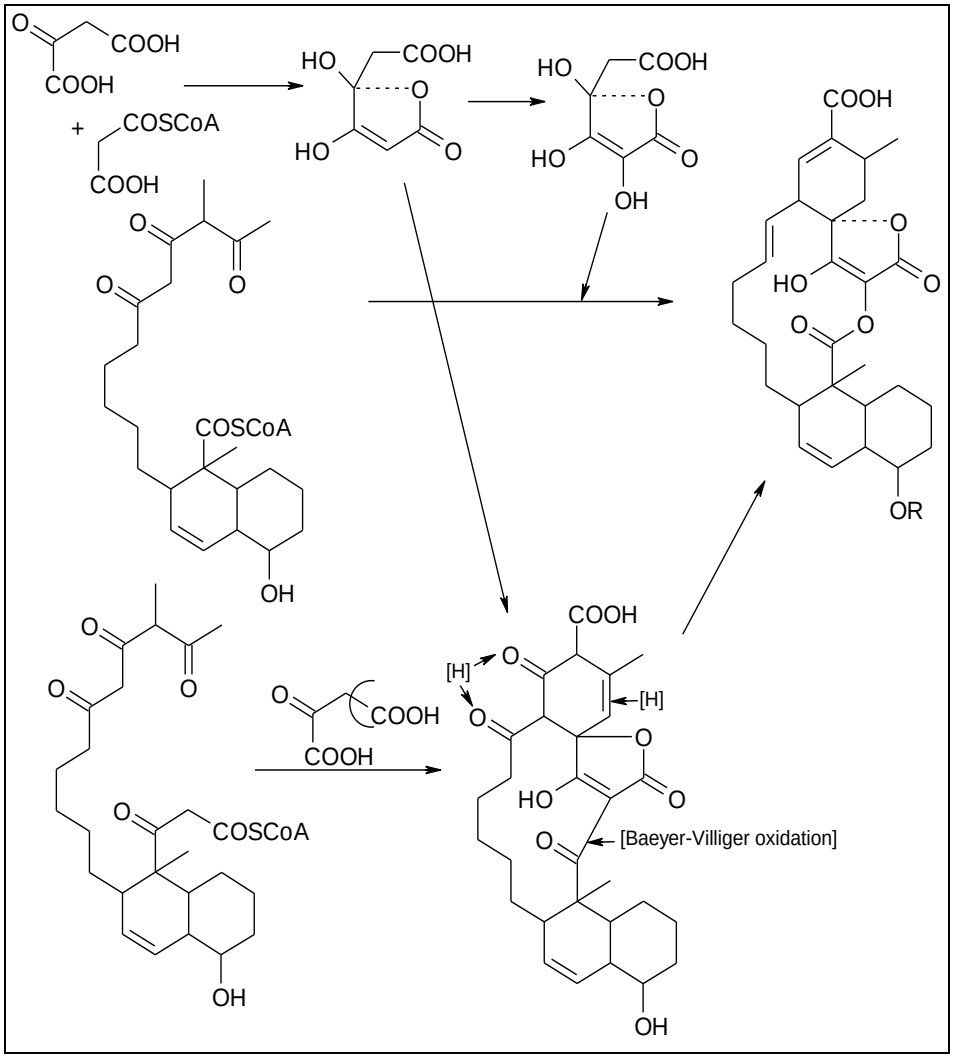


Figure 38: Hypothetical biosynthetic pathway[67] of aglycone of MC-033 (**62**).

4.4 Terrestrial *Streptomyces* Strain GW 51/426

The crude extract of the terrestrial Streptomyces strain GW 51/426 showed moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, it also exhibited high antifungal activity against *Mucor miehei* and *Aphanomyces cochlioides* and 95% cytotoxicity against brine shrimps (*Artemia salina*). A large-scale fermentation of the terrestrial *Streptomyces* isolate delivered a highly green fluorescent band and two orange/dark red components which turned to red/violet on treatment with sodium hydroxide as indication of *peri*-hydroxyquinones. Purification of the extract resulted in the following compounds.

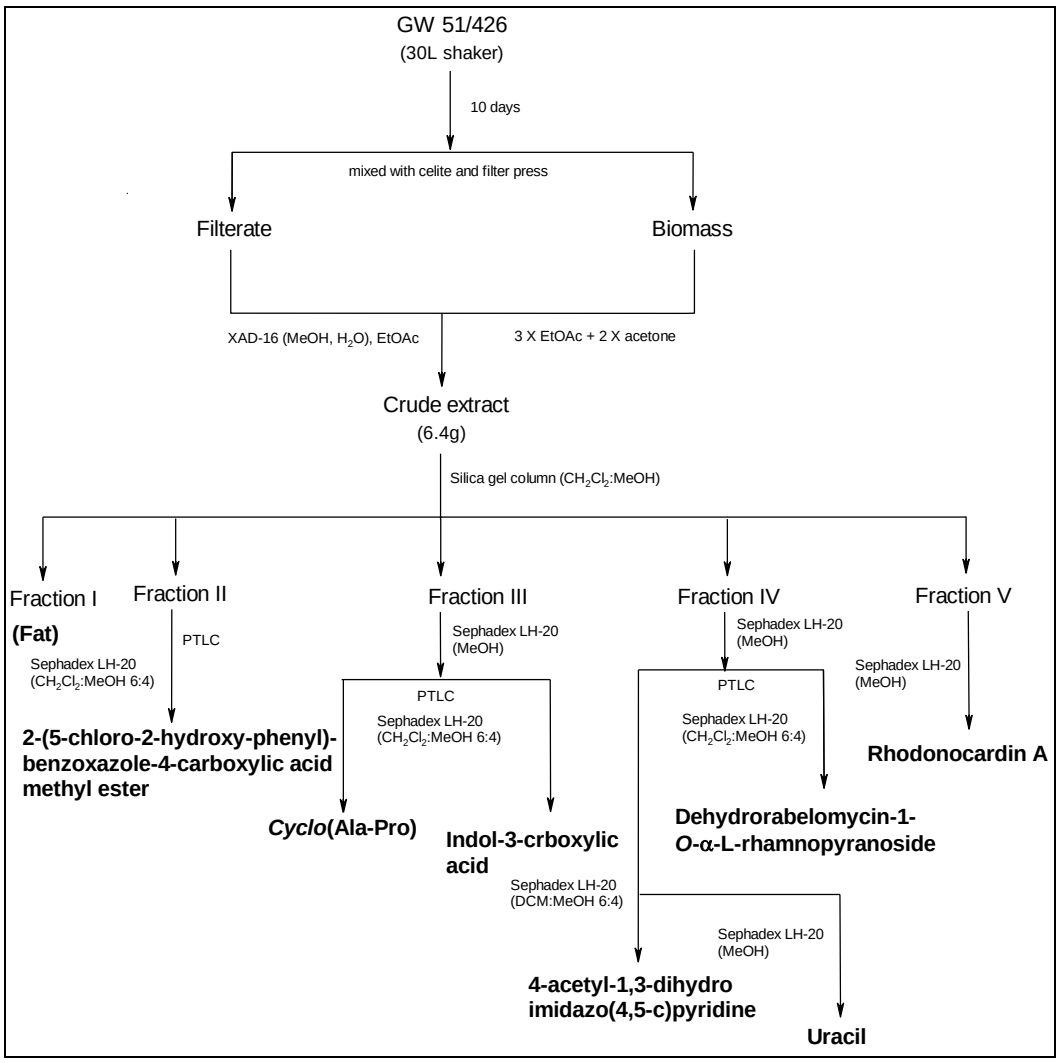


Figure 39: Work up scheme of terrestrial *Streptomyces* sp. GW 51/426

4.4.1 Dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside

Compound **64** was obtained from fraction IV through PTLC and Sephadex LH-20 (CH_2Cl_2/40%MeOH) as a middle polar brown solid, which turned to green on TLC by spraying with anisaldehyde/sulphuric acid, and red with diluted sodium hydroxide, as indication of a *peri*-hydroxy quinone. The molecular weight of **64** was determined by both modes of ESIMS as 466 Dalton, and its corresponding molecular formula was recognized as $C_{25}H_{22}O_9$ by HRESIMS.

The ^{1}H NMR spectrum of **64** revealed six aromatic methine signals, assigned to three aromatic patterns: being of 1,2,3-trisubstituted (δ 7.73, 7.54, 7.22, J = 7.4 Hz), 1,3-disubstituted (δ 7.14, 7.08, J = 1.3) and pentasubstituted (δ 7.34, s) aromatic residues, respectively. In the sugar and aliphatic regions, two 3H signals, an aromatic- bound methyl singlet (δ 2.44) and methyl doublet (δ 1.28, J = 6.2) of the sugar system were observed. Five sp^3 oxygenated methines were displayed, among them one anomeric (δ 5.46, J = 1.7 Hz), confirming the existence of a sugar system. The sugar system was also fixed by tandem ESI mass spectrometry; (+)-ESI-MS^2, which exhibited an ion peak at *m/z* 342 due to the loss of sugar moiety from the main structural ion peak

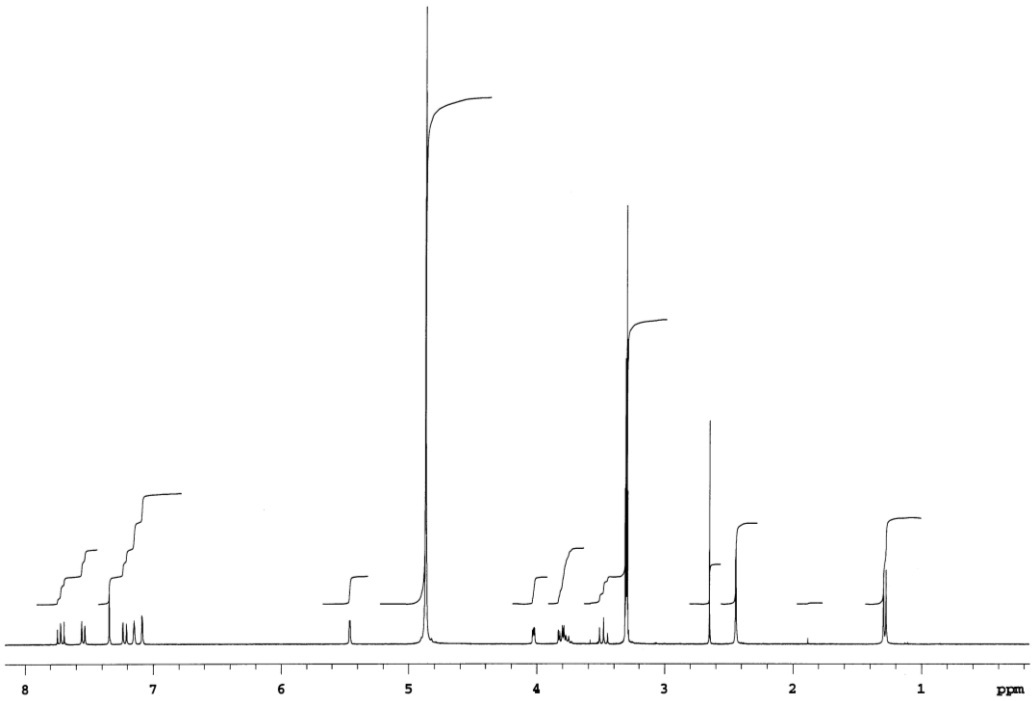


Figure 40: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside (**64**).

Correspondingly, the ^{13}C/HMQC spectra displayed the presence of 25 carbon signals, which were classified into the following categories: two quinone carbonyls (δ 193.9 and 187.1), three sp^2 oxygenated carbons (δ 162.3~155.4), five sp^2 methine carbons together with another seven sp^2 quaternary carbons. In the aliphatic region, four oxygenated methines were visible between δ 74.0~71.2 together with an anomeric carbon (δ 100.8), and two methyl carbon signals (δ 22.3 and 18.1). Based on these spectroscopic data and the molecular formula, a search in AntiBase, the Dictionary of Natural Products (DNP) and the Chemical Abstracts, confirmed **64** as a new quinone glycoside.

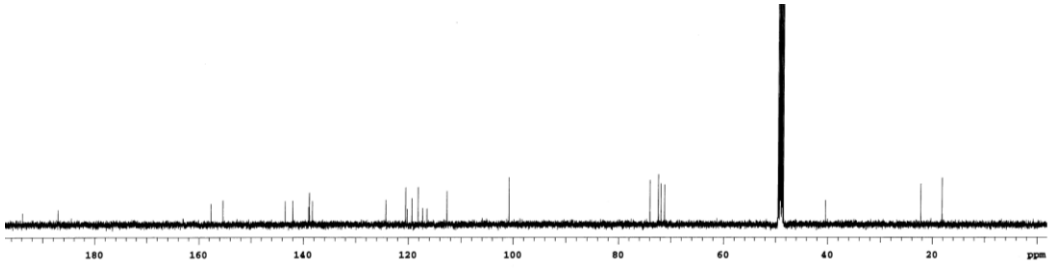


Figure 41: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of Dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside (**64**).

With the help of HMBC and H,H COSY experiments, the trisubstituted aromatic residue was found to be fused with the benzoquinone system in such a way that the methine doublet of H-11 (δ_H: 7.54, δ_C: 119.4) showed a ^3J coupling with the up-field shifted carbonyl C-12 (δ 187.1) together with the two quaternary carbons C-11a (δ 138.3, ^2J) and 7a (δ 116.5, ^3J). On the other hand, the methine doublet of H-9 (δ_H: 7.22, δ_C: 124.3) displayed a coupling with the oxygenated carbon C-8 (δ 163.2, ^2J) and C-7a (δ 116.5, ^3J). By this way, the downfield shifted carbonyl (δ 193.9) was fixed in *peri*-position to the hydroxyl group attached to C-8 (substructure **A**).

Furthermore, the two *m*-coupled protons H-2 (δ 7.08), and H-4 (δ 7.14), and the aromatic-bound methyl 3-CH_3 (δ_H: 2.44) were included in one aromatic ring, containing another oxygenated carbon C-1 (δ 155.1), two fused quaternary carbons C-12b (δ 117.3) and C-4a (δ 142.0), and C-3-CH_3 (δ 143.4). The remaining sp^2 methine singlet CH-5 (118.2) displayed four essential correlations, among them two were obvious to CH-4 (δ_C 120.5) and C-12b (δ 117.3), indicating a fusion between both rings *via* C-12b/C-4a. The respective couplings between CH-5 and the third sp^2 oxygenated carbon C-6 (δ_C 157.7) and C-6a (δ 20.0) confirmed the main skeleton of the quinone system to be one of the two partial structures **B** or **C**. Based on these fea-

tures, compound **64** is an angular hydroxybenzanthraquinone, at where the two *peri*-hydroxyl groups could be on the same side around one carbonyl (C-7) or in opposite directions chelating both carbonyls (C-7 and C-12).

A further observation of the HMBC experiment proved a direct attachment of rhamnose to the quinone system by an *O*-glycosidic bond between C-1 (δ 155.4) and C-1' (δ 100.8), *via* a long rang coupling between the anomeric proton H-1' (δ 5.46) and C-1. In accordance, two alternatives were purposed (**64** and **65**), however, the big difference in chemical shifts between both carbonyls ($\Delta\delta$ = 6) and comparison of the δ_C values of C-7 (193.8) and C-12 (187.1) in ring C with the closely related benzanthrin A (**67**) [68] and its aglycone 6-hydroxy-tetraangulol [69] or dehydrorabelomycin[70] (**66**) established that the two *peri*-hydroxy groups must be on the same side around the carbonyl C-7, and hence the structure **64** has been deduced as 6-hydroxytetrangulol-1-*O*-α-L-rhamnopyranoside (dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside).

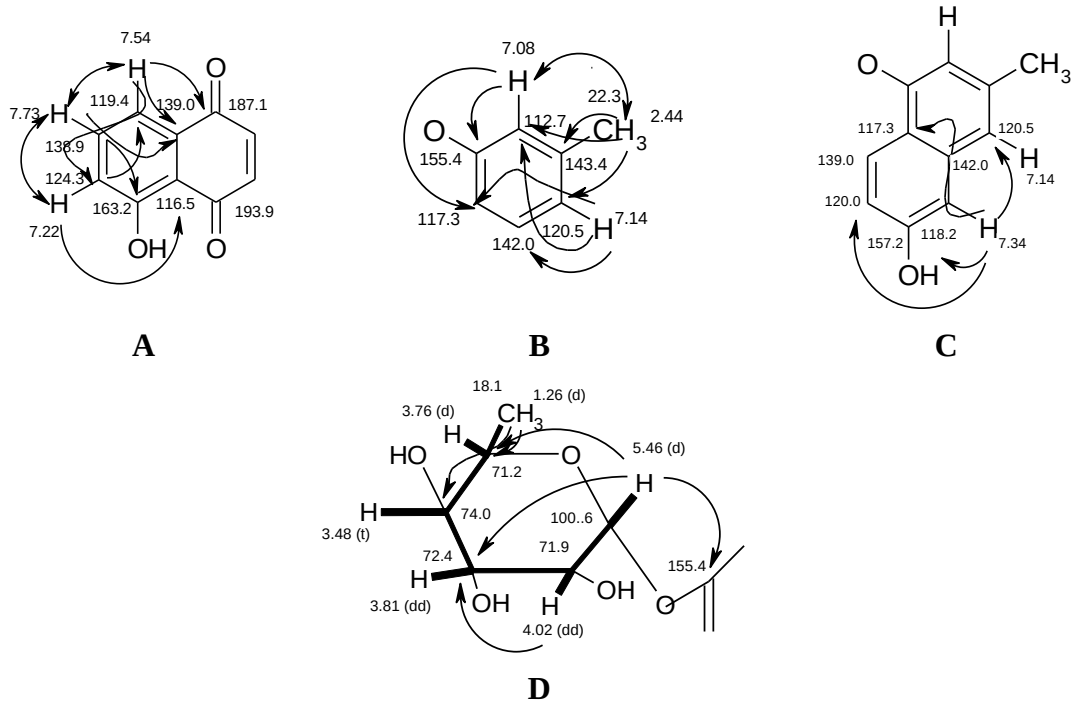
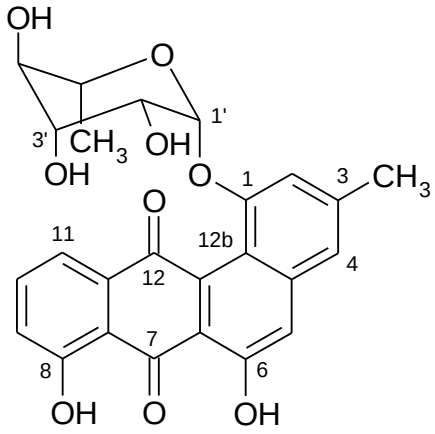
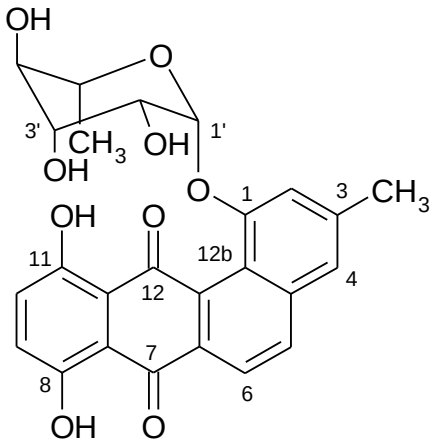


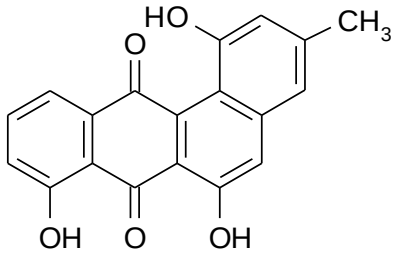
Figure 42: HMBC (→), and H, H COSY (↔, ▬) connectivities of constituted partial structures (**A**, **B**, **C**, **D**) of dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside (**64**)


OH
O
1'
3'
CH3
OH
OH
O
1
3
CH3
O
11
12b
4
12
7
8
6
OH
O
OH

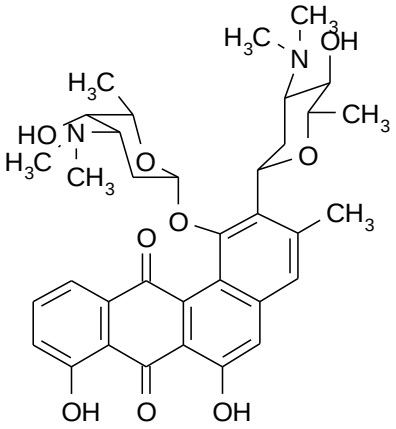
64


OH
O
1'
3'
CH3
OH
OH
O
1
3
CH3
OH
O
11
12b
4
12
7
8
6
OH
O

65


HO
CH3
O
OH
O
OH

66


CH3
H3C
N
OH
H3C
CH3
HO
N
H3C
CH3
O
O
O
CH3
O
OH
O
OH

67

Table 7: NMR (CD_3OD, (*J* in Hz) assignments of dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside (**64**)

No.	Dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside (**64**)	
	δ_C [a]	δ_H [b]
1	155.4	-
2	112.7	7.08 (d, 1.3)
3	143.4	-
3-CH_3	22.6	2.44 (s)
4	120.5	7.14 (d, 0.9)
4a	142.0	-
5	118.2	7.34 (s)
6	157.7	-
6a	120.0	-
7	193.8	-
7a	116.5	-
8	163.2	-
9	124.3	7.22 (d, 7.4)
10	138.9	7.72 (t, 7.4)
11	119.4	7.54 (d, 7.4)
11a	138.3	-
12	187.1	-
12a	139.0	-
12b	117.3	-
1'	100.8	5.46 (d, 1.7)
2'	71.9	4.02 (dd, 3.4, 1.8)
3'	72.4	3.81 (dd, 9.5, 3.4)
4'	74.0	3.48 (t, 9.4)
5'	71.2	3.76 (m)
6'	18.1	1.28 (d, 6.2)

[a]125 MHz; [b]300 MHz

Compound **64** belongs to the angucycline class of compounds which represent a large class of polyketides in nature having diverse biological activities. The compound **64** was tested in our lab against a number of pathogenic microorganisms and it showed moderate activity against *Staphylococcus aureus* (inhibition zone: 18 mm) and *Escherichia coli* (inhibition zone: 16 mm) at 40 µg/disk.

The aglycone of compound **64**, dehydrorabelomycin can be biosynthesized by 10 acetate units through a minimal polyketide synthase complex (minPKS), which

contains various enzymatic domains and an acyl carrier protein (ACP) to which the growing polyketide chain is attached. The polyketide chain is successively folded by different enzymes to form the respective compound.[71] The hypothetical biosynthetic pathway is drawn in Figure 43.

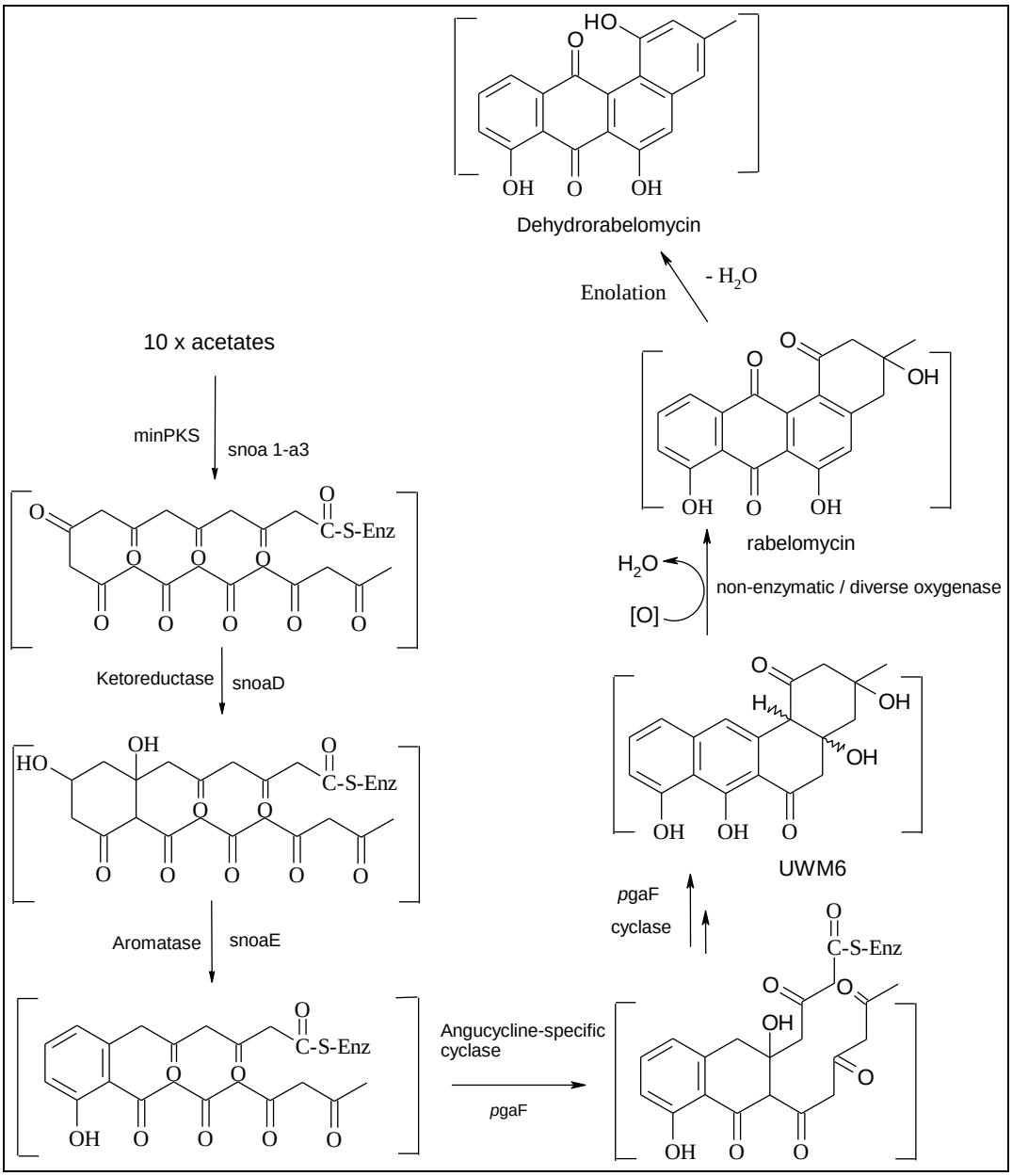


Figure 43: Hypothetical biosynthetic pathway[71] of dehydrorabelomycin (**66**).

4.4.2 Rhodonocardin

The compound **68** was isolated from fraction V as highly polar, water soluble dark red solid, which turned to green with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of **68** showed four signals in the aromatic region, attributed to 1,2,3-trisubstituted (δ 7.71, 7.57, 7.29; J ~ 8.0 Hz) and pentasubstituted (δ 6.77, s) aromatic residues, respectively. In the sp^3 region, three anomeric protons (δ 5.37, 5.30, 5.15; J ~0-3.6 Hz) corresponding to three α-configurated glycosides were observed, together with several oxygenated methines and methylenes located in the region of δ 4.25-3.36. Finally, two signals, representing two methyls, were displayed at δ 1.19 (s) and 0.52 (d).

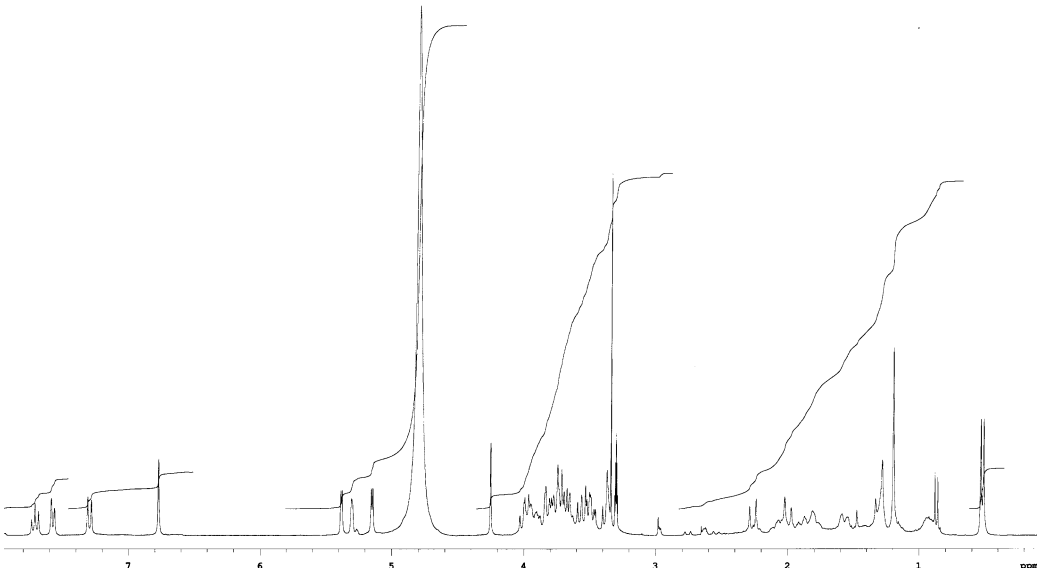


Figure 44: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of rhodonocardin A (**68**).

The ^{13}C NMR spectrum of **68** displayed 37 carbon signals, which were classified into two methyls, three methylenes, twenty methines and twelve quaternary carbon signals; five down-field quaternary sp^2 carbons among them were attributed to three carbonyls (δ 204.0, 189.4, 183.8) and two oxygenated carbons (δ 164.5 and 162.4). Three anomeric carbon signals were displayed at δ 95.5, 95.0 and 93.6 along with many oxy-methine/methylene carbons, as a further confirmation for the existence of three sugar moieties.

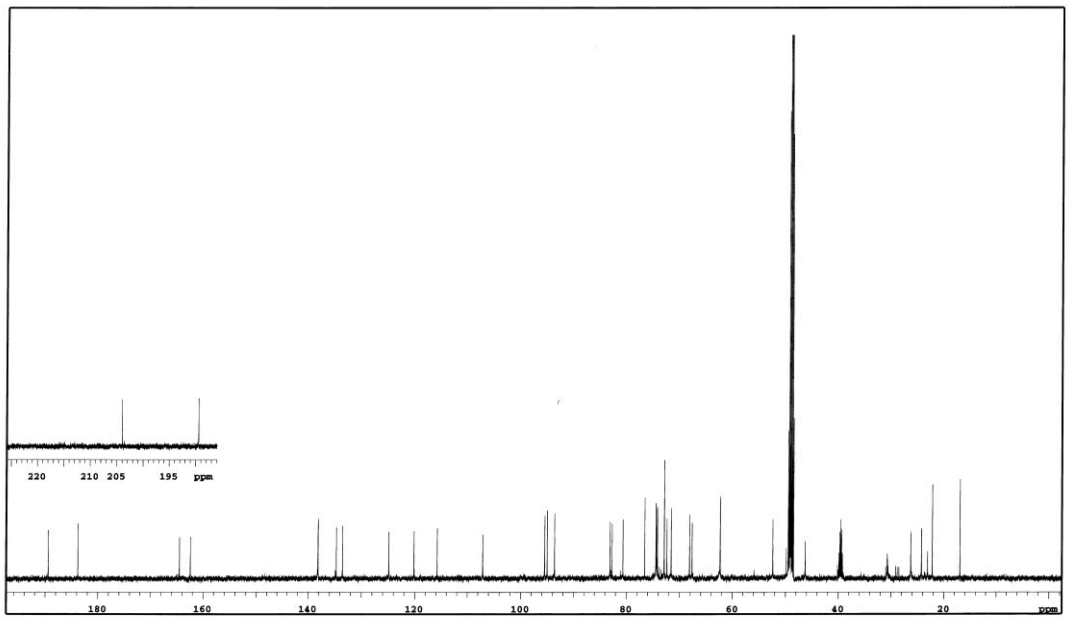
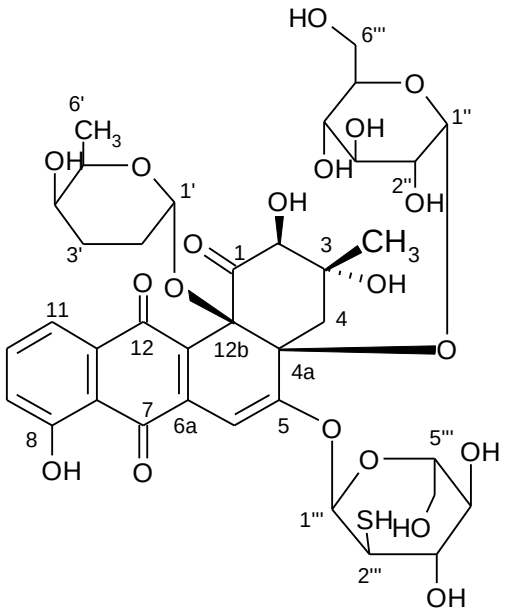


Figure 45: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of rhodonocardin A (**68**).

The molecular formula $C_{37}H_{46}O_{20}S$ was deduced by HRESIMS showing a molecular ion peak at *m/z* 865.2191 $[M+Na]^+$. Searching with the above spectroscopic data in AntiBase resulted in rhodonocardin A (**68**), which was previously reported only as its decaacetate; the structure was further confirmed by 2D NMR data (Table 8) and by ESI MS^2 (Figure 46).



68

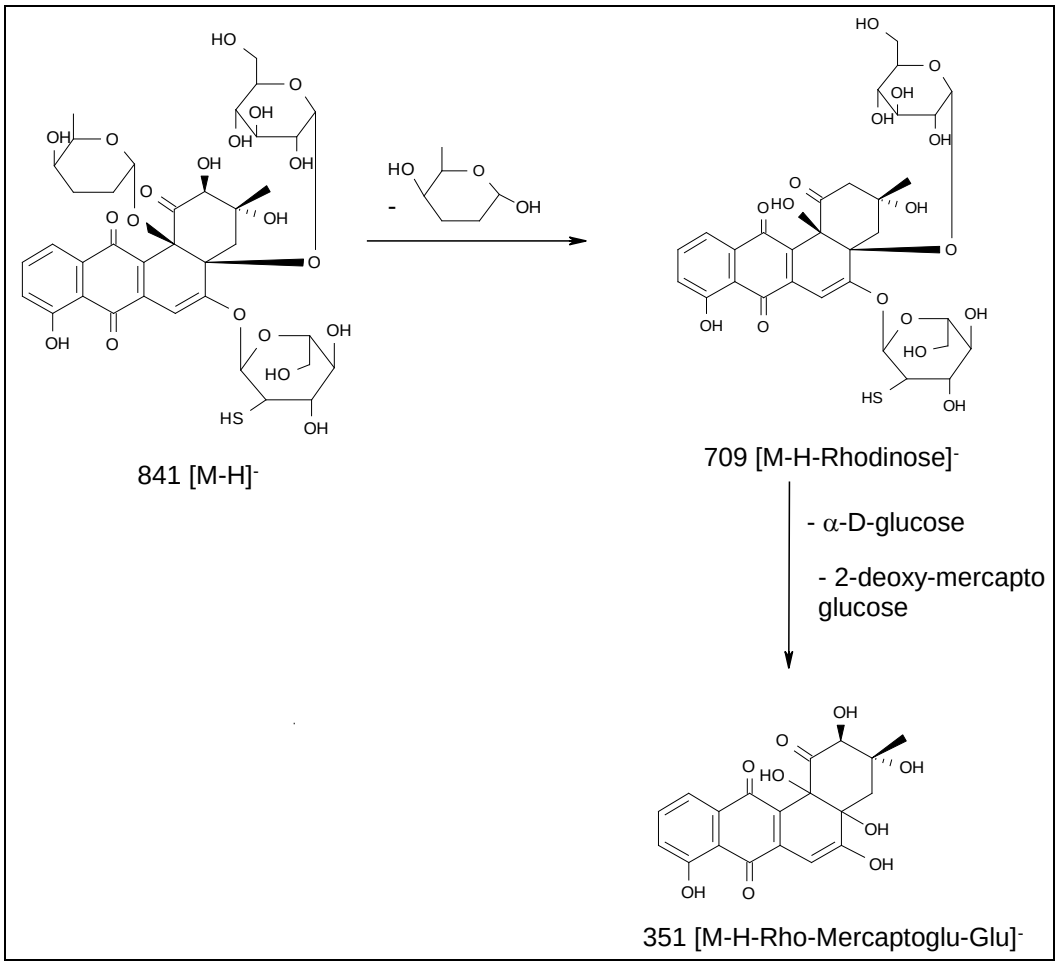


Figure 46: (-)-ESI MS-MS of rhonocardin A (**68**).

The three sugars in rhodonocardin A (**68**) are rhodinose, α-D-glucose and α-2-deoxy-2-mercaptoglucose. Rhodonocardin A and B were firstly isolated from *Nocardia* sp. as wine-coloured water soluble pigments by Etoh *et al.*[72] but it was reported as its decaacetate form due to its high polarity. So the complete NMR assignments of the compound were reported here first time (Table 8). The compound **68** did not exhibit any antimicrobial activity in our tests.

Table 8: NMR (CD_3OD, (J in Hz)) assignments of rhodonocardin A (**68**)

Position	Rhodonocardin A (**68**)		
	δ_C [a]	δ_H [b]	HMBC[c] (H→C)
1	204.0	-	-
2	83.1	4.25 (s)	1, 3, 3-CH_3
3	76.65	-	-
3-CH_3	22.0	1.19 (s)	2, 3, 4
4	46.3	2.26 (d,14.0), 2.06 (d, 14.0)	2, 3, 3-CH_3, 12b
4a	80.7	-	-
5	164.5	-	-
6	107.1	6.77 (s)	7, 5, 4a, 12a
6a	138.2	-	-
7	189.4	-	-
7a	115.8	-	-
8	162.4	-	-
9	124.9	7.29 (d, 8.3)	11, 7a
10	138.1	7.72 (t, 7.8)	8, 11a
11	120.0	7.57 (d, 7.2)	7a, 9, 12
11a	133.5	-	-
12	183.8	-	-
12a	134.7	-	-
12b	82.7	-	-
1'	95.5	5.30 (s)	4a, 3'
2'	24.2	1.82 (m)	4'
3'	26.2	1.58 (m)	2'
4'	67.7	3.36 (m)	2'
5'	68.2	4.25 (s)	4'
6'	17.0	0.52 (d, 6.6)	4'
1"	95.0	5.15 (d, 3.6)	5, 5"
2"	72.9	3.53 (m)	4"
3"	72.4	3.95 (m)	1", 5"
4"	74.4	3.72 (m)	2", 6"
5"	71.6	3.40 (m)	1", 3"
6"	62.3	3.67 (t, 6.9)	4"
1"'	93.6	5.37 (d, 3.0)	12b, 5"'
2"'	52.3	3.50 (m)	4"'
3"'	72.4	3.97 (m)	1‴,5"'
4"'	74.2	3.95 (m)	2"',6"'
5"'	72.9	3.53 (m)	1"'
6"'	62.3	3.67 (t, 6.9)	4"'

[a,c]125 MHz; [b]300 MHz

4.4.3 *Cyclo*(Ala-Pro)

Compound **69** was isolated as colourless solid, exhibiting UV absorbance and staining to blue by spraying with anisaldehyde/sulphuric acid. The molecular formula $C_8H_{12}N_2O_2$ was deduced by HRESIMS. The ^{1}H NMR spectrum of **69** displayed one methyl doublet at δ1.43, two oxygenated and/or amide-bounded methines (δ 4.09), six 1H multiplets in the region of δ3.55~1.87 and an exchangeable proton at δ6.70 (NH or OH).

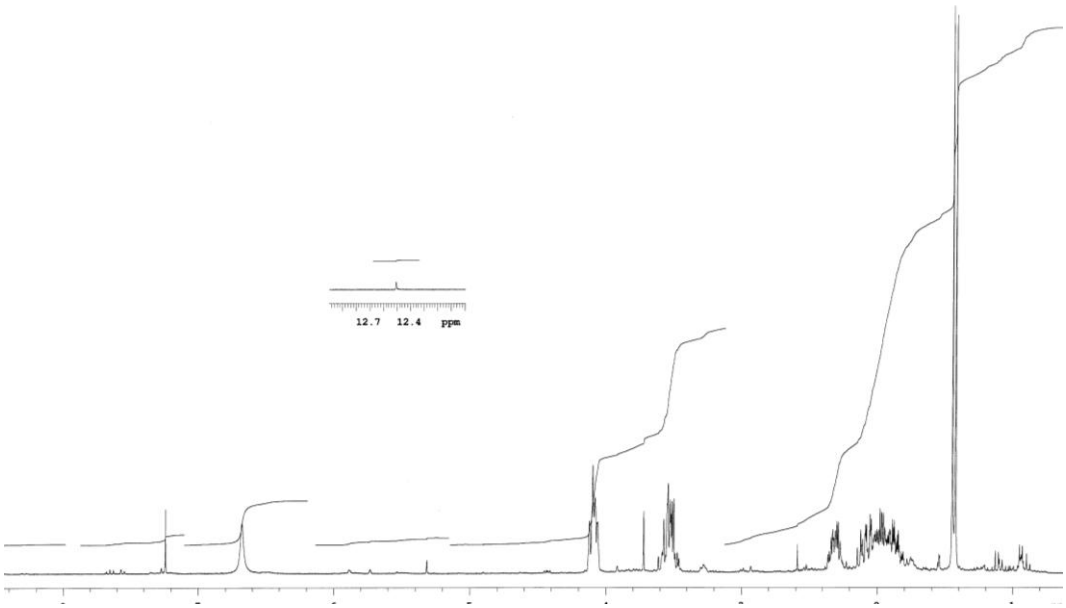


Figure 47: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of *cyclo*(Ala-Pro) (**69**).

The ^{13}C NMR/HSQC spectrum showed eight carbon signals, classified into one methyl (δ 16.0), three methylenes (δ45.5, 28.2, 22.2), two amide-linked methines (δ 59.3, 51.2) and two carbonyls (δ170.3, 166.2).

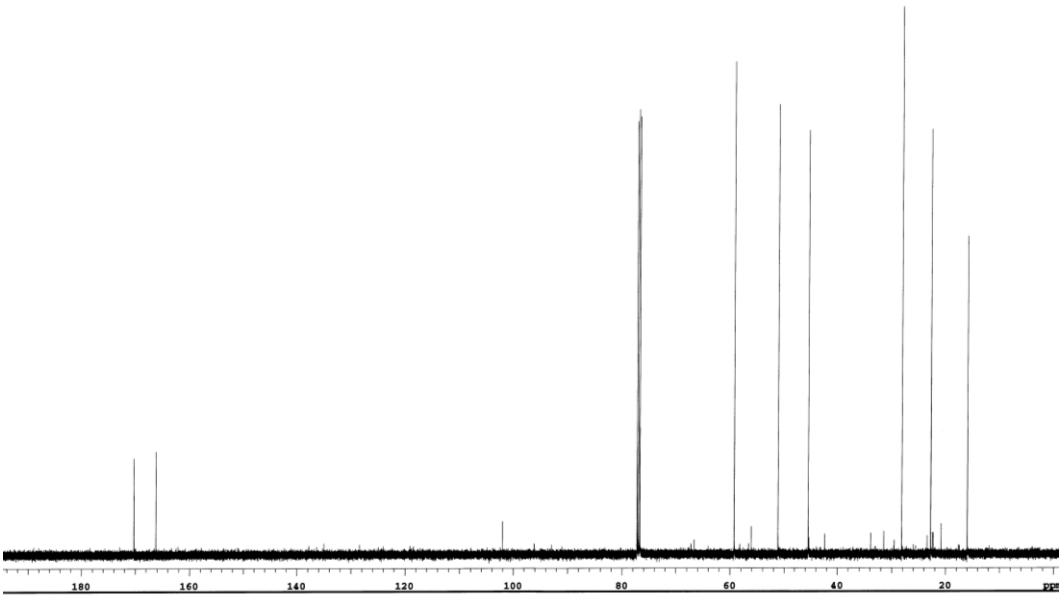


Figure 48: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of *cyclo*(Ala-Pro) (**69**).

Based on these spectroscopic data and a search in AntiBase, compound **69** was recognized as *cyclo*(Ala-Pro), isolated from marine sponge and plants, however, without completely reported spectroscopic data.[73, 74] Therefore, a full assignment for *cyclo*(Ala-Pro) **69** is reported here on the basis of HMBC and COSY experiments.

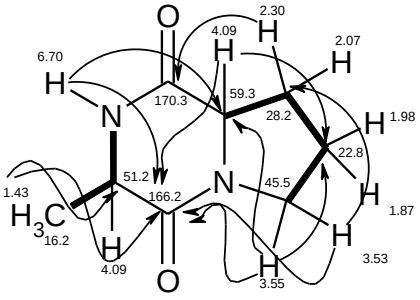
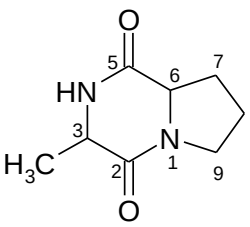


Figure 49: HMBC (→), and H,H COSY (▬) connectivities of *cyclo*(Ala-Pro) (**69**).



69

The diketopiperazines are characterised by the presence of two chiral centres at positions 3 and 6 to afford four potential stereoisomers[75]. The relative configuration for compound **69** could not be assigned by NOE or NOESY experiments due to the overlapping signals of chiral protons at the chiral centres C-3 and C-6 . The absolute configuration of both amino acids could be solely determined by hydrolysis of the parent molecule with HCl followed by application to chiral HPLC analysis; however, the insufficient available quantity restricted that.

4.5 Terrestrial *Streptomyces* sp. WO 463

The terrestrial *Streptomyces* sp. WO463 was selected due to its high activity against plant pathogen fungi. It also showed 100% activity against *Artemia salina*. On agar plates, it showed well-defined white colonies, which were used to inoculate 100 of 1L Erlenmeyer flasks each containing 300 ml of M_2 medium. These flasks were placed on linear shaker; the well-grown culture was harvested after 9 days and filtered by filter press using Celite. The mycelium was extracted with ethyl acetate and acetone and the water phase was passed through an XAD-16 column, washed with water and extracted with methanol. Both extracts were combined based on their similar chromatograms and fractionated by silica gel column using a dichloro-methane/methanol gradient. The fractions were further purified by different chroma-tographic techniques to get the pure compounds.

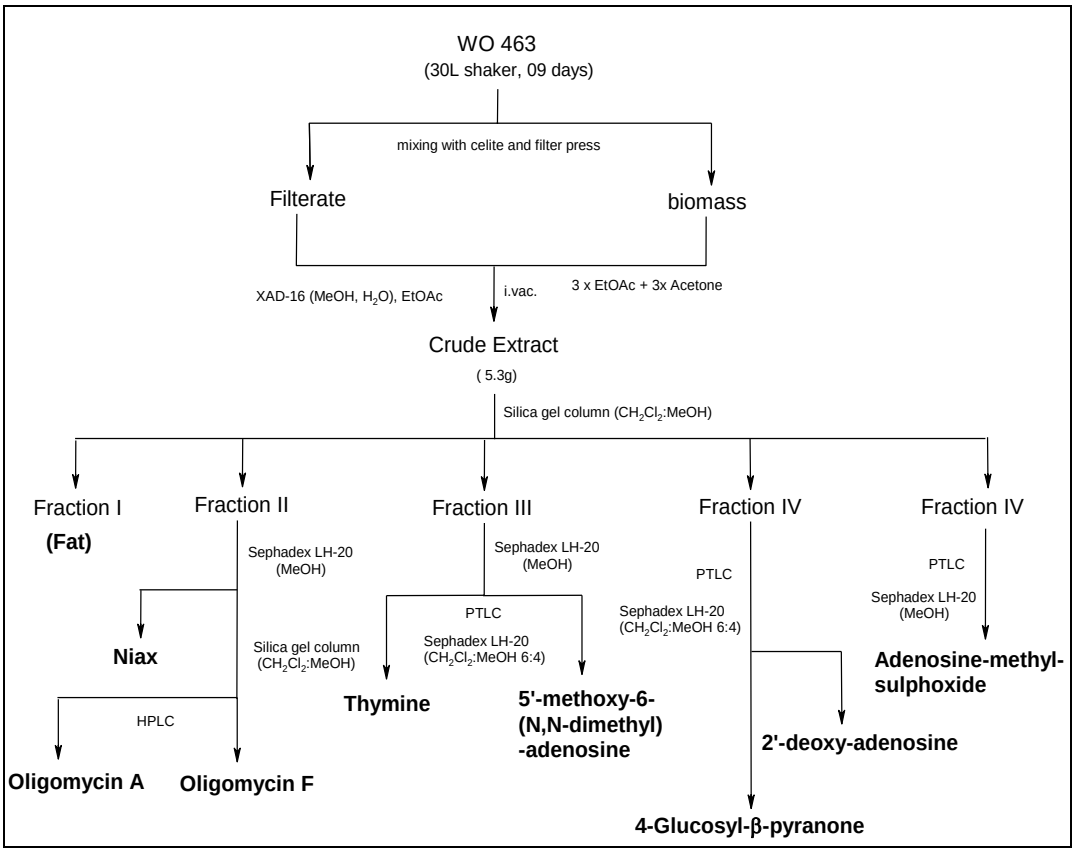


Figure 50: Work scheme of terrestrial *Streptomyces* strain WO 463

4.5.1 Oligomycin A

Oligomycin A (**70**) was firstly isolated from fraction II by silica gel column chromatography using CH_2Cl_2/MeOH as a mixture with oligomycin F, which was further purified by *semi*-preparative reversed phase HPLC (RP_{18}, MeOH:H_2O, 85:15). It was a colourless, UV absorbing solid, which turned to reddish brown and later to green after spraying with anisaldehyde/sulphuric acid.

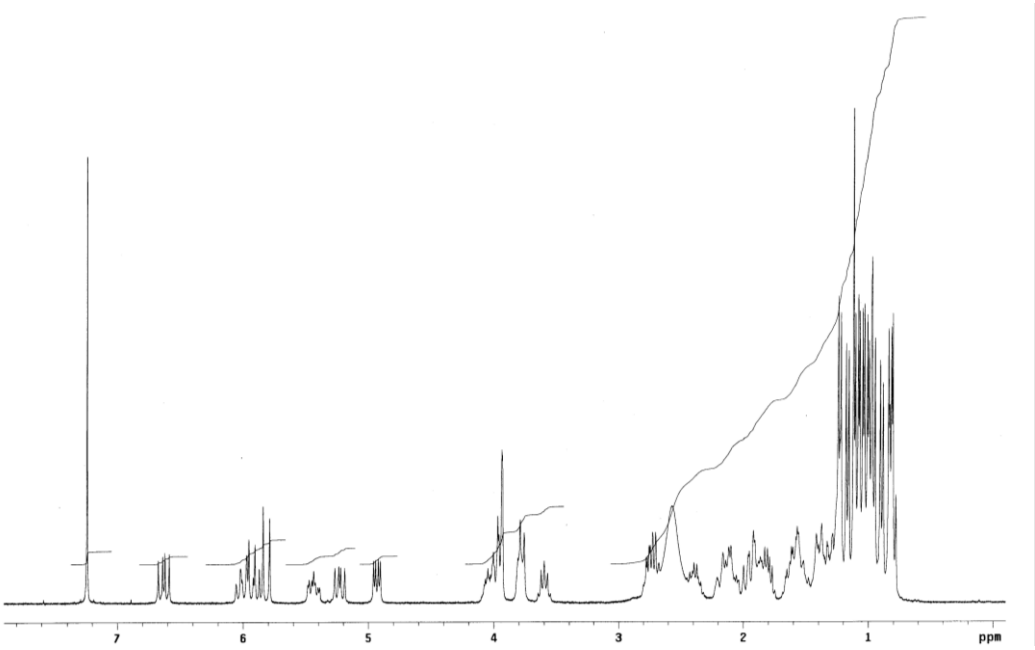


Figure 51: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of oligomycin A (**70**).

The ^{1}H NMR of compound **70** showed six olefinic signals between δ 4.8-6.8. Moreover, many methyl doublets in the region of δ 0.8-1.3 were also observed owing to a complex structure. The ^{13}C NMR spectrum revealed 45 carbon signals including two carbonyls at δ 220.0 and 219.7, an acidic or amide signal at δ 165.0 and six olefinic carbons. It also exhibited nine oxymethine carbons between δ 60-100, seven methylene and eleven methyl signals in the aliphatic region.

The ESI mass spectrum showed a *quasi*-molecular ion peak at *m/z* 813 $[M+Na]^+$, which delivered the molecular mass of 790 Dalton. The molecular formula $C_{45}H_{74}O_{11}$ was established by HRESIMS. The above spectral data was used to search the compound in AntiBase, which gave a hit for oligomycin A (**70**). It was further confirmed by comparing the ^{13}C NMR data with the literature [76] (Table 9).

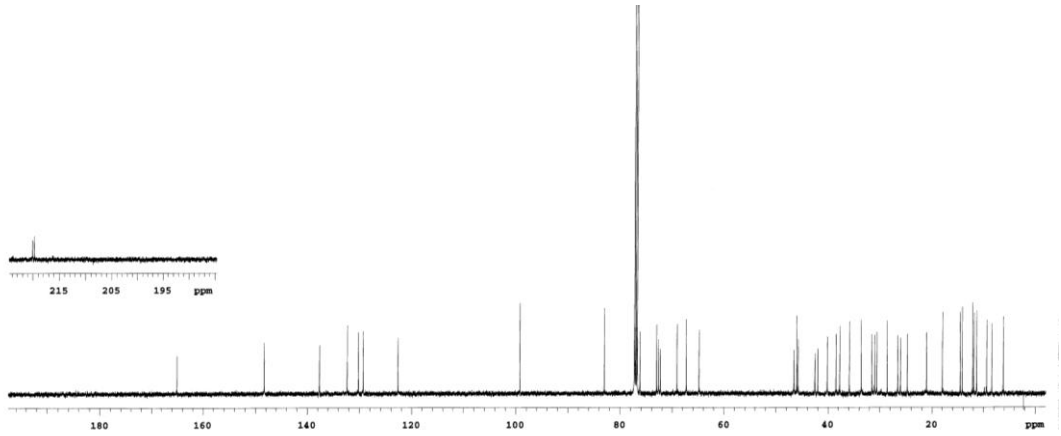
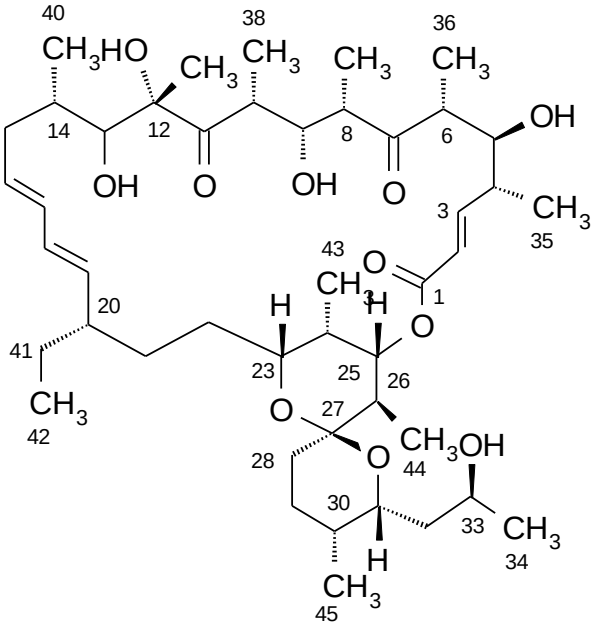


Figure 52: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of oligomycin A (**70**).



70

Oligomycin A (**70**) was for the first time isolated as complex containing oligomycin A, B and C from a *Streptomyces* strain in 1954 and showed broad spectrum antifungal activity.[77] The structure of oligomycin A was fully elucidated in 1985 [78] and later in 1986.[76] Oligomycins are not in clinical use as antifungal agents because of their high toxicity, however, these are highly interesting compounds as ATP synthesis inhibitors by inhibiting the enzyme ATP synthase *via* blocking the phosphorylation of ADP to ATP and are used in understanding the process of oxidative phosphorylation.[79]

Table 9: ^{13}C NMR data of oligomycin A (**70**) and its comparison with the literature.[76]

Position	Exp.[a]	Lit.[a]	Position	Exp.[a]	Lit.[a]
	δc[b]	δc[c]		δc[b]	δc[c]
1	165.0	165.1	24	35.9	35.9
2	122.7	122.7	25	76.2	76.3
3	148.3	148.5	26	37.8	37.8
4	40.2	40.3	27	99.2	99.2
5	73.0	73.0	28	26.1	26.0
6	46.6	46.5	29	26.6	26.6
7	220.0	220.1	30	30.6	30.5
8	42.0	42.1	31	67.3	67.3
9	72.7	72.8	32	42.6	42.6
10	45.8	46.0	33	64.8	64.7
11	219.7	219.9	34	24.8	24.7
12	83.0	83.0	35	18.0	17.9
13	72.3	72.5	36	8.4	8.4
14	33.6	33.6	37	14.2	14.1
15	38.5	38.5	38	9.4	9.4
16	129.3	129.4	39	21.1	21.2
17	132.4	132.4	40	14.6	14.6
18	130.2	130.6	41	30.6	30.5
19	137.7	137.7	42	12.2	12.1
20	46.1	46.0	43	6.2	6.1
21	31.5	31.5	44	11.9	11.8
22	31.0	31.0	45	11.3	11.3
23	69.1	69.0	-	-	-

[a]$CDCl_3$, [b]125 MHz, [c]150 MHz

4.5.2 Oligomycin F

Oligomycin F (**71**) was also isolated with oligomycin A (**70**) by *semi*-preparative HPLC. It showed the same colour reaction with anisaldehyde/sulphuric acid. The ^{1}H and ^{13}C NMR spectrum of the compound showed high similarity with compound **70** but ^{13}C NMR spectrum of the compound **71** showed an additional methylene signal at δ 30.9. It was also confirmed by the molecular weight of the compound, which was obtained by ESIMS as 804 Dalton and the molecular formula $C_{46}H_{76}O_{11}$ as established through HRESIMS. The search in AntiBase with the spectroscopic data and molecular formula resulted in two oligomycin derivatives, oligo-

mycin F (**71**) and 44-homooligomycin B (**72**). The difference between two compounds is the position of methyl or ethyl groups at C-26 and C-33.

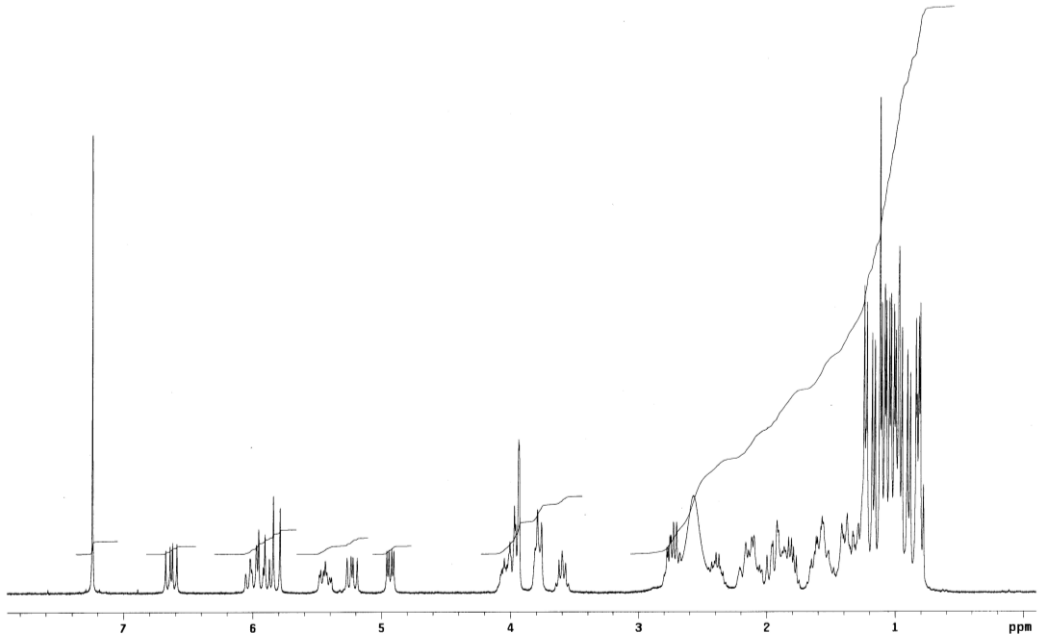
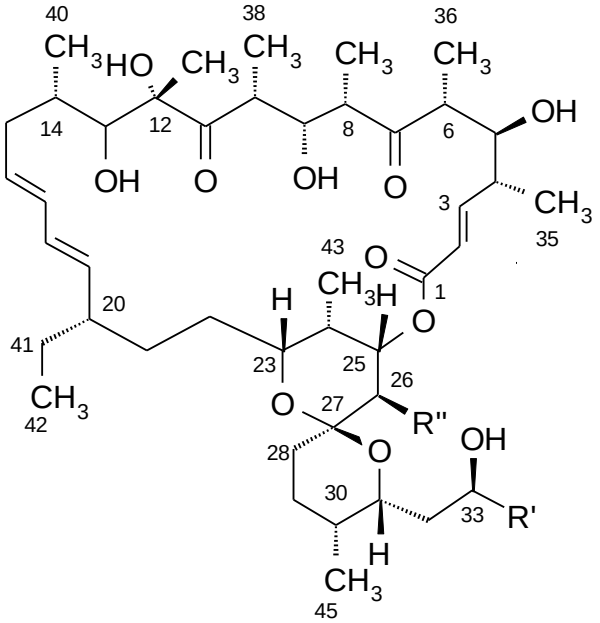


Figure 53: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of oligomycin F (**71**).



71: R' = CH_2CH_3, R" = CH_3

72: R' = CH_3, R" = CH_2CH_3

Therefore, the complete structure was confirmed by measuring 2D NMR spectra of the compound. In the HMBC spectrum, methyl protons (δ 0.95, d, J = 7.1 Hz) of

C-44 exhibited ^{3}J correlations with C-25 and C-27 confirming the attachment of methyl group at C-26. Moreover, methylene protons (δ 1.38, m) of C-34 showed ^{3}J correlation with C-32 and methyl protons (δ 0.94, t, J = 7.0 Hz) of C-34' showed ^{3}J coupling with C-33 and ^{2}J coupling with C-34, hence confirming the position of ethyl group at C-33. Furthermore, the complete structure of oligomycin F (**71**) was confirmed by comparing ^{1}H and ^{13}C NMR data with literature values.[80] Oligomycin F (**71**)was first time isolated from *Streptomyces* strain. A 171 in our group in 1993 and is reported to possess high activity against plant pathogen fungi and as an immunosuppressive agent.[80] The plant pathogenic activity of the strain can be considered due to oligomycin A and F.

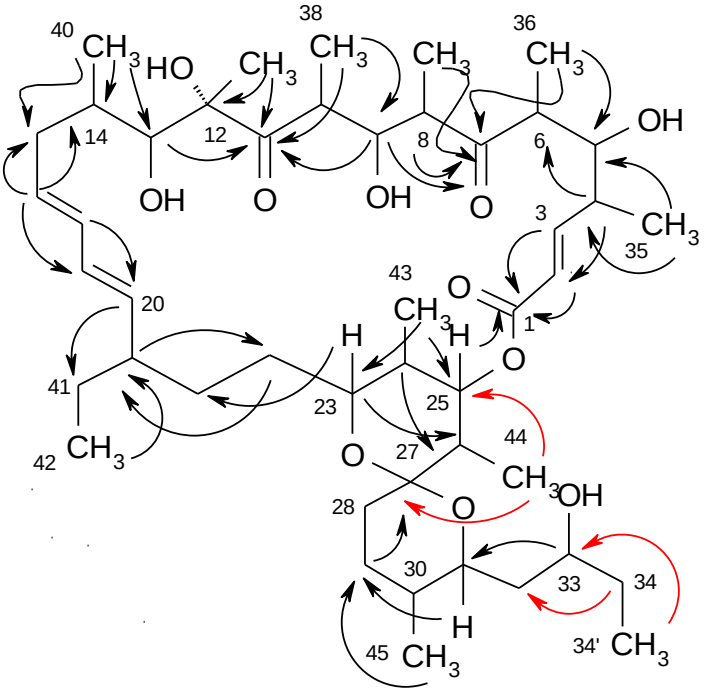


Figure 54: Selected HMBC (→) correlations of oligomycin F (**71**). The key correlations were shown in red (→).

Table 10: ^{1}H and ^{13}C NMR data of oligomycin F (**71**) in comparison with the literature.[80]

Position	Literature[a]		Experimental[a]	
	δ_C[b]	δ_H[c] (*J* in [Hz])	δ_C[b]	δ_H[c] (*J* in [Hz])
1	165.1	-	165.2	-
2	122.8	5.83 (dd, 15.5, 0.7)	122.7	5.80 (d, 15.6)
3	148.4	6.65 (dd, 15.5, 10.0)	148.4	6.63 (dd, 15.7, 10.1)
4	40.2	2.40 (dd, ~ 5)	40.2	2.37 (m)
5	73.0	3.79 (d, ~ 10)	73.1	3.78 (d, 10.0)
6	46.6	2.73 (dq, 8.7, 6.8)	46.6	2.70 (m)
7	220.4	-	220.3	-
8	42.0	3.62 (dq, 8.7, 6.8)	42.0	3.62 (m)
9	72.7	3.97 (m)	72.7	3.94 (dd, (8.6, 2.9)
10	45.7	2.78 (dq, 6.9, 3.0)	45.7	2.75 (dd, 6.9, 2.9)
11	220.0	-	219.9	-
12	83.1	-	83.0	-
13	72.2	3.96 (m)	72.3	3.92 (d, 1.8)
14	33.5	1.92 (dt, 9.5, 4.5)	33.5	1.38 (m)
15	38.5	2.21,1.96 (dm, 14.3)	38.5	2.18, 1.96 (m)
16	129.4	5.46 (ddd, 15.0, 10.5, 3.5)	129.4	5.44 (ddd, 14.8, 10.7, 3.8)
17	132.4	6.04 (ddd, 15.0, 10.5, 1.8)	132.4	6.01 (ddd, 16.0, 10.8, 1.8)
18	130.3	5.94 (dd, 15.0, 10.5)	130.3	5.91 (dd, 15.0,10.4)
19	137.8	5.26 (dd, 15.0, 9.7)	137.8	5.22 (dd, 15.0, 9.6)
20	46.1	1.86 (dm, ~3)	46.0	1.82 (m)
21	31.5	1.25, 1.41	31.5	1.38 (m)
22	31.0	1.55, 1.49	31.0	1.50 (m)
23	69.1	3.79 (dt, 11.5, 2.5)	69.1	3.62 (m)
24	35.9	2.15 (dd)	35.8	2.10 (m)
25	76.3	4.95 (dd, 11.5, 5.0)	76.3	4.91 (dd, 11.4, 5.0)
26	37.8	1.83 (dq, 11.5, 6.3)	37.7	1.79 (m)
27	99.2	-	99.3	-
28	26.1	1.96, 1.25 (dd)	26.1	1.80, 1.23 (m)
29	26.6	2.12, 1.41 (dd)	26.5	1.40 (m)
30	30.7	1.59	30.6	1.55 (m)
31	67.3	4.02 (dt, 10.5, 2.5)	67.3	3.98 (dt, 10.2, 2.3)
32	40.3	1.66, 1.25 (dd)	40.3	1.63, 1.25 (m)
33	69.8	3.75	69.9	3.76 (d, 9.4)
34	31.2	1.49, 1.41, (d)	31.5	1.38 (m)
34'	9.8	0.97 (t,7.0)	9.7	0.94 (t, 7.0)
35	18.0	1.19 (d, 6.6)	17.9	1.16 (d, 6.5)

36	8.3	1.08 (d, 7.3)	8.3	1.09(d, 6.8)
37	14.1	1.12 (d, 6.8)	14.1	1.05 (d, 7.3)
38	9.3	1.04 (d, 6.9)	9.3	1.01 (d, 6.9)
39	21.0	1.14 (s)	21.0	1.11 (s)
40	14.5	1.01, (d, 6.7)	14.5	0.98 (d, 6.7)
41	28.6	1.38, 1.30 (dq, d)	28.5	1.36, 1.23 (m)
42	12.1	0.83 (t, 7.5)	12.1	0.79 (t, 7.4)
43	6.1	0.85 (d, 6.9)	6.1	0.80 (d, 7.4)
44	11.9	0.98 (d, 6.3)	11.8	0.95 (d, 7.1)
45	11.3	0.92 (d, 7.0)	11.3	0.89 (d, 6.9)

[a]$CDCl_3$, [b]125MHz, [c]300 MHz

4.5.3 5'-Methoxy-6-(N,N-dimethyl)-adenosine

The compound **73** was isolated as colourless solid from fraction V using PTLC followed by Sephadex LH-20 (CH_2Cl_2/40%MeOH). It showed UV absorption at 254 nm and stained to green after spraying with anisaldehyde/sulphuric acid. The ^1H NMR revealed two 1H singlets at δ 8.22 and 8.28, five oxygenated proton signals between δ ~ 4.60-3.50 and one anomeric proton (δ 5.93, d, J = 5.0 Hz) corresponding to a sugar moiety. In addition, a methoxy signal at δ 3.30 was also visible.

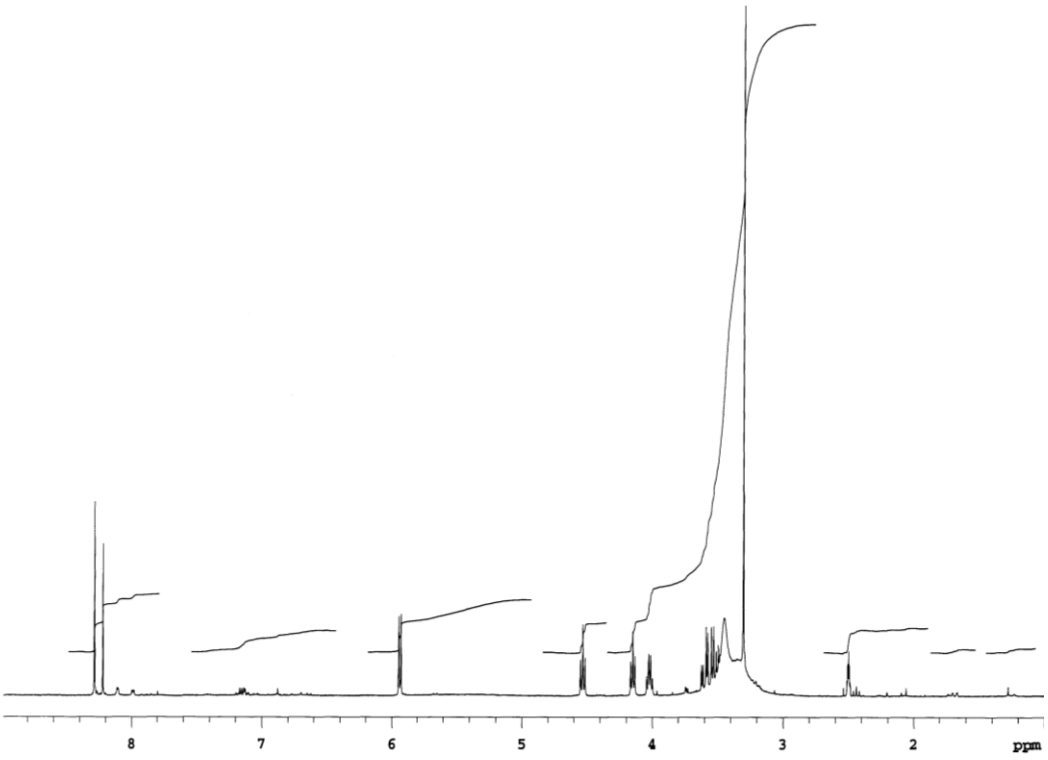


Figure 55: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, RT) of 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**).

^{13}C NMR/HSQC spectrum showed 11 carbon signals including two CH signals (δ151, 137) and three quaternary carbons at δ154.1, 151.7 and 119.3. Moreover five carbons at δ87.2, 82.8, 73.4, 72.2 and 70.3 belonging to sugar moiety and a methoxy signal at δ58.5 were also revealed. According to HMBC and COSY correlations, the structure was proposed as 5'-methoxy-adenosine.

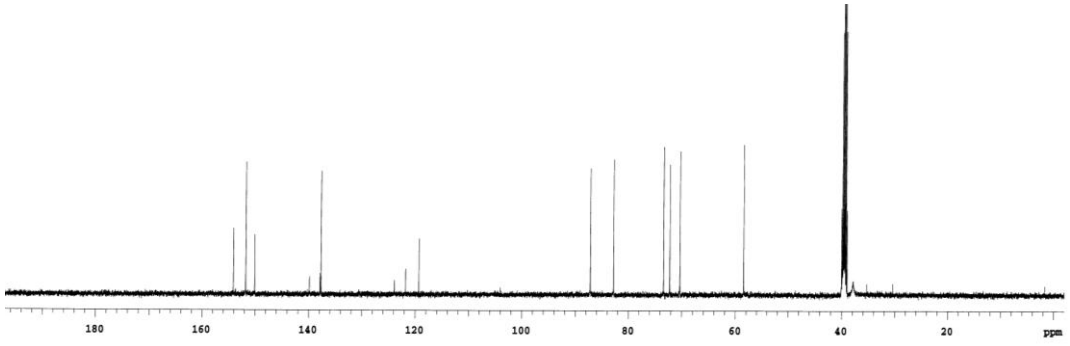


Figure 56: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz, RT) of 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**).

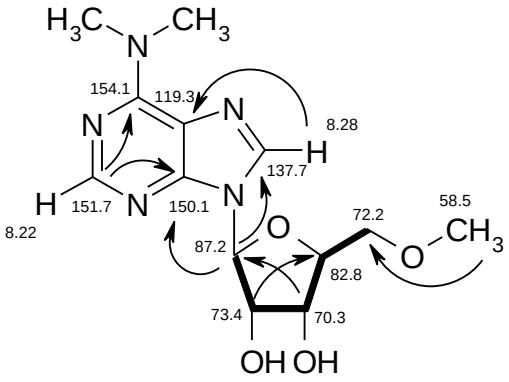


Figure 57: HMBC (→), and H,H COSY (▬) connectivities of 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**).

The molecular weight was determined by ESIMS as 309 Dalton, and the molecular formula $C_{13}H_{19}N_5O_4$ was obtained by HRESIMS. However, 5'-methoxy-adenosine has molecular formula $C_{11}H_{15}N_5O_4$, what means it contains two additional methyl signals, which could be attached to N-6. To confirm this possibility, the spectra were repeated at 100 °C: Indeed, the two methyl signals appeared in the ^{1}H NMR at δ 3.46 (s) and in the ^{13}C NMR spectrum at δ 37.5. The reason for their invisibility at room temperature is obviously a coalescence phenomenon; it means that these methyl groups flip between two orientations at room temperature closely with the frequency of the spectrometer at higher temperature, the two frequencies differ sufficiently.

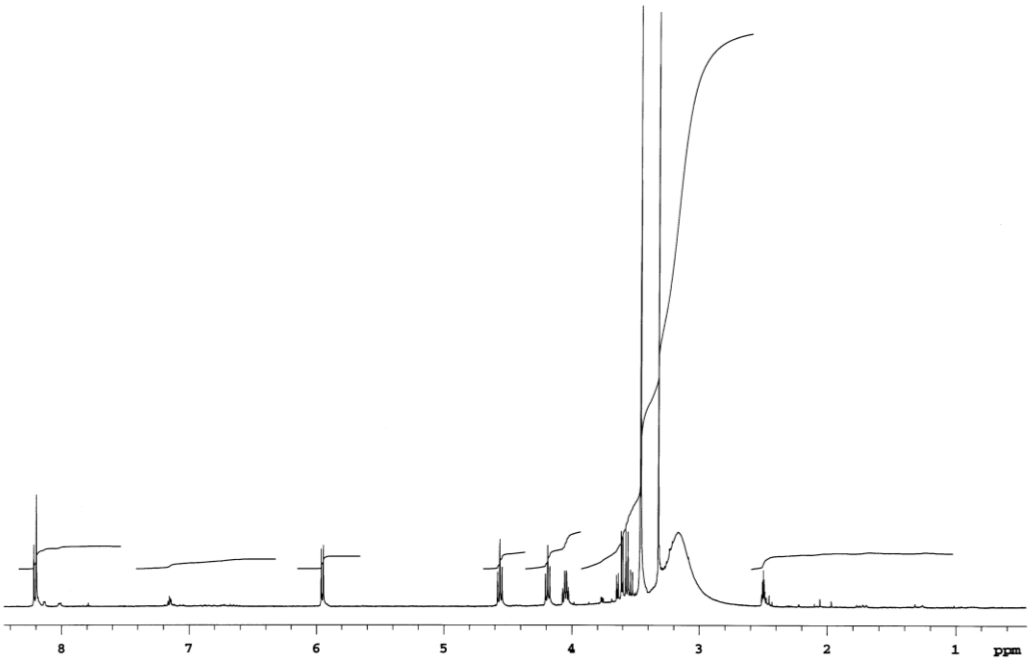


Figure 58: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, 100 °C) of 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**).

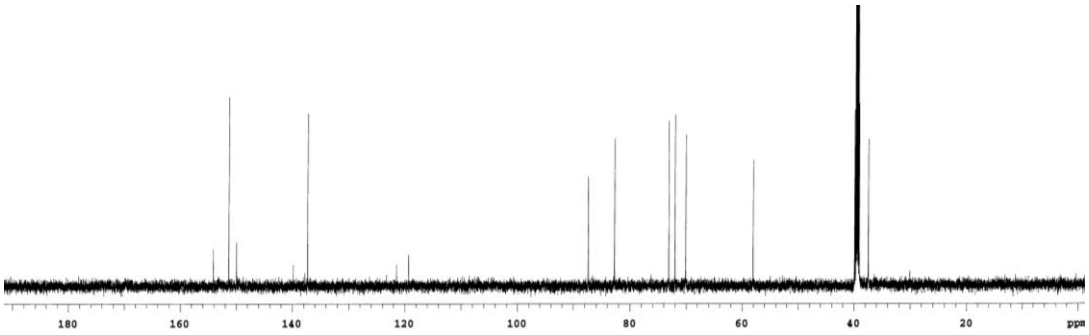
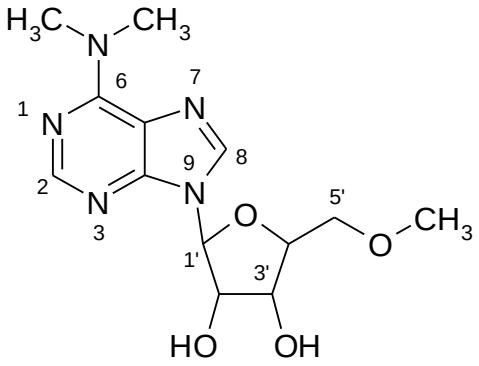


Figure 59: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz, 100 °C) of 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**).

On the basis of above spectroscopic data, the structure was fully elucidated as 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**), which is a new natural product, as searching in the different data bases (AntiBase, DNP, the Chemical Abstracts) indicated.



73

The primary metabolites such as adenosine or adenine, thymine or thymidine guanosine and uridine are frequently isolated from *Streptomyces*. However, many new nitrogenous bases derivatives were also isolated previously in our group such as 5'-methyl-thioinosine[81] and 3'-2'-deoxythymidine.[82]

4.5.4 4-*α*-L-Glucosyl-*β*-pyranone

From fraction IV, the compound **74** was isolated as UV-absorbing, colourless oil which showed dark green colour after spraying with anisaldehyde/sulphuric acid. The ^{1}HNMR spectrum showed an olefinic proton at δ 6.61 (m). It also delivered many oxymethine and oxymethylene signals between δ 3.20-4.80 and an anomeric proton at δ 5.28 (d, J = 3.6 Hz) corresponding to a sugar moiety. It also exhibited a methyl singlet at δ 1.46. The mass of the compound **74** was determined by ESIMS as 306 Dalton showing a *pseudo*molecular ion peak at *m/z* 329 $[M+Na]^+$. The corresponding molecular formula was determined by HRESIMS as $C_{12}H_{18}O_9$.

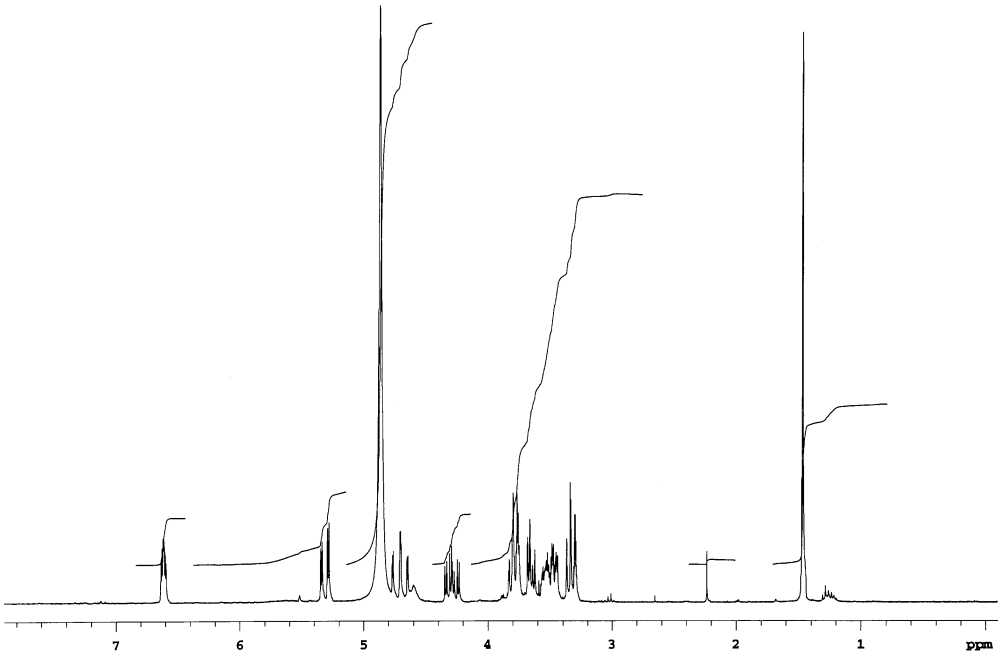


Figure 60: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 4-α-glucosyl-β-pyranone (**74**).

The ^{13}C NMR spectrum showed 12 carbon signals, but each signal appeared two times, so the compound seemed to be mixture of two isomers containing a carbonyl at δ 190.4/190.3, an oxymethine at δ 144.9/144.6, and an olefinic carbon at δ 125.0/124.0. It also revealed an anomeric carbon at δ 99.4/99.1, a quaternary acetal carbon at δ 97.5/97.4, four oxy-methines, two oxy-methylenes and a methyl at δ 23.0/22.9.The search with above spectral data in AntiBase gave no exact hit. The complete structure of compound **74** was elucidated with the help of 2D NMR spectra.

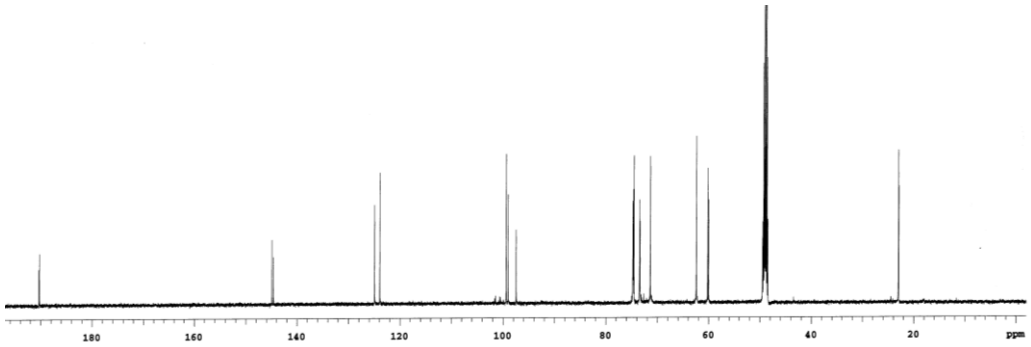
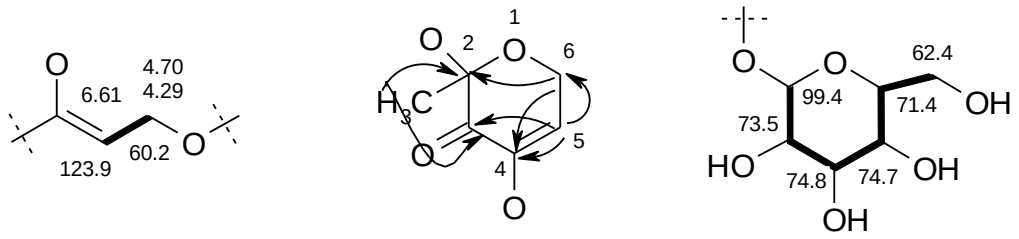


Figure 61: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 4-α-glucosyl-β-pyranone (**74**).

In the H,H COSY spectrum, the olefinic proton H-4 (δ 6.61) showed ^3J correlation with oxy-methylene protons at δ 4.29 and 4.70, which in turn showed ^3J long range coupling with C-2 and C-4 in the HMBC spectrum. The methyl protons (δ 1.46) also showed ^2J coupling with C-2 (δ 97.4) to which it is directly attached, and ^3J correlation with the carbonyl (δ 190.0). On the basis of these correlations a β-pyranone moiety was constructed (substructure **A** and **B**). Moreover, a hexose sugar with α-configuration (J = 3.6 Hz) was also constructed on the basis of H,H COSY correlations. The sugar could be attached with C-2 or C-4, but an obvious ^3J correlation was observed from the anomeric proton δ 5.28 to C-4 (δ 144.9), which confirmed the attachment of the sugar at C-4, and µL of MeOH. The structure was further confirmed by acidic hydrolysis and silylation with MSTFA: For hydrolysis, a solution of about 0.01 mg of **74** in 50μL of MeOH and 50μL of 1 M HCl was kept at 80 °C. After 4 hours, the sample was dried at 0.1mbar at r.t., and the residue was derivitized with 50μL of MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) at 40 °C for 1 h. For comparison several sugars were treated in the same way and anlysed by GC/MS.[83] The retention times of the silylated sugar were compared with the reference sugars. After hydrolysis and silylation, the sugar of **74** revealed peaks of α/β-pyranone/furanone derivatives which were identical within the error limits with peaks delivered by TMS-glucose. All other investigated sugars were clearly different and hence the complete structure was confirmed as 4-α-L-glucosyl-β-pyranone (**74**), as according to Klyne rule,[84] the α-configuration indicated L-sugar. The search in AntiBase with this structure gave no hit, however, searching in the Chemical Abstracts confirmed that 4-α-L-glucosyl-β-pyranone was earlier isolated from red Ginseng as a mixture of two structural isomers, 4-α-L-glucosyl-β-pyranone (**74**) and 2-α-L-glucosyl-β-pyranone (**75**).[85] Such a mixture was obviously also obtained from *Streptomyces* sp. WO463, as the NMR data of both isomers agreed very well with the published data.

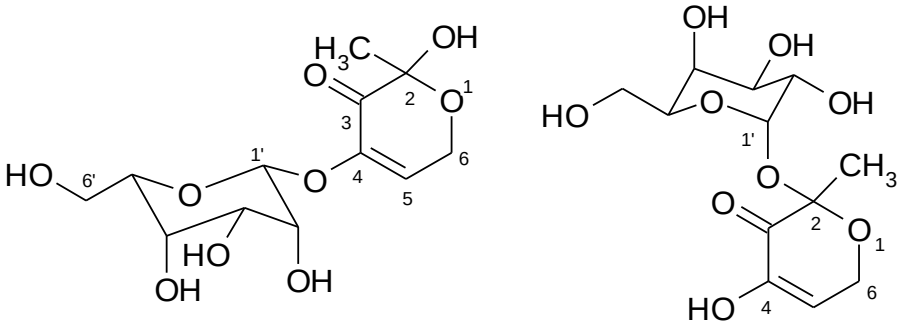
Table 11: Retention times of silylated sugars.

	R_t [min]		
Sugar	signal I	signal II	signal III
Sugar of **74**	18.05	19.01	19.85
Glucose	18.05	19.06	19.85
Galactose	18.22	18.71	19.14
Mannose	18.10	19.15	19.61



A **B** **C**

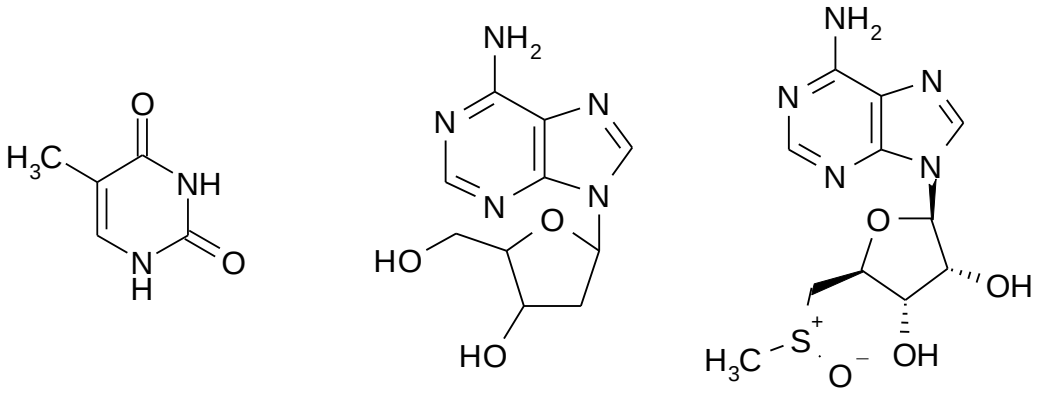
Figure 62: HMBC (→), and H, H COSY (—) connectivities of partial structures **A**, **B**, **C** of 4-*α*-glucosyl-*β*-pyranone (**74**).



74 **75**

4.5.5 Other metabolites

The other metabolites isolated from the strain were thymine (**76**), 2'-deoxy-adenosine (**77**) and 5'-methyl-thioadenosine (**78**). These compounds are very common from bacteria and are frequently isolated. The compounds **76** and **77** are primary metabolites and essential bases of DNA.



76 **77** **78**

4.6 Terrestrial *Streptomyces* sp. Ank 351

The terrestrial *Streptomyces* sp. Ank 351 was selected due to its high biological activity and the many UV absorbing zones in the crude extract, which exhibited violet, brown and green colours on spraying with anisaldehyde/sulphuric acid. Large scale (30 L) cultivation of the strain on the shaker for 7 days, filtration and extraction with different organic solvents resulted in a dark brown extract, which was subjected to silica gel column chromatography (see work scheme).

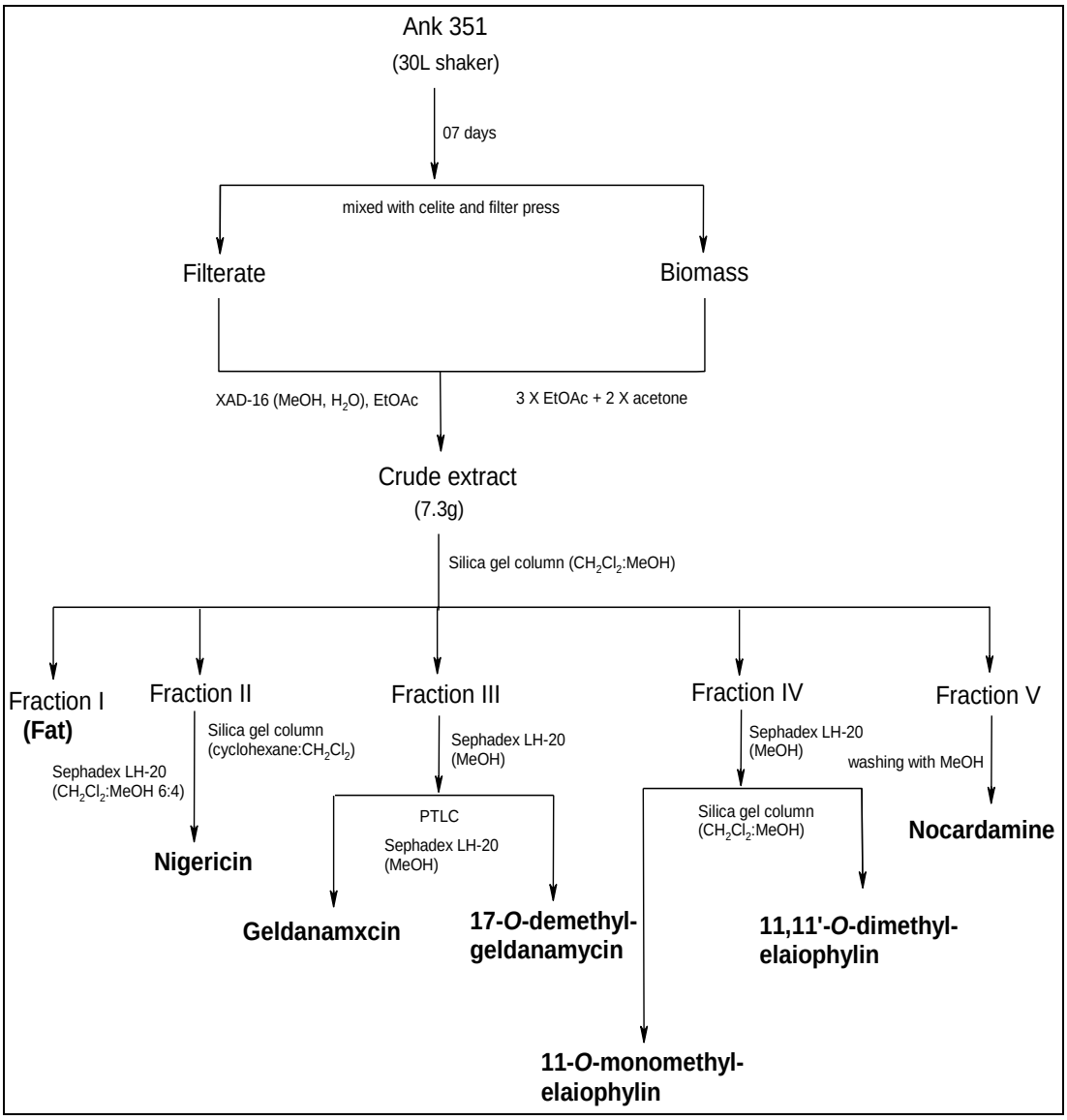


Figure 63: Work up scheme of terrestrial *Streptomyces* sp. Ank 351.

4.6.1 Geldanamycin

Geldanamycin (**79**) was isolated from fraction III as a yellow crystalline solid, which showed UV absorbance at 254 nm and a reddish brown colour after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed a broad singlet (NH or OH) at δ 9.16, five olefinic protons comprising of two doublets, two triplets and a singlet between δ 5.45-7.02. Additionally two oxy-methine protons at δ 4.88 (s) and 4.35 (d, J = 8.1 Hz), three methoxy signals at δ 3.96 and 3.20 and four methyl signals were also visible.

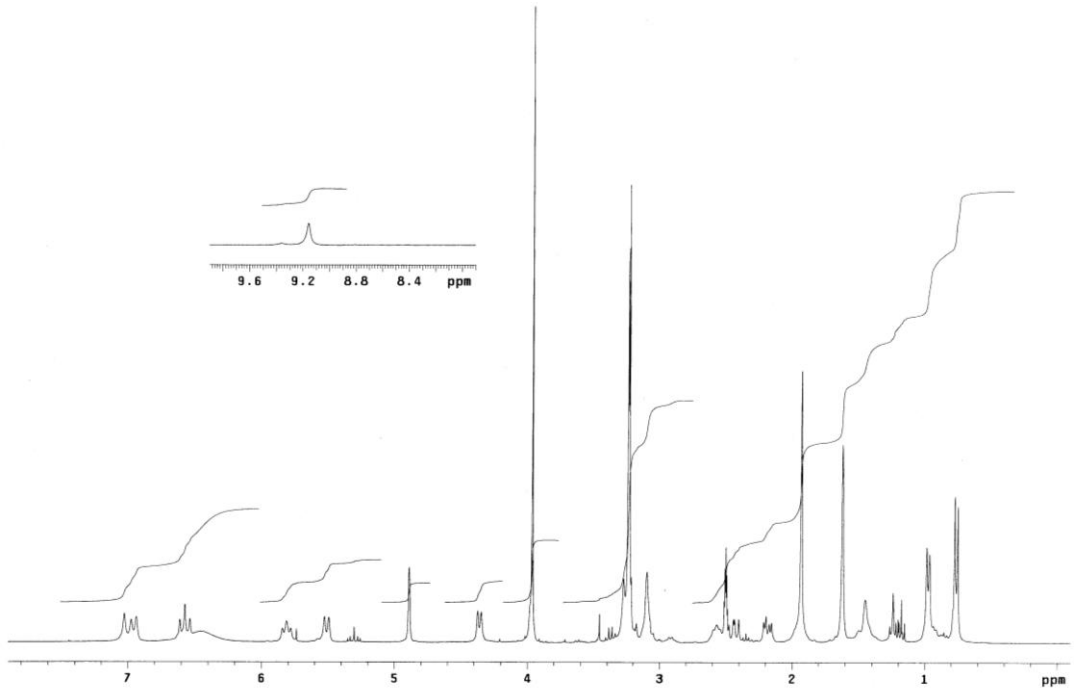


Figure 64: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of geldanamycin (**79**).

The ^{13}C NMR/HSQC spectrum revealed 29 carbon signals including two carbonyls (δ 183.7 and 183.1), one carbonyl of ester or amide (δ 179.0), eleven sp^2 carbons (six quaternary and five CH signals), four oxymethine, three methoxy signals, two methylene signals, two methine signals and four methyls.

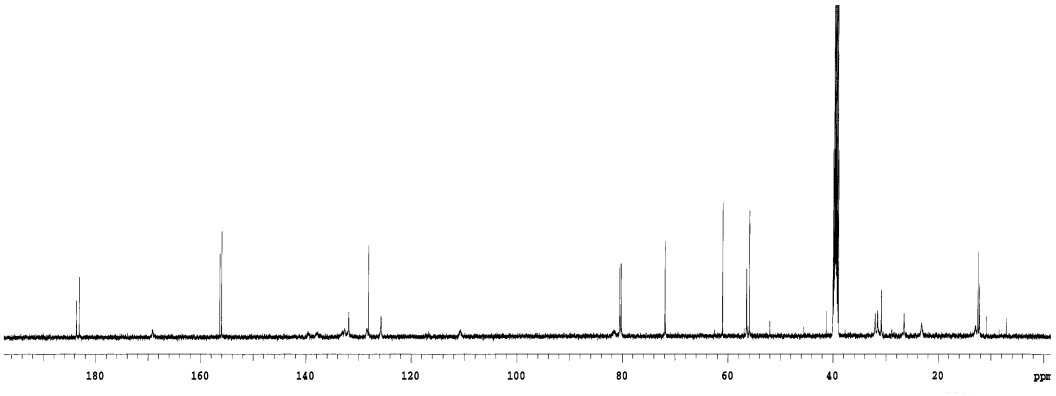


Figure 65: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of geldanamycin(**79**).

The mass of the compound was determined by ESIMS as 560 Dalton containing a *pseudo*molecular ion peak at *m/z* 583 $[M+Na]^+$; HRESIMS established the molecular formula as $C_{29}H_{40}N_2O_9$. The search in AntiBase with the help of above spectroscopic data and the molecular formula gave 6 hits; all belong to the ansamycin group of antibiotics.

Therefore the complete structure was elucidated with the help of 2D NMR spectroscopy and comparison with the literature data. According to H,H COSY and HMBC correlations, three sub-structures **A**, **B** and **C** were constructed.

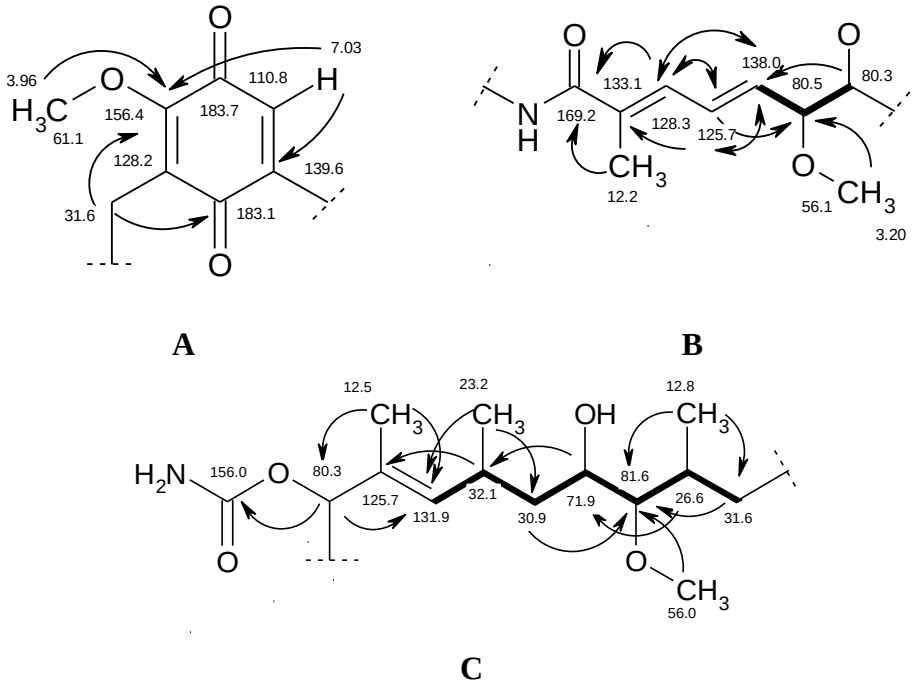
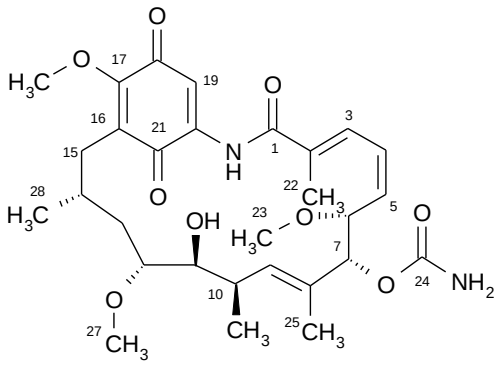


Figure 66: H,H COSY (▬)and HMBC (→) correlations of geldanamycin (**79**)

The ^{13}C chemical shift of C-20 (δ 139.6) suggested the attachment of fragment B to the benzoquinone ring; hence, the complete structure was deduced as geldanamycin (**79**) which was further confirmed by comparison with the literature data.[86, 87]



79

Table 12: ^{1}H and ^{13}C NMR data of geldanamycin (**79**) in comparison with the literature.[86, 87]

Position	Experimental		Literature	
	δ_C [a]	δ_H [b] (J in [Hz])	δ_C [a]	δ_H [b] (J in [Hz])
1-CONH	-	9.16 (s)	-	8.67 (s)
1	169.2	-	169.1	-
2	133.1	-	133.2	-
3	128.3	6.95 (d, 11.6)	128.4	7.36 (d, 11.5)
4	125.7	6.57 (t, 11.4)	125.7	6.54 (t, 11.5)
5	138.0	5.81 (t, 9.0)	137.8	5.79 (q, 9.5)
6	81.6	4.35 (d, 8.1)	81.6	4.38 (d, 9.5)
7	80.5	4.88 (s)	80.6	5.07
8	132.4	-	132.6	-
9	131.9	5.51 (d, 9.8)	131.9	5.12 (d, 9.0)
10	32.1	2.58 (t, 6.8)	32.1	3.0 (m)
11	71.9	3.09 (s)	71.9	4.87 (q, 4.0)
12	80.3	3.10 (m)	80.2	3.6 (m)
13	30.9	1.45 (m), 1.20 (m)	31.0	1.50
14	26.6	1.94 (m)	26.6	1.90 (m)
15	31.6	2.42 (m)	31.7	2.47(q, 7.0)
		2.18 (dd, 12.6, 5.2)		
16	128.2	-	128.1	-
17	156.4	-	156.4	-
18	183.7	-	183.6	-
19	110.8	7.02 (s)	110.9	7.35 (s)

20	139.6	-	139.6	-
21	183.1	-	183.1	-
22	12.2	1.93 (s)	12.2	2.02 (s)
23	56.0	3.23 (s)	56.0	3.36 (s)
24	156.1	-	156.0	-
25	12.5	1.62 (s)	12.5	1.76 (s)
26	23.2	0.97 (d, 6.4)	23.3	0,94 (d, 6.5)
27	56.4	3.24 (s)	56.5	3.33 (s)
28	12.8	0.76 (d, 6.8)	13.0	1.11 (d, 6.0)
29	61.1	3.96 (s)	61.0	4.13 (s)

[a]125 MHz; [b]300 MHz

Geldanamycin (**79**) is a first member of ansamycin antibiotics (rifamycins, streptovaricins) containing a benzoquinone nucleus, originally isolated from *Streptomyces hygroscopicus* in 1970.[88] In the same year, the structure of geldanamycin was elucidated by Sasaki *et al*.[86] Besides of its antibacterial activity like other ansamycin antibiotics, it specifically possessed antiprotozoal activity against *Tetrahymena pyriformis* and *Crithidia fasciculata* and antifungal activity against several plant pathogenic fungi.[88] Geldanamycin is also reported as antitumor agent and inhibits the cancer proliferating leukaemia cells by inhibiting the RNA-dependant DNA polymerase.[89] Later on, it was discovered that geldanamycin selectively binds with Hsp90 (heat-shock proteins) chaperone and inhibits its action and destabilizes it. Hsp90 is a molecular chaperon important for folding, assembly and activity of mutated and over-expressed signal proteins which promote the growth of tumor cells.[90]

The biosynthetic pathway of geldanamycin (**79**) and its derivatives as suggested by Patel *et al*.[91] is shown in Figure 67. The skeleton of geldanamycin is assembled by a modular polyketide synthase (PKS) consisting of seven active sites known as modules; each module has a catalytic site for a single round of polyketide-chain elongation. The starter unit is 3-amino-5-hydroxybenzoic acid (AHBA), which is extended by successive condensation of two-carbon units in seven chain elongation steps using one malonyl, four methylmalonyl and two methoxymalonyl extender units, which is then cyclized to pro-geldanamycin through intramolecular lactamization by another enzyme. Progeldanamycin is then converted to geldanamycin through different chemical modifications catalysed by a set of enzymes.

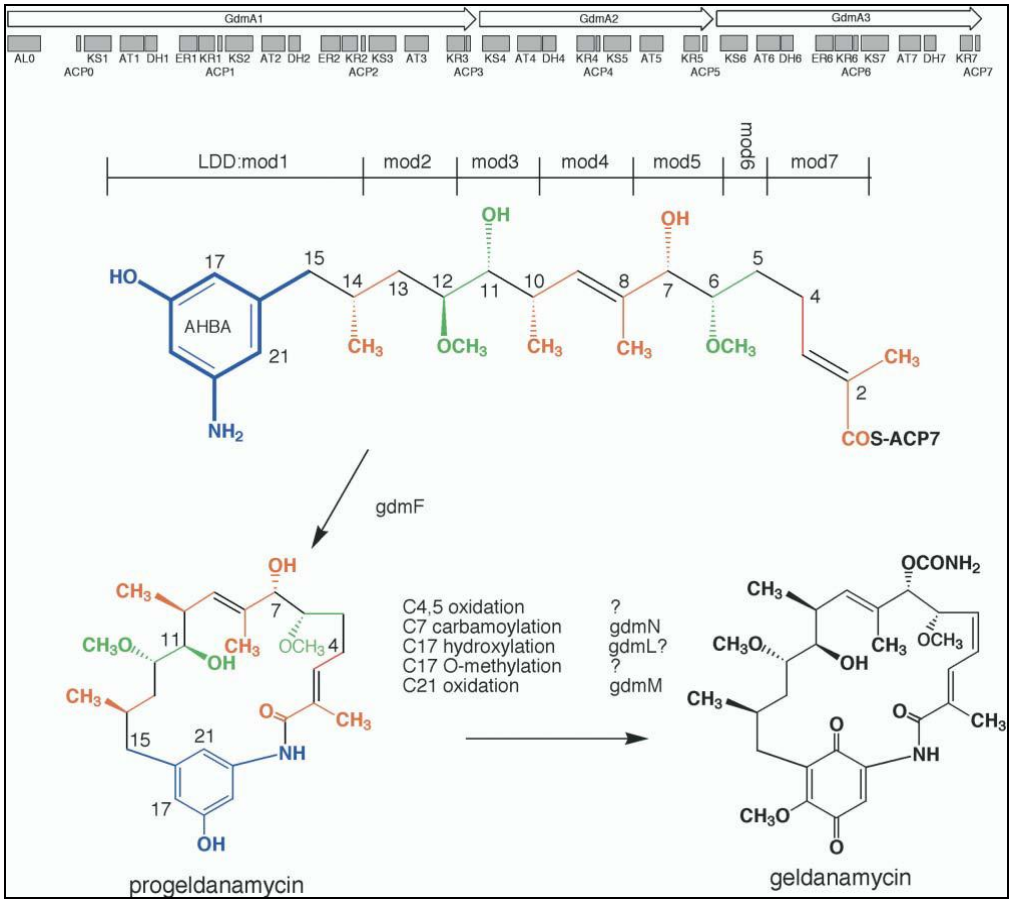


Figure 67: Proposed biosynthetic pathway[91] of geldanamycin (**79**).

4.6.2 17-*O*-Demethylgeldanamycin

17-*O*-Demethylgeldanamycin **82** was isolated as a dark purple powder from fraction III, which showed UV absorbance at 254 nm and changed to brown colour after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of the compound showed high similarity with that of compound **79**, however, it was different by the absence of the methoxy signal at δ 3.96. This was also confirmed by the ^{13}C NMR spectrum, where the carbon signal at δ 61.0 was missing.

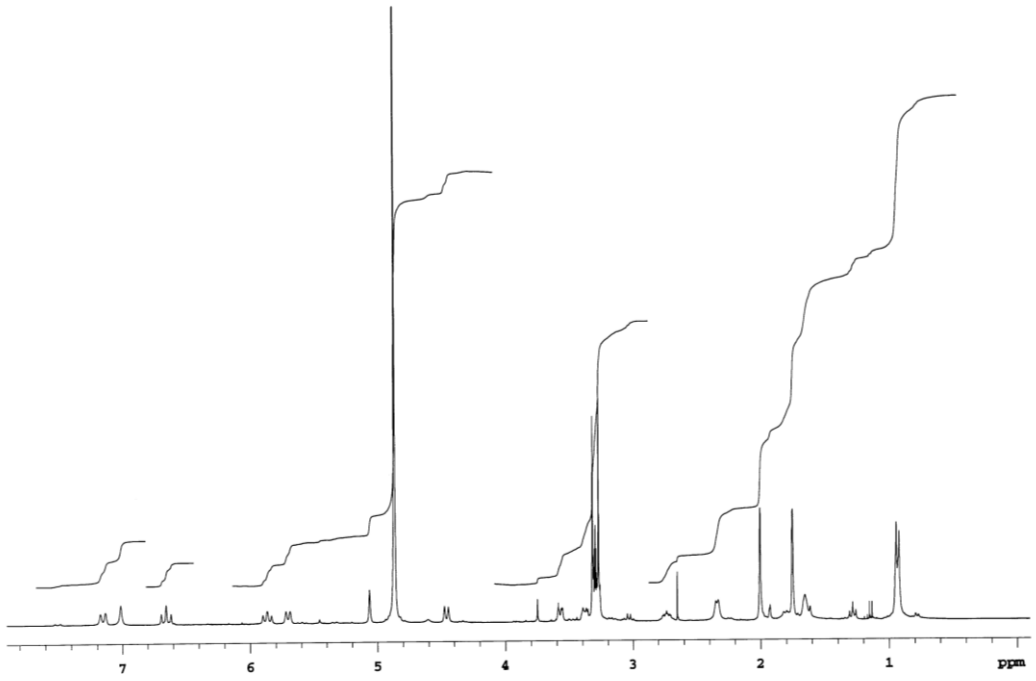


Figure 68: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 17-*O*-demethylgeldanamycin (**82**).

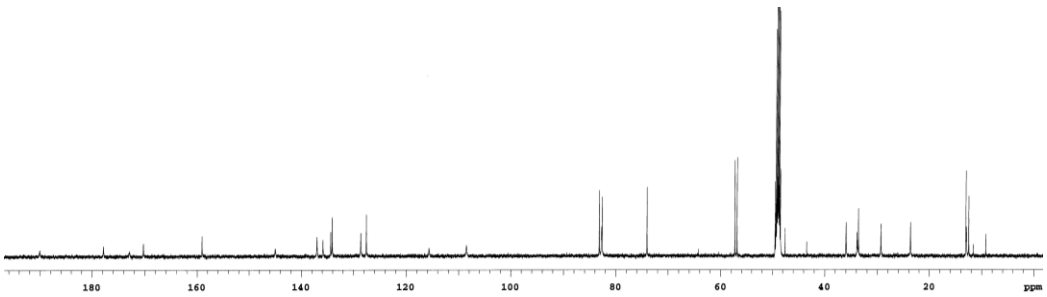
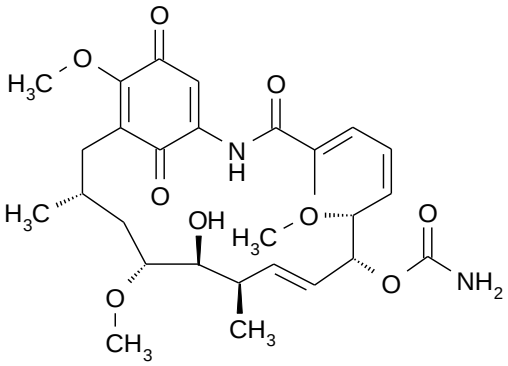


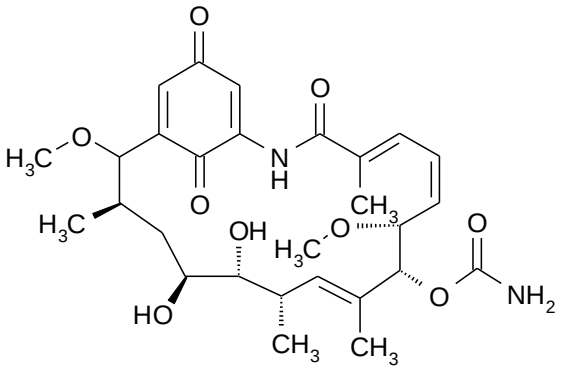
Figure 69: ^{13}C NMR spectrum (CD_3OD, 125MHz) of 17-O-demethylgeldanamycin (**82**).

The ESI mass spectrum showed a *pseudo*molecular ion peak at *m/z* 569 $[M+Na]^+$, which exhibited the molecular mass as 546 Dalton (14 amu lesser than geldanamycin) and molecular formula as $C_{28}H_{38}N_2O_9$. A search in AntiBase with the above spectroscopic data provided three geldanamycin derivatives: 8-demethyl-geldanamycin (**80**), TAN-420-B (**81**), and 17-*O*-demthyl-geldanamycin (**82**). The possibility of 8-demethylgeldanamycin (**80**) was excluded as the latter contains three methoxy groups while NMR data of compound **82** showed only two. TAN-420-B (**81**) was excluded as the olefinic proton at δ 6.93 appeared as singlet in **82** and the compound contained a hydroxyl group at C-17. The complete structure was then de-

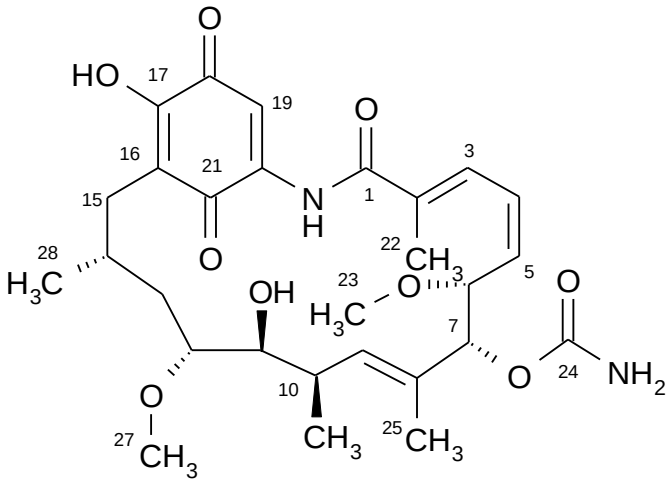
termined as 17-*O*-demethylgeldanamycin (**82**) by comparison of spectroscopic data with authentic spectra.



80

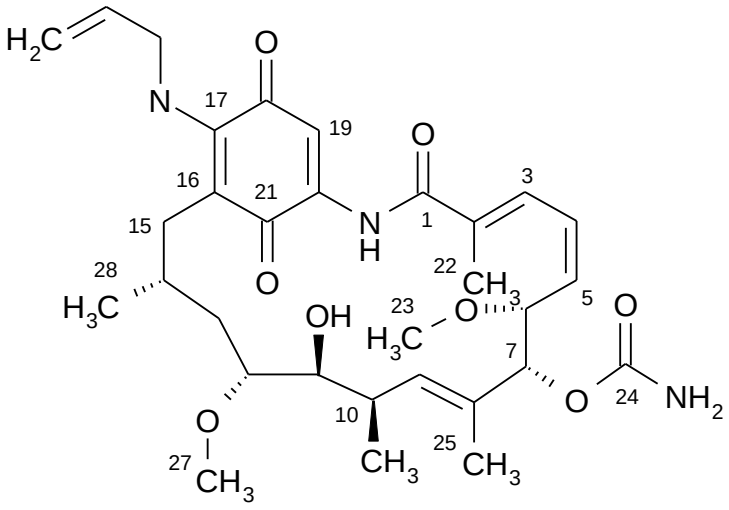


81



82

17-*O*-Demethylgeldanamycin (**82**) is a derivative of geldanamycin (**79**), isolated from *Streptomyces hygroscopicus* strain. B-434 in 1979 along with geldanamycin.[92] Due to high hepatotoxicity and low stability of geldanamycin, several derivatives were produced synthetically and *semi*-synthetically by alteration on position 17. Among them 17-allylamino-17-demothoxygeldanamycin (**83**) was found to possess lower cytotoxicity and equally activity as antitumor agent.[93] Recently, a novel class of geldanamycin derivatives was synthesized *via* substitution with different aliphatic cyclic groups and polar phosphate groups at position 17 of geldanamycin, which inhibited the Hepatitis C virus (HCV) replication in GS4.3 HCV replicon cells.[94] However, the activity of 17-*O*-demethylgeldanamycin (**82**) as compared to geldanamycin (**79**) is not yet reported.



83

4.6.3 Nigericin

Nigericin (**84**) was isolated as pale yellow powder from fraction II by purification with silica gel column chromatography using CH_2Cl_2/MeOH and Sephadex LH-20, which showed no UV absorbance and changed to dark brown with anisaldehyde/sulphuric acid spray reagent.

The ^{1}H NMR spectrum of the compound **84** revealed no signal in the aromatic region. However, it showed an anomeric or olefinic proton at δ 5.25 and several oxymethine/methylene protons were present between δ 4.50-3.20 with a methoxy signal at δ 3.30. Additionally, nine methyl signals were also visible; two of them appeared as singlets, and seven as doublets.

The ESIMS spectrum of the compound **84** exhibited a peak at *m/z* 747 $[M+Na]^+$ which gave the molecular mass of the compound as 724 Dalton and HRESIMS revealed the molecular formula as $C_{40}H_{68}O_{11}$.

As expected from the molecular formula, the ^{13}C NMR spectrum also showed 40 carbon signals, out of which one was carbonyl (δ 177.4) of ester, amide or acid, two were acetal carbons (δ 108.1, 97.0), thirteen oxycarbons appeared in the region of δ 85.7-53.4 and 24 carbons signals between δ 44.1-10.7 were also visible. Based on the spectroscopic data, the compound could be a polyether derivative.

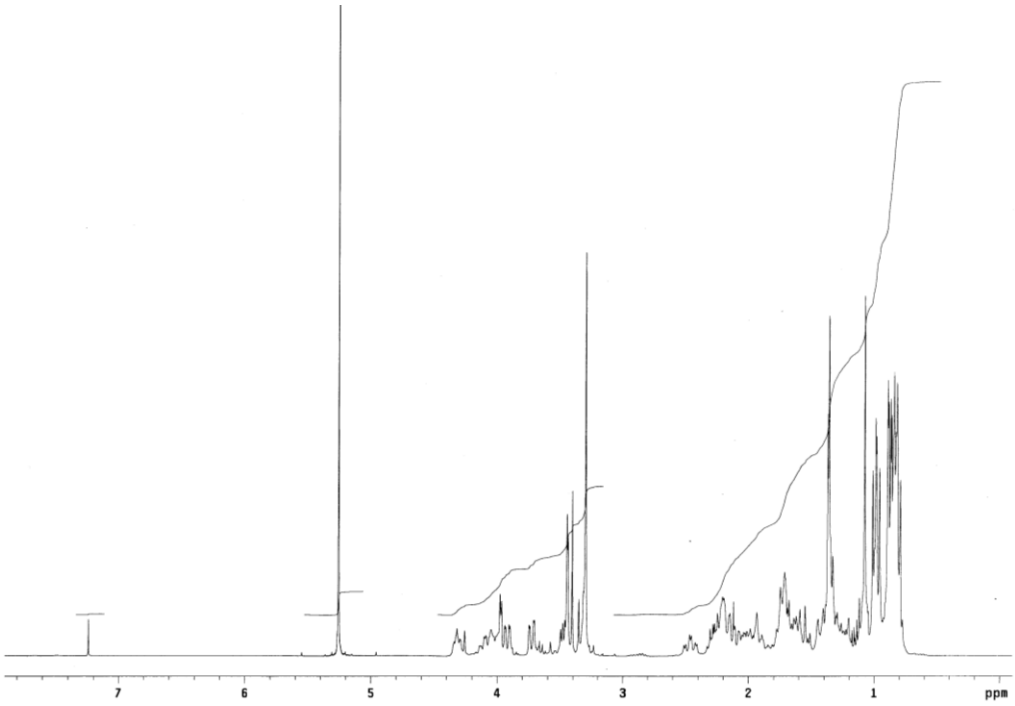


Figure 70: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of nigericin (**84**).

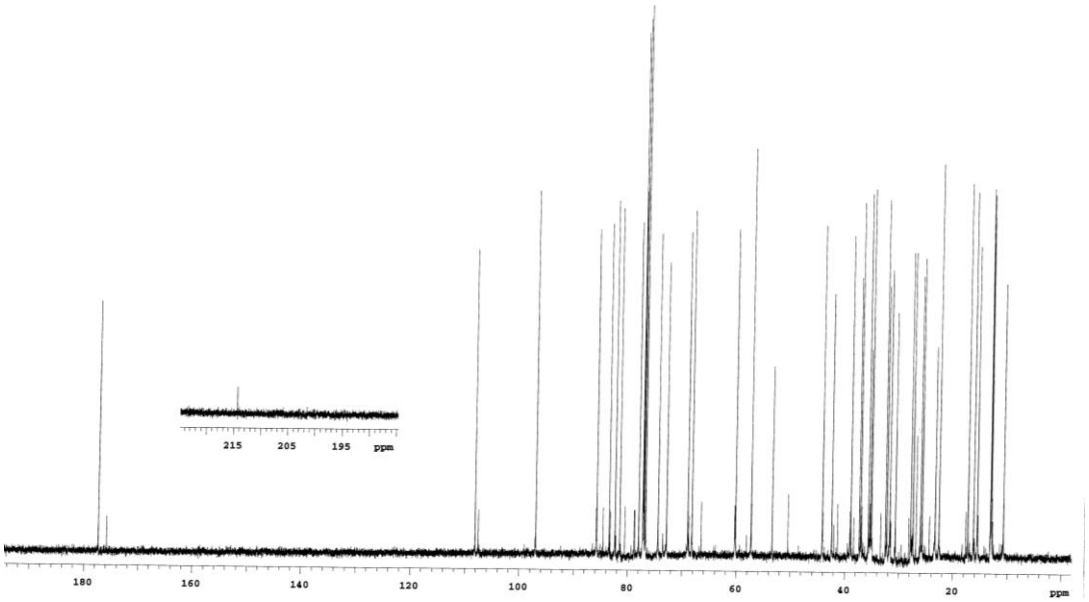
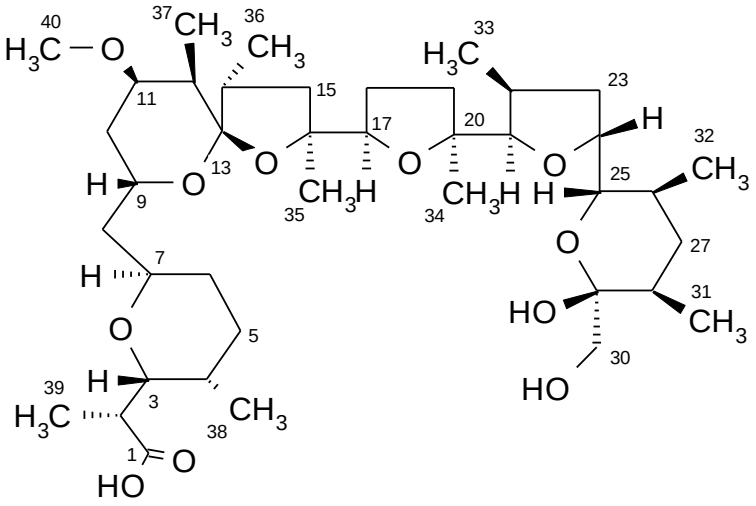
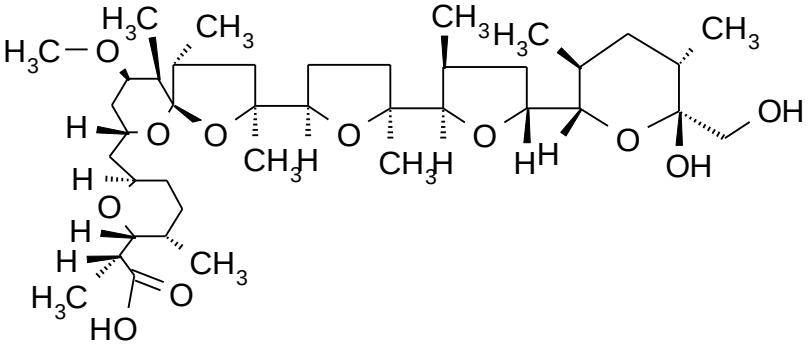


Figure 71: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of nigericin (**84**).

By searching in AntiBase, the compound was identified as nigericin (**84**) or its isomer epinigericin (**85**). The compound was finally confirmed as nigericin by comparison of ^{13}C NMR data with authentic spectra and the literature.[95]



84



85

Table 13: ^{13}C NMR data of nigericin (**84**) in comparison with literature.[95]

position	Lit.[a]	Exp.[a]	Position	Lit.[a]	Exp.[a]
	δ_C[b]	δ_C[c]		δ_C[b]	δ_C[c]
1	177.5	177.4	21	85.3	85.8
2	44.2	44.1	22	35.8	35.3
3	72.9	72.9	23	32.6	32.5
4	27.5	27.8	24	78.0	78.0
5	26.1	26.0	25	74.5	74.4
6	25.7	25.7	26	33.0	35.1
7	69.0	68.9	27	38.4	37.3
8	36.9	36.9	28	37.2	37.3
9	60.4	60.2	29	97.0	97.0
10	32.2	32.2	30	68.3	68.2
11	78.0	77.3	31	16.4	16.2
12	37.4	37.1	32	17.3	17.3
13	108.2	108.1	33	16.3	15.6
14	39.0	38.9	34	22.7	22.6
15	42.5	42.4	35	27.5	27.4
16	81.5	81.5	36	13.2	13.1
17	82.4	82.4	37	13.1	13.2
18	26.1	26.0	38	10.8	10.7
19	31.8	31.7	39	15.6	13.0
20	83.4	83.4	40	57.4	57.3

[a]CDCl3, [b]50 MHz, [c]125 MHz

Nigericin (**84**) belongs to polyether antibiotics. It was firstly isolated in the 1950s from a *Streptomyces* strain.[96] After many years in 1968, its complex structure was for the first time determined by X-ray crystallography.[97] Nigericin acts as ionophore that binds with cations (Na^+, K^+, H^+) and affects the ion transport and ATPase activity in mitochondria.[98] Nigericin has a broad spectrum of biological activities as antibacterial, antifungal,[99] herbicidal,[100] antitumor[101] as well as antiviral compound, including HIV.[102]

4.6.4 11, 11'-*O*-Dimethylelaiophylin

The compound **86** was obtained from fraction IV as a colourless crystalline solid, which showed UV absorbance at 254 nm and changed to dark brown after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum showed four olefinic protons between δ 6.95-5.60 as doublets of doublets (J = ~15, 11.2); the large coupling constant indicated their *trans* configuration. Moreover, six oxy-methine protons between δ ~5.11-3.48 and a methoxy signal at δ 3.04 were also revealed. Several methine/methylene protons were also visible between δ ~ 2.60-1.2. Additionally, it also exhibited six methyl signals, out of which five were doublets and one was a triplet.

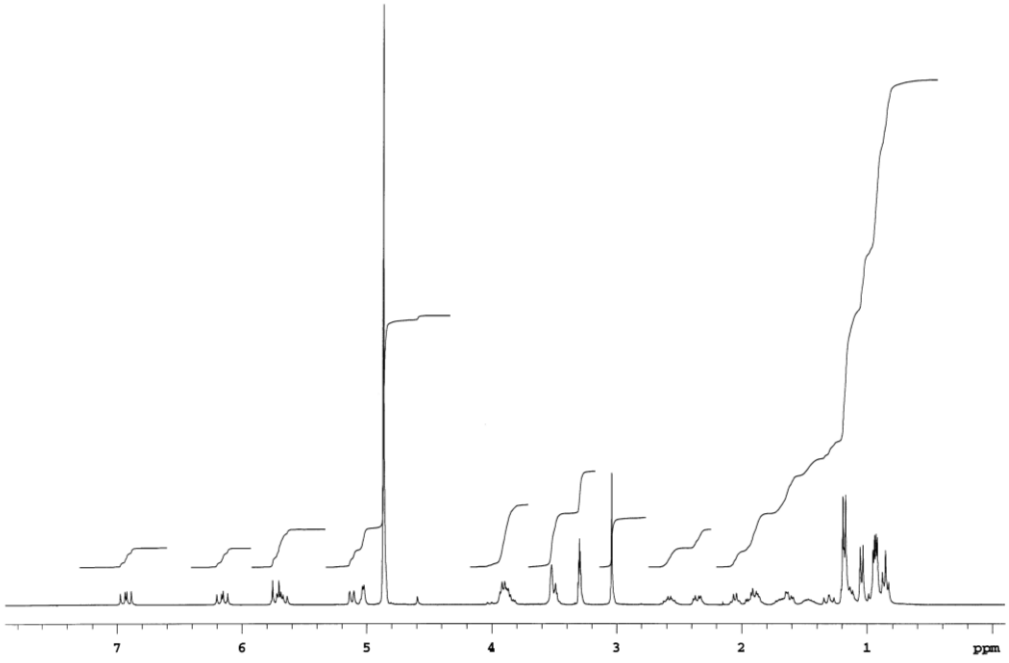


Figure 72: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 11,11'-*O*-dimethylelaiophylin (**86**).

The ^{13}C NMR/HSQC spectrum revealed twenty-eight carbon signals, one as carbonyl of ester, acid or amide at δ 170.2, four aromatic/olefinic carbons (δ 146.8, 146.2, 132.6, 122.6) and a quaternary carbon signal at δ 104.5. It also showed eight oxymethine, four methine, one methoxy, three methylene and six methyl carbon signals.

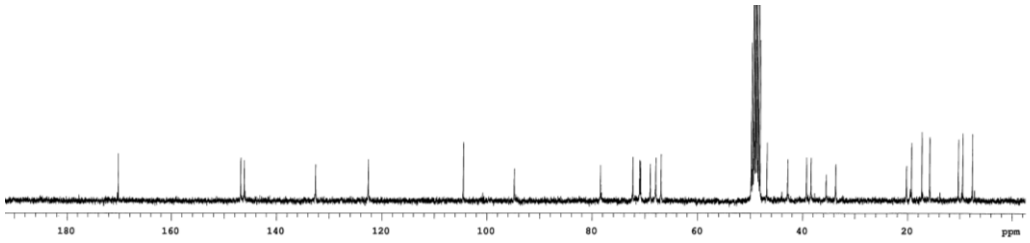
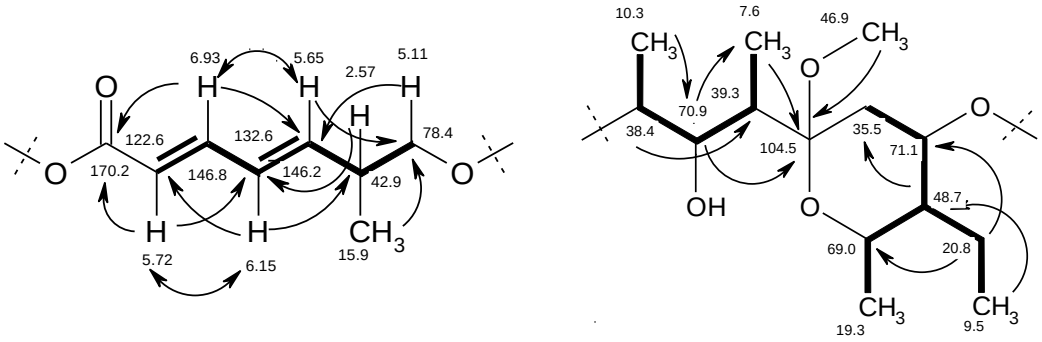


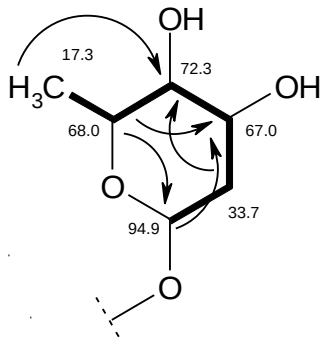
Figure 73: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 11,11-*O*-dimethylelaiophylin (**86**).

The mass of compound **86** was determined by ESIMS as 1052 Dalton, which showed *quasi*molecular ion peaks at *m/z* 549 $[M+Na]^{+2}$ and 1075 $[M+Na]^{+}$. The molecular formula $C_{56}H_{92}O_{18}$ was depicted by HRESIMS. However, ^{13}C NMR spectrum showed only 28 carbon signals, so the compound could be a symmetrical dimer.



A

B



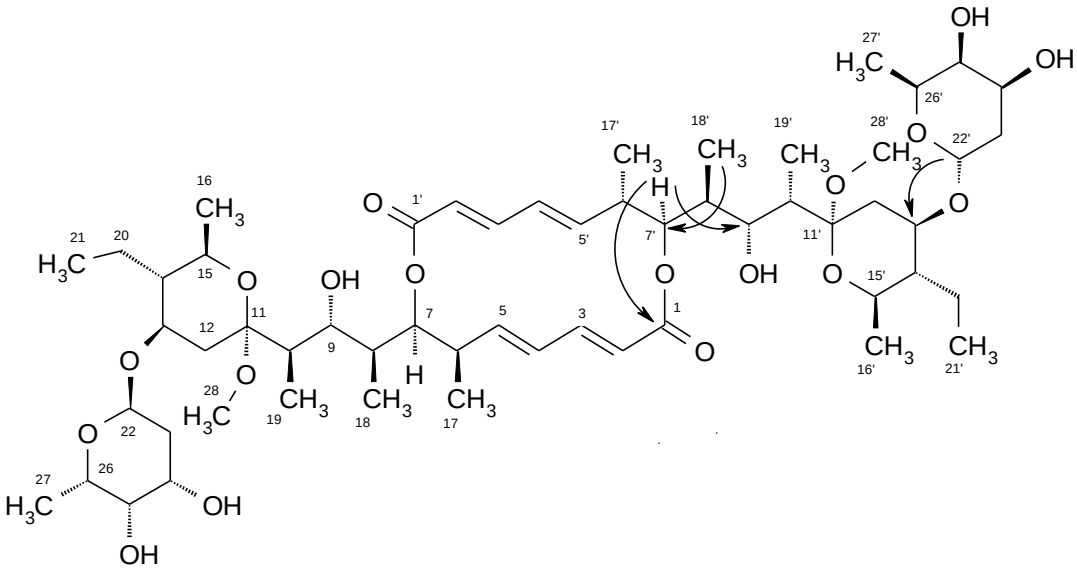
C

Figure 74: H,H-COSY (↔, ▬) and HMBC (→) correlations of 11,11-*O*-dimethylelaiophylin (**86**).

The H,H COSY spectrum showed strong correlations among the olefinic protons; H-5 (δ 5.65, ddd) also gave a correlation to H-6, which in turn showed correlation with oxymethine H-7 (δ 5.11, d). Moreover, in the HMBC spectrum, H-2 (δ

5.72) showed ^{2}J and H-3 (δ 6.93) showed ^{3}J correlation with the ester carbonyl (δ 170.0) of (C-1) (fragment **A**). Further ^{3}J correlations were observed from the C18-methyl protons (δ 0.95) to CH-10 (δ 2.05) which in turn showed COSY correlation with C19-methyl (δ 0.92). The CH-9 (δ 3.52), C-19 methyl protons (δ 0.92) and C-28 methoxy protons (δ 3.04) showed ^{3}J correlations with the ketal carbon (δ 104.5) as constructed in the fragment **B**. The presence of a 2-deoxysugar moiety was confirmed by COSY correlations between the anomeric proton at δ 5.09 (d, J = 3.02) and methylene (δ 1.92, 1.62) and oxymethine protons. The ^{3}J HMBC correlations between anomeric proton (δ 5.09) and C-13 (δ 71.2) supported the connectivity of the sugar moiety and fragment **B**. The C-18 methyl protons (δ 0.94) also showed ^{3}J HMBC correlations with C-7 (δ 78.5) confirming the connection between fragment **A** and **B**. Furthermore, ^{3}J correlation was observed between H-7 (δ 5.11) and the ester carbonyl (δ 170.0) in the HMBC spectrum, which was the connection point of two monomers of the molecule.

The search in the AntiBase with the help of above spectroscopic data resulted in 11,11'-*O*-dimethylelaiophylin (**86**), which was further confirmed by comparison with literature data and 2D NMR spectroscopy.



86

Elaiophylin is a glycosylated macrodiolide isolated firstly from *Streptomyces melanosporus* in 1959,[103] and later on in 1960, with the name of azalomycin B from a *Streptomyces* strain.[104] Its structure was fully elucidated by Keller-Schierlein *et al*. in 1981 by spectroscopic and chemical degradation methods.[105] It is also a highly

characteristic metabolite of *Streptomyces hygroscopicus*; producer of geldanamycin (**79**) and nigericin (**84**). It exhibits promising antibacterial, antifungal, cytocidal, and anticoccoidal activities.[106] It is also reported to enhance rumen efficiency[107] and possess antiulcer properties. It also inhibits testosterone 5-α-reductase. [108]

Two derivatives of elaiophylin; 11-*O*-monomethylelaiophylin (**87**) and 11,11'-*O*-dimethylelaiophylin (**86**) were isolated from *Streptomyces* strain in 1999 and reported to exhibit antimicrobial and moderate cytotoxic activities against several cancer cell lines.[109] Further compounds structurally related to elaiophylin are the efomycins.[110]

Table 14: ^{1}H and ^{13}C NMR data (CD_3OD) of 11,11'-*O*-dimethylelaiophylin (**86**) in comparison with the literature.[109]

Position	Literature		Experimental	
	δ_C [a]	δ_H [b] (J in [Hz])	δ_C [a]	δ_H [b] (J in [Hz])
1	170.2	-	170.2	-
2	122.6	5.73(d, 15.2)	122.6	5.72 (15.3, 10.2)
3	146.9	6.99 (dd, 15.2, 11.1)	146.8	6.93 (dd, 14.9, 11.2)
4	132.6	6.15 (dd, 15.1,11.1)	132.6	6.15 (dd, 14.9, 11.2)
5	146.2	5.67(dd, 15.1,9.8)	146.2	5.65 (d, 9.8)
6	42.9	2.58 (m)	42.9	2.57 (m)
7	78.5	5.12 (dd, 10.4, 2.0)	78.4	5.11 (d, 10.3)
8	38.4	1.87(m)	38.4	1.88 (m)
9	70.9	3.51 (m)	70.9	3.52 (m)
10	39.3	2.05 (q, 6.9)	39.3	2.05 (q, 6.9)
11	104.5	-	104.5	-
12	35.5	2.35 (dd, 13.1, 4.5)	35.5	2.35 dd (13.3, 4.6)
		1.31 (m)		1.30 dd (13.0, 11.0)
13	71.2	3.85 (dt, 10.7, 4.7)	71.1	3.85(m)
14	48.7	1.31 (m)	48.7	1.30 (dd, 13.0, 11.0)
15	69.1	3.51 (m)	69.0	3.48 (t, 2.5)
16	19.3	1.18 (d, 6.4)	19.3	1.18 (d, 6.4)
17	15.1	1.04 (d, 6.7)	15.9	1.04 (d, 6.6)
18	10.3	0.94 (d, 6.6)	10.3	0.95 (d, 3.1)
19	7.6	0.94 (d, 6.8)	7.6	0.92 (d, 3.3)
20	20.3	1.46 (m),1.66(m)	20.3	1.47 (m), 1.70 (m)
21	9.5	0.86 (t, 6.5)	9.5	0,85 (t, 7.3)
22	94.9	5.02 (d, br, 3.5)	94.9	5.09 (d, 3.3)
23	33.7	1.62 (dd, 12.7, 4.9),	33.7	1.61(dd, 12.7, 4.9),

		1.92 (dd, 12.7, 3.8)		1.94 (m)
24	67.0	3.90 (m)	67.0	3.89 (m)
25	72.5	3.52 (m)	72.3	3.52 (m)
26	68.0	3.91 (m)	68.0	3.91 (m)
27	17.3	1.18 (d, 6.8)	17.3	1.18 (d, 6.4)
28	46.9	3.04 (s)	46.9	3.04 (s)

[a]125 MHz; [b]300 MHz

4.6.5 11-*O*-Monomethylelaiophylin

The compound **87** was also isolated from fraction IV as a UV-absorbing, colourless solid, which showed same colour as compound **86** with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum was also very similar to that of compound **86**, however the intensity of the methoxy signal (δ 3.04) was smaller as compared to compound **86**. The ESI mass spectrum revealed the mass of the compound as 1038 Dalton showing a *quasi*molecular ion peak at *m/z* 1061 $[M+Na]^+$, which is 14 amu lesser than for compound **86**.

The ^{13}C NMR spectrum also showed high similarity to compound **86** but all the carbon signals were split into two signals and it also contained an additional carbon signal at δ 100.9. This indicated that the compound was not longer a symmetrical dimer, rather than that both halves were slightly different from each other.

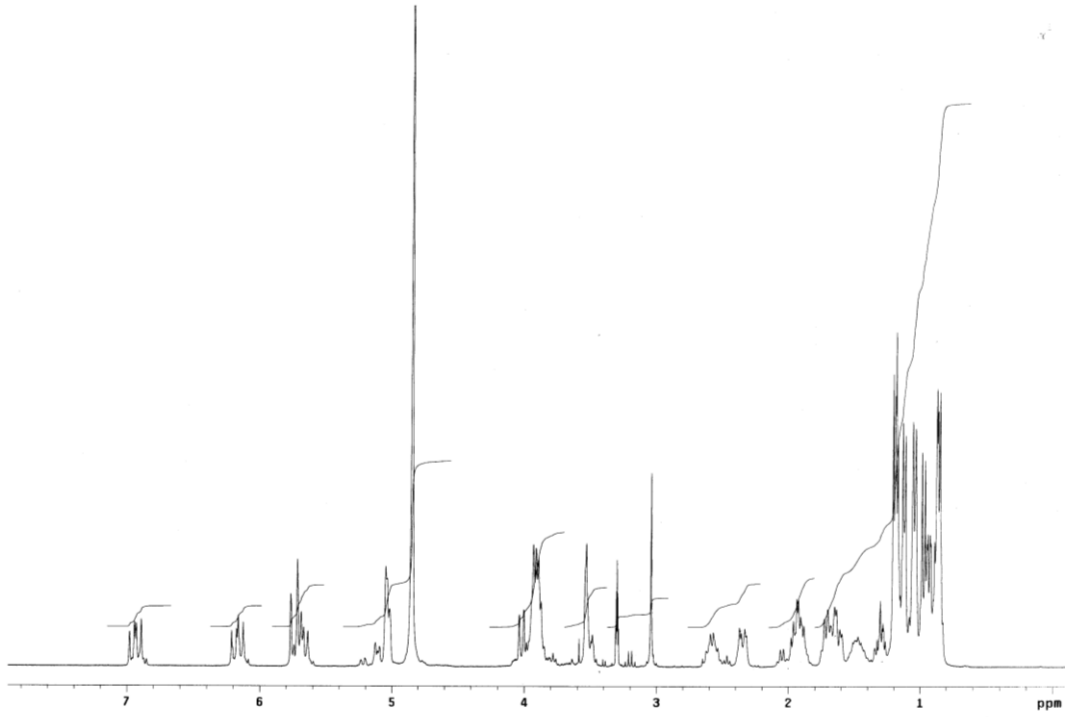


Figure 75: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 11-*O*-monomethylelaiophylin (**87**).

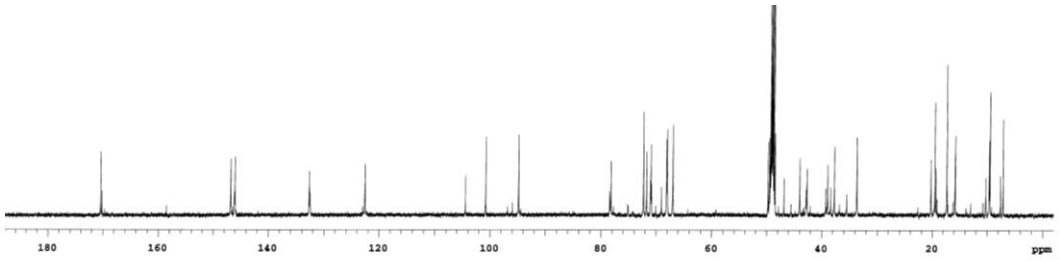
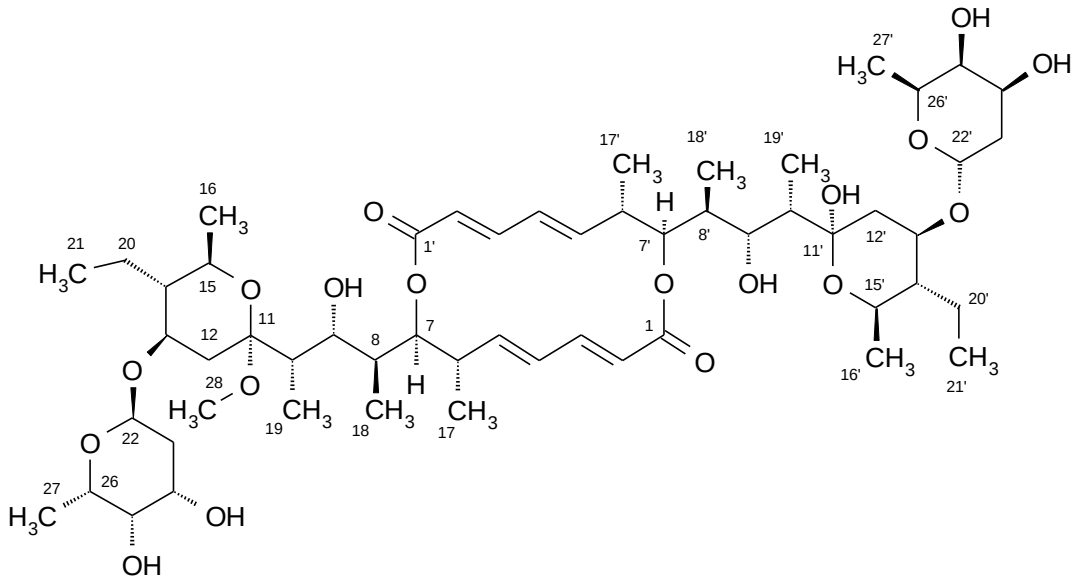


Figure 76: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 11-*O*-monomethylelaiophylin (**87**).

The search in AntiBase with the above spectroscopic data and comparison with literature data confirmed the compound to be 11-*O*-monomethylalaiophylin (**87**), which differs from compound **86** in an OH instead an OMe group at position 11'.



87

4.6.6 Nocardamine

Nocardamine (**88**) was isolated from fraction V as a colourless powder after washing the fraction with methanol. The ^{1}H NMR spectrum displayed seven methylene signals in the aliphatic region, in addition to two replaceable protons (NH, OH) at δ 9.45 and δ 7.60. The ESIMS spectrum showed a *quasi*molecular ion peak at *m/z* 623 $[M + Na]^{+}$. The search in AntiBase with the mass and NMR data led to the known cyclic peptide nocardamine (**88**), which possesses a characteristic 3-fold symmetry and was confirmed by comparing the data with authentic spectra.

Nocardamine (**88**) was firstly isolated from *Nocardia* sp., from *Pseudomonas stutzeri* and later also from *Streptomyces* sp.[111] It belongs to the siderophore antibiotics and shows a high binding affinity to ferric ions.[112, 113] It is reported as antibacterial agent against mycobacteria.[114]

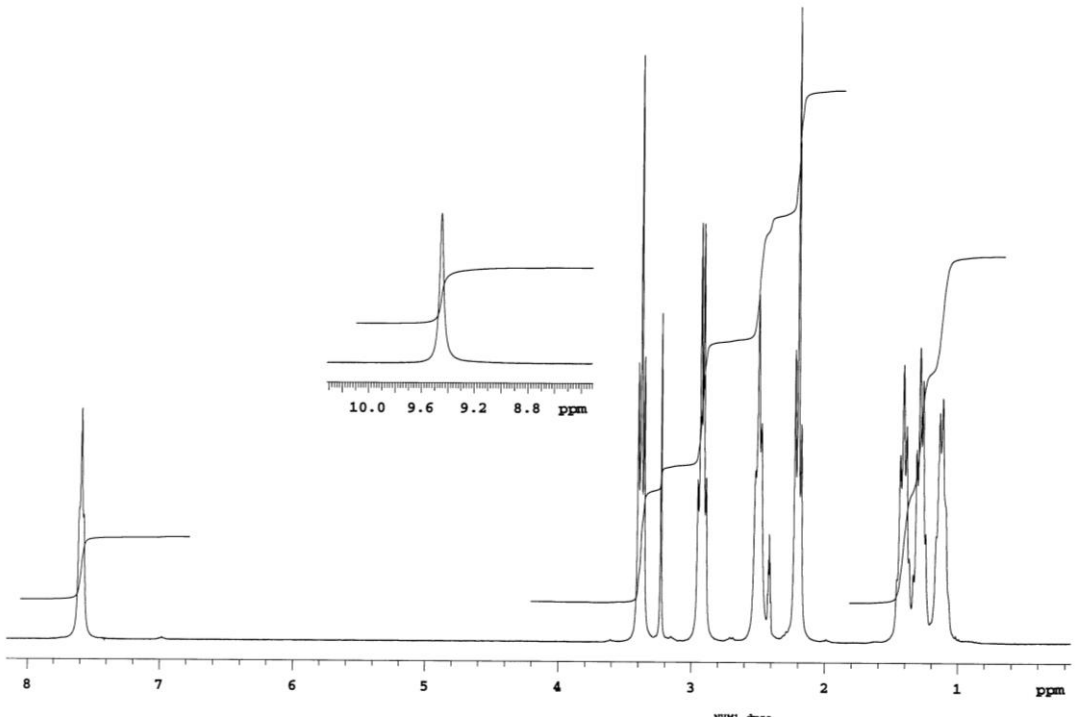
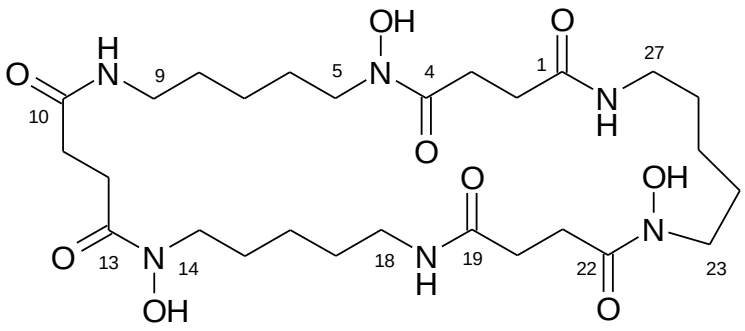


Figure 77: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of nocardamine (**88**).



88

4.7 Terrestrial *Streptomyces* sp. Ank 202

In the primary screening, the crude extract of terrestrial *Streptomyces* sp. Ank 202 exhibited high activity against *Mucor miehei* (Tü 57) and moderate activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. It had only very weak activity (9.0%) against *Artemia salina*. On TLC, it showed a highly green fluorescent band along with other UV absorbing zones, which turned to yellow and green after spraying with anisaldehyde/sulphuric acid.

The strain was fermented as 30L shaker culture using M_2 medium for 7 days. The brown culture broth was applied to the usual extraction process giving two separate extracts from mycelial cake and filtrate. TLC of both extracts showed identical results, and so they were combined and concentrated *in vacuo* to get 3.2g of brown crude extract.

The extracted was subjected to silica gel column and eluted with dichloromethane-methanol gradient to afford four fractions, which were further purified by different chromatographic techniques to get pure compounds (see work-up scheme).

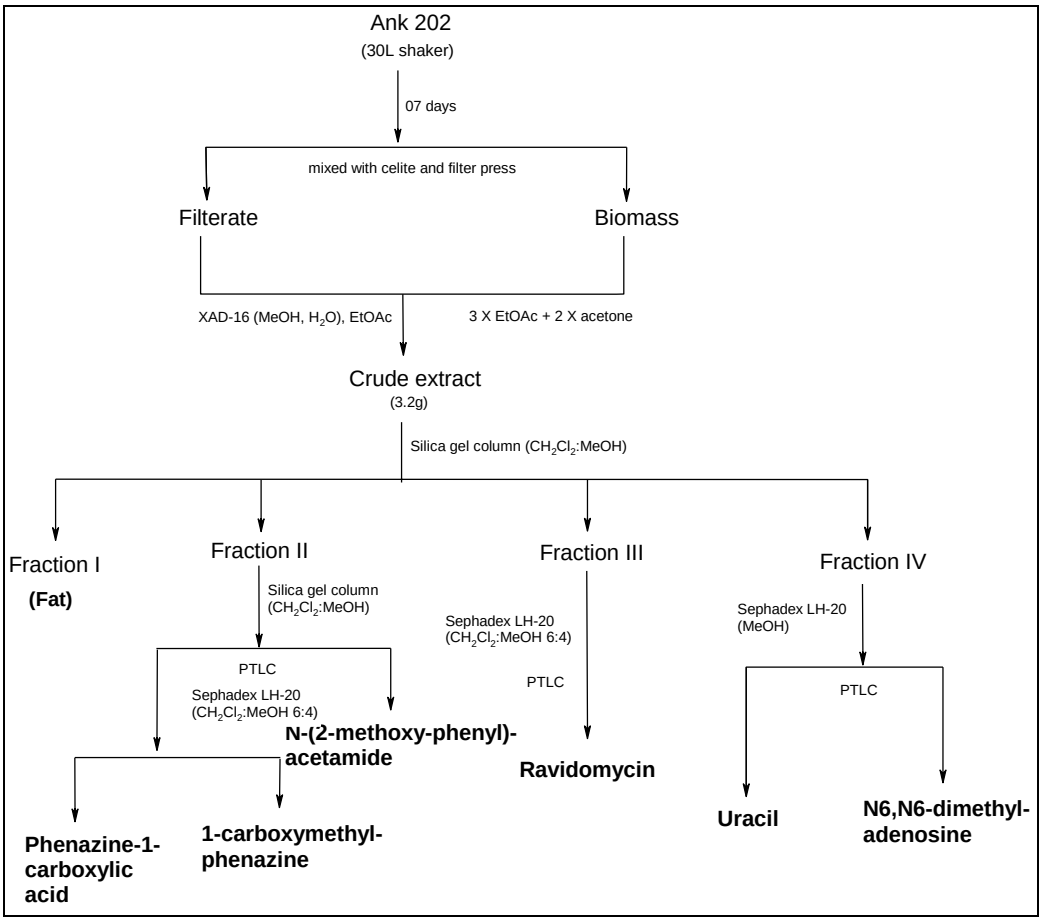


Figure 78: Work up scheme of terrestrial *Streptomyces* sp. Ank202.

4.7.1 Ravidomycin

Ravidomycin (**89**) was isolated from fraction III using PTLC followed by Sephadex LH-20 as a bright yellow solid, which showed a strong green fluorescence under UV light and turned to green after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum afforded in the aromatic region two *ortho*-coupled protons at δ 7.95 (J = 8.4 Hz) and δ 7.01 (J = 8.3 Hz) and two *meta*-coupled protons at δ 7.99 (J = 1.5 Hz) and δ 7.22 (J = 1.5 Hz). It also showed a singlet at δ 8.25 and a broad singlet at δ 9.76. Moreover, it also exhibited three *cis* and *trans* coupled protons (J = 17.6, 10.9 Hz) in the olefinic region. Additionally it revealed two methoxy signals at δ 3.98 and δ 3.97, two N-dimethyl protons at δ 2.48, an acetyl at δ 2.09 and a methyl doublet at δ 1.02 (J = 6.4 Hz).

The ^{13}C NMR spectrum revealed 31 carbon signals, one ester, acid or amide carbonyl at δ 170.7, four sp^2 oxy-carbons between δ 160-150, fourteen olefinic carbons between δ 145-100, and five oxy-methine carbons between δ 80-60. It also exhibited two methoxy carbons at δ 56.2 and δ 56.1, two N-dimethyl carbons at δ 40.7 and two methyl carbons at δ 21.7 and δ 16.8.

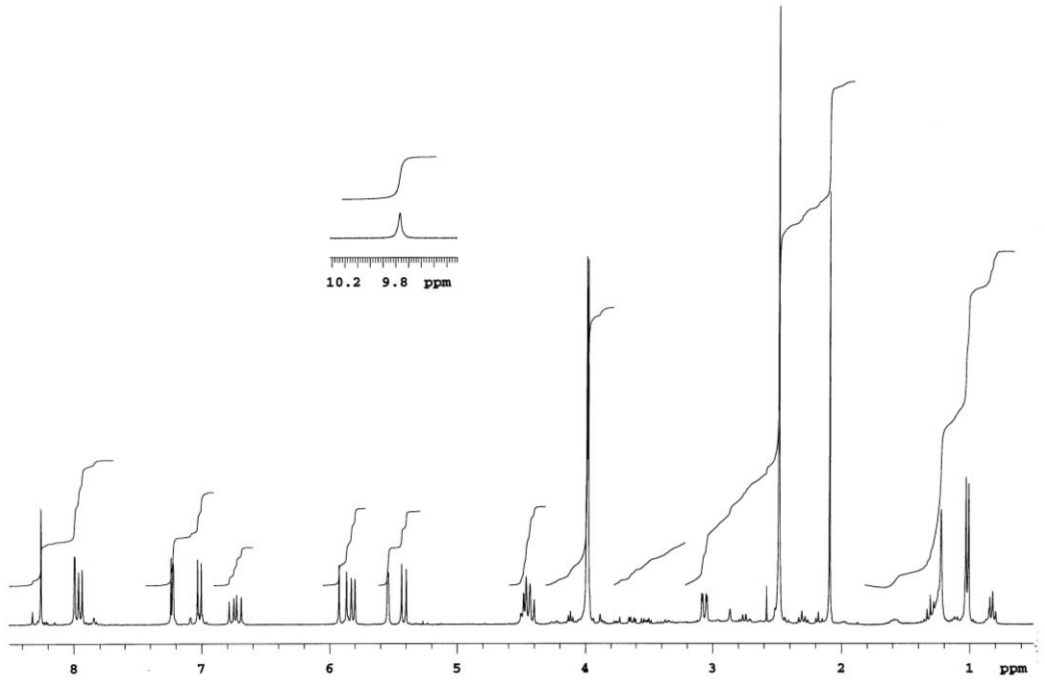


Figure 79: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of ravidomycin (**89**).

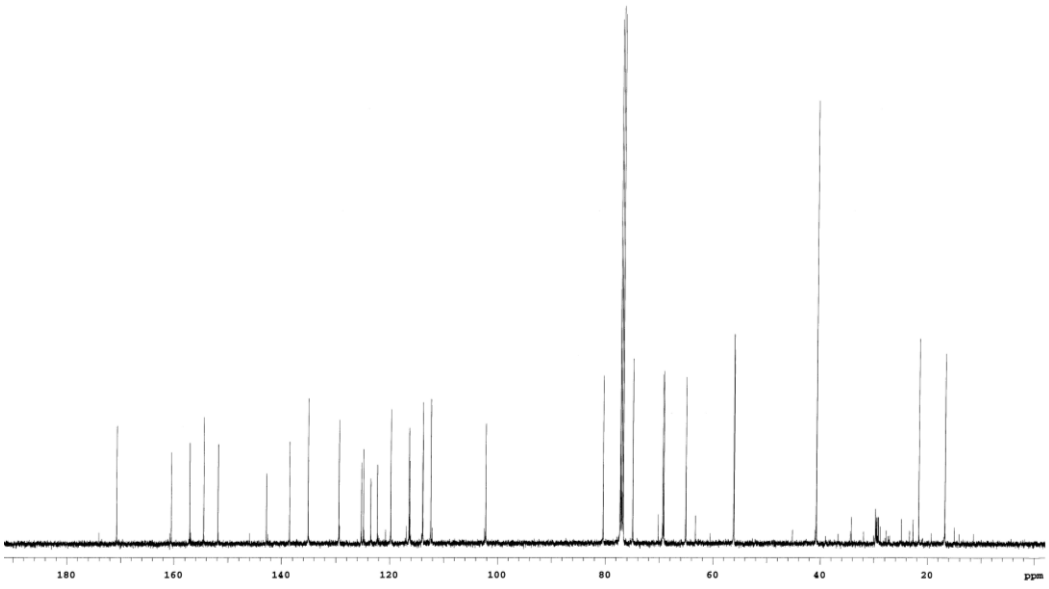
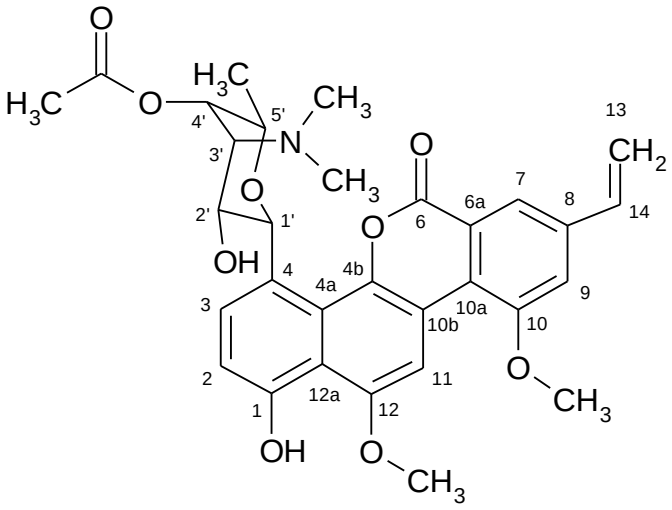


Figure 80: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of ravidomycin (**89**).

The ESIMS spectrum showed a *pseudo*molecular ion peak at *m/z* 564 $[M+H]^+$ which afforded the molecular formula by HRESIMS as $C_{31}H_{33}N_1O_9$. The search in AntiBase with the above spectroscopic data fixed the compound as ravidomycin (**89**). This was also confirmed by comparing the data with the literature.[115]



89

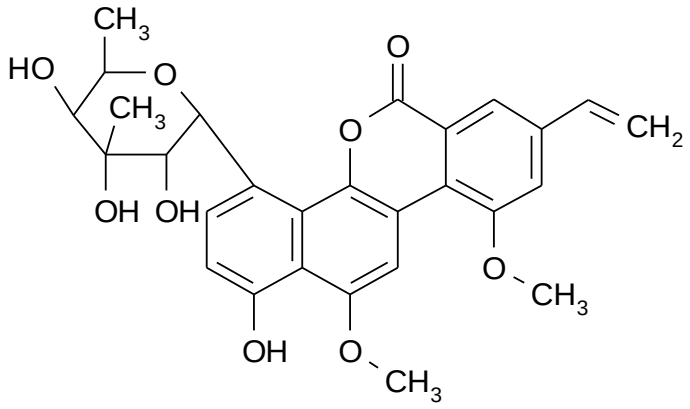
Table 15: NMR data of ravidomycin (**89**) in comparison with literature.[115]

Position	Literature[a]		Experimental[a]	
	δ_C	δ_H (J in [Hz])	δ_C[b]	δ_H[c] (J in [Hz])
1	154.7	-	154.5	-
2	112.4	7.04 (d, 8.3)	112.4	7.01 (d 8.3)
3	129.5	7.98 (d, 8.3)	129.4	7.95 (d, 8.6)
4	125.2	-	125.2	-
4a	142.9	-	142.9	-
4b	160.6	-	160.6	-
6	122.2	-	122.3	-
7	119.7	8.02 (d,1.6)	119.8	7.99 (d, 1.5)
8	138.6	-	138.6	-
9	113.8	7.25 (d, 1.6)	113.8	7.22 (d, 1.5)
10	157.1	-	157.1	-
10a	123.4	-	123.5	-
10b	113.9	-	113.9	-
11	102.1	8.28 (s)	102.2	8.25 (s)
12	151.8	-	151.9	-
12a	116.3	-	116.4	-
13	135.2	6.76 (dd, 17.2, 10.5)	135.1	6.74 (dd, 17.6, 10.9)
14	116.2	5.91 (d, 17.2)	116.3	5.89 (d, 17.6)
		5.44 (d, 10.5)		5.41 (d, 10.9)
10-OCH_3	56.0	4.00 (s)	56.2	3.98 (s)
12-OCH_3	55.9	4.01 (s)	56.1	3.97 (s)
1'	80.5	5.85 (d, 9.4)	80.4	5.81 (d, 8.9)
2'	65.4	4.44 (dd, 10.2, 9.4)	65.1	4.44 (dd, 14.8, .9)
3'	69.6	3.08 (dd, 10.2, 3.0)	69.2	3.06 (dd, 10.2, 2.4)
4'	69.4	5.56 (d, 3.0)	69.4	5.54 (d, 1.7)
5'	75.0	4.49 (q, 6.4)	75.0	4.44 (m)
5'-CH_3	16.8	1.05 (d, 6.4)	16.8	1.02 (d,6.4)
3'-$N(CH_3)_2$	40.8	2.51 (s)	40.8	2.48 (s)
4'-$COCH_3$	21.6	2.11 (s)	21.7	2.09, (s)
4'-C=O	170.8	-	170.8	-

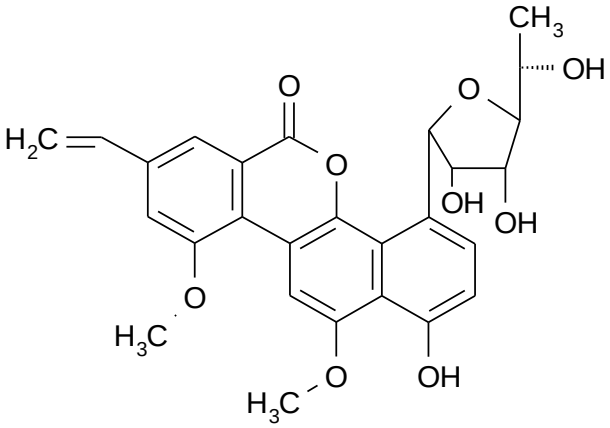
[a]CDCl3, [b]300 MHz, [c]125 MHz

Ravidomycin (**89**) was isolated in 1980 from the fermentation broth of *Streptomyces ravidus*.[116] Its structure was elucidated by NMR methods by Findley *et al*.[117] It is a C-glycosidic polycyclic aromatic hydrocarbon containing an amino sugar, ravidosamine. The aglycone moiety of ravidomycin is same as in albacarcin V (**90**), gilvocarcin V (**91**) and chrysomycin. Ravidomycin (**89**) is reported to possess anti-

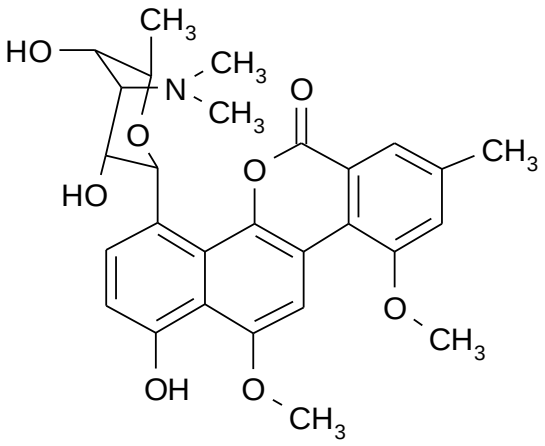
bacterial properties mainly against Gram-positive bacteria including mycobacteria. It is also a potent antitumor agent against P-388 lymphocytic leukaemia, colon 38 tumor and CD8F1 mammary tumor.[118] Until now, several derivatives of ravidomycin have been isolated from *Streptomyces* sp. and synthesized. Higher antibacterial activity was found in deacylated derivatives of ravidomycin.[119] Recently, another derivative of ravidomycin, deacetylravidomycin M (**92**) was isolated from *Streptomyces* sp. which showed inhibitory effect on IL-4-induced CD-23 expression in U937 cells without any cytotoxic effects. Inhibitors of IL-4 signal transduction prevent allergic reactions.[120]



90



91



92

4.7.2 N-(2-Methoxyphenyl)-acetamide

The compound **93** was isolated as a UV absorbing, colourless solid from fraction II after purification with silica gel column (CH_2Cl_2/MeOH). It showed no colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum exhibited two 1H doublets of doublets at δ 8.38 and 6.83 and two 1H triplets of doublets at δ 7.04 and 6.98, an indication of a 1,2-disubstituted aromatic pattern. Additionally, a broad singlet of an exchangeable proton at δ 7.80, a methoxy (δ 3.84) and a methyl singlet at δ 2.18 also appeared.

The mass of the compound determined by EIMS was 165 Dalton. A search in AntiBase and comparing the data with authentic spectra confirmed the compound as N-(2-methoxyphenyl)-acetamide (**93**). It is known from synthesis but also previously isolated for the first time in our group from *Streptomyces* sp.[121]

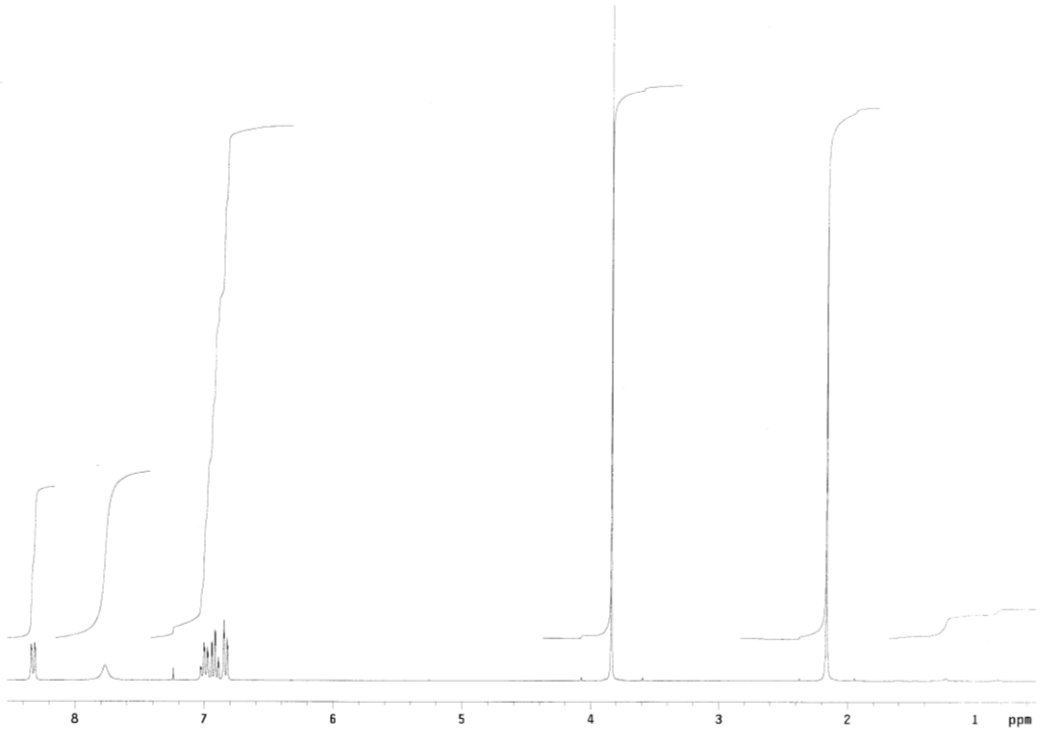
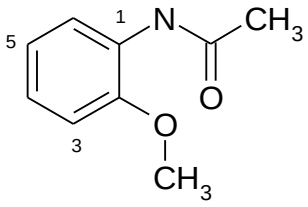


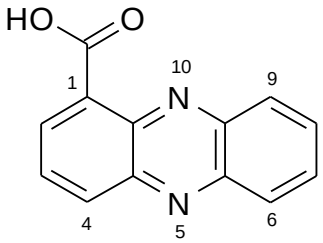
Figure 81: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of N-(2-methoxyphenyl)-acetamide (**93**).



93

4.7.3 Phenazine-1-carboxylic acid

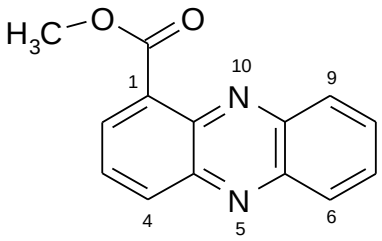
The compound **94** was also isolated from fraction II using PTLC followed by Sephadex LH-20 as a UV absorbing yellow solid. The mass of the compound was determined by ESIMS as 224 Dalton. The ^{1}H NMR showed seven protons in the aromatic region between δ 9.00-7.90, clearly indicating the presence of mono-substituted phenazine moiety. The search in AntiBase and comparison with authentic spectra confirmed the compound to be phenazine-1-carboxylic acid (**94**).



94

4.7.4 1-Carboxymethyl-phenazine

The compound **95** was also isolated from the same fraction by PTLC as a UV absorbing yellow solid with lower polarity than **94**. The ^{1}H NMR spectrum showed the same pattern in the aromatic region as compound **94** but an additional methoxy signal appeared at δ 4.10. The mass of the compound was determined by ESIMS as 238 Dalton. The compound was confirmed as 1-carboxymethyl-phenazine (**95**) by searching in AntiBase and comparison with authentic spectra.



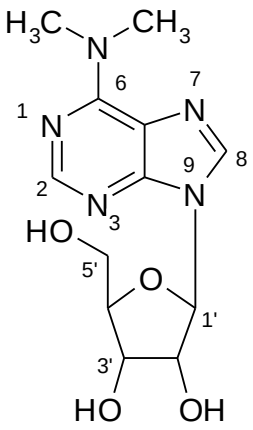
95

Many phenazine derivatives are frequently found in nature and mostly produced by bacteria such as *Pseudomonas* spp. and *Streptomyces* spp. They have potent anti-bacterial and antifungal activities.

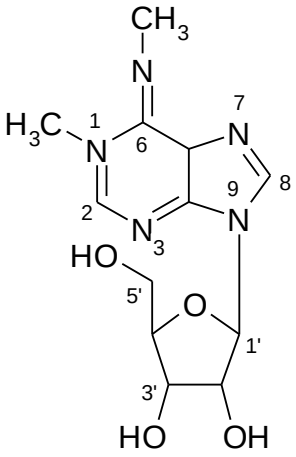
4.7.5 N6, N6-Dimethyladenosine

The compound **96** was isolated from fraction IV as a UV absorbing colourless solid after purification with PTLC. It showed green colour with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum revealed two downfield shifted aromatic singlets at δ 8.27 and 8.20, five oxygenated proton signals between δ 3.60 and 4.70, along with an anomeric proton at δ 5.93 (d, J = 5.6 Hz). The molecular weight of **96** was established by ESIMS as 295 Dalton and HRESIMS afforded the molecular formula $C_{12}H_{17}N_5O_4$. In contrast to molecular formula, the ^{13}C NMR spectrum exhib-

ited only 10 carbon signals. The search in AntiBase with the spectroscopic data and molecular formula provided two possible adenosine analogues, N6,N6-dimethyladenosine (**96**) and 1,6-dimethyladenosine (**97**), however, two methyl signals were absent in both ^{1}H and ^{13}C NMR spectra. The NMR measurements of the compound (**96**) was again performed at 100 °C where then two overlapped methyl signals were observed in the ^{1}H NMR spectrum at δ 3.47 and in the ^{13}C NMR spectrum at δ 37.5. The disappearance of methyl signals at room temperature is obviously due to coalescence phenomenon: it seems that two methyl groups flip between two orientations at room temperature nearly with the spectrometer frequency. The compound was finally identified by comparison of the NMR data with authentic spectra as N6,N6-dimethyladenosine (**96**), which was previously isolated in our group.



96



97

4.8 Terrestrial Streptomyces sp. Ank 291

The terrestrial *Streptomyces* isolate Ank 291 showed a dark red aerial mycelium on agar. In the pre-screening, its crude extract exhibited high activity against *Bacillus subtilis* and *Staphylococcus aureus*, moderate activity against *Mucor miehei* (Tü 57) and 100% activity against brine shrimps (*Artemia salina*). Moreover on TLC, it showed several red zones, which turned to violet/blue with sodium hydroxide, indicating the presence of *peri*-hydroxyquinones.

A large-scale fermentation of the strain using linear shaker for seven days followed by extraction and purification of the compounds using different chromatographic techniques afforded five compounds, including three *peri*-hydroxyquinones.

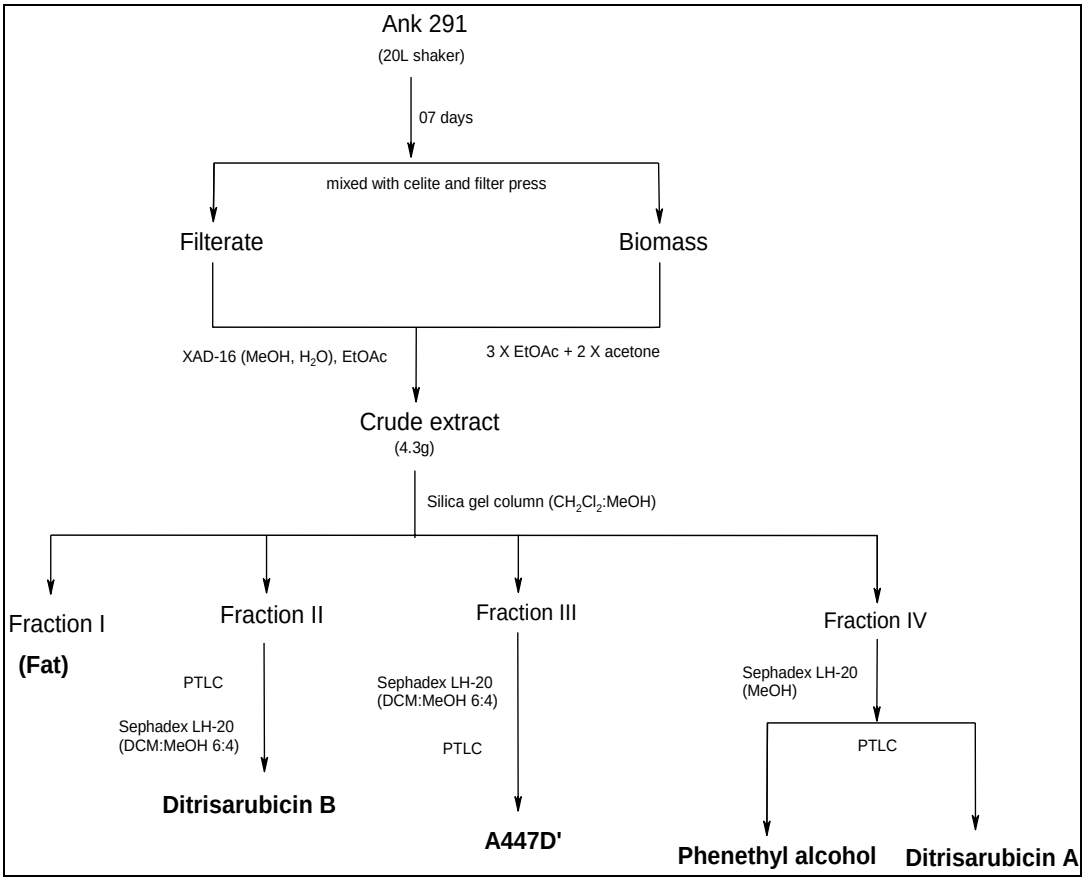


Figure 82: Work-up scheme of terrestrial *Streptomyces* sp. Ank 291

4.8.1 Ditrisarubicin A

Ditrisarubicin A (**98**) was isolated as a red solid from fraction II which showed orange UV fluorescence and turned to violet with sodium hydroxide, as indication of *peri*-hydroxyquinones.

The ^{1}H NMR spectrum of **98** exhibited three broad singlets at δ 13.73, 12.85 and 12.12, which are characteristic of chelated *peri*-hydroxy groups, and three aromatic protons as doublet, triplet and doublet for a 1,2,3-trisubstituted aromatic ring. The oxymethine/methylene signals between δ 3.0-5.50 represented several sugar moieties. Moreover, it also revealed many overlapping methyl/methylene signals in the upfield region between δ 2.60-0.80. The ESI mass spectrum of **98** showed a *quasi*-molecular ion peak at *m/z* 1181 $[M-H]^-$. The search in AntiBase and comparing the spectroscopic data with the literature confirmed the compound as ditrisarubicin A (**98**) which contained six sugar moieties: rhodosamine, 2-deoxyfucose, cinerulose B attached at C-7 and rhodosamine, 2-deoxyfucose, cinerulose A attached at C-10.

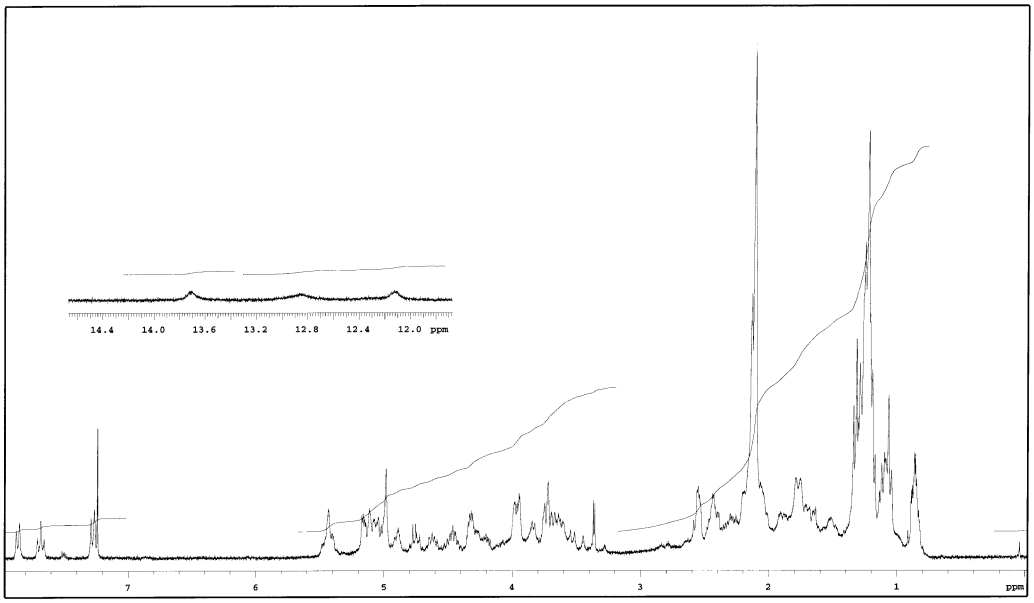
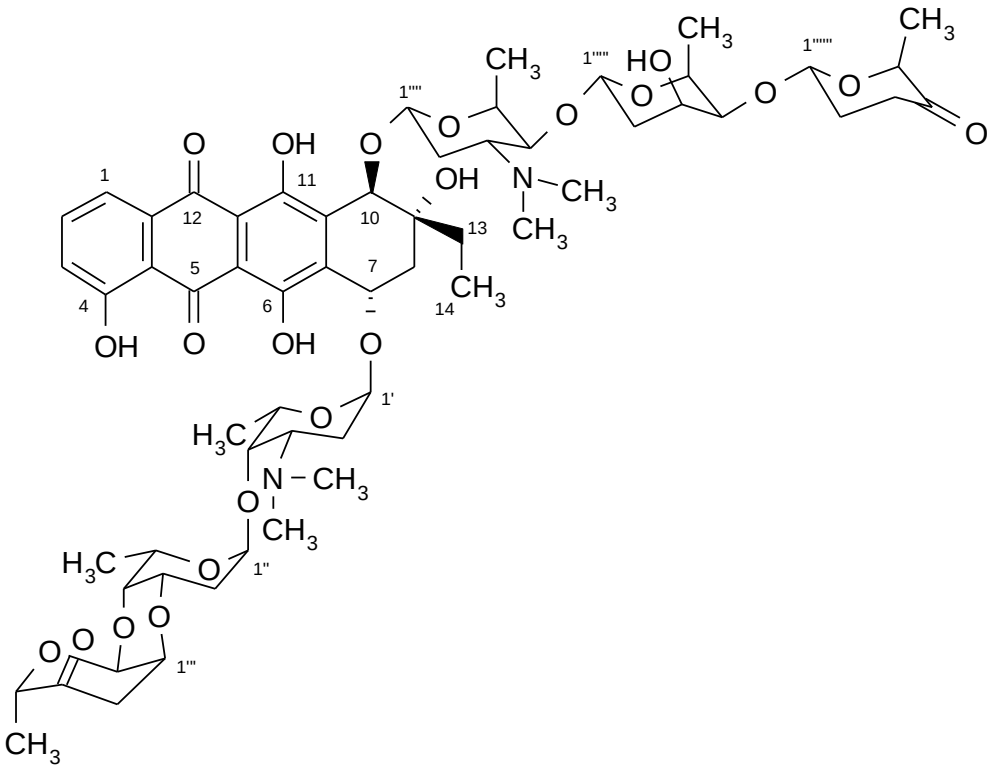
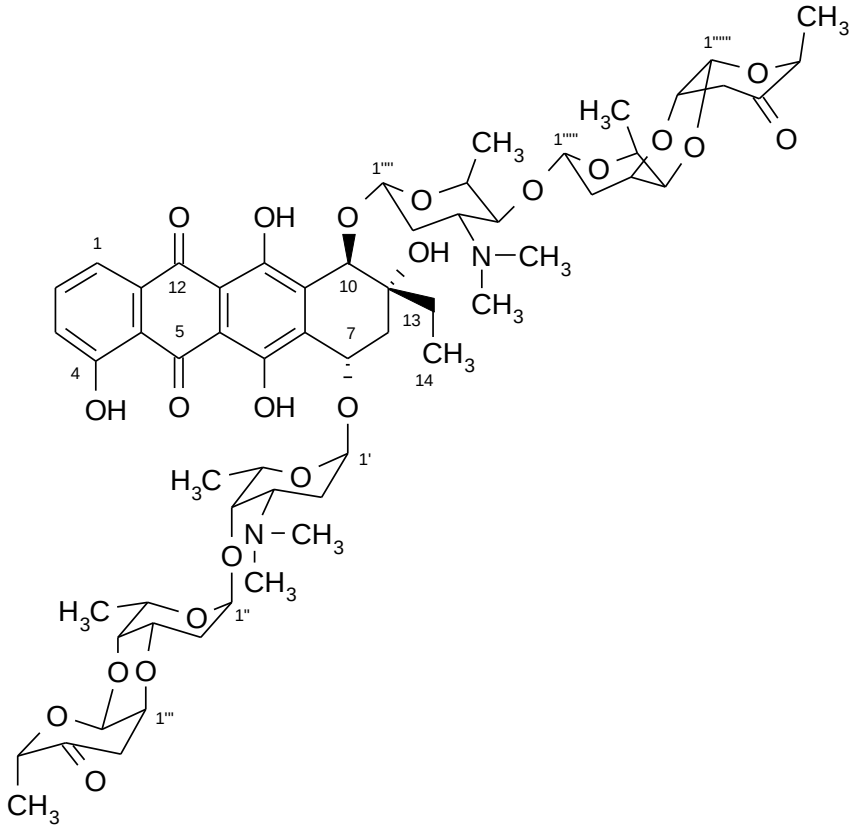


Figure 83: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of ditrisarubicin A (**98**).



4.8.2 Ditrisarubicin B

The compound **99** was also isolated as red powder, which showed similar properties and ^{1}H NMR spectrum as **98**. The only difference was in the mass, which showed a *quasi*molecular ion peak at *m/z* 1179 $[M-H]^-$ that is 2 amu lesser than compound **98**. A search in AntiBase fixed the compound as ditrisarubicin B (**99**), which was further confirmed by comparison with authentic spectra.



99

Ditirisarubicin A (**98**) and B (**99**) are members of anthracycline antibiotics. Anthracyclines are among the most effective chemotherapeutic agents ever developed against a number of cancer types.[122] However these compounds also inhibit bacterial growth but are not used against other infections due to their high toxicity. Ditirisarubicin A (**98**) and B (**99**) were firstly isolated by Uchida *et al*. as highly active antitumor antibiotics, which inhibited the growth of Gram-positive bacteria and prolonged the survival period of mice bearing leukaemia L-1210.[123]

4.8.3 A447 D'

The compound **100** was isolated as a dark red powder by fraction III and purified by PTLC. It showed orange fluorescence at 311 nm and blue colour with dilute NaOH as indication of *peri*-hydroxy quinone moiety.

The ^{1}H NMR spectrum exhibited three chelated hydroxyl groups appearing as broad singlets at δ 13.84, 12.72 and 12.14, respectively, and three aromatic protons as a doublet, triplet and doublet for a 1,2,3-trisubstituted aromatic system similar as in ditrisarubicins A and B. Moreover, a doublet; δ 5.30 (J = 3.4), a triplet; δ 5.02 (J = 5.3), a broad singlet (δ 4.91) and signals between δ 4.44-1.23 including three methyl doublets indicated the presence of three sugar moieties. The molecular weight of the compound was revealed by ESIMS as 753 Dalton and the molecular formula by HRESIMS as $C_{40}H_{51}NO_{13}$.

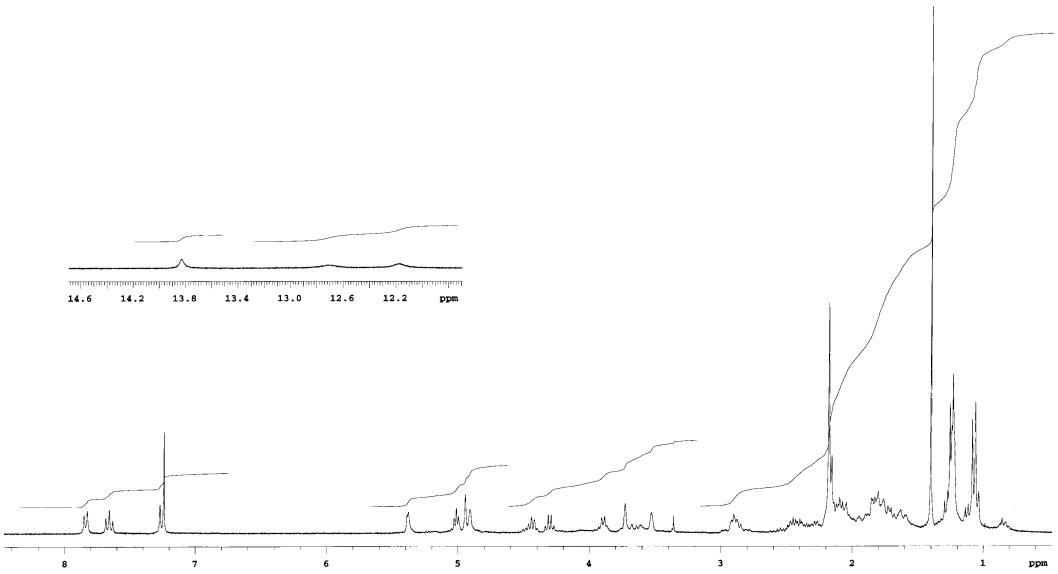


Figure 84: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of A447 D' (**100**).

The ^{13}CNMR/HSQC spectra showed 40 carbon signals including one carbonyl at δ 210.9 and two carbonyls at δ 190.7 and 185.5, clearly indicating the quinone moiety. It also showed three anomeric carbons at δ 98.9, 98.6 and 97.0 and in addition, several oxymethine signals between δ 60.2-80.0 representing three sugar moieties. Many methyl/methylene signals were also observed in the upfield region.

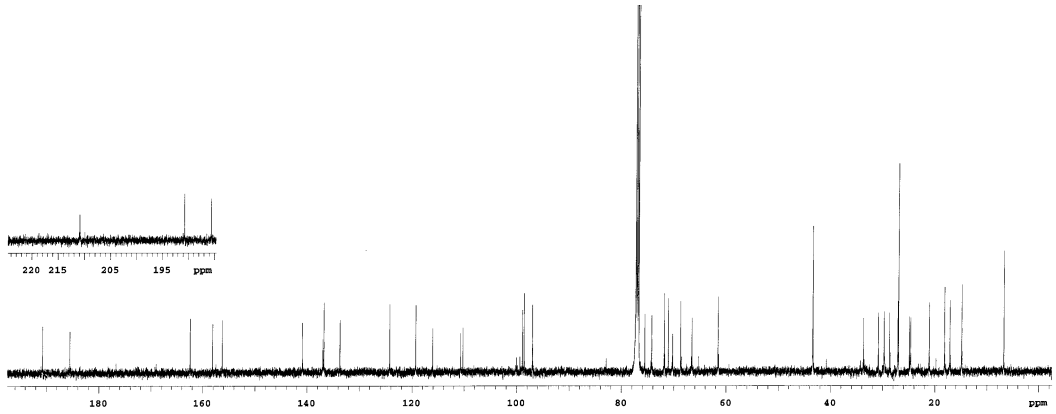
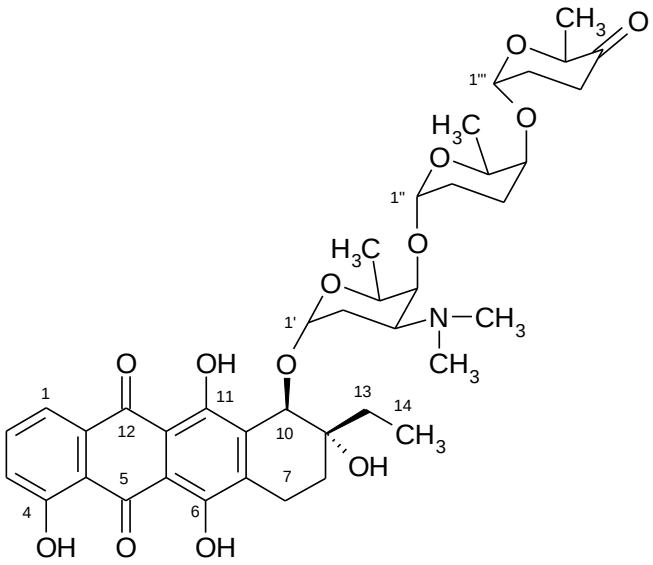


Figure 85: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of A447 D' (**100**).

The search in AntiBase with the mass peak *m/z* 754 $[M + H]^+$ resulted in 17 compounds including three *peri*-hydroxyquinones, but the NMR data matched with only one compound: A447 D' (**100**) is a hydrolysed product of the anthraquinone glycoside A447 D (**101**) isolated from the soil streptomycete strain A447.[124] The compound was finally confirmed by 2D NMR spectroscopic data and comparison with literature data (Table **16**) as well as by the fragmentation pattern of $ESIMS^2$ (

Figure 86). Compound **100** could be a natural product isolated for first time from nature now, or an artefact of A 447D (**101**), which was hydrolysed during purification.



100

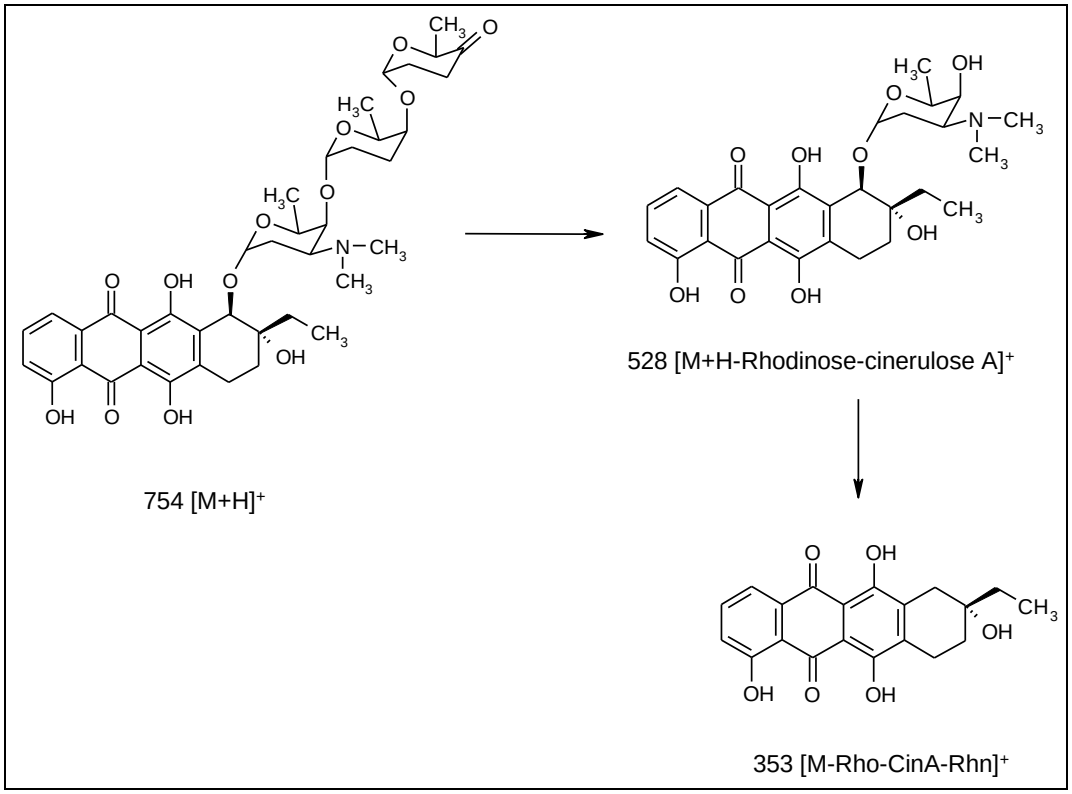
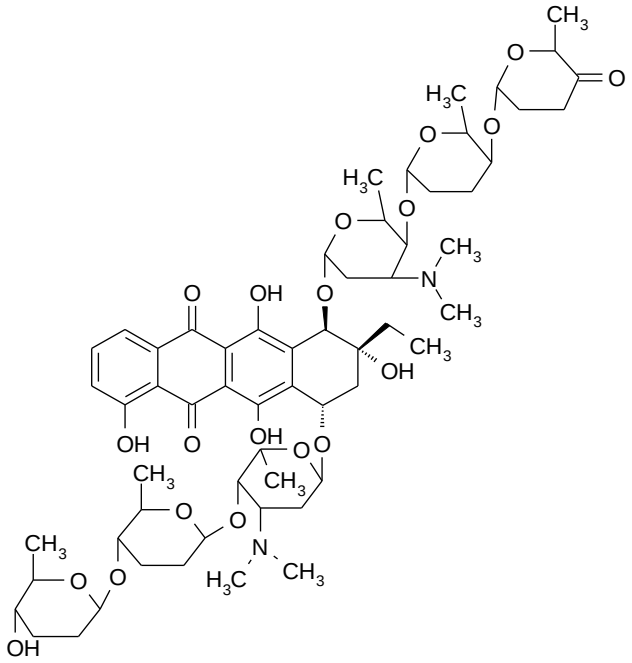


Figure 86: (+) ESI-MS-MS of A447 D' (**100**).



101

Table 16: NMR data of A447D' (**100**) in comparison with literature [124]

Position	Literature[a]		Experimental[a]	
	δ_C[b]	δ_H[c] (*J* in [Hz])	δ_C[d]	δ_H[e] (*J* in [Hz])
1	119.6	7.84 (dd, 7.2, 1.1)	119.3	7.84 (d, 7.1)
2	137.0	7.65 (dd 7.4, 7.2)	136.7	7.67 (t, 8.2)
3	124.7	7.25 (dd, 7.2, 1.1)	124.3	7.26 (d, 8.4)
4	162.6	-	162.4	-
4a	116.1	-	116.1	-
5	190.8	-	190.7	-
5a	112.0	-	110.2	-
6	157.1	-	156.3	-
6a	136.5	-	137.0	-
7	70.9	5.15 (m)	33.7	2.41 (m)
8	33.0	not assigned	30.9	1.76 (m), 1.66 (m)
9	71.8	-	71.8	-
10	70.5	5.01 (s)	70.2	4.94 (s)
10a	138.3	-	140.9	-
11	157.6	-	158.1	-
11a	111.7	-	110.7	-
12	186.0	-	185.5	-
12a	133.5	-	133.6	-
13	30.7	not assigned	29.7	1.73 (m), 1.60 (m)
14	6.6	1.10 (t, 7.4)	6.7	1.07 (t, 6.8)
1'	97.4	5.46 (d, 3.5)	97.0	5.39 (d, 3.4)
2'	29.8	not assigned	27.1	
3'	61.5	not assigned	61.6	
4'	74.3	3.73 (br s)	74.2	3.73 (br s)
5'	68.7	3.88 (q, 6.7)	68.7	3.89 (q, 6.6)
6'	18.0	1.24 (d, 6.7)	18.2	1.23 (d, 5.8)
3'-$N(CH_3)_2$	43.3	2.17 (s)	43.4	2.18 (s)
1"	98.6	4.92 (br s)	98.6	4.91 (br s)
2"	24.6	not assigned	26.9	2.01 (m)
3"	24.8	not assigned	21.1	2.90 (m)
4"	75.6	3.55 (br s)	75.6	3.53 (br s)
5"	66.5	4.44 (q, 6.3)	66.6	4.43 (q, 5.8)
6"	17.0	1.09 (d, 6.3)	17.1	1.10
1"'	98.9	5.03 (t, 5.3)	98.9	5.02 (t, 5.3)
2"'	28.6	not assigned	26.9	2.01 (m)
3"'	33.6	2.51 (ddd, 16.0, 6.7, 5.3)	28.7	2.32 (m), 2.07 (m)

4'''	211.0	-	210.9	-
5'''	71.1	4.33 (q, 6.6)	71.1	4.30 (q, 6.6)
6'''	14.8	1.27 (d, 6.6)	14.9	1.24 (d, 6.8)

[a]$CDCl_3$, [b]100 MHz, [c]500MHz, [d]125 MHz,[e]300 MHz

4.9 Terrestrial *Streptomyces* sp. GW 13/475

The terrestrial *Streptomyces* strain GW 13/475 was selected due to the interesting TLC pattern of its crude extract, which showed several UV absorbing zones, which stained to reddish brown with anisaldehyde/sulphuric acid. Moreover, during the pre-screening, the extract showed promising biological activities.

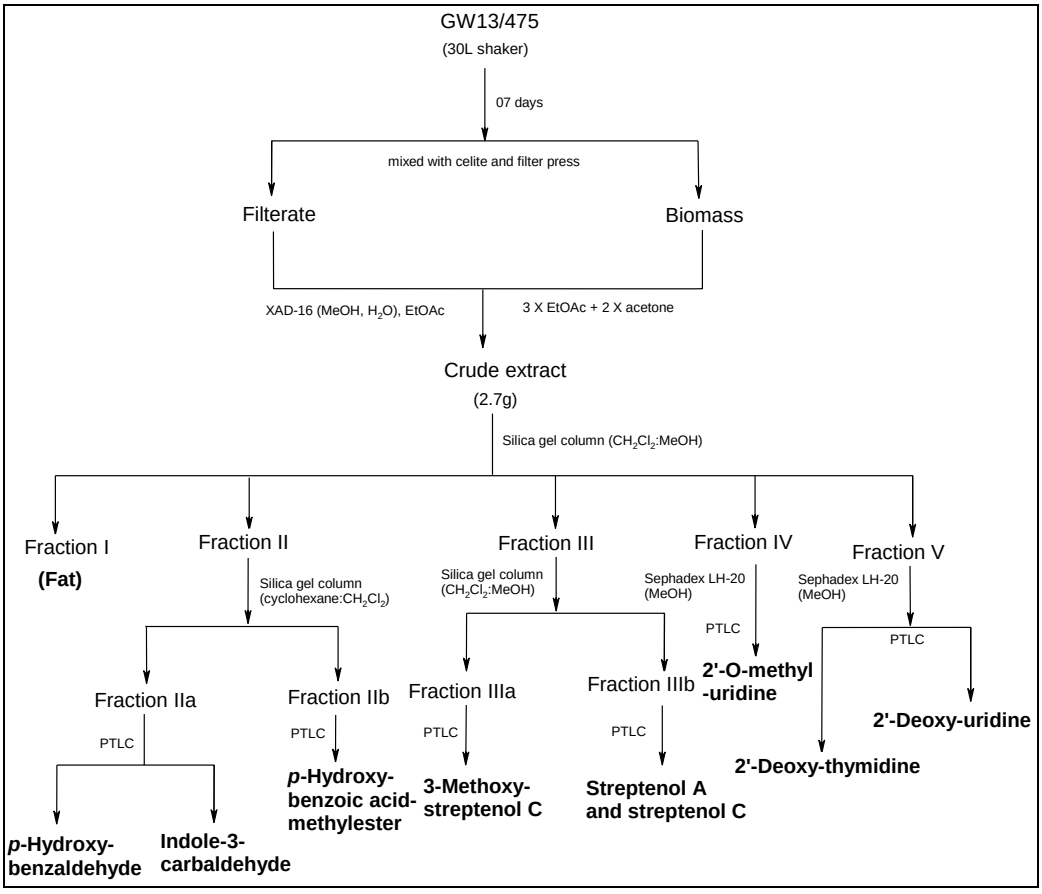


Figure 87: Work-up scheme of terrestrial *Streptomyces* strain GW 13/475

4.9.1 3-Methoxy-streptenol C

The compound **102** was isolated from fraction IIIa by PTLC as UV-absorbing, light yellow oil, which stained to dark brown with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum revealed four olefinic protons out of which two protons (δ 7.11, dd, J = 15.5, 9.9 Hz and δ 6.05, d, J = 15.6) showed *trans* coupling, and two protons

appeared at the same position as multiplet (δ 6.17). Additionally, an oxymethine, an oxymethylene and methoxy protons appeared at δ 3.92 (m), δ 3.71 (t, J = 5.7 Hz) and δ 3.31 (s) respectively. Moreover it showed an ABX pattern methylene protons at δ 2.91 and 2.61 (dd, J = 15.9, 6.0), a methyl doublet at δ 1.83 (J = 5.1) and two methylene protons at δ 1.74 (m).

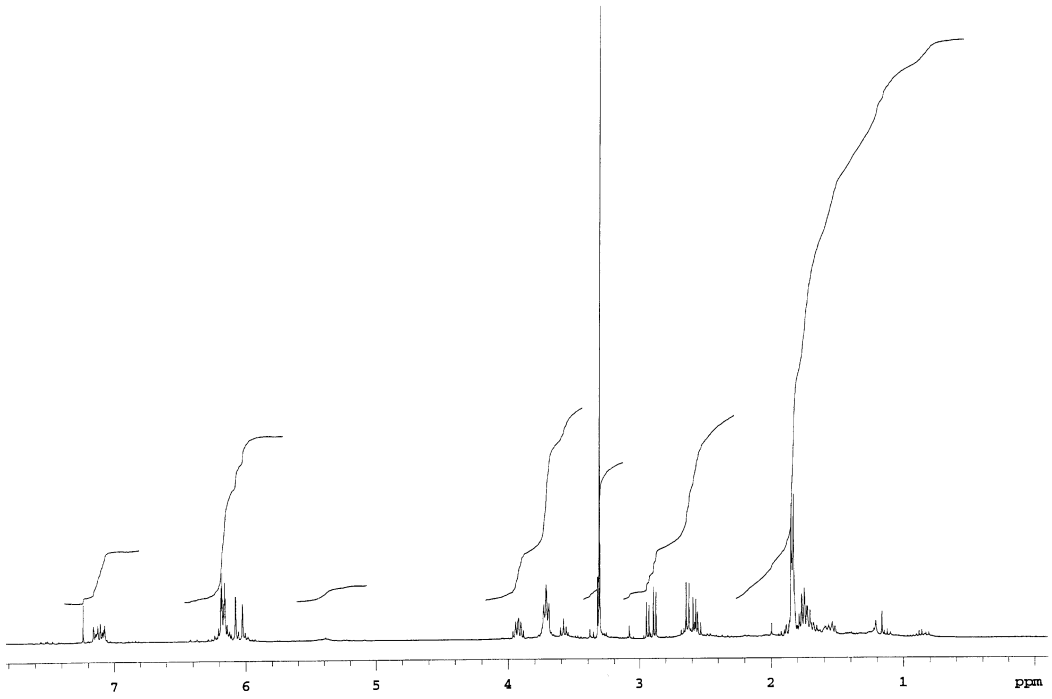


Figure 88: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-methoxy-streptenol C (**102**).

The ^{13}C/HSQC spectrum indicated 11 carbon signals including one carbonyl (δ 199.0), four olefinic carbons, an aliphatic oxymethine carbon at δ 76.7, an oxymethylene carbon at δ 59.9, a methoxy signal at δ 57.5, two methylene signals at δ 44.5 and 36.5 and a methyl carbon at δ 18.7.

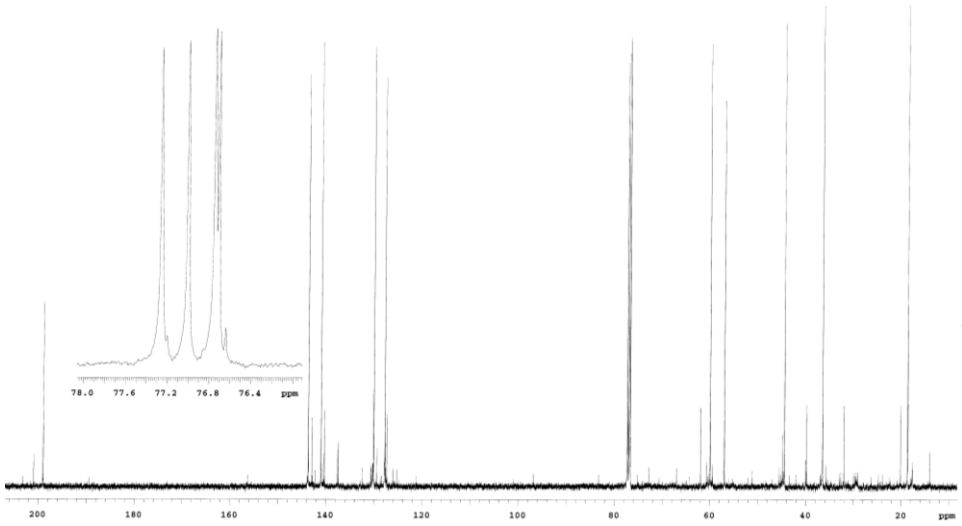
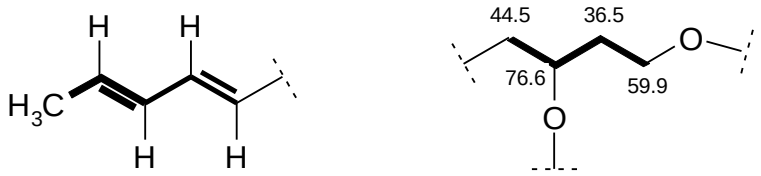


Figure 89: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 3-methoxy-streptenol C (**102**).

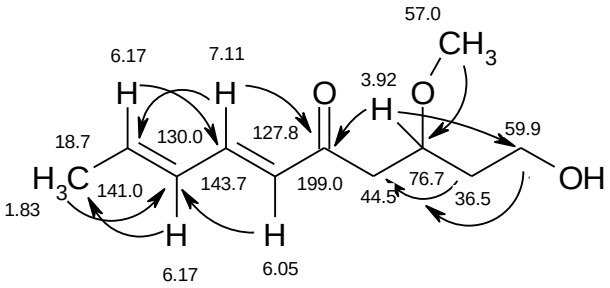
The mass of the compound **102** was determined by ESIMS as 198 Dalton and HRESIMS confirmed the molecular formula as $C_{11}H_{18}O_3$. A search in AntiBase with the above spectroscopic data provided no exact hit. So, the compound was subjected to 2D NMR measurements to confirm the complete structure. The $^1H,^1H$ COSY correlations verified the presence of two substructures **A** and **B**.

The two subunits **A** and **B** were combined by long range HMBC correlations, where the olefinic proton at δ 6.05 and methylene protons at δ 2.91 and 2.60 showed a ^2J correlation, while the olefinic proton at δ 7.11 and oxymethine proton at δ 3.92 exhibited ^3J correlation with the carbonyl at δ 199.0. On the other hand, the methoxy protons showed ^3J correlation only with the oxymethine carbon at δ 76.7 (substructure **C**). Hence the complete structure was confirmed as 3-methoxy-streptenol C (**102**), which is a new metabolite from nature.



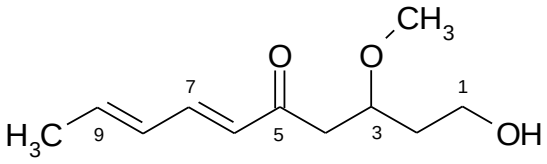
A **B**

Figure 90: H,H COSY correlations (—) of 3-methoxy-streptenol C (**102**)



C

Figure 91: HMBC correlations (→) of 3-methoxy-streptonol C (**102**).



102

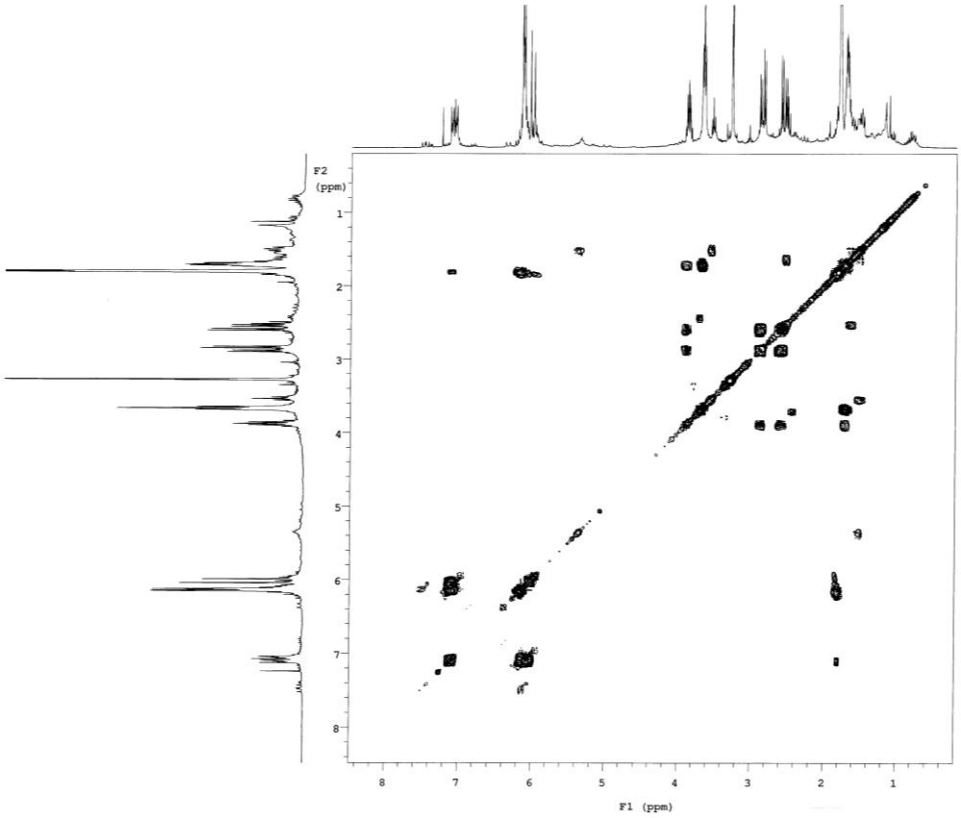


Figure 92: COSY spectrum ($CDCl_3$, 600 MHz) of 3-methoxy-streptenol C (**102**).

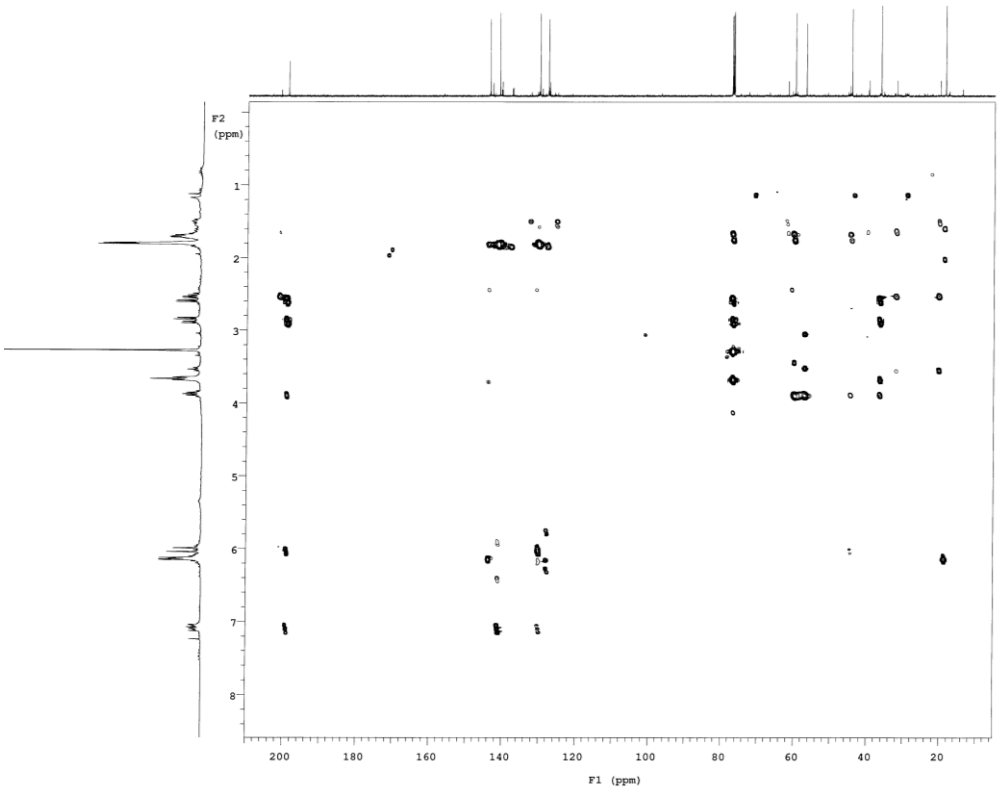


Figure 93: HMBC spectrum ($CDCl_3$, 600 MHz) of 3-methoxy-streptenol C (**102**).

4.9.2 Streptenol A and C

The non-separable mixture of streptenol A (**103**) and C (**104**) was obtained during the purification of fraction IIIb by PTLC as UV absorbing, light yellow oil, which showed dark brown colour with anisaldehyde/sulphuric acid. The ESI mass spectrum of the mixture displayed two *pseudo*-molecular ion peaks at *m/z* 207 $[M + Na]^+$ and 209 $[M + Na]^+$, corresponding to the molecular formulas $C_{10}H_{16}O_3$ and $C_{10}H_{18}O_3$ in the HRESIMS spectrum respectively. The ^{13}C NMR spectrum revealed 20 carbon signals. According to the HR-ESIMS and NMR data it was clear that it was a mixture of two compounds having the same carbon skeleton.

The ^1H NMR spectrum showed partial similarity with that of 3-methoxy-streptenol C (**102**), however, the methoxy signal at δ 3.31 was absent. In addition, it showed an olefinic proton at δ 5.37 (m), an oxymethine proton at δ 4.25 (m) and a methyl doublet at δ 1.55.

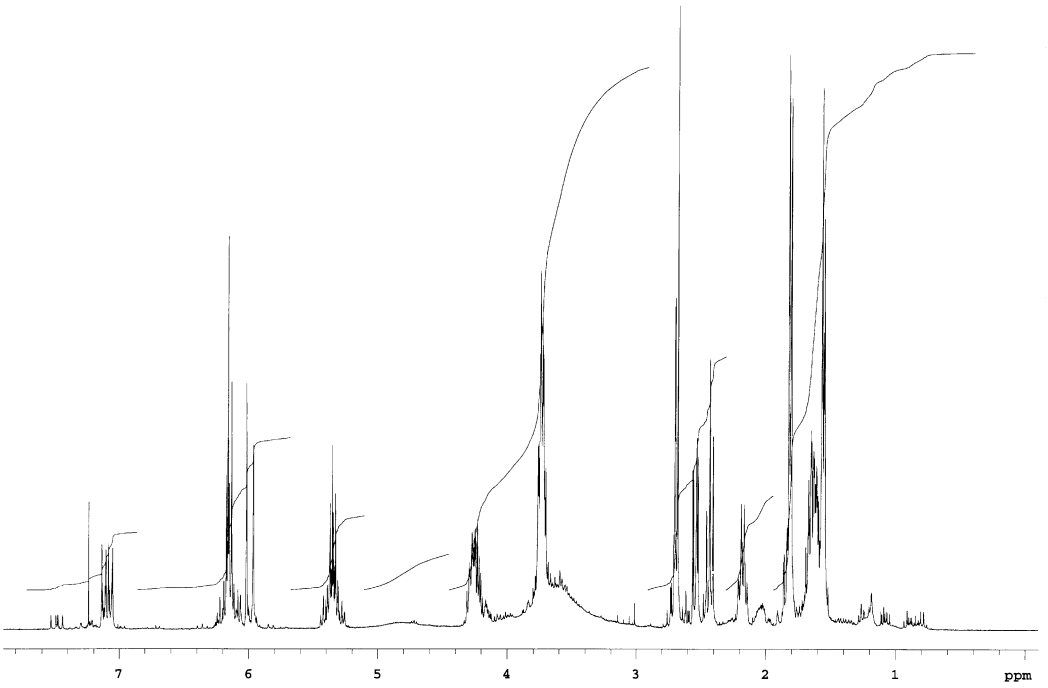


Figure 94: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of the mixture of streptenol A (**103**) and streptenol C (**104**).

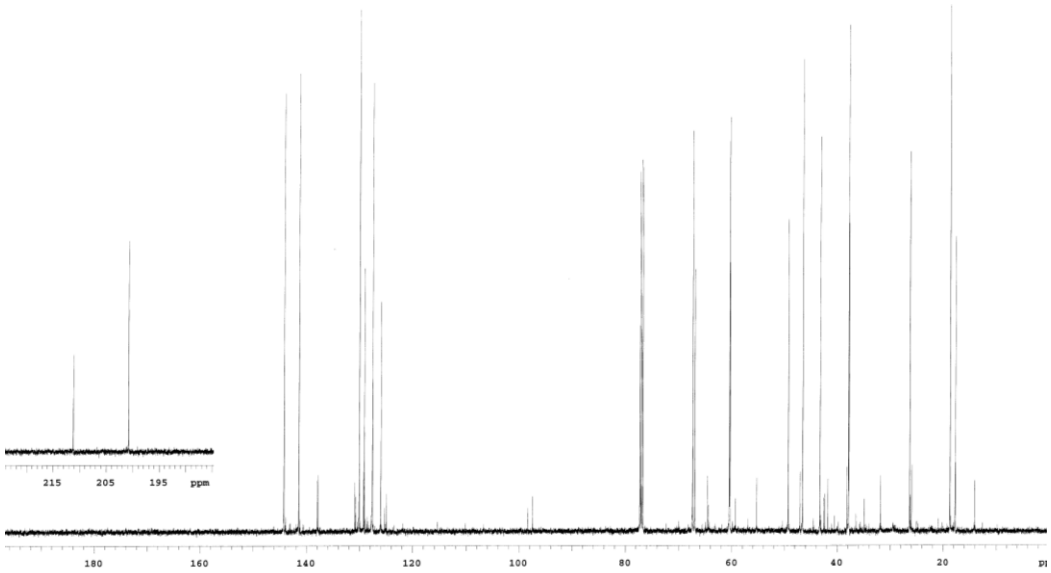
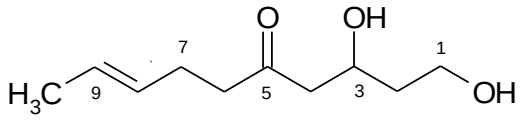
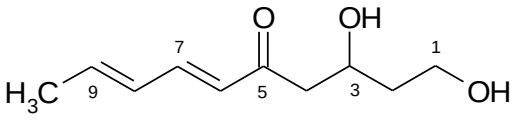


Figure 95: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of mixture of streptenol A (**103**) and streptenol C (**104**).

The search in AntiBase with both molecular formulas and NMR spectra resulted in streptenol A (**103**) and streptenol C (**104**); the sole difference between two compounds is that the former is reduced at C-6 and C-7.

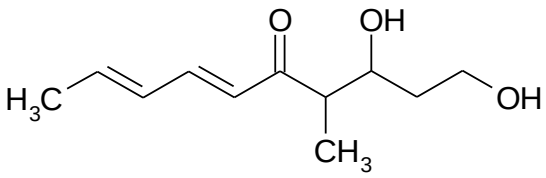


103



104

Streptenol A (**103**, (3S,8E)-1,3-dihydroxy-8-decen-5-on) was firstly obtained from the cultures of *Streptomyces fimbriatus* strain Tü 2335 [125] and later on, streptenol C (**104**, 1,3-dihydroxy-6E,8E-decadien-5-one) was isolated from another *Streptomyces* strain.[126] However, the name "Streptenol" was given to these compounds by Zeeck *et al*. who have isolated streptenol A, B, C and D from *Streptomyces luteogriseus*.[127] All streptenols have cholesterol biosynthesis inhibition activity, while streptenol A and C were reported to possess anti-tumor activity against mouse lymphoma cell line P388D.[128] Streptenol A is also reported as immunomodulatory agent.[129] Another closely related compound SS 7313A (**105**) was patented as a remedy for autoimmune diseases such as chronic rheumatoid arthritis or systemic lupus erythromatosus.[130]



105

4.10 Terrestrial *Streptomyces* sp. GW 12/459

During the pre-screening, the terrestrial *Streptomyces* isolate GW12/459 showed only a low activity against *Escherichia coli* but on TLC, the extract showed several UV absorbing bands that turned to pink/violet after spraying with anisaldehyde/sulphuric acid.

The strain was cultivated on M_2 medium in the scale of 25 L on the shaker for seven days. The brown culture broth was mixed with Celite and filtered through a filter press. While the water phase was passed through Amberlite XAD-16 column and eluted with methanol, the mycelium phase was extracted with ethyl acetate and acetone and then brought to dryness under reduced pressure. The crude extract was applied to silica gel column with a gradient system of dichloromethane/methanol,

which afforded three fractions. The obtained fractions were further purified by different chromatographic techniques to get the pure metabolites.

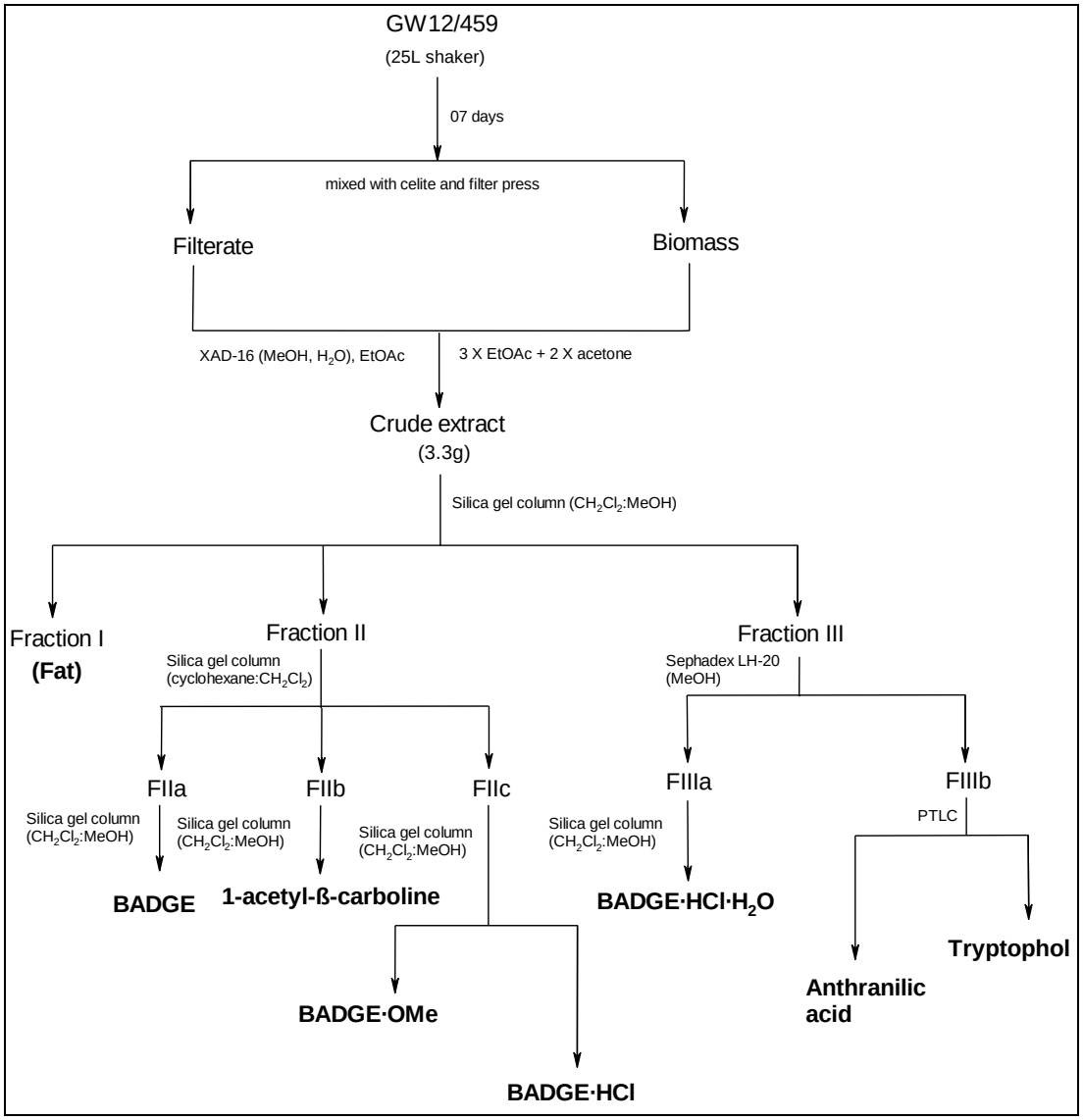


Figure 96: Work up scheme of terrestrial *Streptomyces* sp. GW12/459

4.10.1 Bisphenol A diglycidyl ether (BADGE)

The compound **107** was obtained as low polar, UV absorbing yellow oil from fraction IIa, which turned to dark pink with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **107** showed two doublets at δ 7.12 (J = 8.8 Hz), 6.80 (J = 8.8 Hz) suggesting the presence of a *p*-substituted aromatic ring. It also showed two doublets of doublets at δ 4.16 (J = 11.0, 3.3 Hz) and 3.92 (J = 11.0, 5.6 Hz), a multiplet at δ

3.32 along with a triplet at δ 2.87 (J = 4.9) and a doublet of doublet at δ 2.72 (J = 4.9, 2.6 Hz). A singlet at δ 1.61 for a methyl group was also visible.

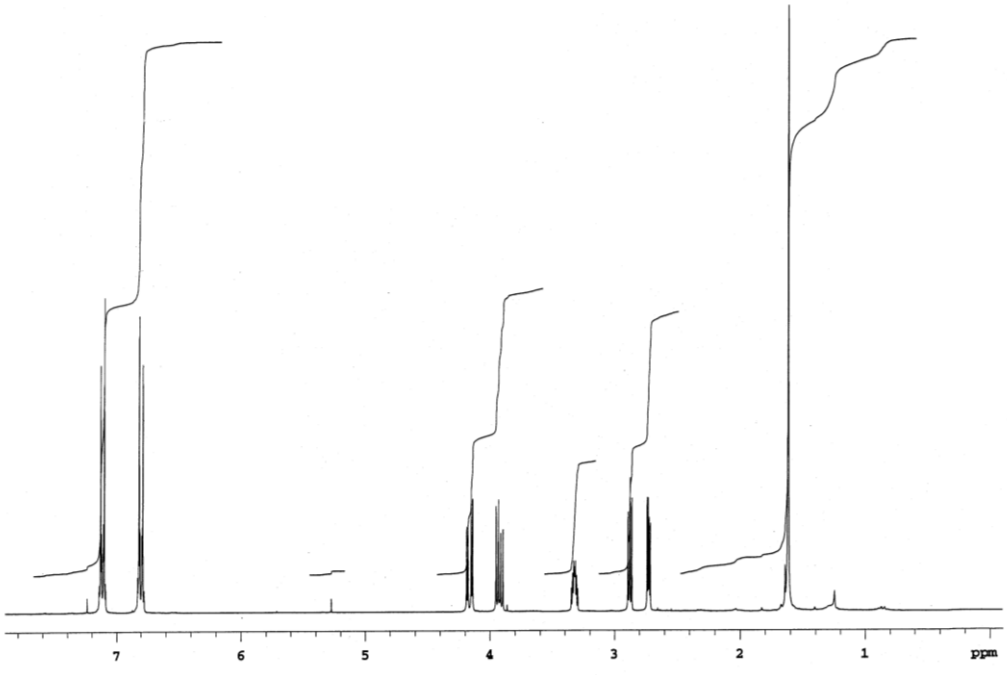


Figure 97: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of bisphenol A diglycidyl ether (**107**).

The ^{13}C NMR/HSQC spectrum of **107** disclosed nine carbon signals corroborating the presence of one methyl (δ 31.0), two methylene (δ 44.6 and 68.7), three methine (δ 127.6, 113.8 and 50.1) and three quaternary (δ 156.1, 143.4 and 41.6) carbon atoms. The molecular formula $C_{21}H_{24}O_4$ was established through the data of HRESIMS. The conflict between molecular mass (340 amu) and ^{13}C NMR resonances (nine carbon signals in the spectrum) supported the symmetrical dimeric nature of the molecule. The search in AntiBase with the help of above spectroscopic data gave no exact hit.

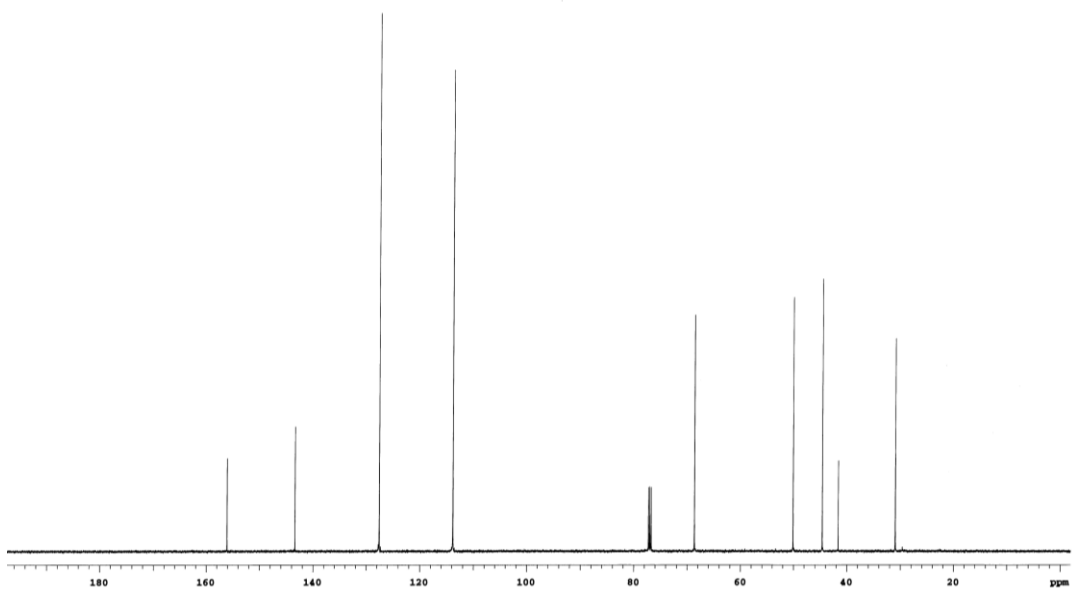


Figure 98: ^{13}C NMR spectrum ($CDCl_3$, 300 MHz) of bisphenol A diglycidyl ether (**107**).

The molecule was finally constructed with the help of HMBC and COSY correlations. In HMBC, the proton appearing at δ 1.61 showed a ^2J correlation with δ 41.6 and ^3J correlations with δ 143.4 and 31.0 confirmed the presence of an isopropyl group between two aryl rings. The presence of a *p*-substituted aryl ring on one side with oxymethylene was confirmed through HMBC correlations, in which oxymethylene protons (δ 4.16, 3.92) showed ^3J correlation with δ 156.1. The remaining connections were established with the help of COSY correlations, in which the protons at δ 4.16, 3.92 showed cross peak with δ 3.32, with an extended correlation of δ 3.32 with δ 2.87 and 2.72. Because of the lower ^{13}C shift of methine (δ 50.1) and methylene (δ 44.6), the presence of terminal glycerol moiety was excluded. Additionally, a 1,4-dioxane ring was also constructed (compound **106** and considered that the unusual resonances of both methylene and methine were due to the placement of the dioxane moiety into the shielded zone of both aryl rings. A search of the related compounds in the Chemical Abstracts resulted to be the compound as bisphenol A diglycidyl ether (**107**) abbreviated as BADGE, which contains two epoxy rings; all the spectral data of the compound were confirmed by comparison with the literature.

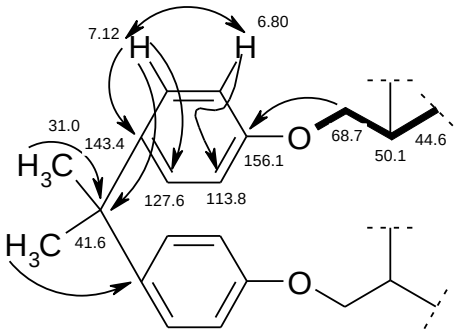
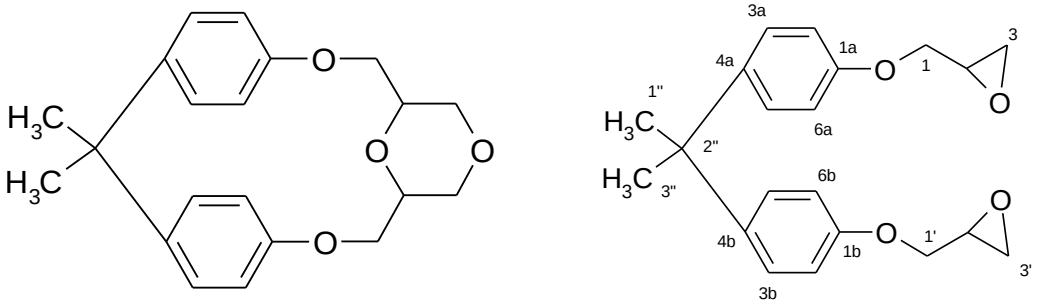


Figure 99: COSY and HMBC correlations of bisphenol A diglycidyl ether (**107**).



106 **107**

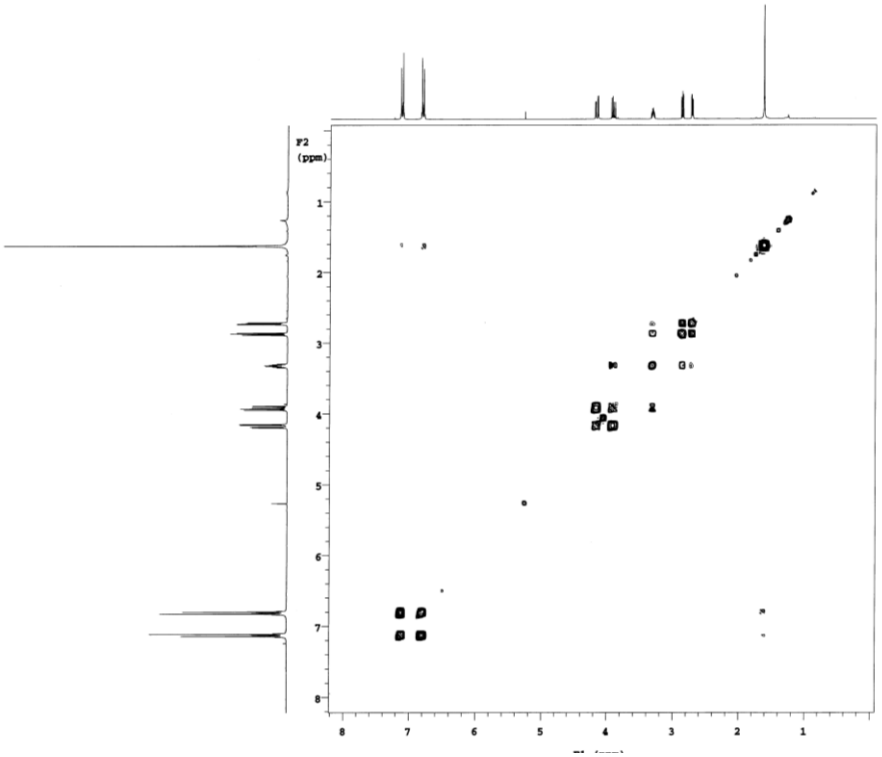
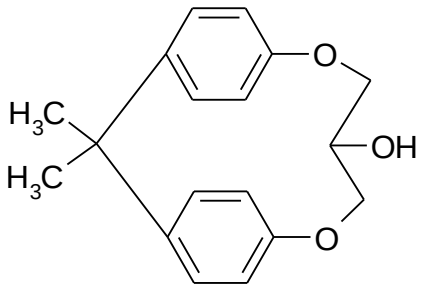


Figure 100: COSY spectrum ($CDCl_3$, 600 MHz) of bisphenol A diglycidyl ether (**107**).

Glycidyl ethers are basic and important components of epoxy resins and bisphenol A diglycidyl ether (BADGE) (**107**) and its polymers are used as cross linking agents in commercially available epoxy resins. These epoxy resins are used in the coating of food cans, in reinforced plastic laminates and as bonding and adhesive materials. In the mild acidic conditions, BADGE can easily be hydrolysed. Due to their lipophilic nature, BADGE and its hydrolysed products have tendency to migrate from coating of food cans into the fat containing food stuffs.[131] The food contaminant BADGE and its derivatives were frequently tested for their mutagenicity. The reports showed mutagenic potential of BADGE and its hydrolysed products in mice.[132, 133] In contrast, BADGE is also reported to induce peroxisome proliferator activated receptors (PPARγ) activation, nuclear localization of the receptor and induces cell death by apoptosis.[134]

The natural occurrence of BADGE (**107**) was not yet reported. As the technical product is racemic, it is surprising, that we observed a positive optical rotation: An abiotic origin cannot be excluded therefore, although also an enzymatic degradation of **107** is plausible (kinetic resolution). A structurally related compound to BADGE, 1,3,di-O-[2',2'-di-(*p*-phenylene)isopropylidene] glycerol (**108**) was reported from a brown algae *Sargassum pervivesiculosum*.[135] However, as compound **107** could not be isolated from other bacterial strains or reproduced by fermentation of the same strain again, considering compound **107** as a bacterial metabolite or fermentation contaminant is still to be verified by biosynthetic studies.



108

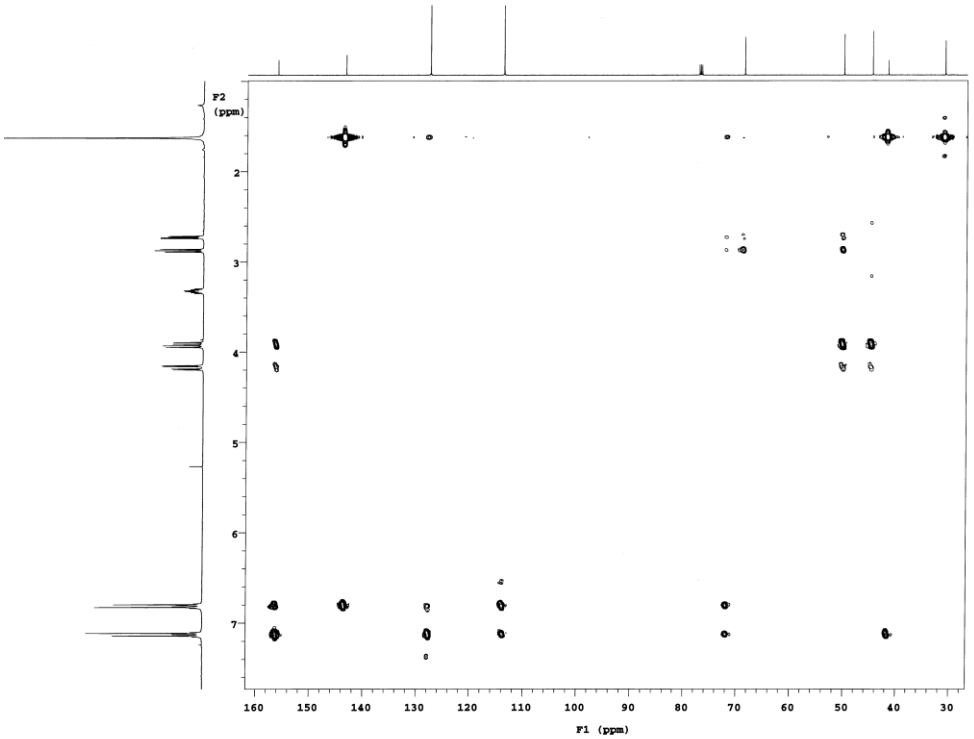


Figure 101: HMBC spectrum ($CDCl_3$, 600 MHz) of bisphenol A diglycidyl ether (**107**).

4.10.2 3-Methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]- 2-propanol (BADGE·OMe)

The compound **109** was isolated as middle polar, UV absorbing yellow oil from fraction IIc and purified by silica gel column chromatography. The molecular formula $C_{22}H_{28}O_5$ was deduced by HRESIMS. The ^{1}H NMR spectrum of **109** showed two doublets at δ 7.11 and 6.79 (J = 8.9 Hz) indicating a *p*-substituted aryl ring similar as in **107**. It also exhibited the presence of four oxymethylenes between δ ~ 4.18-2.72, and two oxymethines at δ 3.90 and 3.32 as multiplets. A singlet at δ 3.38 indicating the presence of a methoxy group and another singlet at δ 1.61 like that of **107** were also visible. The ^{13}C NMR/HSQC spectrum of **109** showed 17 carbon signals for two methyl (δ 59.2 and 31.0), four methylenes (δ 73.5, 69.0, 68.7 and 44.8), six methine and five quaternary carbons. Comparing the spectral data with compound **107** indicated that one epoxy ring of compound **107** was opened by the nucleophilic attack of MeOH resulting in an increase of 32 amu in the mass which was also confirmed by the presence of a singlet at δ 3.38 in ^{1}H NMR and δ 59.2 in ^{13}C NMR spectra respectively and the compound became no more symmetrical. This hypothesis was finally confirmed through 2D NMR measurements. The position of the meth-

oxy group was confirmed by HMBC correlations, in which the proton appearing at δ 3.38 showed correlation with δ 73.5, but on the other side the epoxy ring remained the same. The compound **109** is also known as hydrolysed product of BADGE (**107**).

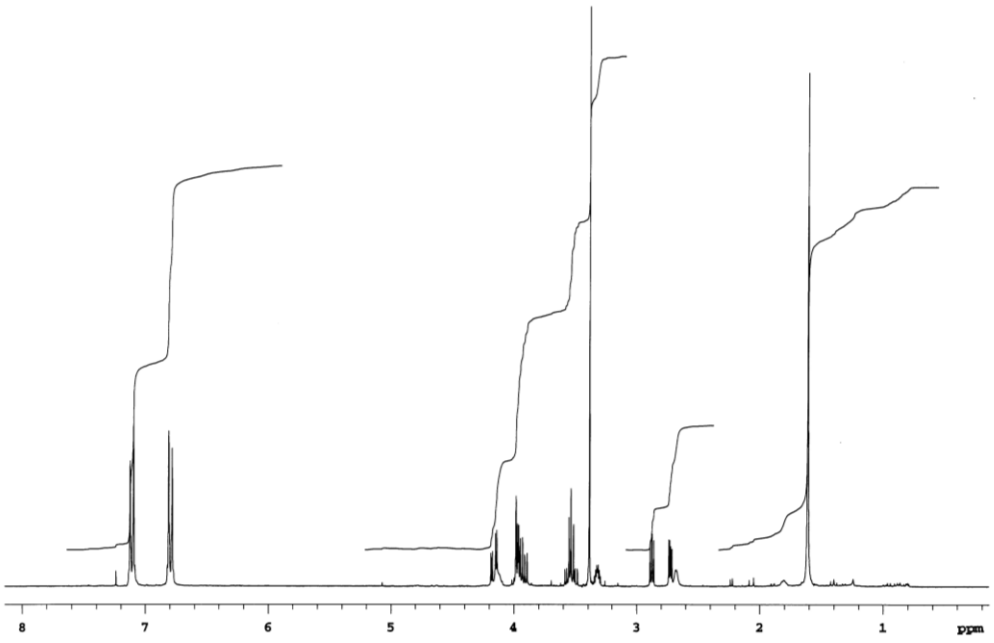


Figure 102: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]-2-propanol (BADGE·OMe) (**109**).

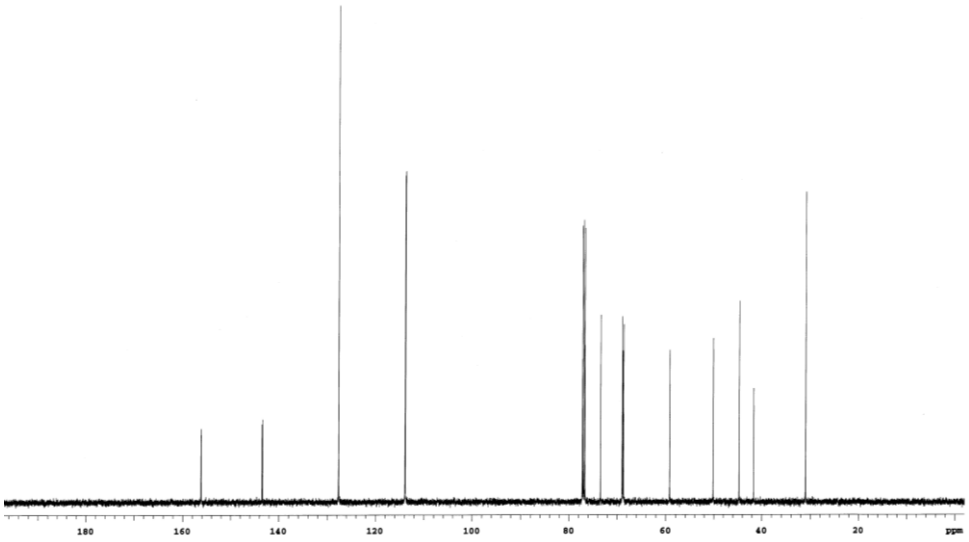


Figure 103: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 3-methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]-2-propanol (BADGE·OMe) (**109**).

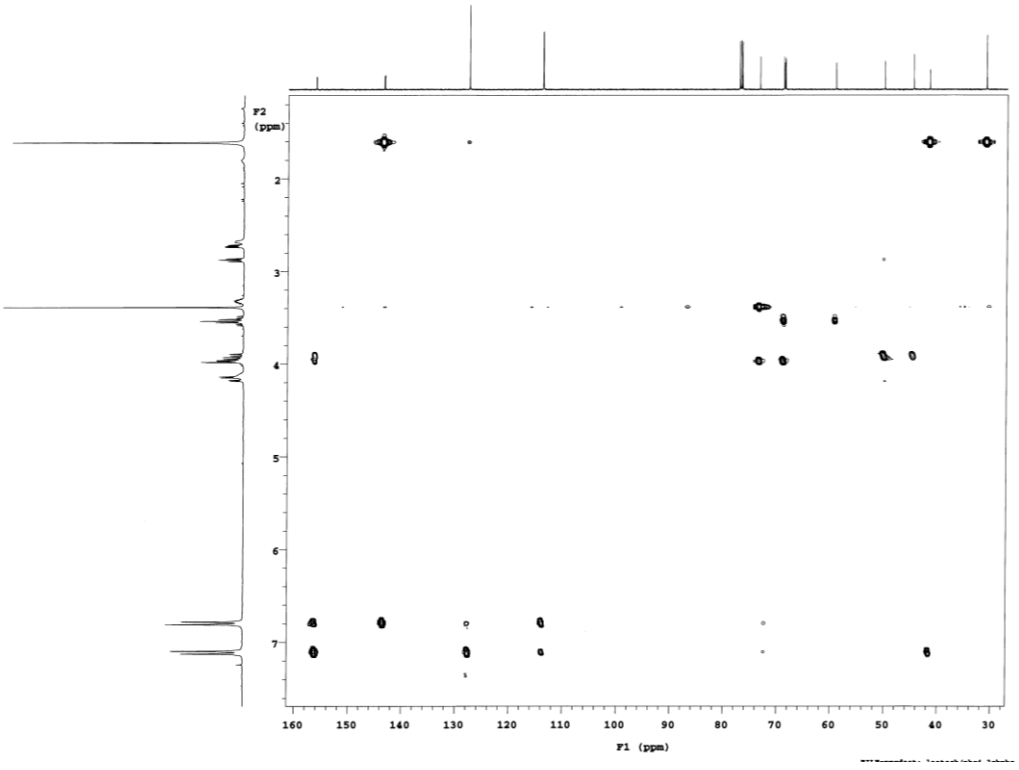
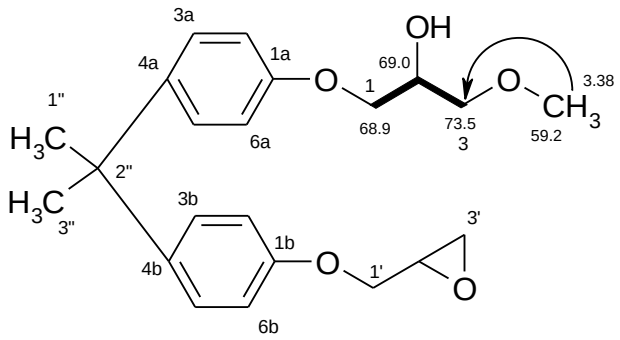


Figure 104: HMBC spectrum ($CDCl_3$, 600 MHz) of 3-methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]-2-propanol (BADGE·OMe) (**109**).



109

Table 17: ^{1}H and ^{13}C NMR data, of compounds **107** and **109** ($CDCl_3$).

Position	Bisphenol A diglycidyl ether (**107**)		3-methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]- 2-propanol (BADGE·OMe) (**109**)	
	δ_C [a]	δ_H [b] (*J* in [Hz])	δ_C [a]	δ_H [b] (*J* in [Hz])
1	68.7	4.16 (ABX, 11.0, 3.3)	68.9	4.18 (dd, 11.0, 3.3)
		3.92 (ABX, 11.0, 5.6)		3.97 (dd,11.0, 5.6)
2	50.1	3.32 (m)	69.0	4.12 (m)
3	44.6	2.87 (ABX, 4.9, 4.9)	73.5	3.54 (m)
		2.72 (ABX, 4.9, 2.6)		
1a	156.1	-	156.2	-
2a, 6a	113.8	6.80 (d; 8.8)	113.9	6.79 (d, 8.9)
3a, 5a	127.6	7.12 d (8.8)	127.63	7.11 (d, 8.9)
4a	143.4	-	143.5	-
1'	68.7	4.16 (dd, 11.0, 3.3)	68.7	3.95 (dd, 11.0, 3.3)
		3.92 (dd, 11.0, 5.6)		3.92 (dd, 11.0, 5.6)
2'	50.1	3.32 (m)	50.2	3.32 (m)
3'	44.6	2.87 (t, 4.9)	44.8	2.88 (t, 4.9)
		2.72 (dd, 4.9, 2.6)		2.78 (dd, 4.9, 2.6)
1b	156.1	-	156.1	-
2b, 6b	113.8	6.80 (d, 8.8)	113.8	6.79 (d, 8.9)
3b, 5b	127.6	7.12 (d, 8.8)	127.62	7.11 (d, 8.9)
4b	143.4	-	143.4	-
1",3"	31.0	1.61 (s)	31.0	1.61 (s)
2"	41.6	-	41.7	-
3-OCH_3	-	-	59.2	3.38 (s)

[a]125 MHz, [b]300 MHz

4.10.3 3-Chloro-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]-ethyl]phenoxy]- 2-propanol (BADGE·HCl)

Compound **110** was obtained as colourless oil from fraction III. It was UV absorbing at 254 nm and showed dark pink colour with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of **110** was very similar to that of **109** showing two doublets at δ 7.11 and 6.79 (J = 8.7 Hz), sp^3-oxymethylenes at δ 4.16 (dd, J = 11.0, 3.4 Hz), 4.04 (m), 3.92 (dd, J = 11.0, 5.6 Hz), 3.72 (m), 2.87 (t, J = 4.9 Hz) and 2.72 (dd, J = 4.9, 2.6 Hz) and oxymethines at δ 4.14 (m), δ 3.32 (m) and a singlet at δ 1.61. The molecular formula $C_{21}H_{25}O_4Cl$ was deduced by HRESIMS.

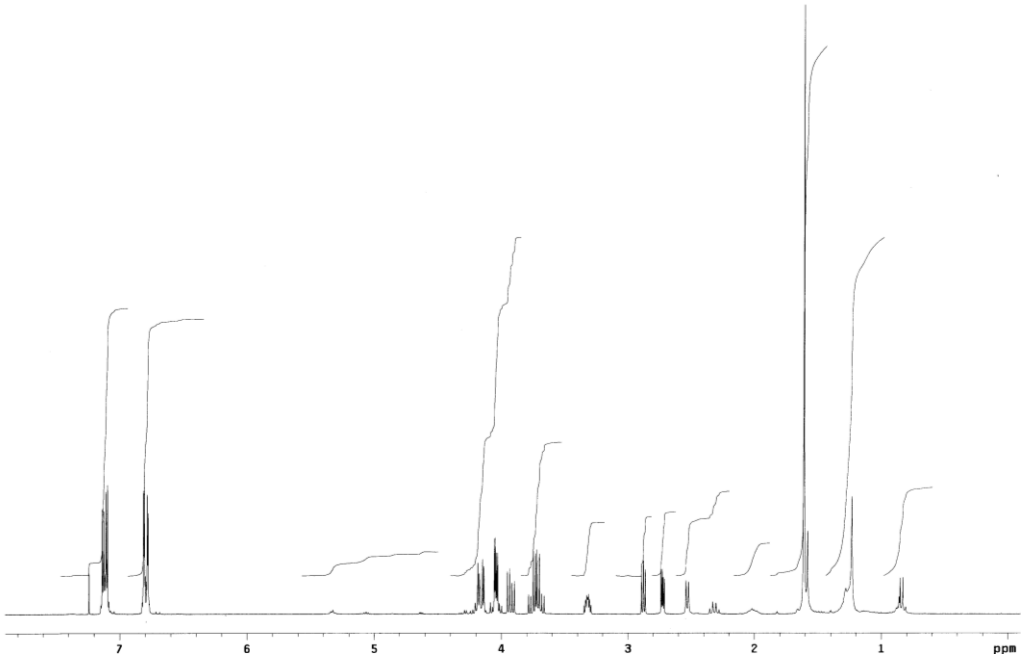
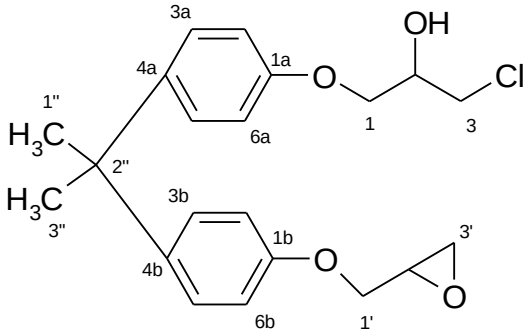


Figure 105: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-chloro-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]-ethyl]phenoxy]-2-propanol (BADGE·HCl) (**110**).

The missing of methoxy signal in ^{1}H NMR spectrum and the appearance of chlorine by HRESIMS spectrum indicated that the methoxy group of **109** was now replaced by chlorine. The ^{13}C NMR/HMQC spectra of compound **110** (Table 18) disclosed sixteen carbon signals for one methyl, four methylene, six methine and five quaternary carbon atoms. The ^{13}C NMR confirmed the missing methoxy signals and the appearance of a methylene signal at δ 44.8 indicated the opening of epoxy ring

of **107** now through chlorine. The structure was finally derived by HMBC and COSY correlations.



110

4.10.4 3-[4-[1-[4-(3-Chloro-2-hydroxypropoxy)phenyl]-1-methylethyl]phenoxy]-1,2-propanediol (BADGE·HCl·H_2O)

The compound **111** was obtained as polar, UV absorbing colourless oil from fraction IIc. The aromatic region of ^{1}H NMR spectrum of **111** showed two doublets at δ 7.11 and 6.79 (J = 8.8 Hz) for a *p*-substituted aryl ring like in **107 - 110**. It showed several multiplets between δ 4.17-3.70 indicating the presence of oxymethylenes and oxymethines in the molecule. A methyl singlet appeared at δ 1.61. The ^{13}C NMR/HMQC spectra of **111** disclosed 16 carbon signals for one methyl (δ 31.1), four methylene, six methine and five quaternary carbon atoms.

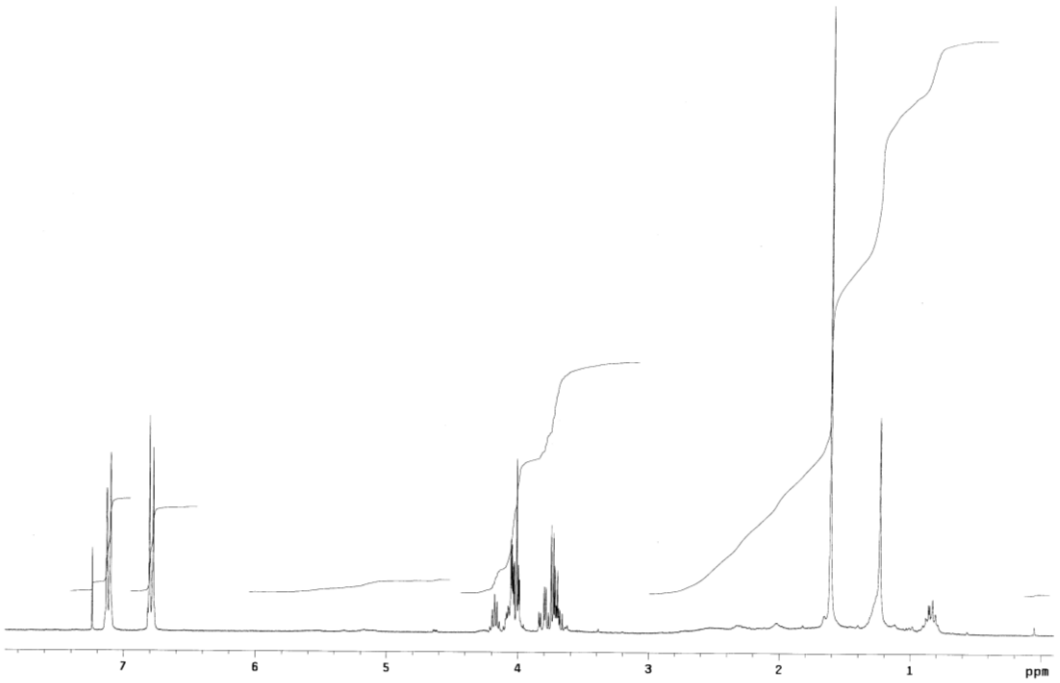


Figure 106: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-[4-[1-[4-(3-chloro-2-hydroxypropoxy)phenyl]-1-methylethyl]phenoxy]-1,2-propanediol (BADGE·HCl·H_2O) (**111**).

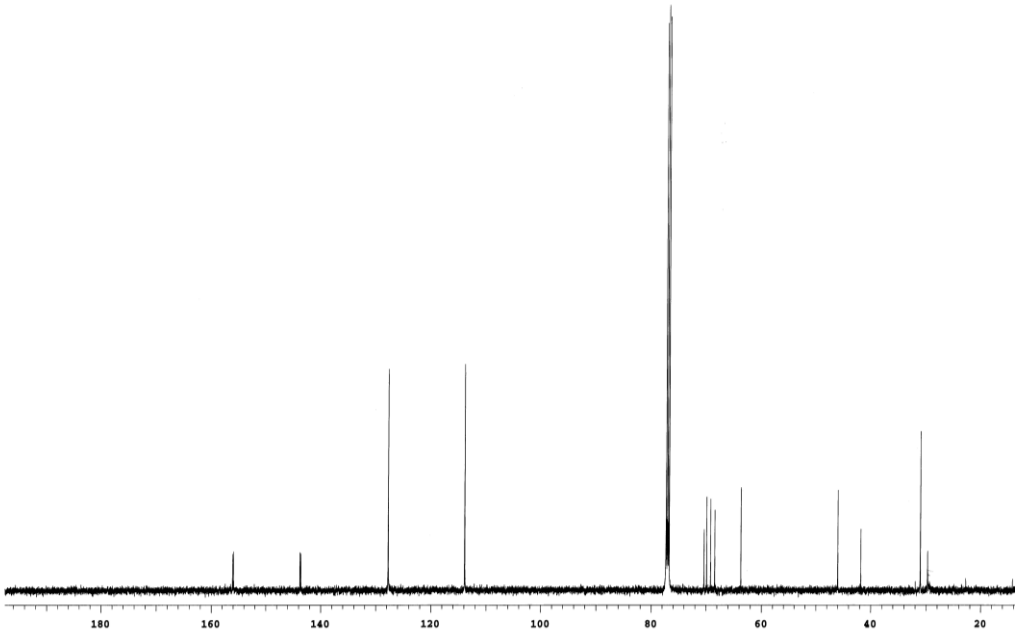
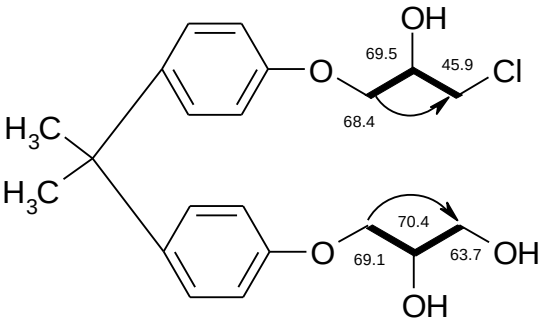


Figure 107: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 3-[4-[1-[4-(3-chloro-2-hydroxypropoxy)phenyl]-1-methylethyl]phenoxy]-1,2-propanediol (BADGE·HCl·H_2O) (**111**).

Comparing the above data (mass, ^{1}H and ^{13}C NMR) with those of **107**, **109** and **110** indicated the opening of both epoxy rings in the form of two free glycerol units out of which one contains chlorine at its terminal.



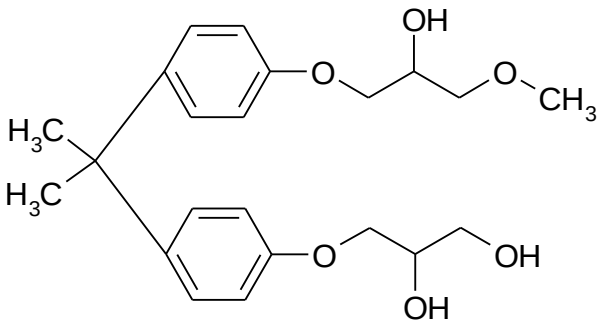
111

Table 18: ^{1}H and ^{13}C NMR data of compounds **110** and **111** in $CDCl_3$

Position	1-chloro-3-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]-ethyl]phenoxy]- 2-propanol (BADGE·HCl) (**110**)		3-[4-[1-[4-(3-chloro-2-hydroxypropoxy)phenyl]-1-methylethyl]phenoxy]-1,2-propanediol (BADGE·HCl·H_2O) (**111**)	
	δ_C [a]	δ_H [b] (*J* in [Hz])	δ_C [a]	δ_H [b] (*J* in [Hz])
1	68.4	4.04 (m)	68.4	4.01 (m)
2	69.9	4.16 (m)	69.9	4.17 (m)
3	46.0	3.76 (ABX, 11.2, 5.2) 3.68 (ABX, 11.2, 5.6)	45.9	3.70 (m)
1a	156.2 [c]	-	155.9	-
2a, 6a	114.0 [c]	6.79 (d, 8.7)	113.8	6.79 (d, 8.8)
3a, 5a	127.8[c]	7.11 (d, 8.7)	127.7	7.11 (d, 8.8)
4a	143.8 [c]	-	143.6	-
1'	68.7	4.14 (dd, 11.0, 3.4) 3.92 (dd, 11.0, 5.6)	69.2	3.99 (m)
2'	50.2	3.32 (m)	70.4	4.04 (m)
3'	44.0	2.87 (t, 4.9) 2.72 (dd, 4.9, 2.6)	63.7	3.81 (dd, 11.3, 3.7) 3.75 (dd,11.3, 5.2)
1b	155.9 [c]	-	156.1	-
2b, 6b	113.9 [c]	6.79 (d, 8.7)	113.9	6.79 (d, 8.8)
3b, 5b	127.7 [c]	7.11 (d, 8.7)	127.8	7.11 (d, 8.8)
4b	143.5 [c]	-	143.8	-
1",3"	31.1	1.61 (s)	31.1	1.61 (s)
2"	41.8	-	41.8	-

[a]125 MHz, [b]300 MHz, [c] values may be exchanged pairwise

Compound **111** is also obtained as hydrolysis product of BADGE (**107**) in presence of HCl. However, recently, it was also reported from extracts of a marine sponge and along with other synthetic BADGE derivatives were also reported as modulators of androgen receptor activity in mammalian tissues inhibiting the *in vivo* and *in vitro* tumour growth either in the presence or absence of androgens. So these compounds can be used in the study of indications or as the treatment of prostate cancer, breast cancer, ovarian cancer, endometrial cancer, hair loss, acne, hirsutism, ovarian cysts and age-related macula degeneration.[136] Another closely related compound (**112**) was also recently published as new natural product from *Streptomyces platensis*.[137]



112

4.11 Terrestrial *Streptomyces* sp. GW 8594

The crude extract of the terrestrial *Streptomyces* strain GW 8594 showed moderate activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. It also showed activity against *Mucor miehei*, *Candida albicans*, *Aphanomyces cochlioides* and *Pythium ultimum* and was also active against the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*. Moreover, it exhibited 100 % cytotoxicity against *Artemia salina*.

Table 19: Antimicrobial activity of the crude extract of the strain GW 8594

Test microorganisms	Inhibition zone ∅ [mm]
Bacillus subtilis	12
Staphylococcus aureus	12
Escherichia coli	10
Candida albicans	14
Mucor miehei	15
Aphanomyces cochlioides	13
Pythium ultimum	12
Chlorella vulgaris	10
Chlorella sorokiniana	9
Scenedesmus subspicatus	10

The fermentation of the isolate GW 8594 on a 30 L scale using M_2 medium for six days on a shaker culture resulted in a brown culture broth. Filtration and extraction delivered 2.6g crude extract which was chromatographed on silica gel and the obtained fractions were further purified by various methods to get pure metabolites.

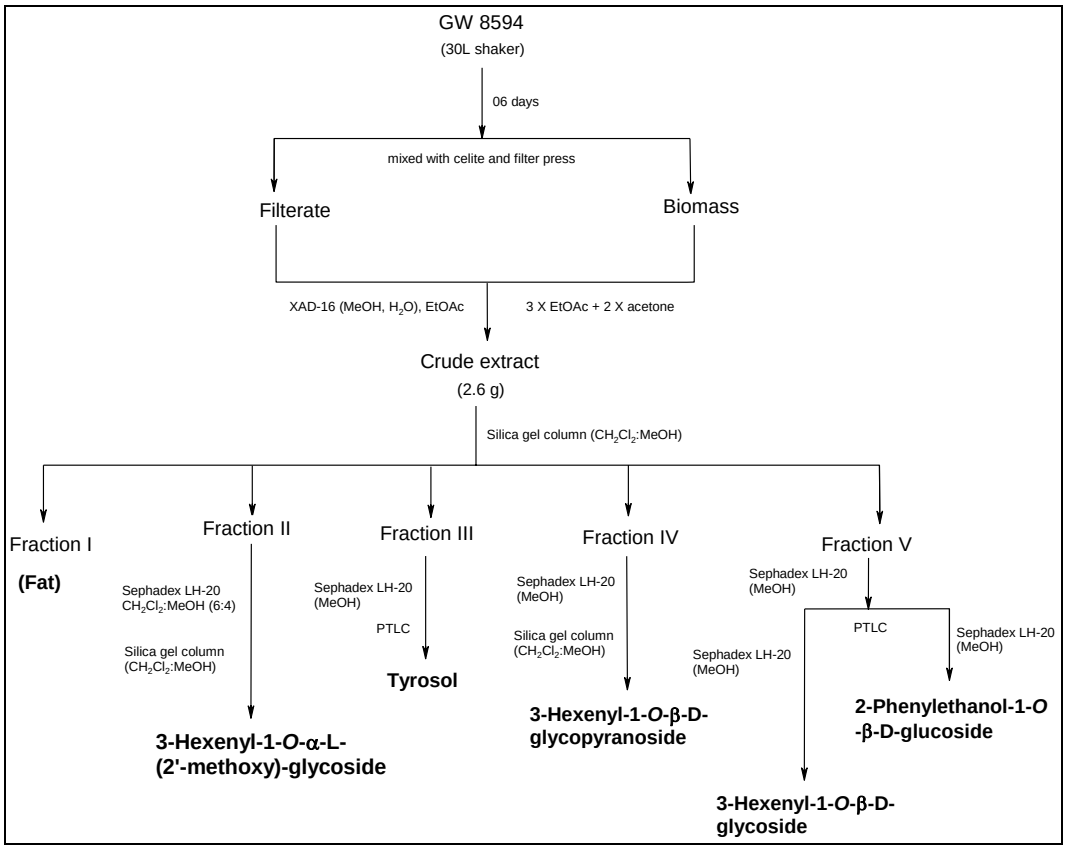


Figure 108: Work up scheme of terrestrial *Streptomyces* sp. GW 8594

4.11.1 3-Hexenyl-1-*O*-*β*-D-glycopyranoside

The compound **113** was isolated from fraction IV as a non-UV absorbing light yellow oil, which turned to green with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum revealed two olefinic protons at δ 5.43 and δ 5.29 as multiplet. Moreover, four sp^3oxygenated methine protons were observed (δ 3.83-3.16) along with one anomeric proton at δ 4.22 (d, J = 7.6 Hz) representing the presence of a sugar moiety. Additionally, in the aliphatic region, it also exhibited an oxygenated methylene, two sp^3-linked methylene groups together with two methyl groups as doublet and triplet at δ 1.28 and δ 0.94, respectively.

The ^{13}C/HSQC experiments indicated the presence of 12 signals including two olefinic carbons at δ 134.1 and 124.1, five oxygenated methines, three methylene carbons being one oxygenated (δ 69.5) and two methyl carbons at δ 17.6 and δ 14.2.

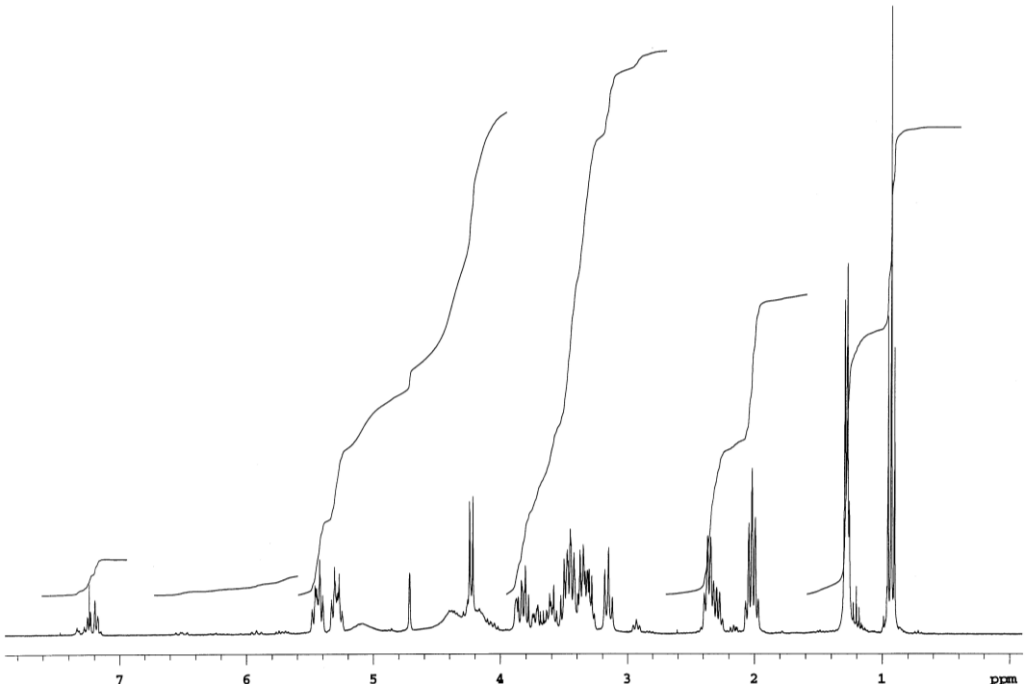


Figure 109: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-hexenyl-1-*O*-β-D-glycopyranoside (**113**).

ESIMS delivered the mass of compound **113** as 246 Dalton and the corresponding molecular formula was obtained from HRESIMS as $C_{12}H_{22}O_5$. A search with the help of above spectroscopic data in different databases such as AntiBase, DNP and the Chemical Abstracts did not give an exact hit confirming the compound **113** as a new metabolite. The 3-hexenol moiety and a 6-deoxyhexose were drawn based on the H,H COSY and HMBC correlations (Figure 110). Moreover, a strong ^{3}J correlation was observed between anomeric proton and methylene carbon at δ 69.5 clearly indicating the attachment of hexose sugar at C-1. Hence the compound was identified as 3-hexenyl-1-*O*-β-D-glycopyranoside (**113**), which is a new natural product. The large coupling constant (J = 7.6 Hz) of the anomeric proton (δ 4.22) pointed to a sugar with β configuration. According to the Klyne rule,[84] the β-configuration indicated a D-sugar. However, the sugar moiety could not be further confirmed after hydrolysis and silylation by comparison with standard sugars as well as by comparison of NMR data with literature.

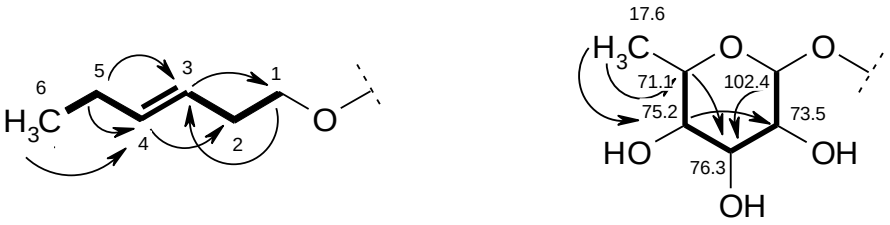
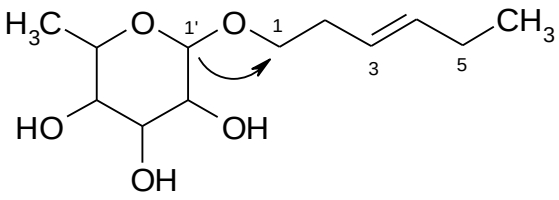


Figure 110: HMBC (→), and H, H COSY (▬) correlations of 3-hexenyl-1-*O*-*β*-D-glycopyranoside (**113**).



113

4.11.2 3-Hexenyl-1-*O*-*α*-2'-methoxy-glycoside

The compound **114** was isolated from the less polar fraction II as colourless oil which showed no UV absorbance and gave a green colour with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum showed high similarity with that of compound **113,** containing two olefinic protons, oxymethine/methylene protons, two methylene and two methyl groups in the aliphatic region. Additionally, a methoxy signal was observed at δ 3.45. However, the anomeric proton appeared at lower chemical shift (δ 4.84, J = 12 Hz) with a small coupling constant compared to compound **113**.

The mass of the compound **114** was determined by ESIMS as 260 Dalton and HRESIMS exhibited the molecular formula as $C_{13}H_{24}O_5$. Correspondingly, ^{13}C NMR spectrum revealed 13 carbon signals showing high similarity to compound **113** with an additional methoxy signal at δ 58.5. The search in AntiBase did not give any exact hit. So the compound was finally confirmed by 2D NMR spectroscopy.

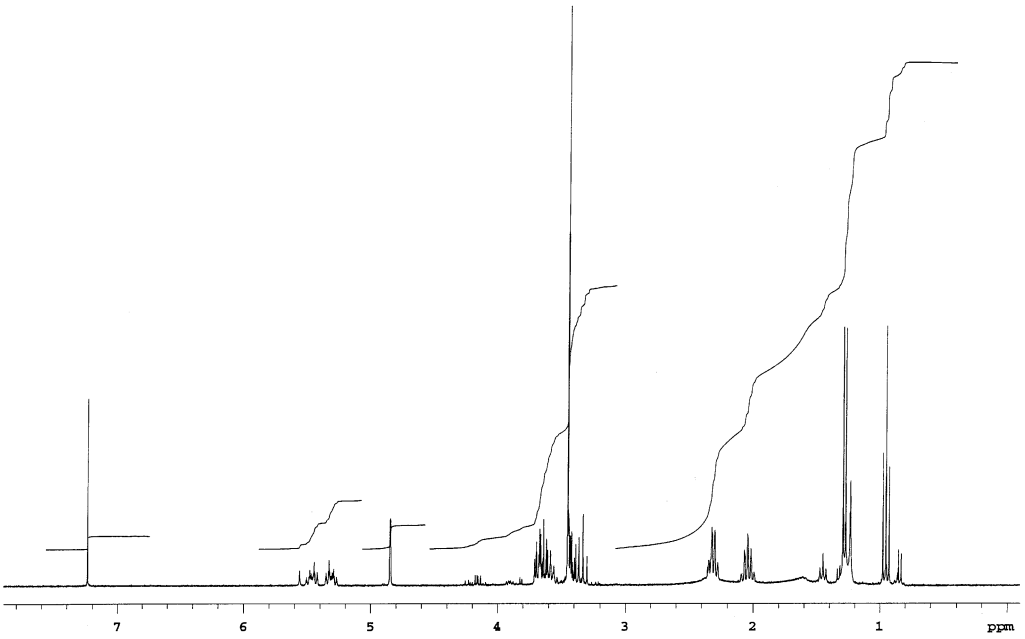


Figure 111: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-hexenyl-1-*O*-α-L-2'-methoxy-glycoside (**114**).

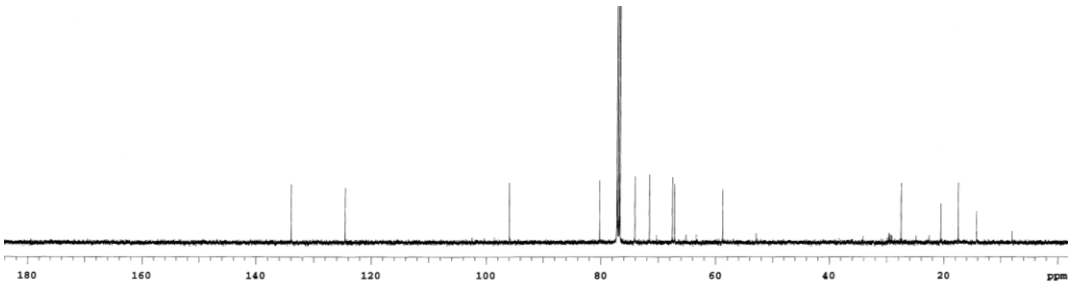


Figure 112: ^{13}C NMR spectrum (CDCl3, 125 MHz) of 3-hexenyl-1-*O*-α-L-2'-methoxy-glycoside (**114**)

The H,H COSY and HMBC correlations confirmed the structure of the compound as 3-hexenyl-1-*O*-α-L-2'-methoxy-glycoside (**114**) which is also a new metabolite from nature. The configuration of compound **114** will be determined later by GC/MS of the persilylated hydrolysis product and comparison with reference sugars.

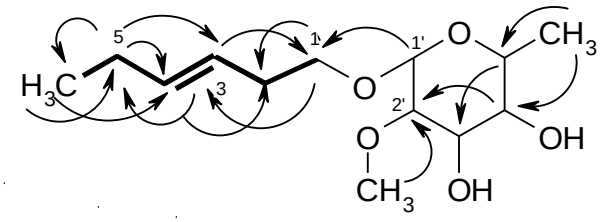


Figure 113: HMBC (→), and H, H COSY (▬) correlations of 3-hexenyl-1-*O*-α-L-2'-methoxy-glycoside (**114**).

4.11.3 3-Hexenyl-1-*O*-β-D-glycoside

3-Hexenyl-1-*O*-β-D-glycoside (**115**) was obtained as colourless oil from a polar fraction V by purification through PTLC and finally with Sephadex LH-20 (MeOH). It was UV non-absorbing colourless oil, which showed green colouration after spraying with anisaldehyde/sulphuric acid.

As in compounds **113** and **114**, the ^{1}H NMR spectrum of compound **115** exhibited the presence of hexenoyl moiety by showing the two olefinic protons at δ 5.43 and 5.31, an oxygenated methylene, two methylenes at δ 2.10 and 2.34 and a triplet methyl at δ 0.92. Additionally, an anomeric proton at 4.30 (d, J = 7.6) along with oxygenated methine/methylene protons between δ 3.81-3.29 indicating the presence of a sugar moiety. However, a methyl doublet at δ 1.28 as observed in compounds **113** and **114** was absent.

ESIMS spectrum revealed a *pseudo*molecular ion peak at *m/z* 285 $[M+Na]^{+}$ corresponding to the molecular mass of 262 Dalton and HRESIMS delivered the molecular formula as $C_{12}H_{22}O_6$. Accordingly ^{13}C/APT NMR spectrum showed 12 signals including two olefinic carbons, one anomeric carbon at δ 102.2, four oxy-methine, two oxy-methylene, two methylene and a methyl carbon.

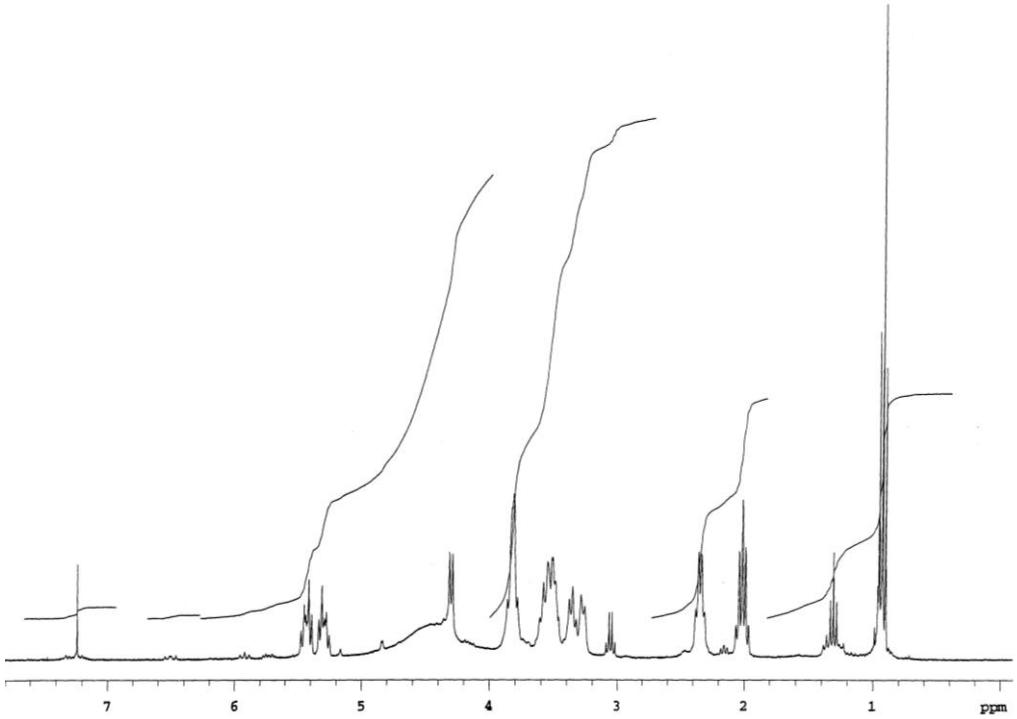


Figure 114: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 3-hexenyl-1-*O*-*β*-D-glycoside (**115**).

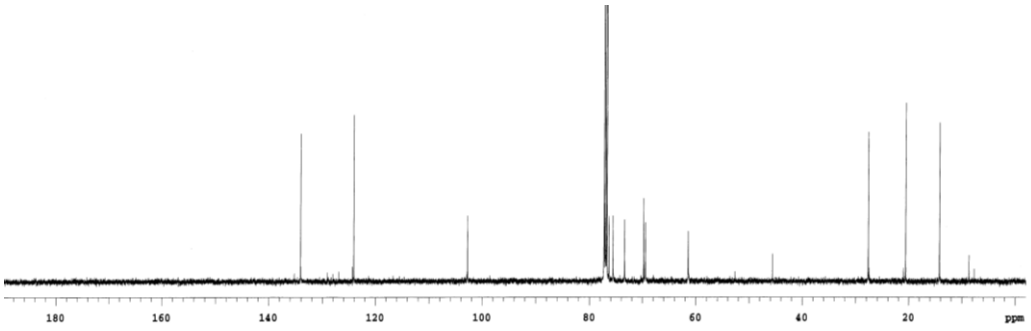


Figure 115: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 3-hexenyl-1-*O*-*β*-D-glycoside (**115**).

From the H,H COSY spectrum, a glycopyran moiety was constructed in addition to 3-hexenol, which were then combined by an obvious correlation from the anomeric proton (δ 4.30) to the oxymethylene carbon (C-1) at δ 69.5. Therefore, the compound **115** was fully elucidated as a 3-hexenyl-1-*O*-*β*-D-glycoside. The configuration will be determined later by GC/MS of the persilylated hydrolysis product and comparison with reference sugars.

Several stereoisomers of **115** (the glucoside, mannoside and galactoside) have been isolated from plants,[138-140] but this is first time that such a compound was isolated from bacteria. They are reported to possess cytotoxicity against human melanoma cells and hepatocellular carcinoma cells [141] and act also as an anti-inflammatory agents.[142]

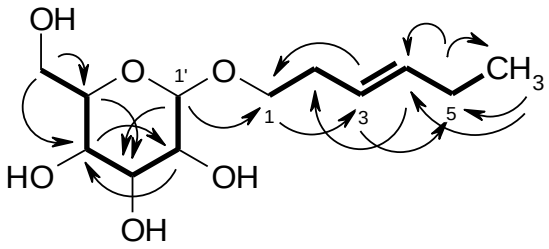


Figure 116: HMBC (→), and H, H COSY (—) correlations of 3-hexenyl-1-*O*-*β*-D-glycoside (**115**).

4.11.4 2-Phenylethanol-1-*O*-*β*-D-glucoside

2-Phenylethanol-1-*O*-*β*-D-glucoside (**116**) was also purified from fraction V as UV-absorbing colourless oil, which turned to green with anisaldehyde/sulphuric acid.

In the ^{1}HNMR spectrum five aromatic protons appeared at δ 7.18 as multiplet. Moreover, nine oxygenated methine/methylene protons were observed in the sugar region between δ 4.30-3.24. It also revealed a methylene triplet at δ 2.88.

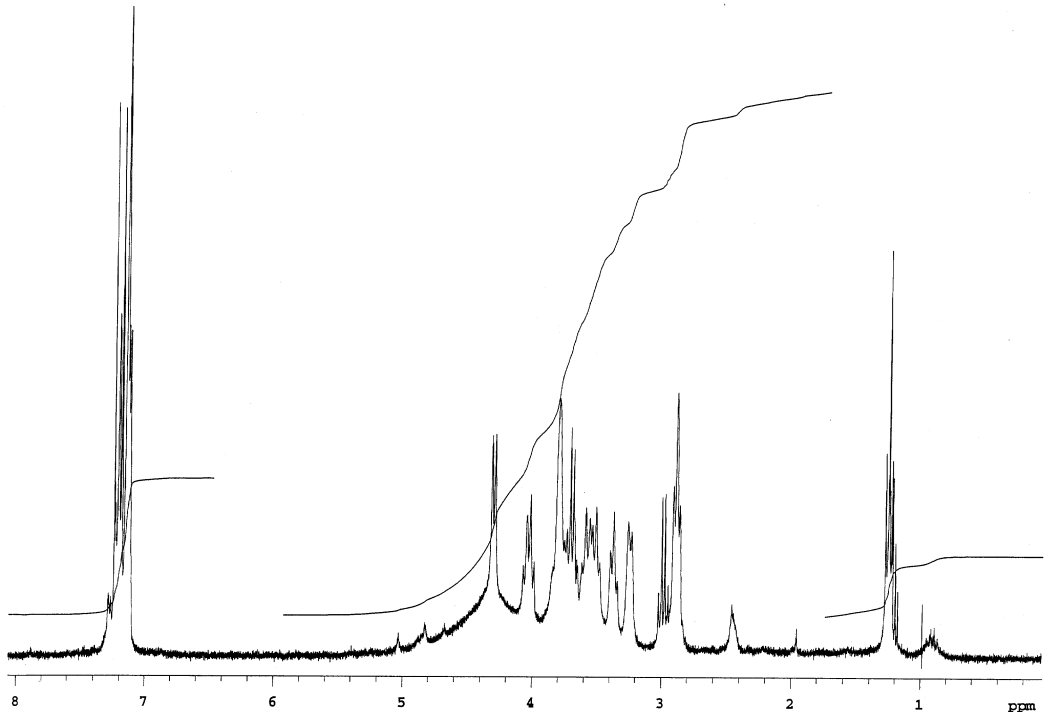


Figure 117: ^{1}H NMR spectrum ($CDCl_3$, 125 MHz) of 2-phenylethanol-1-*O*-*β*-D-glucoside (**116**).

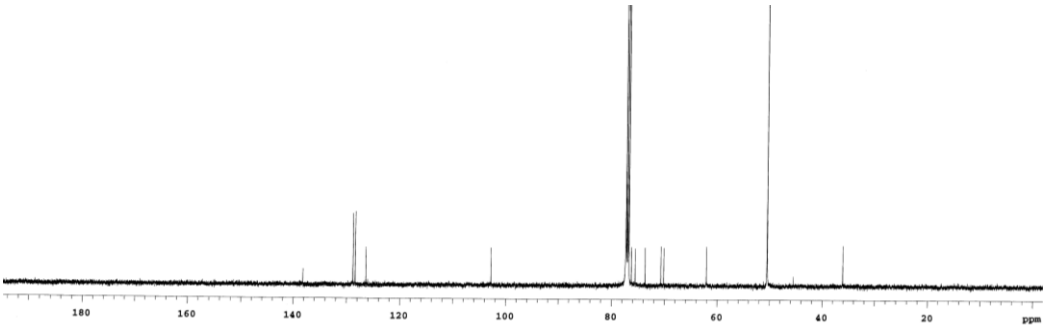


Figure 118: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 2-phenylethanol-1-*O*-*β*-D-glucoside (**116**)

Accordingly, the ^{13}CNMR/APT spectra revealed 14 carbon signals including six aromatic carbons, one anomeric carbon at 102.8, five sp^2 oxymethine/methylene carbons and a methylene carbon at 36.1 probably attached to an aromatic ring.

The mass of the compound **116** was revealed by ESIMS as 284 Dalton and the molecular formula by HRESIMS as $C_{14}H_{20}O_6$. The search in AntiBase with the help of spectroscopic data provided no exact hit pointing to a new metabolite from microorganisms. Finally, the compound was fully elucidated with the help of ^{1}H,^{1}H COSY

and HMBC correlations as 2-phenylethanol-1-*O*-*β*-D-glucoside (**116**). Futhermore, the sugar moiety was confirmed as glucose by GC/MS of the persilylated hydrolysis product and comparison with reference sugars. The search in the Chemical Abstracts indicated that compound **116** is commonly found in plants but now for the first time isolated from bacteria. It showed low activity (zone of inhibition = 12 mm) against the fungus *Mucor miehei* at 40 µg/disk.

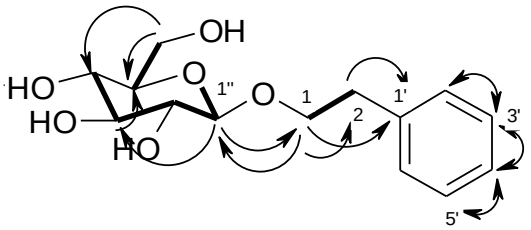


Figure 119: HMBC (→), and H, H COSY (↔,─) correlations of 2-phenylethanol-1-*O*-*β*-D-glucoside (**116**).

4.12 Terrestrial *Streptomyces* sp. Ank 282

During the pre-screening, the terrestrial *Streptomyces* strain Ank 282 was selected due to its high activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Streptomyces viridochromogenes* (Tü 57). It also exhibited 100 % cytotoxicity against *Artemia salina*.

A large-scale fermentation of 25 litres on a linear shaker at 28 °C using M_2 medium for 7 days resulted in a dark brown culture which was filtered over a filter press. The mycelium was extracted with ethyl acetate followed by acetone. The filtrate was extracted by Amberlite XAD-16; the resins were washed with distilled water following elution with methanol. The methanol was evaporated under vacuum and the aqueous residue was again extracted with ethyl acetate. Both extracts were combined based on similar chromatograms and subjected to a silica gel column using CH_2Cl_2/MeOH gradient to yield four fractions. These fractions were further purified by different chromatographic techniques to obtain the metabolites in pure form.

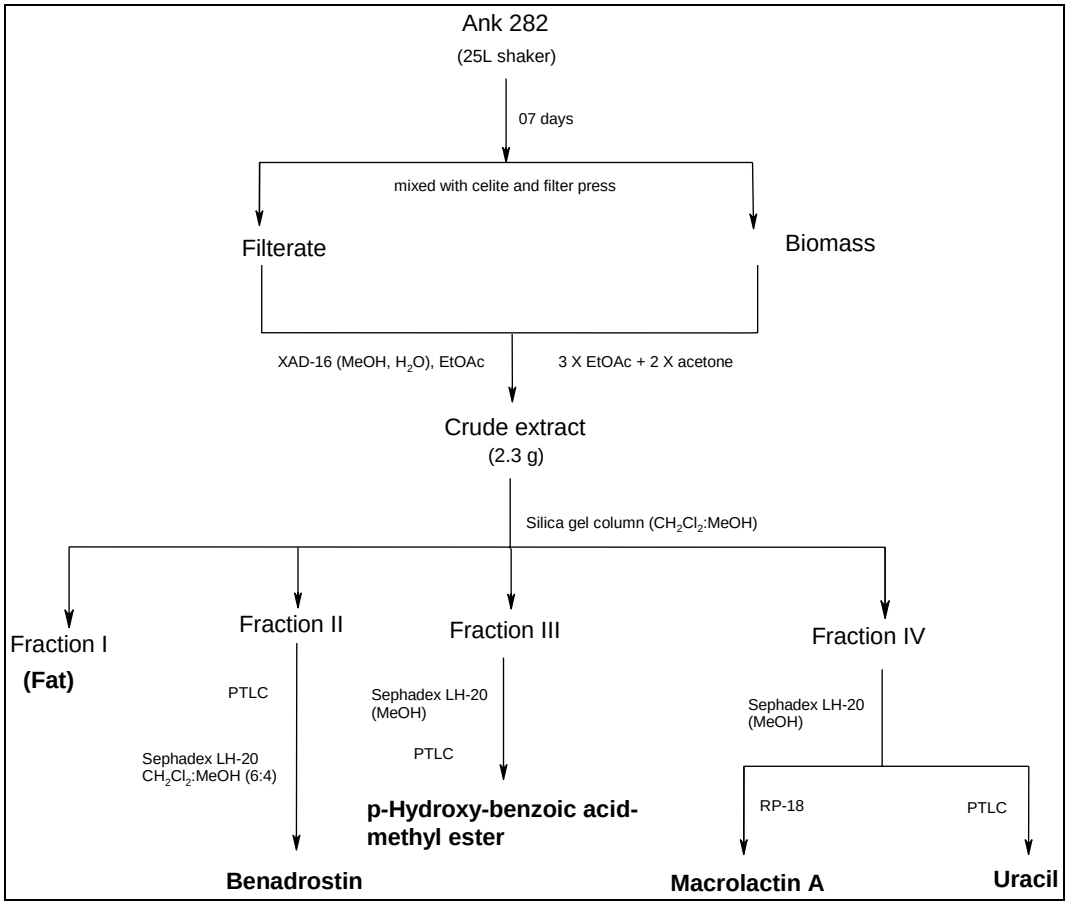


Figure 120: Work up scheme of terrestrial *Streptomyces* sp. Ank 282

4.12.1 Benadrostin

Benadrostin (**117**) was obtained as a colourless powder from fraction II using PTLC followed by Sephadex LH-20 (CH_2Cl_2/40%MeOH) column. It was UV absorbing and showed no colour reaction with anisaldehyde/sulphuric acid reagent. The mass of the compound **117** was determined by ESIMS as 179 Dalton showing a *pseudo*molecular ion peak *m/z* 202 $[M+Na]^+$. No aliphatic proton was observed in the ^1H NMR spectrum, however, in the aromatic region, two protons appeared as doublet of doublet at δ 7.33 and 7.22 and a proton as triplet at δ 7.16 establishing a 1,2,3-trisubstituted benzene ring. Additionally, it also exhibited two exchangeable protons (NH, OH) at δ 11.16. Searching the compound with a 1,2,3-trisubstituted aromatic ring in AntiBase resulted in 2129 compounds, while a search with the mass peak 202.0-203.0 $[M+Na]^+$ resulted in 32 compounds. The intersection of both lists of compounds provided benadrostin (**117**) as the only hit, which was further confirmed

by comparing the spectroscopic data with authentic spectra. Benadrostin (**117**) was found as inhibitor of poly(ADP-ribose)-synthetase.[143]

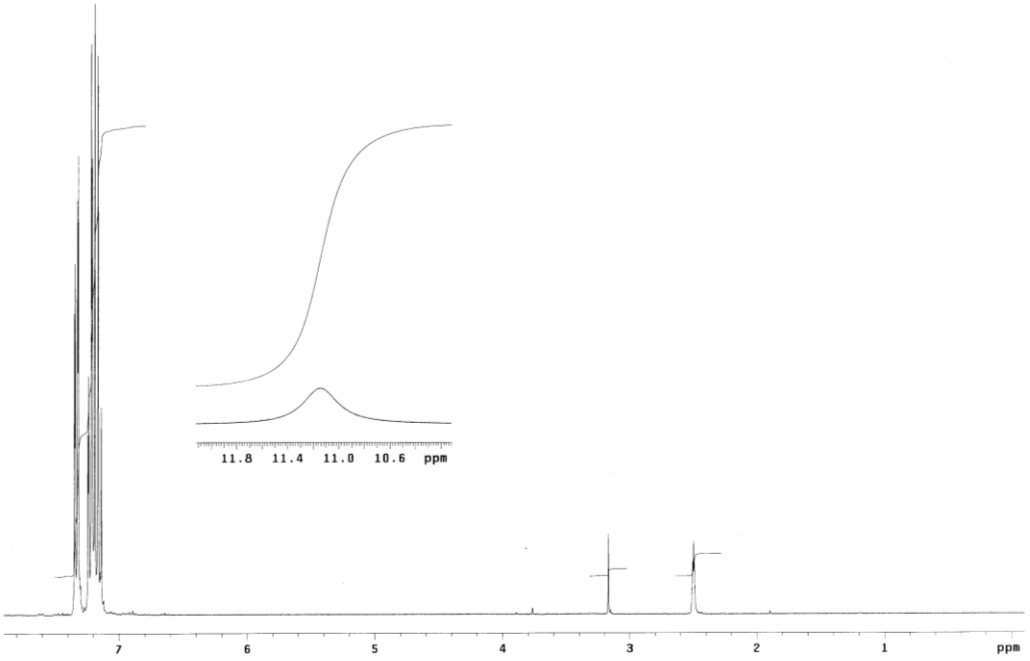
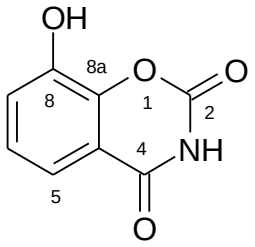


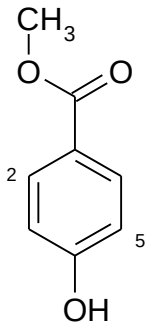
Figure 121: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of benadrostin (**117**).



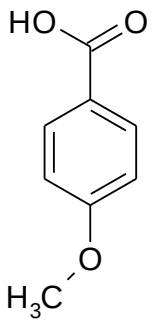
117

4.12.2 *p*-Hydroxybenzoic acid methyl ester

p-Hydroxybenzoic acid methyl ester (**118**) was isolated from fraction III as UV-absorbing, light yellow solid, which turned to pink colour after anisaldehyde/sulphuric acid. In the ^{1}H NMR spectrum, two doublets appeared at δ 7.77 (2H) and 6.80 (2H), in addition to a methoxy signal at δ 3.77. The EIMS mass spectrum of compound **118** exhibited a molecular ion peak [M^{+}] *m/z* at 152. The search with the spectroscopic data provided two hits *p*-hydroxybenzoic acid methyl ester (**118**) and *p*-methoxy benzoic acid (**119**). The compound was finally confirmed as *p*-hydroxybenzoic acid methyl ester (**118**) by comparing with authentic spectra.



118



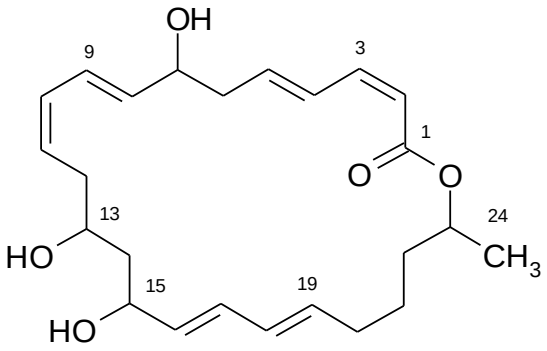
119

4.12.3 Macrolactin A

Macrolactin A (**120**) was isolated from fraction IV using Sephadex LH-20 followed by RP-18. It was a yellow oil, which showed a UV-absorbing band at 254 nm and turned to black with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum exhibited twelve olefinic protons between δ 7.25-5.43. Moreover, it also showed four oxymethine protons at δ 5.02, 4.30, 4.25 and 3.83. Additionally, six methylene groups in the upfield region between δ 2.55-0.98 and a methyl doublet at δ 1.24 were also observed. The molecular weight of compound **120** was obtained by ESIMS as 402 Dalton showing a *pseudo*molecular ion peak at *m/z* 425 $[M+Na]^{+}$. The search in AntiBase with the help of above spectroscopic data resulted in macrolactin A (**120**), which was confirmed by comparing the data with authentic spectra. Macrolactin A (**120**) was reported to possess profound antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* at a concentration of 5 and 20 μg/disk. Moreover, macrolactin A was found to possess significant antiviral and cancer cell cytotoxic properties.[144]

The macrolactins have been firstly isolated by Fenical *et al*. from a deep-sea bacterium; so far, 27 members of this class have been reported. They are often cited as typical examples for metabolites from high-pressure habitats, but obviously also "normal" bacteria can synthesize this type of compounds.



120

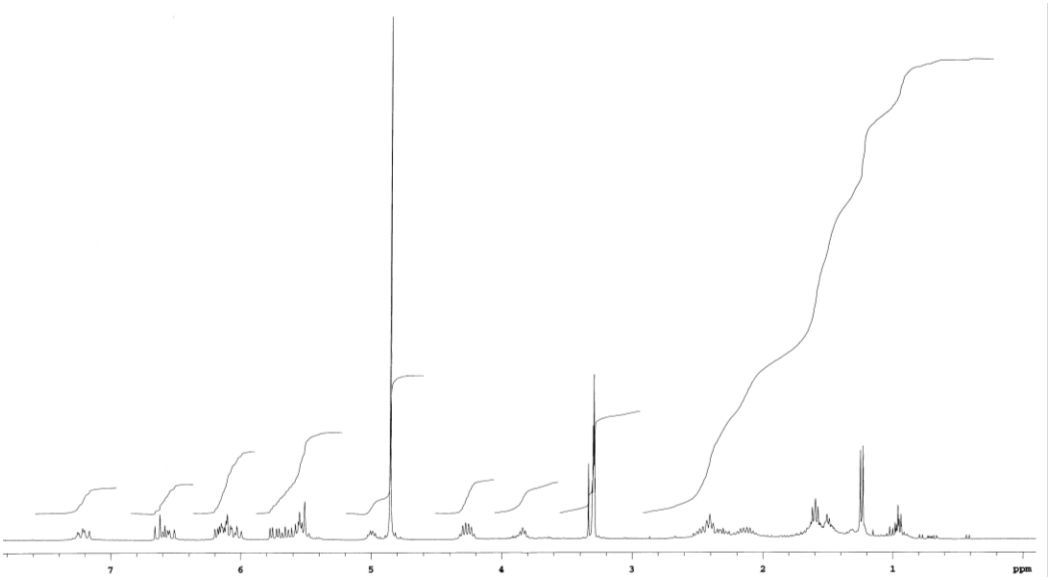


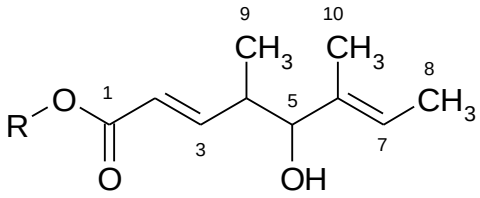
Figure 122: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of macrolactin A (**120**).

5 Summary

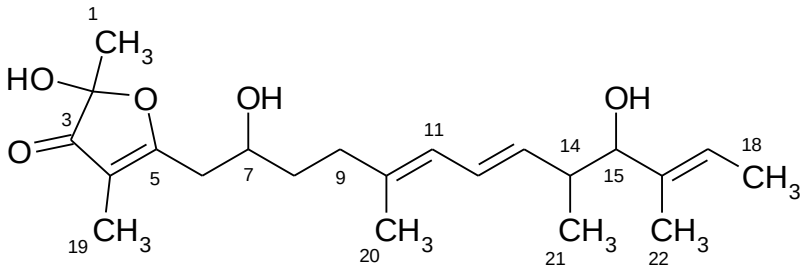
Approximately 30% of the drugs available on the market worldwide are natural products or their derivatives.[145] Natural products have a greater diversity of chemical structures that can not be sometimes accessed even by the most sophisticated synthetic concepts. Moreover, natural products have not only opened up new ways to therapeutic approaches but also contributed to identify and understand novel biochemical pathways and proved to make new valuable drugs available. They are also used as important tools in biochemistry and molecular cell biology.[146]

In our search for new and biologically active natural products, we are continuously performing a screening of extracts of marine and terrestrial bacteria. In the present work, twelve terrestrial *Streptomyces* strains were selected on the basis of biological and chemical screening. Accordingly, fermentation, extraction, isolation and purification of metabolites were performed under standard conditions. The dereplication of isolated compounds was done with the help of different databases such as AntiBase, the Dictionary of Natural Products (DNP) and the Chemical Abstracts. For structure elucidation, NMR and MS techniques were applied. The pure compounds were tested for various biological activities.

The crude extract of the terrestrial *Streptomyces* strain GW54/453 exhibited moderate antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. It also showed 100% activity against *Artemia salina*. From 30 L shaker culture, four compounds were isolated, two were new compounds (2*E*, 6E)-5-hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (**45a**) together with its synthetic methyl ester (**45b**) and actinofuranone C (**46**) and two known compounds 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**49**) and 2,5-furan-dimethanol (**50**). Compounds **45a**, **45b** and **46** were inactive against *Artemia salina*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

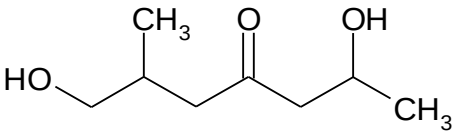


45a: R = H
45b: R = CH_3

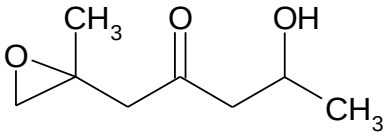


46

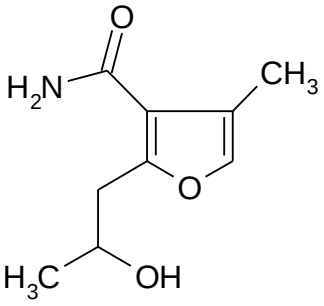
The chemical investigation of terrestrial *Streptomyces* strain Ank 245 afforded three new compounds, 1,6-dihydroxy-2-methyl-heptan-4-one (**51**), 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**52**) and 2-(2-hydroxy-proplyl-4-methyl-furan-3-carboylic acid amide (**53**) as well as two known compounds, 4-vinylphenol (**54**) and 2-methoxy-4-vinylphenol (**55**). All three new compounds showed low cytotoxicity against brine shrimps (*Artemia salina*).



51



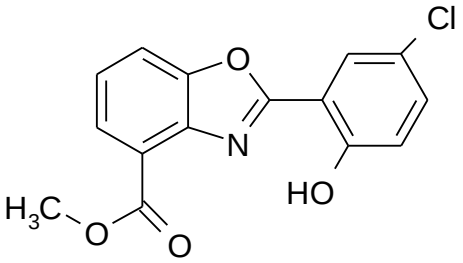
52



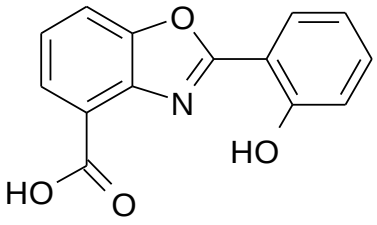
53

The crude extract of terrestrial *Streptomyces* strain Ank 250 in the biological screening showed pronounced antibacterial activity against Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, moderate activity against *Escherichia coli* and *Streptomyces viridochromogenes* (Tü 57) and high cytotoxicity against *Artemia salina*. The chemical investigation led to the isolation of one new benzoxazole derivative, 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**), together with a known cholesterol biosynthesis inhibitor MC-033 (**62**) and a trivial compound 2-phenylethanol. The new benzoxazole derivative was inactive against our test strains, however, recently a closely related structure caboxamycin (**60**) was reported to possess antibacterial activity against Gram-positive bacteria and

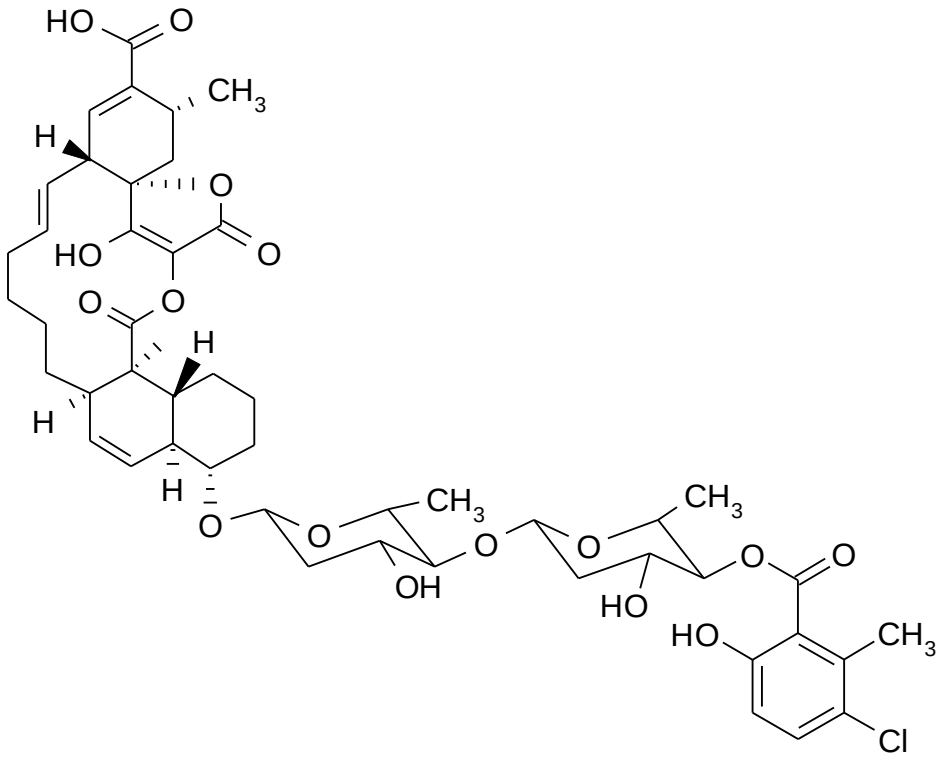
cytotoxic activity against selected human tumor cell lines.[64] The compound **62** displayed high antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*.



56



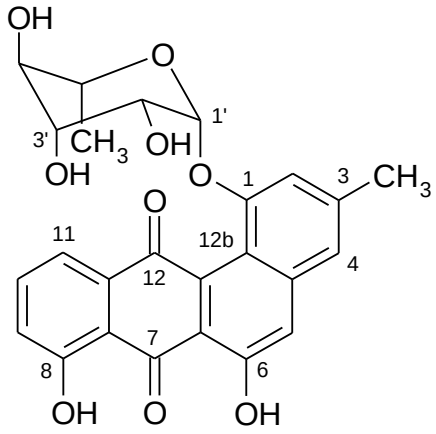
60



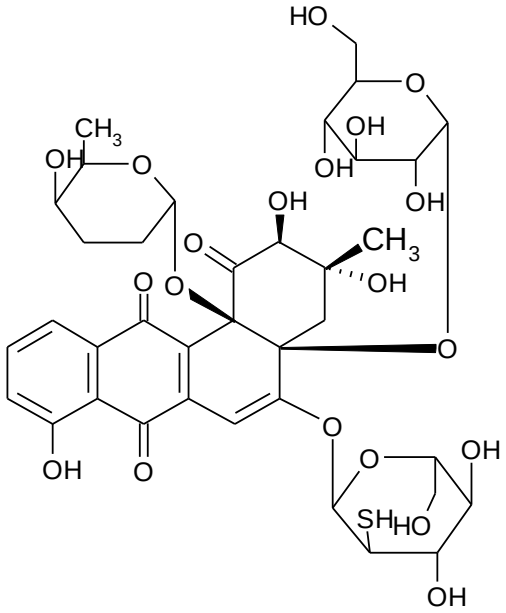
62

During the pre-screening, the terrestrial *Streptomyces* strain GW 51/426 showed moderate activity against *Staphylococcus aureus* and *Escherichia coli*, it also exhibited high antifungal activity against *Mucor miehei* and *Aphanomyces cochlioides* and 95% cytotoxicity against brine shrimps (*Artemia salina*). From a 30 L shaker culture, a new angucycline glycoside; dehydrorabelomycin-1-*O*-α-L-rhamnopyranoside (**64**) and a previously isolated new benzoxazole derivative from Ank 250, 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**), were isolated. It also delivered three known compounds, namely rhodonocardin A (**68**), *cyclo*(Ala-

Pro) (**69**), 4-acetyl-1,3-dihydroimidazo(4,5-c)pyridine (**49**) and two trivial compounds, indole-3-carboxylic acid and uracil. The new compound **64** exhibited moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.



64



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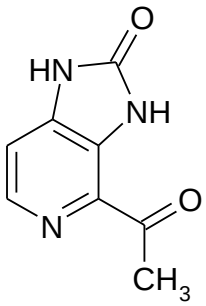
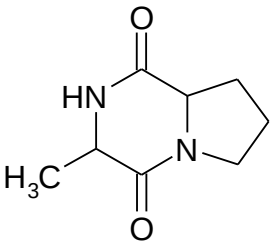
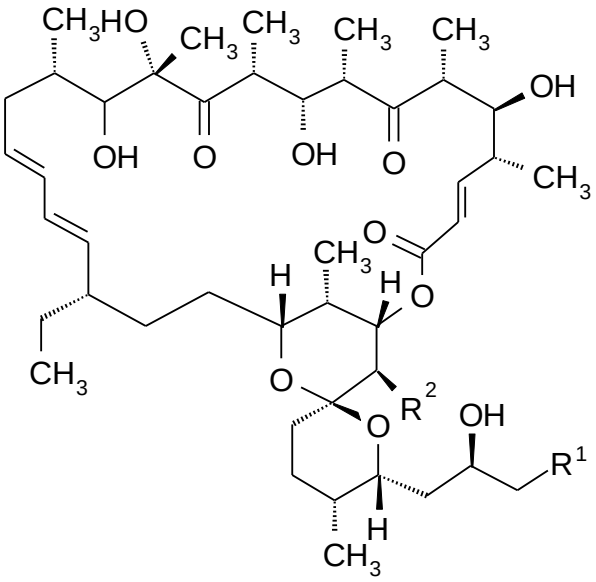


49

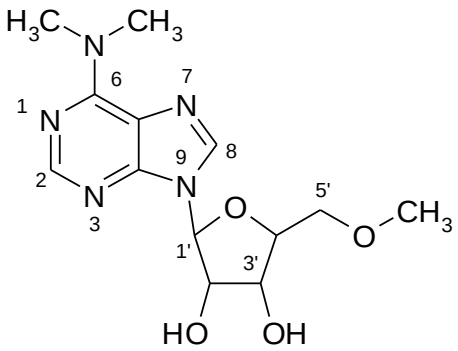


69

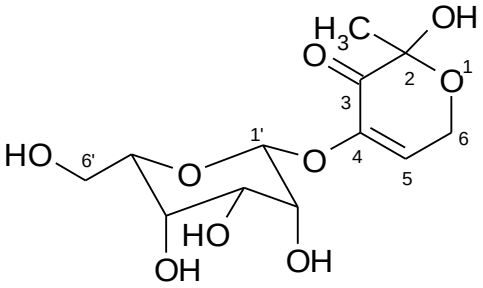
The terrestrial *Streptomyces* strain WO 463 was investigated with the goal to find active compounds against plant pathogen fungi as the crude extract showed high activity in this respect. Fermentation of 30 L, working up and purification of the fractions afforded two known antifungal oligomycin derivatives; oligomycin A (**70**) and oligomycin F (**71**) along with two new metabolites; 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**) and 4-*α*-L-glucosyl-*β*-pyranone (**74**) and three trivial compounds, namely thymine (**76**), 2'-deoxyadenosine (**77**) and adenosine-methyl-sulphoxide (**78**). The new compound **74** is a mixture of two isomers previously isolated from red ginseng but for the first time now isolated from bacteria. It exhibited low inhibitory activity against the fungus *Mucor miehei*.



70: $R^1 = H$, $R^2 = CH_3$
71: $R^1 = CH_3$, $R^2 = CH_3$

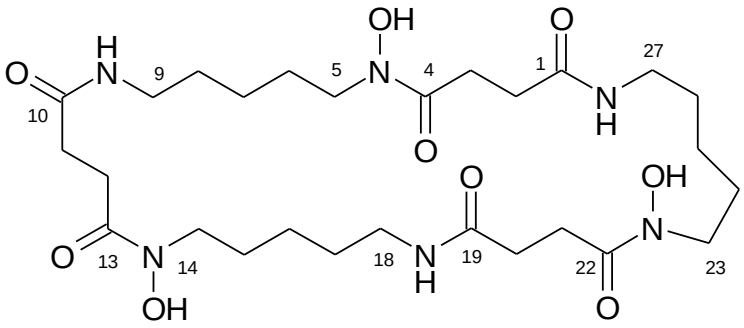


73

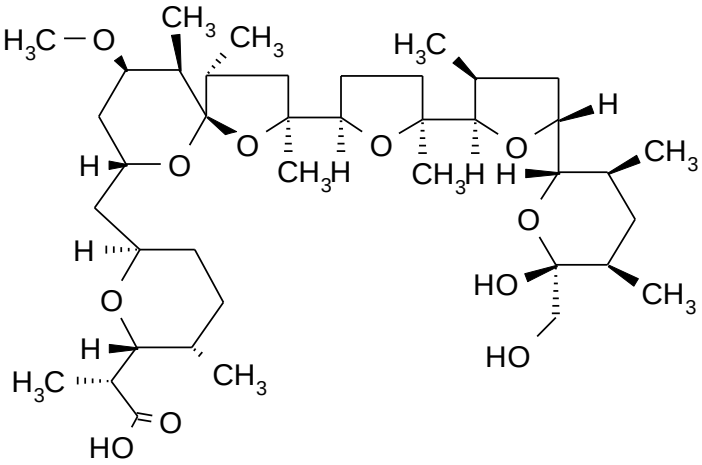


74

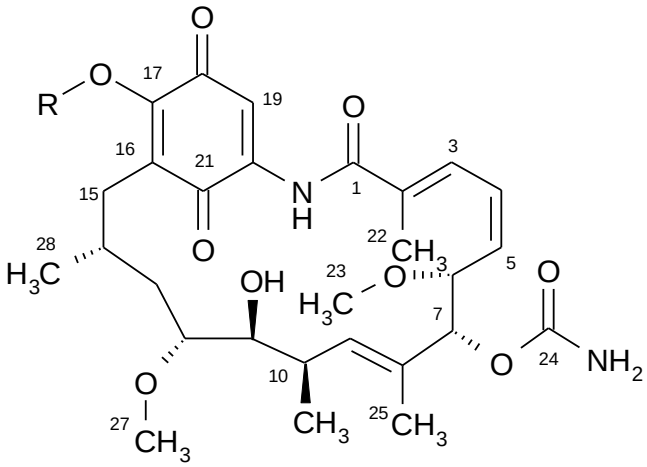
The terrestrial streptomycete strain Ank 351 revealed promising antibacterial activities against *Bacillus subtilis*, *Staphylococcus* aureus, *viridochromogenes* (Tü 57) and high antifungal activity against plant pathogen fungus *Pythium ultimum*. It also exhibited 100% cytotoxicity against *Artemia salina*. From 30 L shaker culture using M_2 medium, six known antibiotics were isolated namely geldanamycin (**79**), 17-*O*-demethyl-geldanamycin (**82**), nigericin (**84**), 11,11'-*O*-dimethyl-elaiophylin (**86**), 11-*O*-monomethyl-elaiophylin (**87**), and nocardamine (**88**).



88

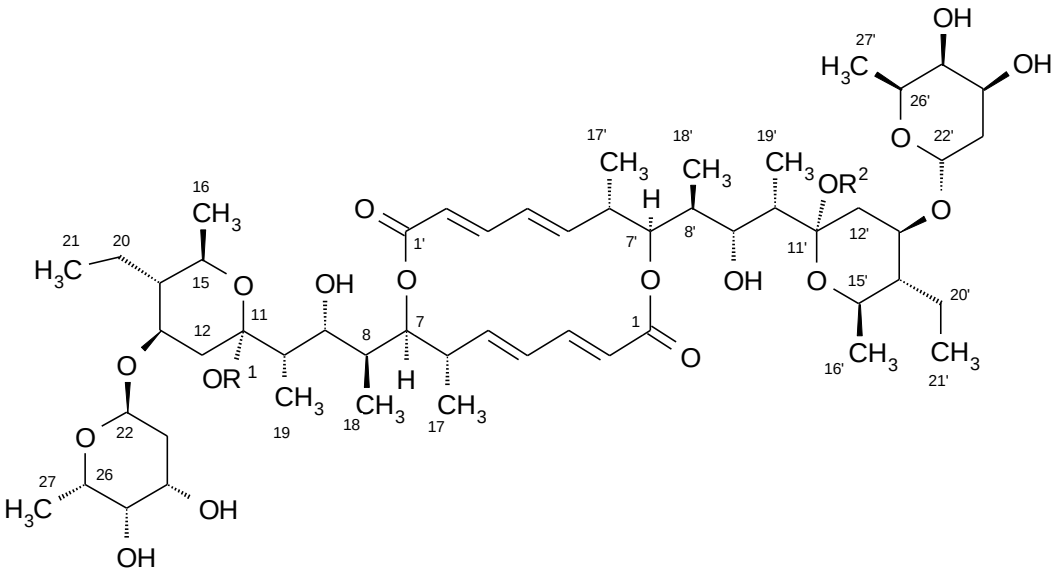


84



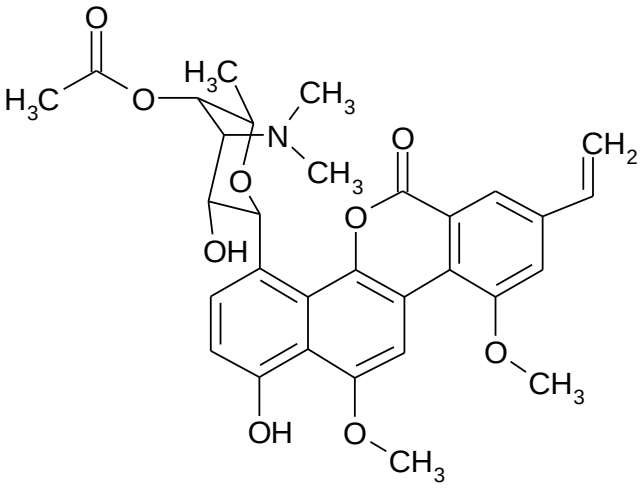
79: R = CH_3

82: R = H



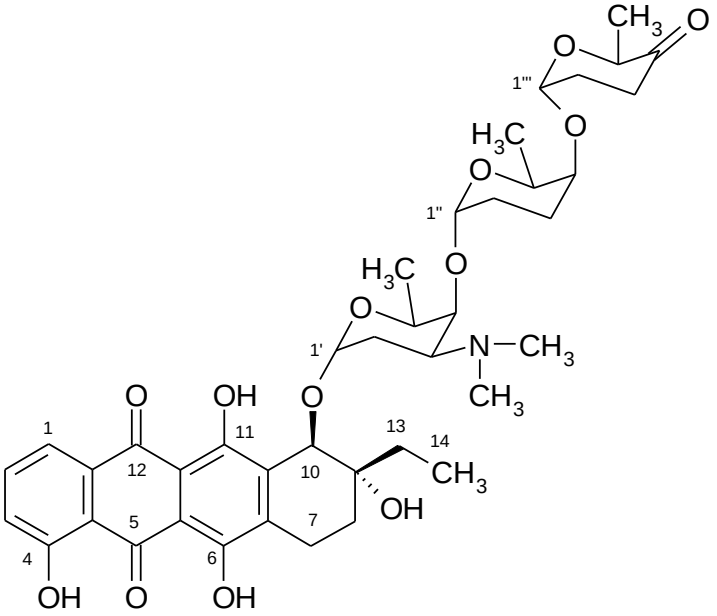
86: $R^1 = CH_3$, $R^2 = CH_3$
87: $R^1 = CH_3$, $R^2 = H$

In the primary screening, the crude extract of the terrestrial *Streptomyces* strain Ank 202 was found to inhibit the growth of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Mucor miehei* in the agar diffusion test. The strain produced six metabolites including ravidomycin (**89**), N-(2-methoxyphenyl)-acetamide (**93**), phenazine-1-carboxylic acid (**94**), 1-carboxymethyl-phenazine (**95**), N6,N6-dimethyladenosine (**96**) and uracil. Compound **89** was reported to have antibacterial and anticancer properties.



89

The red coloured terrestrial *Streptomyces* strain Ank 291 was a producer of three *peri*-hydroxyquinones, ditrisarubicin A (**98**), B (**99**) and A447 D' (**100**). The compound **100** was reported as hydrolysis product of A447 D (**101**). The compound could be a new natural product or an artefact formed during the extraction and purification process.



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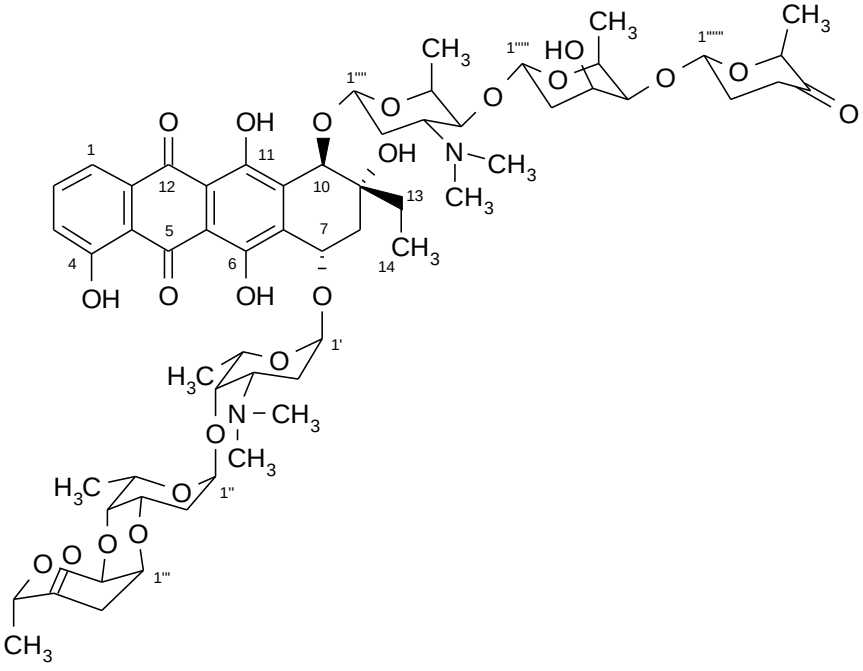
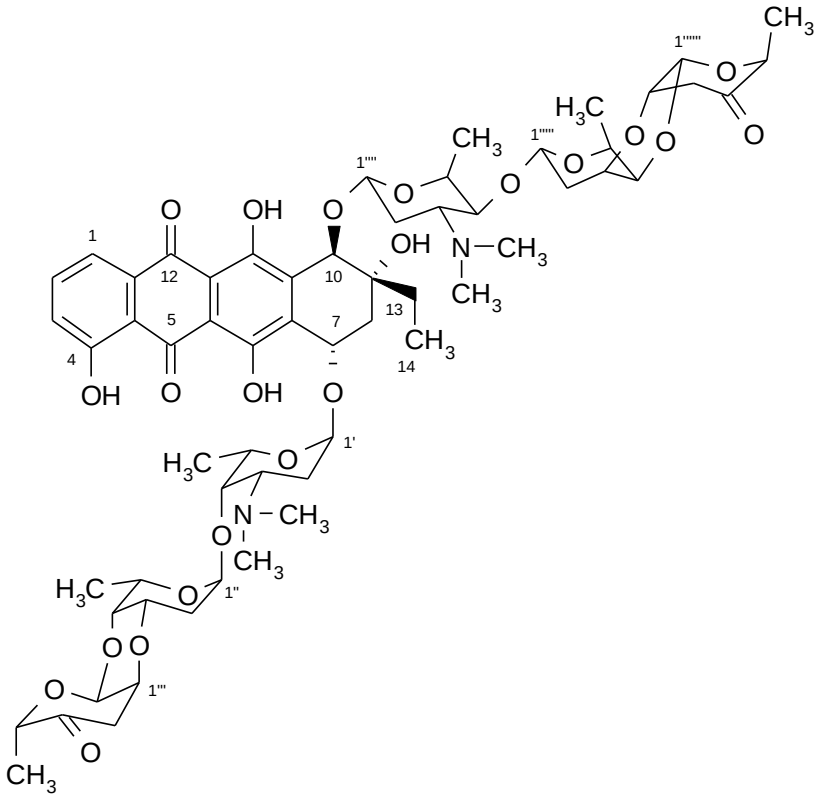
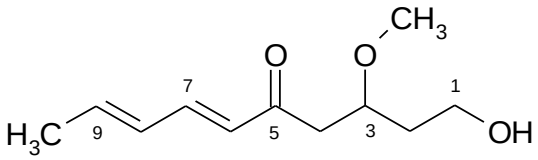


98

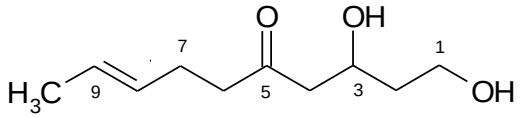


99

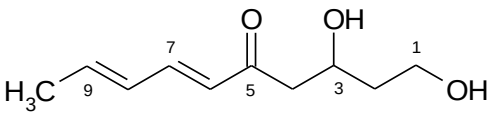
A new metabolite 3-methoxy-streptenol C (**102**) along with a mixture of streptenol A (**103**) and C (**104**) and several known metabolites were isolated form the terrestrial *Streptomyces* strain GW 13/475.



102



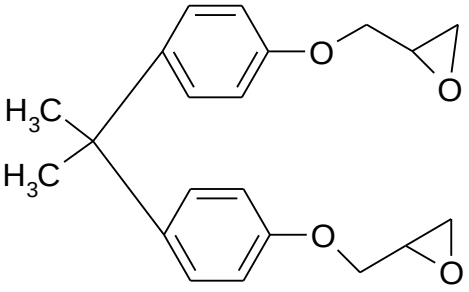
103



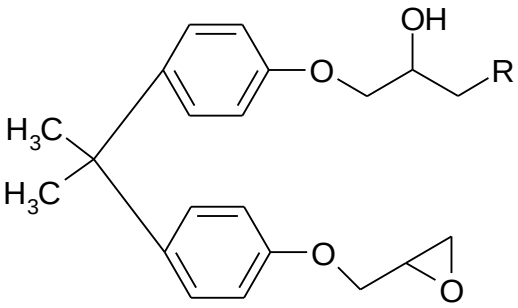
104

The terrestrial *Streptomyces* strain GW 12/459 yielded four new compounds, bisphenol A diglycidyl ether (**107**) abbreviated as BADGE, 3-methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]-2-propanol (BADGE·OMe) (**109**), 3-chloro-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]-ethyl]phenoxy]-2-propanol (BADGE·HCl) (**110**), 3-[4-[1-[4-(3-chloro-2-

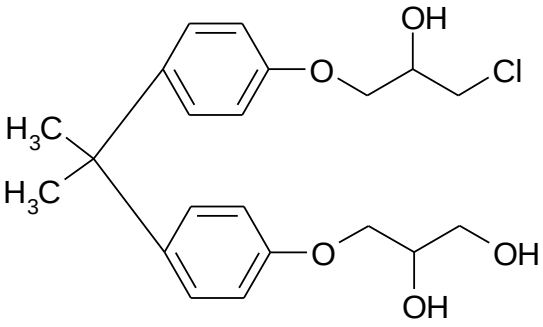
hydroxypropoxy)phenyl]-1-methylethyl]phenoxy]-1,2-propanediol (BADGE·HCl·H_2O) (**111**) as well as anthranilic acid, tryptophol and 1-acetyl-β-carboline. Compound **107** is a component of epoxy resins used in coating of food cans and as bonding and adhesive agent. Its natural occurrence is not reported yet, and also here the true origin needs to be confirmed: Compounds **109**-**111** were also reported as hydrolysis products of BADGE (**107**). However, these compounds were not isolated from other strains and could not be re-isolated from the same strain so considering these compounds as new natural metabolites is still to be confirmed by biosynthetic studies.



107



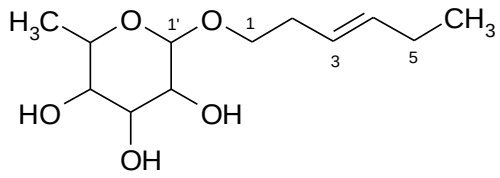
109: R = OCH_3
110: R = Cl



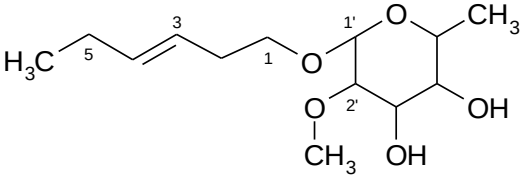
111

The crude extract of the terrestrial *Streptomyces* sp. GW 8594 exhibited moderate activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. It also showed antifungal activity against *Mucor miehei*, *Candida albicans*, *Aphanomyces cochlioides* and *Pythium ultimum* and was also active against the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Chlorella sorokiniana*. The large-scale fermentation, extraction and purification of fractions led to the isolation of four new secondary metabolites, namely 3-hexenyl-1-*O*-*β*-D-glycopyranoside (**113**), 3-hexenyl-1-*O*-*α*-L-2'-methoxy-glycoside (**114**), 3-hexenyl-1-*O*-*β*-D-glycoside (**115**) and 2-phenylethanol-1-*O*-*β*-D-glucoside (**116**). The compounds **115** and **116** were already known from plants but are now for the first time isolated from bacteria.

Among all the isolated compounds, only metabolite **116** showed low activity against the fungus *Mucor miehei* at 40 μg/disk.



113



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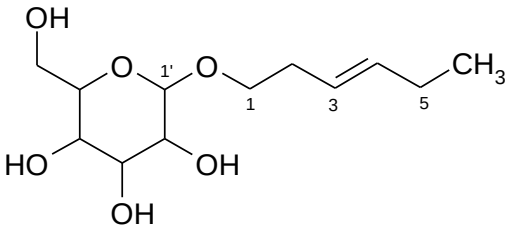
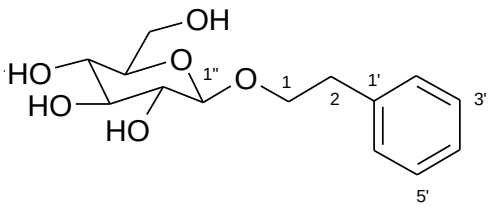
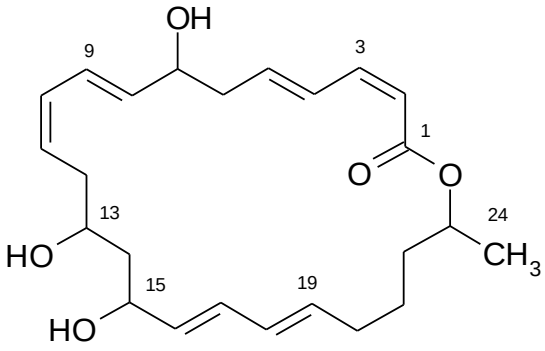


115

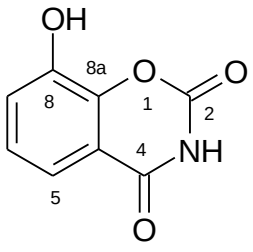


116

The antibacterial assay of the strain Ank 282 showed high activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Streptomyces viridochromogenes* (Tü 57). From this strain, three known compounds; macrolactin A (**120**), benadrostin (**117**), *p*-hydroxy-benzoic acid methyl ester (**118**) and uracil were isolated. Macrolactin A (**120**) is reported as antibacterial agent against Gram-positive bacteria and also possesses anticancer properties.



120



117

Table 20: Total number of isolated compounds from terrestrial *Streptomyces* strains in this thesis

***Streptomyces* strains**	**No of strains**	**No of compounds**	**No of new compounds**
GW strains	5	31	12
Ank strains	6	27	4
WO strain	1	8	2

The present investigation confirmed that the terrestrial *Streptomyces* spp. are a rich source of bioactive and structurally diverse secondary metabolites. However, it is also to be mentioned that most of the new secondary metabolites isolated during this study did not show biological activity against the available test strains. But nature never produces anything useless so it is a strong need to test the isolated compounds against other biological systems. Therefore research cooperation between chemists and biologists is necessary for further research into the discovery of new bioactive compounds.

6 Materials and Methods

6.1 General

IR spectra: Perkin-Elmer 1600 Series FT–IR; Perkin-Elmer 297 infrared spectro-photometer; Beckman DU-640; Shimadzu FT-IR; (KBr tablet and film). - **UV/VIS spectra:** Perkin-Elmer Lambda 15 UV/VIS spectrometer. - **Optical rotations:** Polarimeter (Perkin-Elmer, model 243). – **^{1}H NMR spectra:** Varian Unity 300 (300.145 MHz), Bruker AMX 300 (300.135 MHz), Varian Inova 500 (499.8 MHz), Varian Inova 600 (600 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, br = broad. – **^{13}C NMR spectra:** Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz), Varian Inova 600 (150.7 MHz). Chemical shifts were measured relative to tetramethylsilane as internal standard. Abbreviations: APT (Attached Proton Test): CH/CH_3 up and C_q/CH_2 down. - **2D NMR spectra:** H,H COSY (^{1}H,^{1}H-Correlated Spectroscopy), HMBC (Heteronuclear Multiple Bond Connectivity), HMQC (Heteronuclear Multiple Quantum Coherence) and NOSY (Nuclear Overhauser Effect Spectroscopy). - **Mass spectra**: EI MS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perfluorokerosene as standard. DCI-MS: Finnigan MAT 95 A, 200 eV, Reactant gas NH_3. ESI MS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). ESI HRMS were measured on Micromass LCT mass spectrometer coupled with a HP1100 HPLC with a diode array detector. High-resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). ESI MS/MS was performed with normalized collision energy of 35%. EI MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for EI HRMS, samples were infused with a flow rate of 2 µL/min.– **Filter press:** Schenk Niro 212 B40.

6.2 Materials

Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). – **Glass plates for chemical screening:** Merck silica gel 60 F254, (10 × 20 cm). - **Preparative thin layer chromatography (PTLC):** 55 g Silica gel P/UV_{254} (Macherey-Nagel & Co.) is added to 120 ml of demineralised water with continuous stirring for 15 minutes. 60 ml of the homogenous suspension is poured on a horizontal held (20 × 20 cm) glass plate and the unfilled spaces are covered by dis-

tributing the suspension. The plates are air dried for 24 hours and activated by heating for 3 hours at 130 °C. - **Column chromatography (CC):** MN silica gel 60: 0.05-0.2 mm, 70-270 mesh (Macherey-Nagel & Co); silica gel (230-400 mesh) for flash chromatography: 30-60 μm (J. T. Baker); size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd; purchased from Sigma-Aldrich Chemie, Steinheim, Germany). Amberlite XAD-16 resin was obtained from Rohm and Haas, France.

6.3 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 indole and yellow for other N-heterocycles. **Chlorine/*o*-dianisidin reaction:** The reagent was prepared from 100 ml (0.032%) *o*-dianisidin in 1 N acetic acid, 1.5 g $Na_2WO_4 \cdot 2 H_2O$ in 10 ml water, 115 ml acetone and 450 mg KI. The moistened TLC plate was kept ca. 30 min in a chlorine atmosphere (from 0.5 g $KClO_3$ + 2 ml conc. HCl) and then subjected to drying for ca. 1 h, till the excess of chlorine was evaporated and then dipped into the reagent. The reagent is specific for peptides as universal spraying reagent. **NaOH or KOH:** 2 N NaOH or KOH solutions are used to identify *peri*-hydroxyquinones by deepening of the colour from orange to violet or blue.

6.4 Microbiological Materials

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). - **Ultraturrax:** Janke & Munkel KG. – **Shaker:** Infors AG (CH 4103 Einbach) type ITE. - **Laboratory shaker:** IKA-shaker type S50 (max. 6000 rpm). - **Autoclave:** Fedegari Autoclavi SPA, 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). - **Petri-dishes:** 94 mm diameter, 16 mm height, Fa. Greiner Labortechnik, Nürtingen. – **Celite:** Celite France S. A., Rueil-Malmaison Cedex. - **Sterile filters:** Midisart 2000, 0.2 μm, PTFE-Filter, Sartorius,

Göttingen. - **Laminar-Flow-Box:** Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1. - **Brine shrimp eggs (*Artemia salina*):** SERA Artemia Salinenkrebseier, SERA Heinsberg (brine shrimp eggs can be obtained from aquarist shops).

6.5 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.

6.6 Nutrients

M_2 medium (without sea water)

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Tap water	ad 1 L

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar agar

M_2^+ medium (M_2 medium with sea water)

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Artificial sea water	500 ml
Tap water	500 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar agar.

M_2 100% sea water + $CaCO_3$

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
$CaCO_3$	0.5 g
Artificial sea water	1000 ml

The pH was adjusted to 7.3 using 2N NaOH. Solid medium was prepared by adding 18 g of agar agar.

$CaCl_2$-Medium

Malt extract	40 g
Glucose	5 g
$CaCl_2$	45 g
Tap water	1000 ml

The pH was adjusted to 7.8 usin g 2N NaOH. Solid medium was prepared by adding 18 g of agar agar.

Luria-Bertani-Medium (LB)

Trypton	10 g
Yeast extract	5 g
NaCl	10 g
Tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar agar.

Soja-Mannitol Medium

Soybean meal (defatted)	20 g
D(-)-Mannitol	20 g
Tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar agar.

M Test Agar (for test organisms Escherichia coli, Bacillus subtilis (ATCC 6051), Staphylococcus aureus**,** Mucor miehei (Tü 284):

Malt extract	10 g
Yeast extract	4 g
Glucose	4 g
Bacto agar	20 g
Demineralised water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH.

Sabouraud-Agar (for test organism *Candida albicans*)

Glucose	40 g
Bacto peptone	10 g
Bacto agar	20 g
Demineralised water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH.

Nutritional solution A

Soybean meal (defatted)	30 g
Glycerol	30 g
$CaCO_3$	2 g
Artificial sea water	750 ml
Demineralised water	250 ml

Nutritional solution B

Starch	10 g
NZ-Amine	5 g
Soybean meal	2g
Yeast extract	5 g
KNO_3	3 g
Algal extract	2.5 ml
Artificial sea water	750 ml
Demineralised water	250 ml

Stock Solutions and Media for Cultivation of algae

Fe-EDTA

0.7 g of $FeSO_4 \cdot 7\ H_2O$ and 0.93 g EDTA (Titriplex III) are dissolved in 80 ml of demineralised water at 60 °C and then diluted to 100 ml.

Trace element Solution II:

Solution A:

$MnSO_4 \cdot H_2O$	16.9 mg
$Na_2MoO_4 \cdot 2H_2O$	13.0 mg
$Co(NO_3)_2 \cdot 6H_2O$	10.0 mg

Salts are dissolved in 10 ml of demineralised water.

Solution B:

$CuSO_4 \cdot 5H_2O$	5.0 mg
H_3BO_3	10.0 mg
$ZnSO_4 \cdot 7H_2O$	10.0 mg

Salts are dissolved each in 10 ml of demineralised water. Solutions A is added to B and diluted to 100 ml with demineralised water.

Bold's Basal medium (BBM): (for algae Chlorella vulgaris, Chlorella sorokiniana and Scenedesmus subspicatus.

$NaNO_3$	0.250 g
KH_2PO_4	0.175 g
K_2HPO_4	0.075 g
$MgSO_4 \cdot 7\ H_2O$	0.075 g
NaCl	0.025 g
$CaCl_2 \cdot 2\ H_2O$	0.025 g
Fe-EDTA	1.0 ml
Trace element solution II	0.1 ml

Salts are dissolved in 10 ml of demineralised water and added to Fe-EDTA and trace element solution II. The mixture made to one litre with demineralised water. Solid medium was prepared by adding 18 g of agar agar.

6.7 Microbiological and Analytical Methods

6.7.1 Storage of Strains

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

6.7.2 Pre-screening

The microbial isolates (obtained from culture collections) were cultured in a 1 L scale in 1 L-Erlenmeyer flasks each containing 200~250 ml of M_2 or (for marine strains) M_2^+ medium. The flasks were shaken for 3-5 days at 28 °C after, which the entire fermentation broth was freeze-dried and the residue extracted with ethyl acetate. The extracts were evaporated to dryness and used for the antimicrobial tests in a concentration of 50 mg/ml.

6.7.3 Biological Screening

The crude extract was dissolved in $CHCl_3$/10% MeOH (at concentration of ~100 μg/platelet), in which the paper disks were dipped, dried under sterile conditions (flow box) and put on an agar plates inoculated with the Gram-positive bacteria; *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57), the Gram-negative *Escherichia coli*; the yeast, *Candida albicans*; and the fungi, *Mucor miehei* (Tü 284) along with the three microalgae; *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

The plates were incubated at 37 °C for bacteria (12 hours), 27 °C for fungi (24 hours), and 24-26 °C under day-light for micro-algae (96 hours). The diameter of the inhibition zones were measured by ruler.

6.7.4 Chemical and Pharmacological Screening

Samples of the extracts were separated on silica gel glass plates (10 × 20 cm) with two solvent systems $CHCl_3$/5% MeOH and $CHCl_3$/10% MeOH. After drying, the plates were photographed under UV light 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.

6.7.5 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. The suspension was aerated by bubbling air into the funnel and kept for 24 to 48 hours at room temperature. After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining unhatched eggs dropped. In order to get a higher density of larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. 30 to 40 shrimp larvae were transferred to a deep-well microtiter plate (wells diameter 1.8 cm, depth 2 cm) filled with 0.2 ml of salt water and the dead larvae counted (number N). A solution of 20 μg of the crude extract in 5 to 10 μL DMSO was added and the plate kept at r.t. in the dark. After 24 h, the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of *ca*. 0.5 ml methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in%. Each test row was accompanied by a blind sample with pure DMSO (number B) and a control sample with 1 μg/test actinomycin D. The mortality rate M was calculated using the following formula:

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

M = percent of the dead larvae after 24 h.
A = number of the dead larvae after 24 h.
B = average number of the dead larvae in the blind samples after 24 h
N = number of the dead larvae before starting of the test.
G = total number of brine shrimps

The mortality rate with actinomycin must be 100%.

6.8 Primary Screening

6.8.1 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 (+++).

7 Metabolites from selected bacterial strains

7.1 Terrestrial *Streptomyces* sp. GW 54/453

7.1.1 Pre-screening

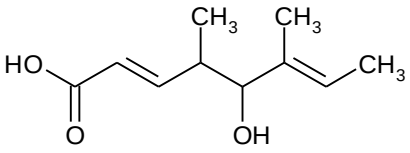
On agar plate, the terrestrial *Streptomyces* sp. GW 54/453 showed white mycelial colonies. A well-grown agar plate was used to inoculate 1L M_2 medium in Erlenmeyer flasks and was incubated 28°C for 96 hours. The resulting culture was extracted with ethyl acetate and the obtained crude extract was used for pre-screening. On TLC, it exhibited several UV absorbing zones which turned blue to green and yellow after spraying with anisaldehyde/sulphuric acid. The crude extract showed 100% cytotoxicity against *Artemia salina*. The antimicrobial screening of the crude extract was tabulated as follows:

Table 21: Antimicrobial activity of the crude extract of the strain GW 54/453

Test microorganisms	Inhibition zone ∅ [mm]
Staphylococcus aureus	12
Bacillus subtilis	11
Escherichia coli	14

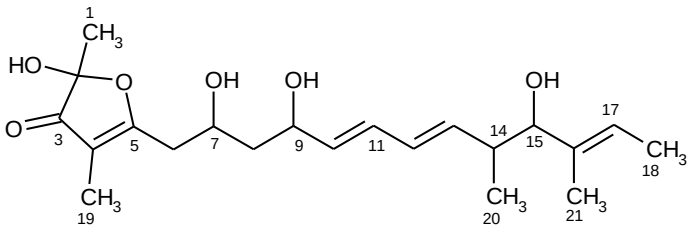
7.1.2 Fermentation and isolation

For large-scale fermentation of 20 L, the well-developed colonies of the agar plates were used to inoculate 80 Erlenmeyer flasks each containing 300 ml of M_2 medium. The fermentation was continued for 10 days at 28°C on a linear shaker (110 rpm). The obtained culture broth (20-liter) was mixed with ca. 1 kg diatomaceous earth and filtered through a filter press. The bacterial cells were extracted (4 times) with ethyl acetate and (3 times) with acetone to give crude extract A (1.5g). The filtrate was passed through XAD-16, and the adsorbent was then eluted by methanol, followed by concentration *in vacuo*. The remaining water residue was re-extracted with ethyl acetate to give extract B (1.7g). Both extracts A and B were identical from TLC, therefore they combined and chromatographed on silica gel using a CH_2Cl_2/MeOH gradient(CH_2Cl_2, 2 L; 3% MeOH, 1.5 L; 5% MeOH, 1 L; 7%MeOH, 0.5 L; 10% MeOH, 0.7 L; 20% MeOH, 0.5 L; 50% MeOH, 0.7 L; 100%MeOH, 0.5 L).The first fraction contained fats and fatty acids, then according to TLC visualisation, fraction II (50.4 mg) was selected and subjected to Sephadex LH-20 column using CH_2Cl_2/MeOH (3:2) and got three sub-fractions. Sub-fraction IIa was purified through silica gel column using CH_2CL_2/MeOH gradient and finally through Sephadex LH-20 column (Methanol) to afford actinofuranone C (**46**). Purification of sub-fraction IIb also by silica gel column using CH_2Cl_2/MeOH gradient and Sephadex IH-20 (Methanol) column delivered (2*E*,6*E*)-5-Hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (**45**). Finally, 2,5,furandimethyl alcohol (**50**) and 4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**49**) were obtained from subfraction IIc by purification through Sephadex LH-20 (Methanol) column and Sephadex LH-20 column using CH_2Cl_2/40%MeOH.



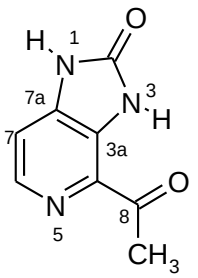
(2*E*,6*E*)-5-Hydroxy-4,6-dimethyl-octa-2,6-dienoic acid (45a): Colourless oil, 11.1 mg, UV non absorbing, dark blue colour with anisaldehyde/sulphuric acid which turned later to green. – *Rf* = 0.35 (CH_2Cl_2/7%MeOH). – $[\alpha]_D^{20}$ = - 3 (c =0.1, MeOH). – **^{1}H** and **^{13}C NMR**, see Table 1. – **H,H COSY** and **HMBC**, see Figure 8. – **EI MS** (70eV) *m/z* % 166 ($[M-H_2O]^+$, 5), 100 (32), 85 (100), 43 (36) – **DCIMS** *m/z* 202.2 $[M+NH_4]^+$ – **HRESIMS** *m/z* 207.09925 $[M+Na]^+$, (calcd. 207.09917 for $C_{10}H_{16}O_3Na$). **Methyl ester 45b**: **HRESIMS** *m/z* 221.11488 $[M+Na]^+$, (calcd. 221.11482 for $C_{11}H_{18}O_3Na$).

Actinofuranone C (46):



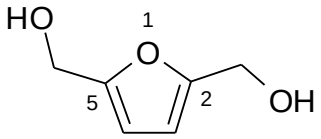
Light yellow solid, 4.2 mg, UV absorbing at 254 nm, dark blue colour with anisaldehyde/sulphuric acid which turned later to green. – R_f = 0.18 (CH_2Cl_2/ 7%MeOH). – **[α]$_D^{20}$** -12 (c = 0.1, MeOH). – **^{1}H** and **^{13}C NMR** see Table **2**. – **H,H COSY** and **HMBC**, see Figure 12. – **(+)-ESIMS:** *m/z* 782 ($[2M+Na]^+$, 58), 403 ($[M+Na]^+$, 100) – **HRESIMS** *m/z* 403.20912 $[M+Na]^+$ (calcd. 403.20911 for$C_{21}H_{32}O_6Na$).

4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (49):



Light yellow solid, 11.2 mg, UV-absorbing, turned as intensive yellow on spraying with anisaldehyde/sulphuric acid. – R_f = 0.66 (CH_2Cl_2/10% MeOH). - **^{1}H NMR** (DMSO-d_6, 300 MHz): δ = 11.05 (br s, 2H, 1,3-NH), δ = 8.22 (1H, d, *J* = 5.0 Hz, H-6), 7.19 (1H, d, *J* = 5.0 Hz, H-7), 2.61 (3H, s, H-9). - **^{13}C NMR** (DMSO-d_6, 125 MHz): δ = 201.4 (Cq-8), 157.6 (Cq-2), 142.7 (CH-6), 139.6 (C_q-7a), 135.0 (C_q-4), 128.5 (C_q-3a), 108.8 (CH-7), 25.9 (9-CH_3). – **EIMS** (70 eV): *m/z* 177 ($[M]^+$, 78), 149 (38), 135 (83), 107 (40), 43 (100). -HREIMS: *m/z* 177.0538 (calcd. 177.05340 for $C_8H_7N_3O_2$).

2,5-Furandimethanol (50):



Colourless oil, 5.4 mg, UV non-absorbing, brown colour with anisaldehyde/sulphuric acid. R_f = 0.28 (CH_2Cl_2/7% MeOH). $C_6H_8O_3$ (128.1), **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 6.22 (s, 2 H, 3,4-H), 4.60(s, 4 H, 2,5-CH_2), 2.45 (br s, 2H, 2,5-CH_2OH).

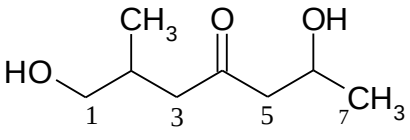
7.2 Terrestrial *Streptomyces* sp. Ank 245

7.2.1 Pre-screening

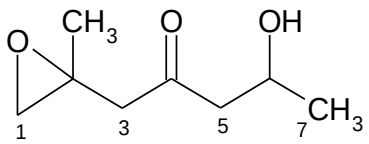
During the pre-screening, on TLC, the crude extract of the terrestrial Streptomyces showed several UV absorbing zones and interesting coloured bands from, violet, pink and blue after spraying with anisaldehyde/sulphuric acid. The crude extract also revealed low toxicity (11.2%) against *Artemia salina*, however, it did not show antimicrobial activity against many pathogenic microorganisms.

7.2.2 Fermentation, workup and isolation

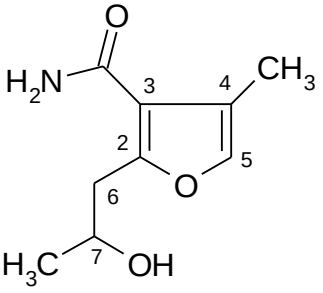
A well-developed agar subculture of the strain Ank 245 was used to inoculate 30L shaker culture on M_2 medium at 28°C. After seven days, the well grown culture broth was mixed with ca. 1 kg Celite and filtered under pressure to separate the water phase and biomass. The mycelium was extracted with 3 times with ethyl acetate and 3 times with acetone and water of the acetone extract again extracted with ethyl acetate and the solvent was evaporated under reduced pressure to get 1.6 g of crude extract. The water phase was passed through XAD-16 column and washed with methanol to get the extract. The water of the methanol extract was again extracted with ethyl acetate and after evaporation of the solvent, 3.2 g of the crude extract was obtained. Based on similar TLC pattern, both the mycelial and methanolic extract were combined together and fractionated by low pressure column chromatography using dichloromethane/methanol gradient as (CH_2Cl_2, 2 L; 2% MeOH, 1.5 L; 5% MeOH, 1.5 L; 7%MeOH, 1.0 L; 10% MeOH, 0.7 L; 20% MeOH, 0.5 L; 50% MeOH, 0.7 L; 100%MeOH, 0.5 L).. Under TLC control, four fractions were obtained, first fraction contained fats and fatty acids and was not further analysed. Fraction II was purified on silica gel column using cyclohexane: dichloromethane gradient followed by Sephadex LH-20 (CH_2Cl_2/40%MeOH) to obtain 2 known compounds; 4-vinyl-phenol (**54**) and 2-methoxy,4-vinyl-phenol (**55**). Fraction III was subjected to Sephadex LH-20 column using CH_2Cl_2/40%MeOH and yielded a new compound, 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**52**). Another new metabolite, 2-(2-hydroxy-proplyl-4-methyl-furan-3-carboxylic acid amide (**53**) was also obtained from fraction III after further purification with silica gel column using CH_2Cl_2/MeOH gradient and Sephadex LH-20 (methanol) column. Purification of fraction IV by silica gel column using CH_2Cl_2/MeOH gradient yielded a further new compound named 1,6-Dihydroxy-2-methyl-heptan-4-one (**51**).



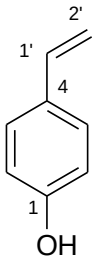
1,6-Dihydroxy-2-methyl-heptan-4-one (51): Colourless oil, 12.8 mg, UV non absorbing, dark green colour with anisaldehyde/sulphuric acid which turned later to blue. – R_f = 0.40 (CH_2Cl_2/7% MeOH). – $[\alpha]_D^{20}$ = -24 (c =0.1, MeOH). – **^{1}H** and **^{13}C NMR**, see Table 3. – **H,H COSY** and **HMBC**, see Figure 20. – **EI MS** (70 eV) *m/z* 143 ([M-H_2O]$^+$, 100), 99 (78), 45 (16), 43 (58), 41 (20), – **DCIMS** *m/z* 160 [M-H_2O+NH_4]$^+$– (+)-**ESIMS** *m/z* – 183 [M+Na]$^+$ – **HRESIMS** *m/z* 183.09910 [M+Na]$^+$, (calcd. 183.09917 for $C_8H_{16}O_3Na$).



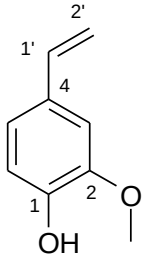
4-Hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (52): Yellow oil, 15.6 mg, UV non absorbing, orange colour with anisaldehyde /sulphuric acid which turned later to violet. – R_f = 0.22 (CH_2Cl_2/7% MeOH). – **[α]$_D^{20}$** = - 30 (c =0.1, MeOH). – **^{1}H** and **^{13}C NMR**, see Table 3. – **H,H COSY** and **HMBC**, see Figure 23. – **EIMS** (70 eV) *m/z* 145 (20), 140 ([M-H_2O]$^+$, 6), 127 (60), 101 (22), 85 (36), 75 (28), 69 (22), 57 (22), 45 (23), 43 (100), 41 (8), – **DCIMS** *m/z* 176 ([M+NH_4]$^+$, 100), 159 ([M+H]$^+$, 32)$^+$– **HRESIMS** *m/z* 181.08368 [M+Na]$^+$, (calcd. 181.08352 for $C_8H_{14}O_3Na$).



2-(2-Hydroxy-proplyl-4-methyl-furan-3-carboxylic acid amide (53): Colourless solid, 7.5 mg, UV absorbing at 254 nm, dark pink colour with anisaldehyde /sulphuric acid. – R_f = 0.33 (CH_2Cl_2/7% MeOH). – **[α]$_D^{20}$** = - 42 (c =0.1, MeOH). – **UV/VIS**: λ_{max} (log ε) = (MeOH): 241 (3.63); (MeOH/HCl): 242 (3.56); (MeOH/NaOH): 240 (3.62) nm. – **^{1}H** and **^{13}C NMR**, see Table 3. – **H,H COSY** and **HMBC**, see Figure 27. – **EI MS** (70eV) *m/z* 139 ([M-$CONH_2$]$^+$, 100), 122 (70), 94 (25), 43 (18) – **DCIMS** *m/z* 367 ([2M+H]$^+$, 10), 201 ([M+NH_4]$^+$, 38), 184 ([M+H]$^+$, 100)$^+$ – (+)-**ESIMS** *m/z* 388 ([2M+Na]$^+$, 100) 206 ([M+Na]$^+$, 44) – **HRESIMS** *m/z* 184.09679 [M+H]$^+$ and 206.07874 [M+Na]$^+$, (calcd. 184.09682 for $C_9H_{14}NO_3$, 206.07876 for $C_9H_{13}NO_3Na$).



4-Vinylphenol (54): Colourless oil, 1.2 mg, UV absorbing at 254 nm, turned pink by anisaldehyde/sulphuric acid, R_f = 0.34 (CH_2Cl_2) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.31 (d, ^3J = 8.7 Hz, 2H, 3/5-H), 6.79 (d, ^3J = 8.7 Hz, 2H, 2/6-H), 6.65 (dd, ^3J = 17.6, 10.7 Hz, 1H, 1'-H), 5.60 (dd, ^3J = 17.6, ^2J = 1.0 Hz, 1H, 2'-H_a), 5.12 (dd, ^3J = 10.9, ^2J = 1.0 Hz, 1H, 2'-H_b).



2-Methoxy-4-vinyl-phenol (55): Colourless oil, 1.0 mg, UV absorbing at 254 nm, turned violet by anisaldehyde/sulphuric acid, R_f = 0.51 (CH_2Cl_2) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 6.97 (d, ^3J = 1.3 Hz, 1H, 5-H), 6.90 (dd, ^3J = 8.7 Hz, ^2J = 1.3 Hz, 1H, 3-H), 6.83 (d, ^3J = 8.7 Hz, 1H, 2-H), 6.65 (dd, ^3J = 17.6, ^2J = 10.7 Hz, 1H, 1'-H), 5.60 (dd, ^3J = 17.6, ^2J = 1.0 Hz, 1H, 2'-H_a), 5.12 (dd, ^3J = 10.9,

^2J = 1.0 Hz, 1H, 2'-H_b), 3.92 (s, 3H, 2-OCH_3). – **EIMS** (70eV) *m/z* 150 ($[M]^+$, 86), 135 ($[M\text{-}CH_3]^+$, 70), 107 (46), 77 (67), 43 (72) – **DCIMS** *m/z* 151 ($[M+H]^+$, 100), 168 ($[M+NH_4]^+$.

7.3 Terrestrial *Streptomyces* sp. Ank 250

The agar plate of the terrestrial *Streptomyces* sp. Ank 250 was used to inoculate 1L culture of M_2 medium and incubated at 28 °C as the shaker culture for 7 days. The well grown culture broth was extracted with ethyl acetate and used for pre-screening.

7.3.1 Pre-screening

The terrestrial *Streptomyces* sp. Ank 250 was selected due to its interesting biological and chemical pre-screening. During the biological screening, the crude extract showed 43% activity against *Artemia salina* and its antimicrobial properties were shown in Table 22.

Table 22: Antimicrobial activity of the crude extract of the strain Ank 250

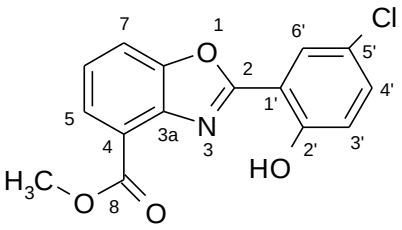
Test microorganisms	**Inhibition zone ∅ [mm]**
Staphylococcus aureus	21
Bacillus subtilis	17
Escherichia coli	15
Streptomyces viridochromogenes	14

In the chemical screening, a highly green fluorescent zone at 311nm and a UV absorbing zone at 254 nm were observed, which stained green with anisaldehyde/sulphuric acid.

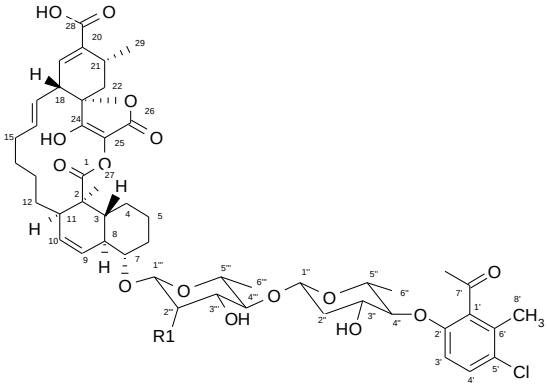
7.3.2 Fermentation and isolation

For large scale fermentation of 30 L, the well-developed agar plates were used to inoculate 100 of 1L Erlenmeyer flasks each containing 300 mL of M_2 medium and placed on linear shaker at 28°C. After 7 days, the culture was harvested and filtered using Celite though filter press to separate the water phase and cell mass. After extraction of mycelium and water phase with organic solvents, 2.5 g of dark brown coloured crude extract was obtained which was subjected to flash chromatography on silica gel column using CH_2Cl_2/MeOH gradient starting from CH_2Cl_2 (100%) up to

CH_2Cl_2/30%MeOH. By TLC visualization, three fractions were obtained; the first fraction contained fat and fatty acids. Fraction II was passed through Sephadex LH-20 (CH_2Cl_2/40%MeOH) column and got 2 sub fractions. The fraction IIa contained a highly green fluorescent zone at 366 nm in UV light, which was separated by PTLC and finally purified through Sephadex LH-20 (CH_2Cl_2/40%MeOH) column as yellow solid (3.6 mg), which was elucidated as a new natural metabolite; 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**). From fraction IIb, a known metabolite 2-phenyl ethanol (1.2 mg) was separated through Sephadex LH-20 (MeOH) column. MC-033 (**62**) was obtained from fraction III using PTLC and Sephadex LH-20 (CH_2Cl_2/40%MeOH) column.



2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (56): Yellow solid, 3.6 mg, green fluorescence at 366 nm, no colour reaction with anisaldehyde/sulphuric acid, R_f = 0.55 (CH_2Cl_2/3% MeOH) – **^{1}H** and **^{13}C NMR**, see Table **5**. – **H,H COSY** and **HMBC**, see Figure 34 and Figure 35. – (+)-**ESIMS** *m/z* 326 ([M+Na]$^+$, 38) – (-)-**ESIMS** *m/z* 302 ([M-H]$^-$, 36) – **HRESIMS** *m/z* 326.018942 [M+Na]$^+$, (calcd. 326.019057 for $C_{15}H_{10}NO_4ClNa$).



MC-033 (62): Colourless amorphous powder, 2.5 mg, UV absorbing at 256 nm, dark green colour with anisaldehyde/ sulphuric acid, *Rf* = 0.42 (CH_2Cl_2/5% MeOH), – **^{1}H** and **^{13}C NMR**, see Table 6. (-)-**ESIMS** *m/z* 939.5 ([M-H]$^-$, 100) – **HRESIMS** *m/z* 963.35353 [M+Na]$^+$, (calcd. 963.35353 for $C_{49}H_{61}ClO_{16}Na$).

7.4 Terrestrial *Streptomyces* sp. GW 51/426

7.4.1 Pre-screening

The terrestrial *Streptomyces* sp. GW 51/426 showed red aerial mycelium on agar plates. The well-grown agar plate was used to inoculate 1L M_2 medium in Erlenmeyer flask and incubated at 28 °C for 10 days. The resulting culture was extracted with ethyl acetate to get red coloured crude extract, which was used for biological

and chemical pre-screening. In the biological screening, the crude extract showed 95 % cytotoxicity against brine shrimp (*Artemia salina*). Its antimicrobial properties are shown in Table 23. During the chemical pre-screening, the crude extract exhibited on TLC, several yellow and red coloured bands which on treating with sodium hydroxide, turned to red as indication of *peri*-hydroxy quinones. It also showed a green fluorescent band and other UV absorbing zones, which showed interesting colour after spraying with anisaldehyde/sulphuric acid.

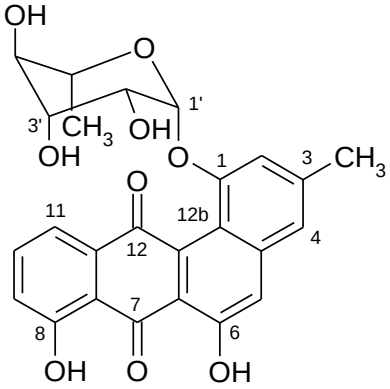
Table 23: Antimicrobial activity of the crude extract of the strain GW 51/426

Test microorganisms	**Inhibition zone ∅ [mm]**
Staphylococcus aureus	13
Escherichia coli	11
Mucor miehei	16
Aphanomyces cochlioides	22

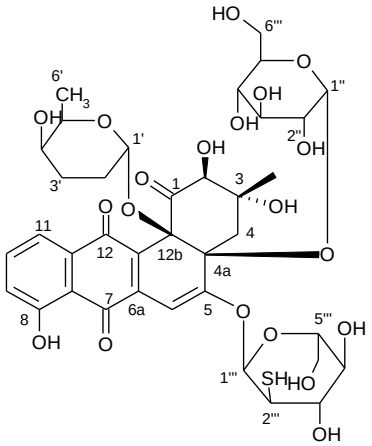
7.4.2 Fermentation and isolation

For large-scale fermentation, the terrestrial *Streptomyces* sp. GW 51/426 subculture was used to incubate 30L shaker culture using M_2 medium (pH 7.8 before sterilisation). The fermented broth was harvested after 10 days and the resulting red culture broth was subjected to filtration on Celite using pressure on filter press. The filtrate and mycelia were subjected to extraction separately by ethyl acetate and acetone. The combined organic phases were evaporated to dryness under vacuum, to give a dark reddish crude extract (6.4 g). This crude extract was subjected to column chromatography under monitoring by TLC (UV and spraying reagent) using dichloromethane-methanol gradient (CH_2Cl_2 100% 2L, CH_2Cl_2/3%MeOH 1.5 L, CH_2Cl_2/5%MeOH 1.5 L, CH_2Cl_2/7%MeOH 1.0 L, CH_2Cl_2/10%MeOH 1.0 L, CH_2Cl_2/15%MeOH 1.0 L, CH_2Cl_2/20%MeOH 0.7 L, CH_2Cl_2/30%MeOH 0.5 L, CH_2Cl_2/50%MeOH 0.5 L, MeOH 100% 0.5L) and obtained five fractions. The first fraction contained mostly fats and fatty acids. From second fraction, a new compound 2-(5-chloro-2-hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (**56**) was purified as green fluorescent yellow powder by PTLC and Sephadex LH-20 (CH_2Cl_2:40%MeOH) column. Fraction III was subjected to Sephadex LH-20 (MeOH) column and two subfractions were obtained. From fraction IIIa, a known diketopiperazine *cyclo*(Ala-Pro) (**69**) was obtained. A trivial compound indol-3-carboxylic acid was delivered from fraction IIIb by purifying with Sephadex LH-20 (MeOH) column. Fraction IVa revealed a new *peri*-hydroxy quinone (**64**) after puri-

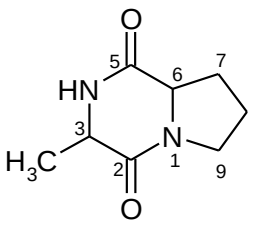
fication with PTLC and Sephadex LH-20 (CH_2Cl_2/40%MeOH) column. From fraction IVb, 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**49**) (1.4 mg) and uracil (2.0 mg) were purified. Moreover, fraction V showed a highly polar red colour spot on TLC which turned to green after spraying with anisaldehyde/sulphuric acid, which was purified through Sephadex LH-20 (MeOH) column as highly red rhodonocardin A (**68**).



Dehydrorabelomycin-1-O-α-L-rhamnopyranoside (64): Dark brown solid, 20.5 mg, UV absorbing at 254 nm, red colour with dilute NaOH, green colour reaction with anisaldehyde/sulphuric acid, R_f = 0.30 (CH_2Cl_2/7% MeOH) – **[α]$_D^{20}$** = + 34 (c = 0.05, MeOH). – **UV/VIS**: λ_{max} (log ε) = (MeOH): basic: 504 (3.70), 288 (4.04), 229 (4.33); acidic: 430 (3.73), 318 (4.02), 296 (3.99), 231 (4.37); neutral: 430 (3.75), 317 (4.05), 298 (4.03), 233 (4.37) – **^{1}H** and **^{13}C NMR**, see Table 7. – **H,H COSY** and **HMBC**, see Figure 42. – (+)-**ESIMS** *m/z* 955 ([2M+Na]$^+$, 43) 489 ([M+Na]$^+$, 100) – (-)-**ESIMS** *m/z* 931 ([2M-H]$^-$, 9) 465 ([M-H]$^-$, 100) – (+)-**ESIMS^2** *m/z* 489 ([M+Na]$^+$, 80), 342 ([M-rhamnose+Na]$^+$, 100) – **HRESIMS** *m/z* 467.133692 [M+H]$^+$, (calcd. 467.13366 for $C_{25}H_{23}O_9$).



Rhodonocardin A (68): Dark red solid, 105.7 mg, UV absorbing at 254 nm, green colour reaction with anisaldehyde/sulphuric acid, R_f = 0.30 (CH_2Cl_2/30% MeOH) – **[α]$_D^{20}$** = + 67.4 (c =0.04, MeOH). – **^{1}H** and **^{13}C NMR**, see Table 8. – (+)-**ESIMS** *m/z* 865 ([M+Na]$^+$, 85) – (-)-**ESIMS** *m/z* 931 ([M-H]$^-$, 80) – **HRESIMS** *m/z* 865.21910 [M+Na]$^+$, (calcd. 865.21952 for $C_{37}H_{46}O_{20}SNa$).



***Cyclo*-(Ala-Pro) (69):** Colourless crystals (1.5 mg), UV absorbing at 254 nm, green fluorescence at 366 nm, blue colour reaction with anisaldehyde/sulphuric acid, R_f = 0.34 (CH_2Cl_2/10 MeOH), – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 6.70 (br s, , 1H, NH), δ 4.09 (m, 2H, 3-H, 6-H), 3.55 (m, 1H, 9-H$_a$), 3.53 (m, 1H, 9-H$_b$), 2.30 (m, 1H, 7-H$_a$), 2.07 (m, 1H, 7-H$_b$), 1.98 (m, 1H, 8-H$_a$), 1.87 (m, 8-H$_b$), 1.43 (d, ^3J = 6.8 Hz, 3H, 3-CH$_3$) – **^{13}C**

NMR ($CDCl_3$, 125 MHz): δ = 170.3 (C_q, CO-5), 166.2 (C_q, CO-2), 59.3 (CH- C6), 51.2 (CH, C-3), 45.5 (CH_2, C-9), 28.2 (CH_2, C-7), 22.8 (CH_2, C-8), 16.0 (3-CH_3) – **DCI MS** *m/z* 354 ($[2M+ NH_4]^+$, 20), 337 ($[2M+ H]^+$, 58), 203 ($[M+ NH_3+NH_4]^+$, 6), 186 ($[M+ NH_4]^+$, 100), 169 ($[M+ H]^+$, 50) – **EIMS** *m/z* 168 ([M]+, 50), 140 (6), 125 (22), 112 (10), 97 (37), 86 (100), 70 (100), 69 (32), 55 (13), 44 (52), 41 (37) – **(+)-HRESIMS** 191.07904 $[M+Na]^+$ (calc: 191.07910 for $C_8H_{12}N_2O_2Na$), 169.09716, $[M+H]^+$ (calc: 169.09715 for $C_8H_{13}N_2O_2$).

7.5 Terrestrial *Streptomyces* sp. WO 463

7.5.1 Pre-screening

The terrestrial *Streptomyces* sp. WO 463 was selected due to its profound activity against *Mucor miehei*, plant pathogen fungi (*Rhizoctonia solani* and *Aphanomyces cochlioides*) micro green algae (*Chlorella vulgaris*) and bacterium *Streptomyces viridochromogenes* (*Tü* 57) (Table 24). It also showed 100% cytotoxicity against *Artemia salina*. Moreover, on TLC, its crude extract showed many UV absorbing zones, which turned to green with anisaldehyde/sulphuric acid.

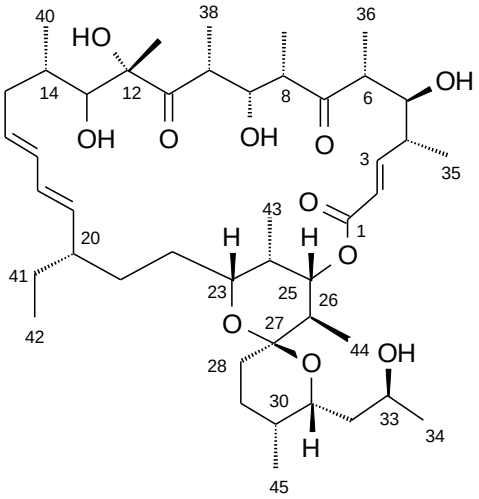
Table 24: Antimicrobial activity of the crude extract of the strain WO 463

Test microorganisms	Inhibition zone ∅ [mm]
Streptomyces viridochromogenes (*Tü* 57)	20
Mucor miehei	15
Rhizoctonia solani	14
Aphanomyces cochlioides	18
Chlorella vulgaris	10

7.5.2 Bacterial culturing and isolation

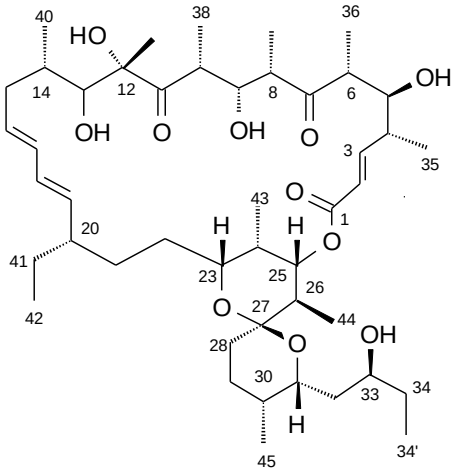
The well-developed white coloured agar colonies were used to inoculate 100 of 1L Erlenmeyer flasks, each containing 300 ml of M_2 medium (pH 7.8). After incubation at 28 °C on linear shaker for 7 days, the well grown culture was harvested and filtered through filter press using ca. 1.2 kg Celite. The biomass was extracted three times with ethyl acetate and similarly by acetone. The filtrate was passed though XAD-16 column, washed with water followed by methanol. The resulting methanolic extract was evaporated under vacuum, and water of methanolic extract was again extracted with ethyl acetate. Based on the similar TLC, both extracts were combined and was subjected to low pressure silica gel column chromatography and eluted with

CH_2Cl_2 followed by stepwise addition of methanol up to 100% methanol and obtained five fractions. Fraction II was subjected to Sephadex LH-20 (MeOH) column, and obtained Niax (5.0 mg), and subfraction IIa, which was subjected to silica gel column using CH_2Cl_2/MeOH gradient and obtained a mixture of oligomycin A (**70**) and F (**71**), which were further purified by semi preparative reversed phase HPLC (RP_{18}, MeOH/H_2O, 85/15). Fraction III was firstly subjected to Sephadex LH-20 (MeOH) column, followed by PTLC and Sephadex LH-20 (CH_2Cl_2/40%MeOH) column to deliver thymine (**76**) and a new primary metabolite; 5'-methoxy-6-(N,N-dimethyl)-adenosine (**73**). Moreover, fraction IV was purified by PTLC and Sephadex LH-20 (CH_2Cl_2/40%MeOH) column to obtain a new metabolite; 4-glucosyl-β-pyranone (**74**) and 2'-deoxy-adenosine (**77**). Fraction V was also purified on PTLC and Sephadex LH-20 (MeOH) column to deliver adenosine-methyl-sulphoxide (**78**).

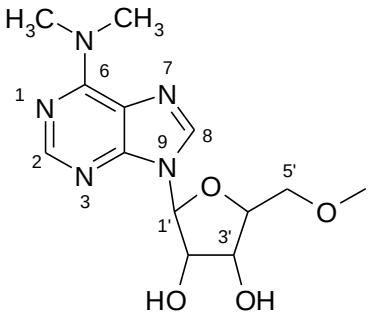


Oligomycin A (70): White powder (2.3mg), UV absorbing at 254 nm, reddish brown which later turned to green with anisaldehyde/sulphuric acid on heating. R_f = 0.41 (CH_2Cl_2/5% MeOH), – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 6.63 (dd, , ^3J = 15.7, 10.1 Hz, 1H, 3-H), 6.00 (ddd, ^5J = 14.9, ^3J = 10.9, ^2J = 3.3, 1H, 17-H), 5.89 (dd, ^3J = 14.4, ^2J = 4.0 Hz, 1H, 18-H), 5.81 (d, ^3J = 15.7, 1H, 2-H), 5.43 (dt, ^3J = 10.3, ^2J = 3.9, 1H, 16-H), 5.22 (dd, ^3J = 14.5, ^2J = 9.6, 1H, 19-H), 4.93 (dd, ^3J = 11.9, ^4J = 4.9, 1H, 25-H), 4.02 (m, 1H, 31-H), 3.96 (m, 1H, 13-H), 3.95 (dd, ^3J = 9.6, ^4J = 2.7, 1H, 9-H), 3.77 (d, ^3J = 9.1, 2H, 5-H, 33-H), 3.76 (m, 1H, 23-H), 3.60 (m, 1H, 8-H), 2.71, d, ^3J = 7.4, 1H, 10-H), 2.70 (m, 1H, 6-H), 2.38 (m, 1H, 4-H), 2.14 (m, 1H, 15_a-H), 2.10 (m, 1H, 24-H), 1.95 (m, 1H, 15_b-H), 1.90 (m, 1H, 28_a-H), 1.80 (m, 1H, 26-H), 1.62 (m, 1H, 32_a-H), 1.57 (m, 3H, CH_2-22, 30-H), 1.39 (m, 2H, CH_2-29), 1.37 (m, 3H, H-14, CH_2-21), 1.35 (d, ^3J = 6.9, 2H, CH_2-41), 1.25 (m, 1H, 28_b-H), 1.24 (m, 1H. 32_b-H), 1.23 (d, ^3J = 6.2, 3H, CH_3-34), 1.16 (d, ^3J = 6.5, 3H, CH_3-35), 1.11 (s, 3H, CH_3-39), 1.07 (d, ^3J = 3.6, 3H, CH_3-36), 1.03 (d, ^3J = 4.0, 3H, CH_3-37), 1.00 (d, ^3J = 3.8, 3H, CH_3-38), 0.98 (d, ^3J = 6.7, 3H, CH_3-40), 0.95 (d, ^3J = 6.7, 3H, CH_3-44), 0.89 (d, ^3J = 6.9, 3H, CH_3-45), 0.83 (d, ^3J = 2.6, 3H, CH_3-43), 0.79 (t, ^3J = 6.7, 3H, CH_3-42) – **^{13}C NMR** see Table 15. – (+)-**ESIMS** *m/z* 813 ($[M+Na]^+$, 85) – (-)-**ESIMS** *m/z* 789 ($[M-H]^-$, 28) – **HRESIMS** *m/z* 791.52968

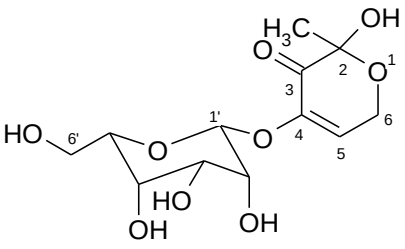
$[M+H]^+$, (calcd. 791.53038 for $C_{45}H_{75}O_{11}$), *m/z* 813.51179 $[M+Na]^+$, (calcd. 813.51233 for $C_{45}H_{74}O_{11}Na$).



Oligomycin F (71): White powder (2.5 mg), UV absorbing at 254 nm, reddish brown which later turned to green with anisaldehyde/sulphuric acid on heating. R_f = 0.41 (CH_2Cl_2/5% MeOH), **^{1}H** and **^{13}C NMR**, see Table 10. – (+)-**ESIMS** *m/z* 827 ($[M+Na]^+$, 83) – (-)-**ESIMS** *m/z* 803 ($[M-H]^-$, 15) – **HRESIMS** *m/z* 805.54626 $[M+H]^+$, (calcd. 805.54603 for $C_{46}H_{77}O_{11}$), *m/z* 827.52747 $[M+Na]^+$, (calcd. 827.52798 for $C_{46}H_{76}O_{11}Na$).



5'-Methoxy-6-(N,N-dimethyl)-adenosine (73): Colorless solid (2.5 mg), UV absorbing at 254 nm, green with anisaldehyde/sulphuric acid on heating. R_f = 0.36 (CH_2Cl_2/7% MeOH), – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ = 8.28 (s, 1H, H-8), 8.22 (s, 1H, H-2), 5.93 (d, ^3J = 5.0 Hz, 1H, H-1'), 4.54 (t, ^3J = 5.0 Hz, 1H, H-2'), 4.15 (t, ^3J = 4.7 Hz, 1H, H-3'), 4.02 (q, ^3J = 3.9 Hz, 1H, H-4'), 3.59 (dd, ^2J = 10.8 Hz, ^3J = 3.7 Hz, 1H, H_a-5'), 3.52 (dd, ^2J = 10.8 Hz, ^3J = 5.0 Hz, 1H, H_b-5'), 3.46 (2 br, 3 H, NMe_2), 3.28 (s, 3 H, OMe). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ = 154.1 (C-6), 151.7 (CH-2), 150.1 (C-4), 137.7 (CH-8), 119.3 (C-5), 87.2 (CH-1'), 82.8 (CH-4'), 73.4 (CH-2'), 72.2 (CH_2-5'), 70.3 (CH-3'), 58.5 (OCH_3-5'), 37.5 ($N(CH_3)_2$) – **H,H COSY** and **HMBC**, see Figure 57. (+)-**ESIMS** *m/z* 310 ($[M+H]^+$, 9) 641 ($[2M+Na]^+$, 85) – (-)-**ESIMS** *m/z* 308 ($[M-H]^-$, 86) 617 ($[2M-H]^-$, 61) – **HRESIMS** *m/z* 310.15085 $[M+H]^+$, (calcd. 310.15098 for $C_{13}H_{20}N_5O_4$).



4-*α*-L-Glucosyl-β-pyranone (74): Colourless oil (6.7 mg), UV absorbing at 254 nm, green with anisaldehyde/sulphuric acid R_f = 0.25 (CH_2Cl_2/10% MeOH), **^{1}H NMR** (CD_3OD, 300 MHz): δ = 6.61 (m, 1H, H-5), 5.28 (d, ^3J = 3.6 Hz 1H, H-1'), 4.70 (dd, ^2J =2.2, ^3J = 7.8 Hz; m,

1H, H_a-6), 4.29 (m, 1H, H_b-6), 3.80 (m, 1H, H-3'), 3.78 (m, 1H, H_a-6'), 3.66 (m, 1H, H_b-6'), 3.52 (m, 1H, H-4'), 3.47 (m, 1H, H-2'), 3.33 (m, 1H, H-5'), 1.46 (s, 3H, 2-CH_3) – **^{13}C NMR** (CD_3OD, 125 MHz): δ = 190.4 (C-3), 144.9 (C-4), 125.0 (CH-5), 99.4 (CH-1'), 97.5 (C-2), 74.8 (CH-3'), 74.7 (CH-4'), 73.5 (CH-2'), 71.4 (CH-5'), 62.4 (CH_2-6'), 60.2 (CH_2-6), 23.0 (2-CH_3) – **H,H COSY** and **HMBC**, see Figure 62 – (+)-**ESIMS** *m/z* 329 ($[M+Na]^+$, 18) 635 ($[2M+Na]^+$, 100) – (-)-**ESIMS** *m/z* 308 ($[M-H]^-$, 59) 611 ($[2M-H]^-$, 100) – **HRESIMS** *m/z* 329.08462 $[M+Na]^+$, (calcd. 329.08430 for $C_{12}H_{18}O_9Na$).

7.6 Terrestrial *Streptomyces* sp. Ank 351

7.6.1 Pre-screening

The terrestrial strain Ank 351 formed a white aerial mycelium on agar plates containing M_2 medium after 4 days of incubation at 28°C. This agar culture was used to inoculate 1L of M_2 medium in four Erlenmeyer flasks and after incubation at 28°C on linear shaker for 7 days resulted in dark brown culture. This culture was filtered under vacuum and extracted with ethyl acetate. The resulting culture was used for pre-screening. In antimicrobial assay, the crude extract showed promising antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus* and *Streptomyces viridochromogenes* (*Tü* 57). It also exhibited high activity against plant pathogen fungus *Pythium ultimum* and 100% cytotoxicity against *Artemia salina*. The antimicrobial activities were tabulated in

Moreover, On TLC, the crude extract exhibited several UV absorbing zones which turned to reddish brown, violet and green with anisaldehyde/sulphuric acid.

Table 24: Antimicrobial activity of the crude extract of the strain Ank 351

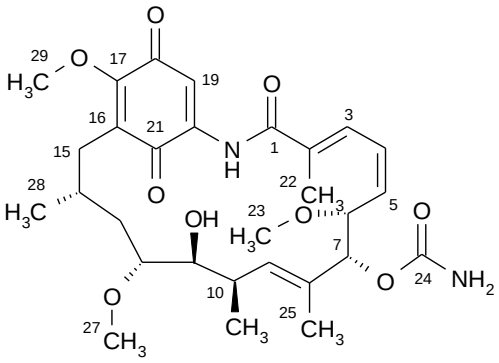
Test microorganisms	**Inhibition zone ∅ [mm]**
Bacillus subtilis	14
Staphylococcus aureus	14
Streptomyces viridochmogenus (*Tü* 57)	11
Pythium ultimum	18

7.6.2 Fermentation, workup and isolation

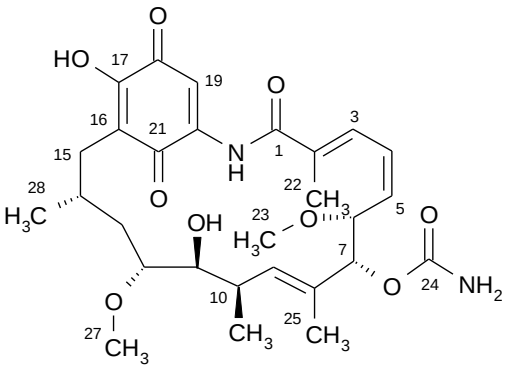
For large-scale fermentation, the well grown agar plates were used to inoculate 30L of M_2 medium in Erlenmeyer flasks each containing 300 ml, and placed on the linear shaker at 28 °C. After 7 days, the well-grown culture was harvested, filtered

through filter press and separately extracted with ethyl acetate (water phase) and acetone (mycelium). The elute of the water phase from XAD-16 column and mycelium extract were combined based on similar TLC and yielded 7.3 g extract was sequentially separated by column chromatography on silica gel, Sephadex LH-20 and PTLC.

The first fraction contained fat and separation of fraction II on silica gel (cyclohexane/CH_2Cl_2) followed by Sephadex LH-20 column yielded nigericin (**84**). Fraction III was firstly subjected to Sephadex LH-20 (MeOH) column and the resulting subfraction was further purified by PTLC and Sephadex LH-20 (MeOH) column to get geldanamycin (**79**) and 17-*O*-demethyl-geldanamycin (**82**). From purification of fraction IV by Sephadex LH-20 (MeOH) column and silica gel column using CH_2Cl_2/MeOH gradient, 11-*O*-monomethyl-elaiophylin (**87**) and 11,11'-*O*-dimethyl-elaiophylin (**86**) were obtained. Washing of fraction V with methanol yielded nocardamine (**88**) as white precipitated amorphous solid.

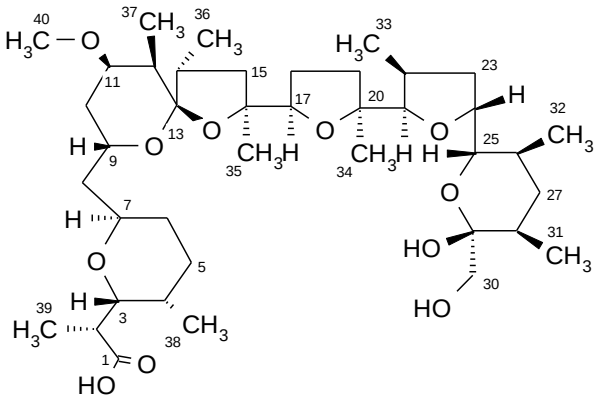


Geldanamycin (79): Yellow crystalline solid (3.3 mg), UV absorbing at 254 nm, reddish brown with anisaldehyde/sulphuric acid, R_f = 0.28 (CH_2Cl_2/3% MeOH), **– ^{1}H** and **^{13}C NMR** see Table **12**. **–** (+)-**ESIMS** *m/z* 583 ($[M+Na]^+$, 51), 1143 ($[M+2Na]^+$, 84), **– HRESIMS** *m/z* 586.26239 $[M+Na]^+$, (calcd. 583.26260 for $C_{29}H_{40}N_2O_9Na$).



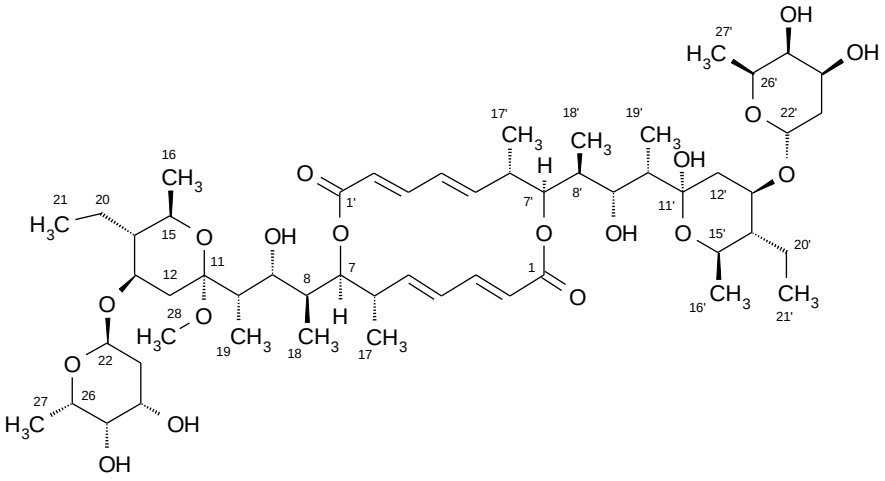
17-*O*-demethyl-geldanamycin (82): Dark purple powder (5.1mg), UV absorbing at 254 nm, brown with anisaldehyde/sulphuric acid, R_f = 0.29 (CH_2Cl_2/5% MeOH), **– ^{1}H NMR** (300 MHz, CD_3OD): δ = 7.14 (d, ^3J = 11.6 Hz, 1H, 3-H), 6.93 (s, 1H, 19-H), 6.66 (t, ^3J = 11.4 Hz, 1H, 4-H), 5.84 (t, ^3J = 10.2 Hz, 1H, 5-H), 5.74 (d, ^3J = 9.7 Hz, 1H, 9-H), 5.02 (s, 1H, 7-H), 4.44 (d, 1H, 6-H), 3.59 (m, 1H, 11-H), 3.40 (d, 8.4 Hz, 1H, 12-H), 3.33 (s, 3H, 23-OCH_3), 3.26 (s, 3H, 27-OCH_3), 2.74 (m, 1H, 10-H), 2.34 (m, 2H, 15-CH_2), 2.00 (s,

3H, 22-CH_3), 1.77 (m, 1H, 14-H), 1.76 (s, 3H, 25-CH_3), 1.69 (m, 2H, 13-CH_2), 0.93 (d, ^3J = 6.8 Hz, 6H, 26-CH_3, 28-CH_3) – **^{13}C NMR** (300 MHz, CD_3OD): δ = 177.9 (C-21), 172.9 (C-18), 170.3 (C-1), 159.1 (C-17,24), 145.0 (C-20), 137.1 (C-5), 135.9 (C-2), 134.4 (C-8), 134.1 (C-9), 128.6 (C-3), 127.6 (C-4), 115.7 (C-16), 108.6 (C-19), 83.2 (C-12), 82.8 (C-7), 82.7 (C-6), 74.0 (C-11), 57.4 (C-27), 56.9 (23-C), 36.0 (C-13), 33.9 (C-15), 33.7 (C-10), 29.4 (C-14), 23.7 (C-28), 13.1 (C-25), 12.9 (C-26), 12.5 (C-22) – (+)-**ESIMS** *m/z* 569 ([M+Na]$^+$, 84), 1115 ([2M+Na]$^+$, 77) – (-)-**ESIMS** *m/z* 545 ([M-H]$^-$, 84) – **HRESIMS** *m/z* 564.29139 [M+NH_4]$^+$, (calcd. 564.29155 for $C_{28}H_{42}N_3O_9$), 569.24678 [M+Na]$^+$, (calcd. 583.26260 for $C_{28}H_{38}N_2O_9Na$).

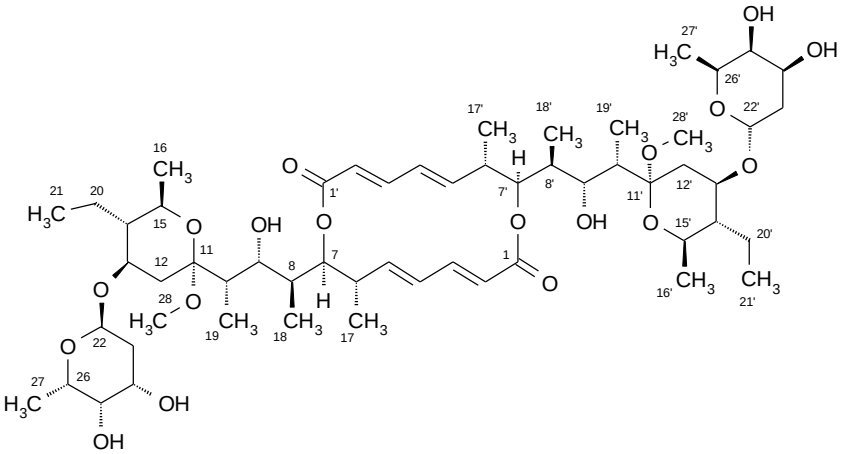


Nigericin (84): Pale yellow powder (40.5 mg), UV non-absorbing, dark brown with anisaldehyde/sulphuric acid, R_f = 0.57 (CH_2Cl_2/3% MeOH), – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.25 (s, 3H, 1, 29, 30-OH), 4.32 (t, ^3J = 8.5 Hz, 1H, 17-H), 4.13 (m, 1H, 3-H), 4.04 (m, 1H, 7-H), 3.99 (m, 1H, 9-H), 3.97 (d, ^3J = 3.6 Hz, 1H, 21-H), 3.91 (dd, ^3J = 10.2 Hz, ^2J = 2.2 Hz, 1H, 25-H), 3.72 (dd, ^3J = 10.2 Hz, ^2J = 2.0 Hz, 1H, 24-H), 3.48 (m, 1H, 11-H), 3.44 (d, 1H, ^3J = 2.2 Hz, 1H, 30_a-H), 3.40 (m, 1H, 30_b-H), 3.30 (s, 3H, 40-OCH_3), 2.45 (ddd, ^3J = 13.4 Hz, ^2J = 4.4 Hz, 1H, 2-H), 2.26 (m, 1H, 22-H), 2.20 (m, 3H, 28-H, 15-CH_2), 2.14 (m, 1H, 4-H), 1.96 (m, 1H, 10_a-H), 1.95 (m, 1H, 12-H), 1.93 (m, 1H, 5_a-H), 1,91 (m, 10_b-H), 1.80 (m, 2H, 19-CH_2), 1.71 (m, 3H, 8-CH_2, 26-H), 1.68 (m, 2H, 27-CH_2), 1.66 (m, 2H, 23-CH_2), 1,59 (m, 1H, 5_b-H), 1.48 (m, 3H, 6-CH_2, 14-H), 1.45 (m, 2H, 18-CH_2), 1.37 (s, 3H, 35-CH_3), 1.07 (s, 3H, 34-CH_3), 1,00 (d, ^3J = 7.0 Hz, 6H, 38, 39-CH_3), 0.89 (d, ^3J = 7.0 Hz, 3H, 37-CH_3), 0.86 (d, ^3J = 7.0 Hz, 3H, 36-CH_3), 0.84 (d, ^3J = 6.7 Hz, 3H, 32-CH_3), 0.82 (d, ^3J = 6.7 Hz, 3H, 33-CH_3), 0.79 (d, ^3J = 6.8 Hz, 3H, 31-CH_3) – **^{13}C NMR** see Table 13. – (+)-**ESIMS** *m/z* (%) 747 ([M+Na]$^+$, 86) – (-)-**ESIMS** *m/z* (%) 723 ([M-H]$^-$, 88) – **HRESIMS** *m/z* (%) 742.50985 [M+NH_4]$^+$, (calcd. 742.50998 for $C_{40}H_{72}NO_{11}$), 747.46522 [M+Na]$^+$, (calcd. 747.46538 for $C_{40}H_{68}O_{11}Na$).

11,11'-*O*-Dimethyle-laiophylin (86): Colourless crystalline solid (20.7 mg), UV absorbing at 254 nm, violet which later turned to green with anisaldehyde/sulphuric acid, R_f = 0.33 (CH_2Cl_2/7% MeOH) – **^{1}H** and **^{13}C NMR** see **Table 14**. – (+)-**ESIMS** *m/z* (%) 549 ([M/2+Na]$^+$, 22), 1075 ([M+Na]$^+$, 94), – **HRESIMS** *m/z* 1075.61722 [M+Na]$^+$, (calcd. 1075.61758 for $C_{56}H_{92}O_{18}Na$).

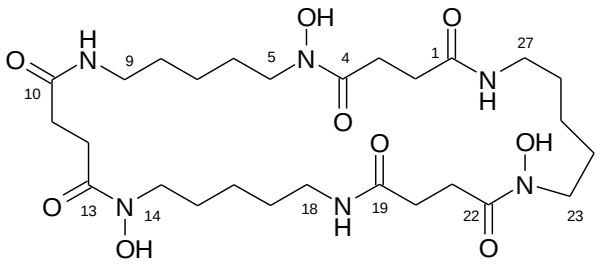


11-O-Monomethyle-laioph-ylin (87): Colourless crystalline solid (34.7 mg), UV absorbing at 254 nm, violet which later turned to green with anisalde-hyde/sulphur-ic acid, R_f = 0.33 (CH_2Cl_2/7% MeOH) – **^{1}H NMR** (300 MHz, CD_3OD): δ = 6.93 (dd, , ^3J = 15.2 Hz, ^2J = 11.1 Hz, 2H, 3, 3'-H), 6.17 (dd, , ^3J = 14.9 Hz, ^2J = 11.2 Hz, 2H, 4, 4'-H), 5.74 (d, , ^3J = 15.1 Hz, 2H, 2, 2'-H), 5.65 (dd, ^3J = 15.2 Hz, ^2J = 9.8 Hz, 2H, 5, 5'-H), 5.11 (d, ^3J = 9.9 Hz, 2H, 22, 22'-H), 5.03 (d, ^3J = 8.2 Hz, 2H, 7, 7'-H), 4.02 (d, ^3J = 10.1 Hz, 2H, 13, 13'-H), 3.90 (dd, ^3J = 11.6 Hz, ^2J = 5.6 Hz, 4H, 24, 24', 26, 26'-H), 3.53 (m, 2H, 25, 25'-H), 3.48 (m, 2H, 15, 15'-H), 3.04 (s, 3H, 11-OCH_3), 2.57 (m, 2H, 6, 6'), 2.34 (dd, ^3J = 12.2 Hz, ^2J = 4.2 Hz, 2H, 12_a, $12'_a$, -H), 2.05 (m, 2H, 8, 8'-H), 1.92 (m, 2H, 23_a, 23_a'-H), 1.70 (m, 2H, 10, 10'-H), 1.63 (m, 4H, 20_a, 20_a', 23_b, 23_b'-H), 1.47 (m, 2H, 20_b, 20_b'-H), 1.30 (m, 2H, 14, 14'-H), 1.19 (d, ^3J = 6.5 Hz, 6H, 27, 27'-H), 1.18 (d, ^3J = 6.5 Hz, 6H, 16, 16'-H), 1.12 (m, 2H, 12_b, 12_b'-H), 1.04 (d, ^3J = 6.5 Hz, 6H, 17, 17'-H), 0,97 (d, ^3J = 7.1 Hz, 6H, 19, 19'-H),



0,93 (d, ^3J = 7.1 Hz, 6H, 18, 18'-H), 0.86 (d, ^3J = 7.1 Hz, 6H, 21, 21'-H) – **^{13}C NMR** (125 MHz, CD_3OD): δ = 170.4 (C-1), 170.2 (C-1'), 146.9 (C-3, 3'), 146.2 (C-5), 146.1 (C-5'), 132.7 (C-4), 132.6 (C-4'), 122.6 (C-2), 122.5 (C-2'), 104.5 (C-11), 100.8 (C-11'), 94.8 (C-22, 22'), 78.4 (C-7), 78.2 (C-7'), 72.3 (C-25, 25'), 71.8 (C-9), 71.1 (C-9'), 70.9 (C-13), 70.8 (C-13'), 69.0 (C-15), 68.1 (C-15'), 68.0 (C-26, 26'), 67.9 (C-24), 66.9 (C-24'), 49.8 (C-14, 14'), 46.8 (C-28, 28'), 43.9 (C-10,10'), 42.7 (C-6, 6'), 38.9 (C-12), 38.3 (C-12'), 37.7 (C-8, 8'), 33.7 (C-23, 23'), 20.3 (C-20), 20.2 (C-20'), 19.5 (C-16), 19.3 (C-16'), 17.3 (C-27, 27'), 15.8 (C-17, 17'), 10.3 (C-18, 18'), 9.6 (C-21, 21'), 7.6 (C-19), 7.1 (C-19') – (+)-**ESIMS** *m/z* (%) 542 ([M/2+Na]$^+$, 21), 1061 ([M+Na]$^+$, 100)

Nocardamine (88): Colourless powder (50.4 mg), UV inactive, turned to pale violet with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.29 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 9.45 (s, 3 H, OH), 7.60 (t, ^3J = 4.9 Hz, 3 H, 9, 18, 27-NH), 3.38 (t, ^3J = 6.6 Hz, 6 H, 5, 14, 23-CH_2), 2.92 (q, ^3J = 6.3 Hz, 6H, 9,18,27-CH_2), 2.51 (m, 6H, 2,11,20-CH_2), 2.21 (t, ^3J = 7.0 Hz, 6H, 3, 12,21-CH_2), 1.42 (t, ^3J = 6.9 Hz, 6H, 6,15,24-CH_2), 1.28 (q, ^3J = 7.2 Hz, 6H, 8,17,26-CH_2), 1.13 (m, 6 H, 7,16,25-CH_2). – (+)-**ESIMS** *m/z* 623 ([M + Na]$^+$, 100). – (-)-**ESIMS** *m/z* 599 ([M - H]$^-$, 100).



7.7 Terrestrial *Streptomyces* sp. Ank 202

7.7.1 Pre-screening

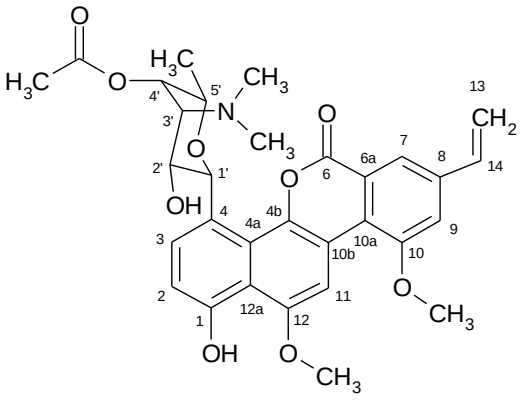
During the pre-screening, the crude extract of the terrestrial *Streptomyces* sp. Ank 202 showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Streptomyces viridochromogenes* (Tü 57) (Table 25). It also exhibited 100% cytotoxicity *Artemia salina*. On TLC, it showed a highly green fluorescent band, which turned to green with anisaldehyde/sulphuric acid, and several UV absorbing zones which turned to yellow and green with anisaldehyde/sulphuric acid.

Table 25: Antimicrobial activity of the crude extract of the strain Ank 202

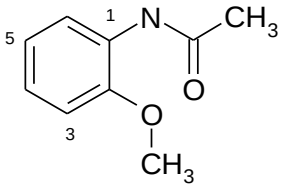
Test microorganisms	**Inhibition zone ∅ [mm]**
Staphylococcus aureus	11
Bacillus subtilis	15
Escherichia coli	15
Streptomyces viridochromogenes (Tü 57)	20

7.7.2 Fermentation and isolation

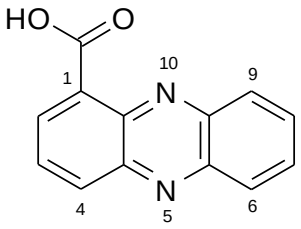
The terrestrial *Streptomyces* sp. Ank 202 showed white mycelium on agar plate. The well-grown agar culture was used to inoculate 100 of 1L Erlenmeyer flasks each containing 300 ml of M_2 medium and placed on a linear shaker at 28°C for 7 days. After harvesting the culture, it was filtered through filter press to separate the mycelium and water phase. The mycelium was extracted three times with ethyl acetate and three times with acetone. The water phase was subjected to XAD-column and extracted with methanol. As the chemical composition of both organic extracts from mycelium and water phase was similar on TLC, they were combined and subjected to silica gel column chromatography (CH_2Cl_2/MeOH). Under TLC monitoring, four fractions were obtained. The first fraction contained fat and fraction II was subjected to silica gel column (CH_2Cl_2/MeOH) followed by PTLC and Sephadex LH-20 column to yield N-(2-methoxy-phenyl)-acetamide (**93**), phenazine-1-carboxylic acid (**94**) and 1-carboxy-methylphenazine (**95**). The purification of fraction III by Sephadex LH-20 column (CH_2Cl_2/40%MeOH) and PTLC revealed ravidomycin (**89**). Fraction V was purified through Sephadex LH-20 column (MeOH) followed by PTLC to obtain uracil (3.5 mg) and N^6-N^6- Dimethyl-adenosine (**96**).



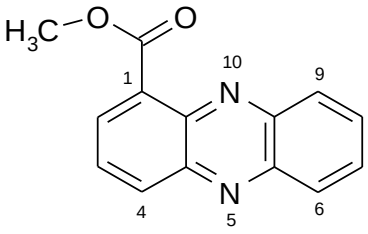
Ravidomycin (89): Bright yellow soild (5.0 mg), UV absorbing at 254 nm and green fluorescent at 311 nm. Green with anisaldehyde/sulphuric acid, R_f = 0.29 (CH_2Cl_2/5% MeOH) – **^{1}H NMR and ^{13}C NMR** see Table 15. – (+)-**ESIMS** *m/z* (%) 564 ($[M+H]^+$, 66), 1127 ($[2M+H]^+$, 42), – **HRESIMS** *m/z* 564.22268 $[M+H]^+$, (calcd. 564.22280 for $C_{31}H_{34}NO_9$).



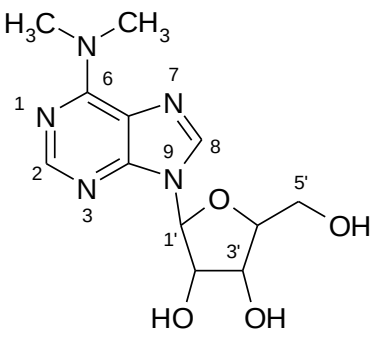
N-(2-Methoxy-phenyl)-acetamide (93): Colourless solid, UV absorbing at 254 nm, no colour reaction with anisaldehyde/sulphuric acid. R_f = 0.41(CH_2Cl_2/3% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.32 (dd, ^3J = 7.8 Hz, ^4J = 1.5 Hz, 1H, 6-H), 7.76 (br s, 1H, NH), 7.00 (dt, ^3J = 7.6 Hz, ^4J = 1.6 Hz, 1H, 4-H), 6.91 (dt, ^3J = 7.8 Hz, ^4J = 1.5 Hz, 1H, 5-H), 6.83 (dd, ^3J = 7.9 Hz, ^4J = 1.4 Hz, 1H, 3-H), 3.83 (s, 3H, -OCH_3), 2.16 (s, 3H, O=C-CH_3) – **EIMS** *m/z* (%) 165.1 (M^+, 92), 123.0 (96), 108.0 (100), 80.0 (75), 65.0 (42), 52.1 (37).



Phenazine-1-carboxylic acid (94): Yellow needles (1.0 mg), UV absorbing at 254 nm, yellow with anisaldehyde/sulphuric acid. R_f = 0.32(CH_2Cl_2/3% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.97 (dd, ^3J = 7.0 Hz, ^4J = 1.3 Hz, 1H, H-2), 8.52 (dd, ^3J = 8.7 Hz, ^4J = 1.4 Hz, 1H, H-4), 8.33 (m, 1H, H-9), 8.28 (m, 1H, H-6), 8.00 (m, 3H, H-3,7,8) – **(+)-ESIMS** *m/z* (%) 247 ($[M+Na]^+$, 80),471 ($[2M+Na]^+$, 44).



1-Carboxymethyl-phenazine (95): Yellow needles (1.2 mg), UV absorbing at 254 nm, yellow with anisaldehyde/sulphuric acid. R_f = 0.50(CH_2Cl_2/3% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.37 (dd, ^3J = 8.7 Hz, ^4J = 1.4 Hz, 1H, H-2), 8.32 (m, 1H, H-4), 8.22 (m, 2H, H-3,9), 7.85 (m, 3H, H-6,7,8), 4.09 (s, 3H, -OCH_3) – **(+)-ESIMS** *m/z* (%) 261 ($[M+Na]^+$, 12),471 ($[2M+Na]^+$, 100).



N6,N6-Dimethyladenosine (96): Colourless oil (1.3 mg), UV absorbing at 254 nm, green with anisaldehyde/sulphuric acid. R_f = 0.20(CH_2Cl_2/7% MeOH) – **^{1}H NMR** (300 MHz, DMSO-D_6): δ = 8.27 (s, 1H, H-8), 8.20 (s, 1H, H-2), 5.93 (d, ^3J = 5.6 Hz, 1H, H-1'), 4.58 (t, ^3J = 5.2 Hz, 1H, H-3'), 4.20 (t, ^3J = 5.2 Hz, 1H, H-3'), 3.98 (q, ^3J = 3.9 Hz, 1H, H-4'), 3.70 (dd, ^3J = 12.8 Hz, ^4J = 4.6 Hz, 1H, H_a-5'), 3.59 (dd, ^3J = 11.9 Hz, ^4J = 3.9 Hz, 1H, H_b-5'), 3.47 (s, 6H, N(CH_3)$_2$) – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ = 154.1 (Cq-6), 151.4 (CH-2), 149.7 (Cq-4), 138.3 (CH-8), 119.6 (Cq-5), 87.7 (CH-1'), 85.6 (CH-4'), 73.5 (CH-2'), 70.3 (CH-3'), 61.4

(CH_2-5'), 37.5 ($N(CH_3)_2$) – (+)-**ESIMS** *m/z* (%) 296 ($[M+H]^+$, 24), 318 ($[2M+Na]^+$, 78), 614 ($[2M+Na]^+$, 100) – (-)-**ESIMS** *m/z* 294 ($[M-H]^-$, 18) 589 ($[2M-H]^-$, 18) – **HRESIMS** *m/z* 296.13544 $[M+H]^+$, (calcd. 296.13533 for $C_{12}H_{18}N_5O_4$).

7.8 Terrestrial *Streptomyces* sp. Ank 291

7.8.1 Pre-screening

The terrestrial *Streptomyces* strain Ank 291 exhibited red aerial mycelium on gar plates after 72 hours of incubation at 28 °C at M_2 medium. The well-grown agar plate was used to inoculate 1L of 4 Erlenmeyer flasks each containing 250 ml of M_2 medium and incubated on the linear shaker for 7 days. The red culture broth was then extracted with ethyl acetate and the resulting extract was used for biological and chemical pre-screening. During the biological pre-screening, the extract showed high antibacterial activity (Table 26) and 100% cytotoxicity against *Artemia salina*.

Table 26: Antimicrobial activity of the crude extract of the strain Ank 291

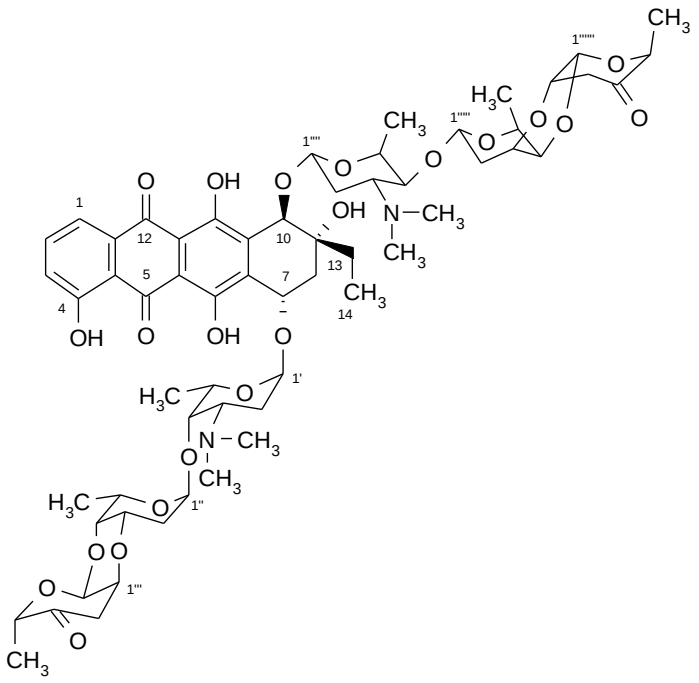
Test microorganisms	**Inhibition zone ∅ [mm]**
Staphylococcus aureus	26
Bacillus subtilis	22
Streptomyces viridochromogenes (Tü 57)	14

Moreover, on TLC, it showed several red zones, which turned to blue with anisaldehyde/sulphuric acid clearly indicating the presence of peri-hydroxy quinones.

7.8.2 Fermentation, isolation and purification

For large-scale fermentation of 20L, the well-developed red agar culture was served to inoculate 80 of 1L Erlenmeyer flasks containing M_2 medium and incubated on the previously described conditions for seven days. The well-grown red broth was mixed with Celite and filtered by pressure filter. The mycelium was exhaustively extracted with ethyl acetate and acetone. The filtrate was subjected to XAD-16 column, after washing with water; the organic phase was eluted with methanol and evaporated under reduced pressure to get dark red crude extract. Based on similar TLC pattern, both extracts from mycelium and filtrate were combined and fractionated on silica gel column using CH_2Cl_2/MeOH (0-100% MeOH) gradient into four fractions. These fractions were further purified by different chromatographic techniques to get pure compounds.

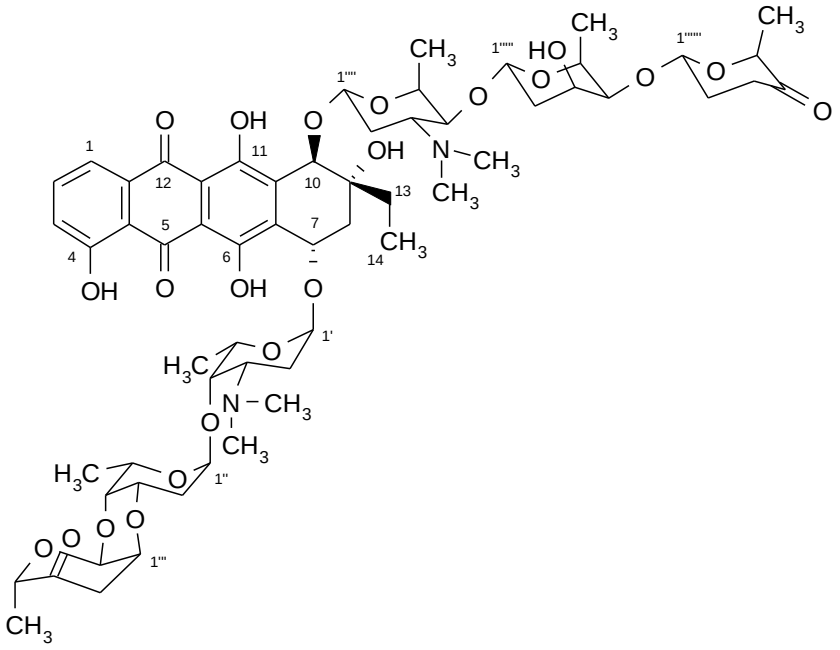
Fraction II was applied to Sephadex LH-20 (MeOH) column and two sub-fractions were obtained. These sub-fractions were further purified through PTLC followed by Sephadex LH-20 (CH_2Cl_2/40% MeOH) to yield ditrisarubicin B (**99**). Purification of fraction III by Sephadex LH-20 (CH_2Cl_2/40% MeOH) and PTLC yielded A447 D' (**100**). Furthermore, a more polar fraction was purified by Sephadex LH-20 (MeOH) following PTLC to obtain a trivial compound phenethyl alcohol (1.1 mg) and ditrisarubicin A (**98**).



Ditrisarubicin B (99): Red solid (1.1 mg), UV absorbing at 254 nm, Orange fluorescent at 366 nm, turned to blue with 2N NaOH, R_f = 0.41(CH_2Cl_2/5% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 13.73 (s br, 1H, OH), 12.86 (s br, 1H, OH), 12.14 (s br, 1H, OH), 7.90 (dd, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H, 1-H), 7.72 (t, ^3J = 8.3 Hz, 1H, 2-H), 7.31 (dd, ^3J = 8.4 Hz, ^4J = 1.1 Hz, 1H, 3-H), 5.45 (s br, 1H, 1'-H), 5.42 (d, ^3J = 2.7 Hz, 1H, 1""-H), 5.18 (dd, , ^3J = 5.8 Hz, ^4J = 3.1 Hz, 2H, 1'"-H, 1"""-H), 5.14 (m, 1H, 7-H), 5.09 (dd, ^3J = 7.0 Hz, ^4J = 3.1 Hz, 2H, 1"-H, 1""-H), 5.01 (s, 1H, 10-H), 4.78 (m, 2H, 5'"-H, 5"""-H), 4.65 (m, 2H, 5"-H, 5""-H), 4.38-4.23 (m, 4H, 2'"-H, 2"""-H, 3"-H, 3""-H), 4.01-3.97 (m, 3-H, 4"-H, 4""-H, 5'-H), 3.86 (q, ^3J = 5.7 Hz, 1H, 5""-H), 3.74 (s br, 1H, 4'-H), 3.70 (s br, 1H, 4""-H), 2.57 (m, 4H, 3'"-H_2, 3"""-H_2), 2.44 (m, 2H, 2"-H_a, 2""-H_a), 2.13 (s, 12H, 2N$(CH3)_2$), 2.24-1.49 (m, 13H, 8-H_2, 13-H_2, 2'-H_2, 3'-H, 2"-H_b, 2""-H_2, 3""-H, 2""-H_b, OH), 1.34 (2 d, ^3J = 6.9 Hz, 6H, 6'"-CH_3, 6"""-CH_3), 1.28-120 (m, 12H, 6'-CH_3, 6"-CH_3, 6""-CH_3, 6"""-CH_3), 1.09 (t, ^3J = 7.2 Hz, 3H, 14-CH_3) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ= 208.1 (2C, Cq-4'", Cq-4"""), 190.7 (Cq-5), 185.9 (Cq-12), 162.5 (Cq-4), 157.5 (Cq-11), 156.9 (Cq-6), 138.0 (Cq-10a), 137.1 (CH-2), 136.3 (Cq-6a), 133.5 (Cq-12a), 124.6 (CH-3), 119.7 (CH-1), 116.1 (Cq-4a), 112.1 (Cq-5a), 111.8 (Cq-11a), 101.8 (CH-1'), 99.0 (2C, CH-1", CH-1""), 97.1 (CH-1""), 91.5 (2C, CH-1'", CH-1"""), 77.9 (CH-5'"), 77.86 (C-5"""), 74.4 (CH-4'"), 74.1 (CH-4'), 71.8 (Cq-

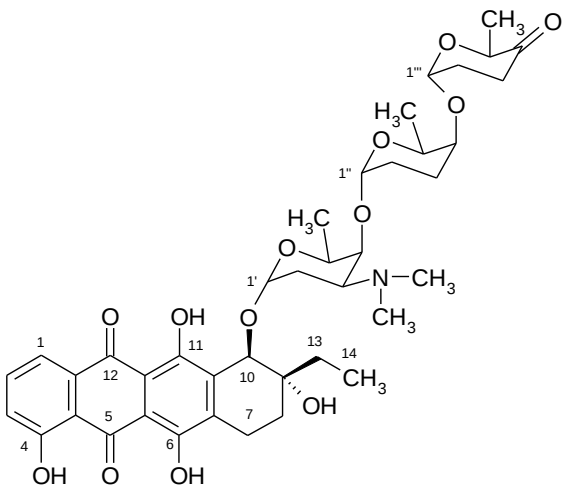
9), 71.0 (CH-7), 70.4 (CH-10), 68.4 (CH-5""), 67.3 (CH-3"), 67.2 (CH-3"""), 66.9 (2C, CH-4", CH-4"""), 65.3 (CH-5"), 65.2 (C-5"""), 63.0 (CH-2'"), 62.9 (CH-2"""), 61.4 (CH-3'), 61.37 (CH-3""), 43.3 (2C, 3""-$N(CH_3)_2$), 43.2 (2C, 3'-$N(CH_3)_2$), 39.8 (CH_2-3'"), 39.7 (CH_2-3"""), 33.0 (CH_2-8), 29.8 (CH_2-13), 29.76 (CH_2-2""), 29.3 (CH_2-2'), 27.0 (CH_2-2"), 26.9 (CH_2-2"""), 18.1 (CH_3-6""), 17.9 (CH_3-6'), 16.3 (CH_3-6'"), 16.2 (CH_3-6"""), 16.1 (2C, CH_3-6", CH_3-6"""), 6.7 (CH_3-14) – (+)-**ESIMS** *m/z* (%) 1181 ($[M+H]^+$, 75) – (-)-**ESIMS** *m/z* 1179 ($[M-H]^-$, 62).

Ditrisarubicin A (98): Red solid (1.1 mg), UV absorbing at 254 nm, Orange fluorescent at 366 nm, turned to blue with 2N NaOH, R_f = 0.58(CH_2Cl_2/7% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$):



δ = 13.78 (s br, 1H, OH), 12.91 (s br, 1H, OH), 12.16 (s br, 1H, OH), 7.86 (d, ^3J = 7.5 Hz, 1H, 1-H), 7.69 (t, ^3J = 8.6 Hz, 1H, 2-H), 7.27 (d, ^3J = 8.2 Hz, 1H, 3-H), 5.43 (s br, 1H, 1'-H), 5.39 (d, ^3J = 2.8 Hz, 1H, 1'"-H), 5.18 (dd, , ^3J = 7.0 Hz, ^4J = 3.3 Hz, 2H, 1'"-H, 1"""-H), 5.11 (m, 1H, 7-H), 5.07 (m, 2H, 1"-H, 1""-H), 5.04 (s, 1H, 10-H), 4.98 (m, 2H, 5'"-H, 5"""-H), 4.89 (m, 2H, 5"-H, 5""-H), 4.78-4.17 (m, 4H, 2'"-H, 2"""-H, 3"-H, 3""-H), 3.99-3.94 (m, 3-H, 4"-H, 4""-H, 5'-H), 3.84 (m, 1H, 5""-H), 3.74 (s br, 1H, 4'-H), 3.72 (s br, 1H, 4""-H), 3.69-3.33 (m, 4H, 3'"-H_2, 3"""-H_2), 2.55-2.44 (m, 2H, 2"-H_a, 2""-H_a), 2.11 (s, 12H, 2N(CH3)$_2$), 2.24-1.49 (m, 13H, 8-H_2, 13-H_2, 2'-H_2, 3'-H, 2"-H_b, 2""-H_2, 3""-H, 2"""-H_b, OH), 1.34 (2 d, ^3J = 6.9 Hz, 6H, 6'"-CH_3, 6"""-CH_3), 1.28-120 (m, 12H, 6'-CH_3, 6"-CH_3, 6""-CH_3, 6"""-CH_3), 1.06 (t, ^3J = 5.9 Hz, 3H, 14-CH_3) – (-)-**ESIMS** *m/z* 1181 ($[M-H]^-$, 100).

A447 D' (100): Red solid (1.5 mg), UV absorbing at 254 nm, orange fluorescent at 366 nm, turned to blue with 2N NaOH, R_f = 0.35(CH_2Cl_2/5% MeOH) – **^{1}H NMR and ^{13}C NMR** see Table 16, – (+)-**ESIMS** *m/z* (%) 754 ([M+H]$^+$, 85) – **HRESIMS** *m/z* 754.3423 [M+H]$^+$ ((calcd. 754.3415 for $C_{40}H_{52}NO1_3$).



7.9 Terrestrial *Streptomyces* sp. GW 13/475

7.9.1 Pre-screening

On TLC, the crude extract of terrestrial *Streptomyces* strain GW 13/475 showed several UV absorbing zones, which turned to reddish brown and green after spraying with anisaldehyde/sulphuric acid. In the biological screening, the crude extract showed low antimicrobial activity against *Bacillus subtilis*, *Candida albicans* and moderate activity against micro green algae (Table 27). It also showed 75% cytotoxicity against *Artemia salina*.

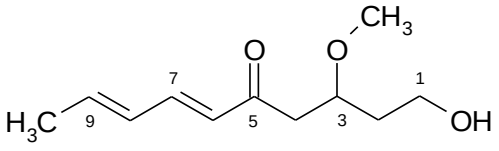
Table 27: Antimicrobial activity of the crude extract of the strain Ank 291

Test microorganisms	**Inhibition zone ∅ [mm]**
Bacillus subtilis	10
Candida albicans	11
Chlorella vulgaris	15
Chlorella sorokiniana	14
Chlorella sorokiniana	15

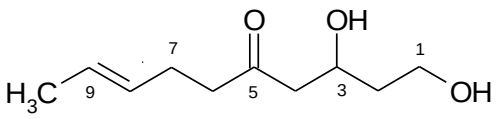
7.9.2 Fermentation and isolation

For up-scaling, the strain GW 13/475 was cultivated as 30 L shaker culture on M_2 medium for 7 days. The well-grown brown coloured culture broth was filtered through filter press by addition of Celite. The filtrate was subjected to XAD-16 column and eluted with methanol. The mycelium was extracted with ethyl acetate and

acetone. The solvents were evaporated under reduced pressure and obtained 2.7 g crude extract, which was pre-separated into five fractions by silica gel column chromatography with a CH_2Cl_2/MeOH. The fraction II was subjected to silica gel column using cyclohexane/CH_2Cl_2 gradient and separated into two sub- fractions IIa and IIb. Fraction IIa was further purified by PTLC into *p*-hydroxy-benzaldehyde (5.5mg) and indole-3-carbaldehyde (7.7 mg). Fraction IIb was purified by PTLC to yield *p*-hydroxy-benzoic acid methyl ester (2.3 mg). Fraction III was separated into two sub-fractions IIIa and IIIb by silica gel column (CH_2Cl_2/MeOH), which were further purified by PTLC into 3-methoxy-streptenol C (**102**) and mixture of streptenol A (**103**) and streptenol C (**104**), respectively. The purification of fraction IV by Sephadex LH-20 (MeOH) column and PTLC delivered 2'-*O*-methyluridine (5.6 mg). The polar fraction V was also subjected to Sephadex LH-20 (MeOH) column, following purification by PTLC yielded two primary metabolites; 2'-deoxy-thymidine (2.1 mg) and 2'-deoxyuridine (1.1 mg).



3-Methoxy-streptenol-C (102): Light yellow oil (25.0 mg), UV absorbing at 254 nm, dark brown with anisaldehyde/sulphuric acid. R_f = 0.45(CH_2Cl_2/5% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.11 (dd, ^3J = 15.5 Hz, ^4J = 9.9 Hz, 1H, 7-H), 6.17 (m, 2H, 8-H, 9-H), 6.05 (d, ^3J = 15.5 Hz, 1H, 6-H), 3.92 (m, 1H, 3-H), 3.71 (t, ^3J = 5.1 Hz, 2H, 1-H_2), 3.31 (s, 3H, 3-OCH_3), 2.91, 2.60 (*AB*X, J_{AB} = 15.9 Hz, J_{AX} = 5.9, J_{BX} = 6.4 Hz; 2H, 4-H_2), 1.83 (d, ^3J = 5.1 Hz, 3H, 10-CH_3), 1.74 (m, 2H, 2-H_2) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 199.0 (C_q-5), 143.7 (CH-7), 141.0 (CH-9), 130.0 (CH-8), 127.8 (CH-6), 76.7 (CH-3), 59.9 (CH_2-1), 57.0 (3-OCH_3), 44.5 (CH_2-4), 36.5 (CH_2-2), 18.7 (CH_3-10) – **H,H COSY** and **HMBC**, see Figure 90 and Figure 91 – (+)-**ESIMS** *m/z* (%) 221 ([M+Na]$^+$, 28), 419 ([2M+Na]$^+$, 100) – **HRESIMS** *m/z* 221.1155 [M+Na]$^+$, (calcd. 221.1148 for $C_{11}H_{18}O_3Na$).



Streptenol A (103): Light yellow oil (25.0 mg), UV absorbing at 254 nm, dark brown with anisaldehyde/sulphuric acid. R_f = 0.32(CH_2Cl_2/5% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.35 (m, 2H, 8-H, 9-H), 4.25 (m, 1H, 3-H), 3.74 (m, 2H, 1-H_2), 2.69 (d, ^3J = 1.5 Hz, 1H, 4_a-H), 2.54 (dd, ^3J = 8.6 Hz, ^4J = 2.0 Hz, 2H, 6-H_2), 2.44 (t, ^3J = 7.3 Hz, 1H, 4_b-H), 2.18 (m, 2H, 7-H_2), 1.63 (m, 2H, 2-H_2), 1.55 (d, ^3J =

1.5 Hz, 3H, 10-CH_3) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 211.2 (Cq-5), 129.1 (CH-8), 125.9 (CH-9), 67.4 (CH-3), 60.5 (CH_2-1), 49.3 (CH_2-4), 43.2 (CH_2-6), 37.9 (CH_2-2), 26.4 (CH_2-7), 17.7 (CH_3-10) – (+)-**ESIMS** *m/z* (%) 209 ($[M+Na]^+$, 11), 395 ($[2M+Na]^+$, 44) – **HRESIMS** *m/z* 209.1148 $[M+Na]^+$, (calcd. 209.1148 for $C_{10}H_{18}O_3Na$).

Streptenol C (104): Light yellow oil (25.0 mg), UV absorbing at 254 nm, dark brown with anisaldehyde/sulphuric acid. R_f = 0.32(CH_2Cl_2/5% MeOH) – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.09 (dd, ^3J = 15.4 Hz, ^4J = 9.6 Hz, 1H, 7-H), 6.16 (m, 2H, 8-H, 9-H), 5.99 (d, ^3J = 16.0 Hz, 1H, 6-H), 4.25 (m, 1H, 3-H), 3.74 (m, 2H, 1-H_2), 2.68 (d, ^3J = 5.3 Hz, 1H, 4_a-H), 2.44 (t, ^3J = 7.3 Hz, 1H, 4_b-H), 1.81 (d, ^3J = 5.4 Hz, 3H, 10-CH_3),1.63 (m, 2H, 2-H_2) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 200.8 (Cq-5), 144.2 (CH-7), 141.5 (CH-9), 130.0 (CH-8), 127.6 (CH-6) 67.0 (CH-3), 60.4 (CH_2-1), 46.5 (CH_2-4), 37.8 (CH_2-2), 18.7 (CH_3-10) – (+)-**ESIMS** *m/z* (%) 207 ($[M+Na]^+$, 17), 391 ($[2M+Na]^+$, 100) – **HRESIMS** *m/z* 207.0990 $[M+Na]^+$, (calcd. 207.0092 for $C_{10}H_{16}O_3Na$).

7.10 Terrestrial *Streptomyces* sp. GW 12/459

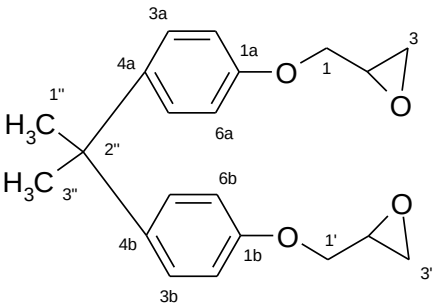
7.10.1 Pre-screening

During the biological pre-screening, the crude extract of terrestrial *Streptomyces* strain GW 12/459 showed only weak activity against *Escherichia coli*. The TLC of the crude extract exhibited several UV absorbing which turned to dark pink and blue with anisaldehyde/sulphuric acid.

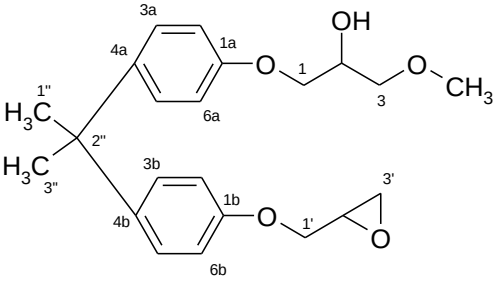
7.10.2 Isolation and purification

The well-grown agar plates of the strain GW 12/459 were served to inoculate 30L of M_2 medium in 100 of 1L Erlenmeyer flasks and kept on linear shaker at 28 °C. The dark brown culture was harvested after seven days and filtered through filter press. The mycelium was exhaustively extracted with ethyl acetate and acetone. The water phase was adsorbed on XAD-16 resins, washed with water and eluted with water-saturated methanol. Methanol was evaporated under reduced pressure and water of methanolic extract was again extracted with ethyl acetate. The extract obtained from mycelium and filtrate were combined based on similar TLC to get 3.3g crude extract. The crude extract was subjected to silica gel column and eluted with

CH_2Cl_2/MeOH gradient (CH_2Cl_2, 2 L; 3% MeOH, 1.5 L; 5% MeOH, 1.5 L; 7%MeOH, 1.0 L; 10% MeOH, 0.7 L; 20% MeOH, 0.5 L; 30% MeOH, 0.7 L; 50%MeOH, 0.5 L) and divided into three fraction by TLC monitoring. The first fraction consisted of fats and fatty acids. Fraction II was further separated into three subfractions by silica gel column using cyclohexane/CH_2Cl_2 gradient. The less polar Fraction IIa was purified on silica gel column (CH_2Cl_2/MeOH) to obtain BADGE (**107**). 1-acetyl-β-carboline (1.2mg) was isolated from fraction IIb using silica gel column (CH_2Cl_2/MeOH). The purification of fraction III through silica gel column using CH_2Cl_2/MeOH gradient yielded 3-methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]- 2-propanol (BADGE·OMe) (**109**) and 3-chloro-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]-ethyl]phenoxy]- 2-propanol (BADGE·HCl) (**110**). Fraction III was subjected to Sephadex LH-20 (MeOH) column and divided into two sub-fractions IIIa and IIIb. Sub-fraction IIIa was purified to 3-[4-[1-[4-(3-Chloro-2-hydroxypropoxy)phenyl]-1-methylethyl]phenoxy]-1,2-propanediol (BADGE·HCl·H_2O) (**111**) by silica gel column (CH_2Cl_2/MeOH). Fraction IIIb afforded anthranilic acid (3.6 mg) and tryptophol (1.6 mg).

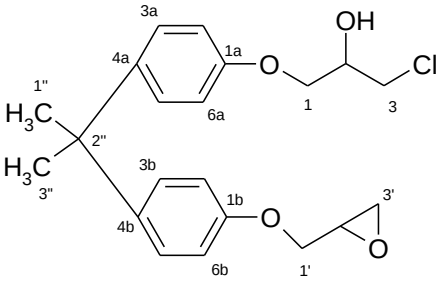


Bisphenol A diglycidyl ether (107): Yellow oil (25.4 mg), UV absorbing at 254 nm, dark pink with anisaldehyde/sulphuric acid. – R_f = 0.55 (CH_2Cl_2/1% MeOH). – $[\alpha]_D^{20}$ = + 55° (*c* = 0.04, $CHCl_3$). – **UV/VIS**: (MeOH): λ_{max} (log ε): 277 (3.72), 242 (3.73) nm – **^{1}H** and **^{13}C NMR**, see Table 17. – **H,H COSY** and **HMBC**, see Figure 99 – **EIMS** (70eV) *m/z* (%) 340 (22), 32 (100), 57.0 (53), 41.0 (12) – (+)-**ESIMS** *m/z* (%) 363 ($[M + Na]^+$, 60) – **HRESIMS** *m/z* 363.15678 $[M + Na]^+$ (calcd. 363.15668 for $C_{21}H_{24}O_4Na$).

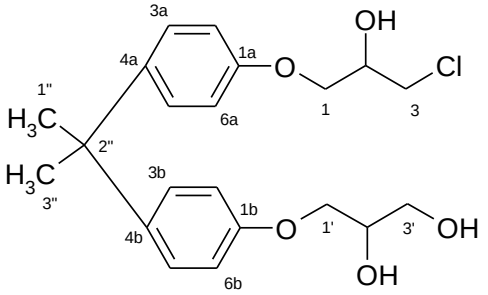


3-Methoxy-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]ethyl]-phenoxy]- 2-propanol (BADGE·OMe) (109): Yellow oil (23.2 mg), UV absorbing at 254 nm, dark pink with anisaldehyde/sulphuric acid. – R_f = 0.32 (CH_2Cl_2/3% MeOH). – $[\alpha]_D^{20}$ = +45.7° (*c* = 0.035, MeOH). – **UV/VIS**: (MeOH): λ_{max} (log ε): 283 (3.22), 276 (3.11), 239 (2.99) nm – **^{1}H** and **^{13}C NMR**, see Table 17. – **EIMS** (70eV) *m/z* (%) 372 (25), 357

(100), 269 (25), 45 (40) – **HRESIMS** *m/z* 395.18293 $[M + Na]^+$ (calcd. 395.18289 for $C_{22}H_{28}O_5Na$).



3-Chloro-1-[4-[1-methyl-1-[4-(2-oxiranylmethoxy)phenyl]-ethyl]phenoxy]-2-propanol (BADGE·HCl) (110): Colourless oil (2.1 mg), UV absorbing at 254 nm, dark pink with anisaldehyde/sulphuric acid. – R_f = 0.20 (CH_2Cl_2/1% MeOH). – $[\alpha]_D^{20}$ = +36.3° (*c* = 0.055, $CHCl_3$) – **UV/VIS**: (MeOH): λ_{max} (log ε): 283 (3.28), 276 (3.34), 240 (3.42) nm – **^{1}H** and **^{13}C NMR**, see Table 18. – **EIMS** (70eV) *m/z* (%) 376 (22), 361 (100), 269 (18), 41.0 (10) – **HRESIMS** *m/z* 399.13338 $[M + Na]^+$ (calcd. 399.13336 for $C_{21}H_{25}O_4NaCl$).



3-[4-[1-[4-(3-Chloro-2-hydroxypropoxy)phenyl]-1-methylethyl]phenoxy]-1,2-propanediol (BADGE·HCl·H_2O) (111): Colourless oil (3.0 mg), UV absorbing at 254 nm, blue with anisaldehyde/sulphuric acid. – R_f = 0.32 (CH_2Cl_2/7% MeOH). – $[\alpha]_D^{20}$ = +18.5° (*c* = 0.055, MeOH) – **UV/VIS**: (MeOH): λ_{max} (log ε): 283 (3.36), 276 (3.37), 240 (3.07) nm – **^{1}H** and **^{13}C NMR**, see Table 18. – **EIMS** (70 eV) *m/z* (%) 394 (30), 379 (100), 305 (10), 213 (15), 135 (18), 119 (15), 43 (22) – **HRESIMS** *m/z* 417.14396 $[M + Na]^+$ (calcd. 417.14392 for $C_{21}H_{27}O_5NaCl$).

7.11 Terrestrial *Streptomyces* sp. GW 8594

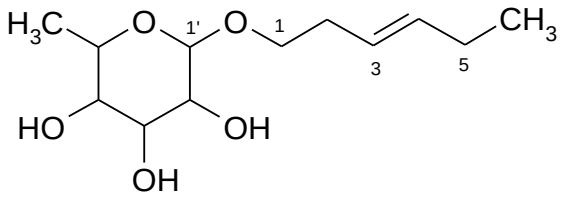
7.11.1 Pre-screening

The well-developed agar colonies of the terrestrial strain GW 8594 were used to inoculate 1L of M_2 medium in Erlenmeyer flasks and incubated at 28 °C on a linear shaker. After 7 days, dark brown agar culture was extracted with ethyl acetate and the resulting crude extract was used for biological and chemical pre-screening. In the biological pre-screening, the crude extract showed promising activity against pathogenic microorganisms (Table 19). On TLC, it showed several green zones after spraying with anisaldehyde/sulphuric acid.

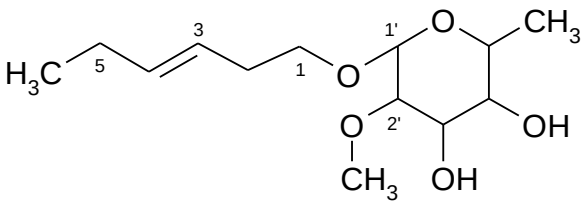
7.11.2 Fermentation, isolation and purification

Hundred of 1L Erlenmeyer flasks containing 300 ml of M_2 medium were inoculated with well grown agar culture cultivated on the pre-described conditions. After 7 days, the dark brown culture was harvested and was filtered using Celite with the aid of filter press. The filtrate was passed over an XAD-16 column (65 × 8), the resin washed with distilled water and eluted with methanol. The biomass was extracted three times with ethyl acetate followed by acetone. On TLC both crude extracts obtained from the water phase and the biomass showed identity, so they were combined. The crude extract (2.6 g) was fractionated over silica gel column chromatography using CH_2Cl_2/MeOH gradient (0 to 40% MeOH) to afford five fractions.

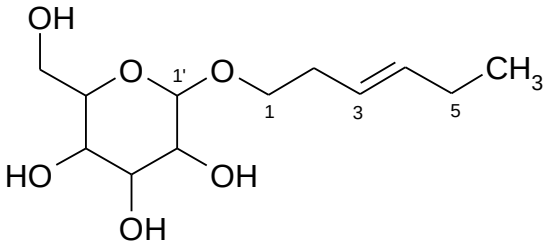
Fraction II was separated by Sephadex LH-20 using CH_2Cl_2/40%MeOH followed by silica gel column (CH_2Cl_2/MeOH) to afford 3-hexenyl-1-*O*-*β*-D-glycopyranoside (**113**). The purification of fraction III through Sephadex LH-20 (MeOH) column and PTLC delivered 1.5mg tyrosol. Fraction IV was purified by Sephadex LH-20 (MeOH) column and silica gel column (CH_2Cl_2/MeOH) and obtained 3-hexenyl-1-*O*-*α*-L-2'-methoxy-glycoside (**114**). Whereas fraction V was subjected to Sephadex LH-20 (MeOH) column and obtained two subfractions, each of which were further purified by PTLC and Sephadex LH-20 (MeOH) column to deliver 3-hexenyl-1-*O*-*β*-D-glycoside (**115**) and 2-phenylethanol-1-*O*-*β*-D-glucoside (**116**).



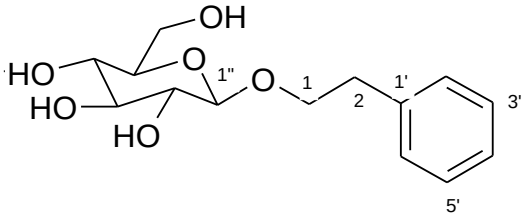
3-Hexenyl-1-*O*-*β*-D-glycoside (113): Light yellow oil (27.5 mg), green with anisaldehyde/sulphuric acid. – R_f = 0.26 (CH_2Cl_2/7% MeOH). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.43 (m, 1H, 4-H), 5.29 (m, 1H, 3-H), 4.22 (d, ^3J = 7.6 Hz, 1H, 1'-H), 3.83 (m, 1H, 1-H_a), 3.47 (m, 1H, 1-H_b), 3.45 (m, 1H, 3'-H), 3.35 (m, 1H, 2'-H), 3.32 (m, 1H, 5'-H), 3.16 (t, ^3J = 8.9 Hz, 1H, 4'-H), 2.34 (m, 2H, 2-H_2), 2.01 (m, 2H, 5-H_2), 1.28 (d, ^3J = 6.0 Hz, 3H, 6'-CH_3), 0.94 (t, ^3J = 7.5 Hz, 3H, 6-CH_3) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 134.1 (CH-4), 124.1 (CH-3), 102.4 (CH-1'), 76.3 (CH-3'), 75.2 (CH-4'), 73.7 (CH-2'), 71.7 (CH-5'), 69.5 (CH_2-1), 27.7 (CH_2-2), 20.6 (CH_2-5), 17.6 (CH_3-6'), 14.2 (CH_3-6) – **H,H COSY** and **HMBC**, see Figure 110. – **ESIMS** *m/z* (%) 269 ($[M+Na]^+$, 22), 515 ($[2M+Na]^+$, 100) – **HRESIMS** *m/z* 269.1362 $[M+Na]^+$, (calcd. 269.1359 for $C_{10}H_{16}O_3Na$).



3-Hexenyl-1-*O*-α-L-2'-methoxy-glycoside (114): Colourless oil (2.3 mg), green with anisaldehyde/sulphuric acid. – R_f = 0.26 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.46 (m, 1H, 4-H), 5.31 (m, 1H, 3-H), 4.84 (d, ^3J = 1.2 Hz, 1H, 1'-H), 3.70 (d, ^3J = 4.0 Hz, 1H, 3'-H), 3.67 (m, 1H, 1-H_a), 3.66 (q, ^3J = 5.6 Hz, 1H, 5'-H), 3.45 (s, 3H, 2'-OCH_3), 3.44 (m, 2H, 1-H_b, 2'-H), 3.42 (m, 1H, 4'-H), 2.32 (m, 2H, 2-H_2), 2.03 (m, 2H, 5-H_2), 1.28 (d, ^3J = 6.2 Hz, 3H, 6'-CH_3), 0.95 (t, ^3J = 7.5 Hz, 3H, 6-CH_3) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 134.0 (CH-4), 124.6 (CH-3), 96.0 (CH-1'), 80.3 (CH-2'), 74.1 (CH-4'), 71.6 (CH-3'), 67.6 (CH-5'), 67.2 (CH_2-1), 58.8 (2'-OCH_3), 27.5 (CH_2-2), 20.6 (CH_2-5), 17.5 (CH_3-6'), 14.3 (CH_3-6) – **H,H COSY** and **HMBC**, see Figure 113 – **ESIMS** *m/z* (%) 283 ([M+Na]$^+$, 23), 543 ([2M+Na]$^+$, 100) – **HRESIMS** *m/z* 283.1519 [M+Na]$^+$, (calcd. 283.1516 for $C_{13}H_{24}O_5Na$).



3-Hexenyl-1-*O*-β-D-glycoside (115): Colourless oil (2.3 mg), green with anisaldehyde/sulphuric acid. – R_f = 0.35 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.43 (m, 1H, 4-H), 5.31 (m, 1H, 3-H), 4.30 (d, ^3J = 7.6 Hz, 1H, 1'-H), 3.81 (m, 3H, 1-H_a, 6'-H_2), 3.54 (m, 2H, 1-H_b, 3'-H), 3.53 (m, 1H, 5'-H), 3.38 (m, 1H, 2'-H), 3.29 (m, 1H, 4'-H), 2.34 (m, 2H, 2-H_2), 2.01 (m, 2H, 5-H_2), 0.92 (t, ^3J = 7.8 Hz, 3H, 6-CH_3) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 134.1 (CH-4), 124.1 (CH-3), 102.8 (CH-1'), 76.4 (CH-3'), 75.6 (CH-4'), 73.4 (CH-2'), 69.8 (CH_2-1), 69.5 (CH-5'), 61.4 (CH_2-6'), 27.7 (CH_2-2), 20.6 (CH_2-5), 14.2 (CH_3-6) – **H,H COSY** and **HMBC**, see Figure 116 – **(+)-ESIMS** *m/z* (%) 285 ([M+Na]$^+$, 17), 547 ([2M+Na]$^+$, 100) – **(-)-ESIMS** *m/z* (%) 261 ([M-H]$^-$, 22), 547 ([2M-H]$^-$, 28) – **HRESIMS** *m/z* 285.1314 [M+Na]$^+$, (calcd. 285.1309 for $C_{12}H_{22}O_6Na$).



2-Phenylethanol-1-*O*-β-D-glucoside (116): Colourless oil (1.1 mg), green with anisaldehyde/sulphuric acid. – R_f = 0.32 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.18 (m, 2H, H-3', H-5'), 7.14 (m, 3H, H-2', H-4', H-6'), 4.30 (d, ^3J = 7.6 Hz, 1H, 1"-H), 4.02 (q, ^3J = 7.8 Hz, 1H, 1-H_a), 3.81 (m, 2H, 6"-H_2), 3.69 (m, 1H, 1-H_b), 3.54 (m, 1H, 4"-H), 3.52 (m, 1H,

3"-H), 3.37 (t, ^3J = 9.6 Hz, 1H, 2"-H), 3.24 (m, 1H, 5"-H), 2.88 (t, ^3J = 9.2 Hz, 2H, 2-H_2) – **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 138.2 (Cq-1'), 128.8 (2C, CH-2', CH-6'), 128.4 (2C, CH-3', CH-5'), 126.4 (CH-4'), 102.8 (CH-1"), 76.3 (CH-3"), 75.6 (CH-5"), 73.7 (CH-2"), 70.7 (CH_2-1), 70.2 (CH-4"), 62.1 (CH_2-6"), 36.1 (CH_2-2). – **H,H COSY** and **HMBC**, see Figure 119 – **(+)-ESIMS** *m/z* (%) 307 ($[M+Na]^+$, 38), 591 ($[2M+Na]^+$, 100) – **HRESI-MS** *m/z* 307.1156 $[M+Na]^+$, (calcd. 307.1152 for $C_{14}H_{20}O_6Na$).

7.12 Terrestrial *Streptomyces* sp. Ank 282

7.12.1 Pre-screening

In the biological pre-screening, the crude extract showed profound antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Streptomyces viridochromogenes* (Tü 57). It also exhibited 100 % cytotoxicity against *Artemia salina*.

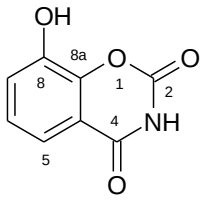
Table 28: Antimicrobial activity of the crude extract of the strain Ank 282

Test microorganisms	**Inhibition zone ∅ [mm]**
Staphylococcus aureus	20
Bacillus subtilis	15
Escherichia coli	26
Streptomyces viridochromogenes (Tü 57)	26

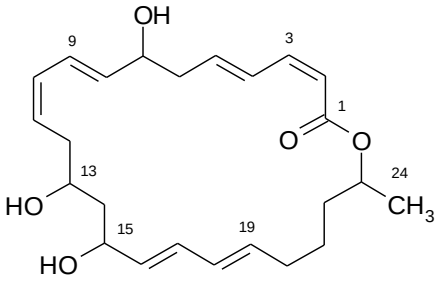
7.12.2 Fermentation, isolation and purification

Well-grown agar plates were used to inoculate 80 of 1L Erlenmeyer flasks each containing 300 ml M_2 medium and cultivated on linear shaker for 7 days. The dark brown culture was harvested and filtered by filter press to separate the mycelium and water phase. The mycelium was extracted three times with ethyl acetate and acetone. The filtrate was subjected to XAD-16 column and extracted with methanol. As the chemical composition of both extracts was same on the TLC, therefore, were combined and the resulting crude extract (2.3 g) was fractionated on silica gel column using CH_2Cl_2/MeOH into four fractions. Benadrostin (**117**) was obtained from fraction II after purification with PTLC followed by Sephadex LH-20 (CH_2Cl_2/40% MeOH). The purification of fraction III yielded *p*-hydroxybenzoic acid methyl ester

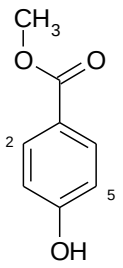
(**118**). Fraction IV was subjected to Sephadex LH-20 (MeOH) column and one of the resulting subfration was further purified by RP-18 column to obtain macrolactin (**120**) and the other subfraction was purified by PTLC to yield uracil (2.5 mg).



Benadrostin (117): Colourless crystalline solid (12.2 mg), UV-absorbing at 254 nm, no colour with anisaldehyde/sulphuric acid. – R_f = 0.41 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (300 MHz, DMSO-d_6): δ = 11.16 (s br, 2H, 3-NH, 8-OH), 7.33 (dd, ^3J = 7.4 Hz, ^4J = 1.8 Hz, 1H, 5-H), 7.22 (dd, , ^3J = 7.9 Hz, ^4J = 1.8 Hz, 1H, 7-H), 7.16 (t, ^3J = 7.6 Hz, 1H, 6-H) – **(+)-ESIMS** *m/z* (%) 202 ([M+Na]$^+$, 100), 381 ([2M+Na]$^+$, 25).



Macrolactin A (120): yellow oil (5.1 mg), UV absorbing, black colour with anisaldehyde/sulphuric acid. − R_f = 0.24 (CH_2Cl_2/10% MeOH), − **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.23 (dd, ^3J = 14.4, ^3J = 11.3 Hz, 1H, 4-H), 6.63 (dd, ^3J = 11.6, ^3J = 11.3 Hz, 1H, 3-H), 6.56 (dd, ^3J = 15.0, ^3J = 11.3 Hz, 1H, 9-H), 6.17 (dd, ^3J = 14.7, ^3J = 10.6, Hz, 1H, 17-H), 6.15 (ddd, ^3J = 14.3, ^3J = 7.9, ^3J = 7.5 Hz, 1H, 5-H), 6.09 (dd, ^3J = 10.6, ^3J = 14.7 Hz, 1H, 18-H), 6.05 (dd, ^3J = 11.5, ^3J = 11.0 Hz, 1H, 10-H), 5.76 (dd, ^3J = 15.2, ^3J = 5.7 Hz, 1H, 8-H), 5.65 (2 m, 2H, 16-H, 19-H), 5.54 (m, 1H, 11-H), 5.53 (d, ^3J = 11.6 Hz, 1H, 2-H), 5.01 (m, 1H, 23-H), 4.29 (m, 1H, 15-H), 4.21 (m, 1H, 7-H), 3.84 (m, 1H, 13-H), 2.48 (m, 1H, 12-H_a), 2.33 (m, 1H, 12-H_b), 2.41 (m, 1H, 6-H), 2.18 (m, 1H, 20-H_a), 2.10 (m, 1H, 20-H_b), 1.63 (m, 1H, 22-H_a), 1.59 (m, 2H, 14-CH_2), 1.52 (m, 1H, 22-H_b), 1.49 (m, 2H, 21-CH_2), 1.24 (d, ^3J = 6.3 Hz, 3H, 24-CH_3) − (+)-**ESIMS** *m/z* (%) 425 ([M + Na]$^+$, 40), 827 ([2 M + Na]$^+$ 100).



***p*-Hydroxybenzoic acid methyl ester (118):** Colourless crystalline solid (3.6 mg), UV-absorbing at 254 nm, no colour with anisaldehyde/sulphuric acid. – R_f = 0.52 (CH_2Cl_2/3% MeOH). – **^{1}H NMR** (300 MHz, DMSO-d_6): δ = 7.77 (d, ^3J = 8.3 Hz, 2H, 2-H, 6-H), 6.80 (dd, , ^3J = 8.3 Hz, 2H, 3-H, 5-H), 3.77 (s, 3H, OCH_3) – **(+)-ESIMS** *m/z* (%) 202 ([M+Na]$^+$, 100), 381 ([2M+Na]$^+$, 25).

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