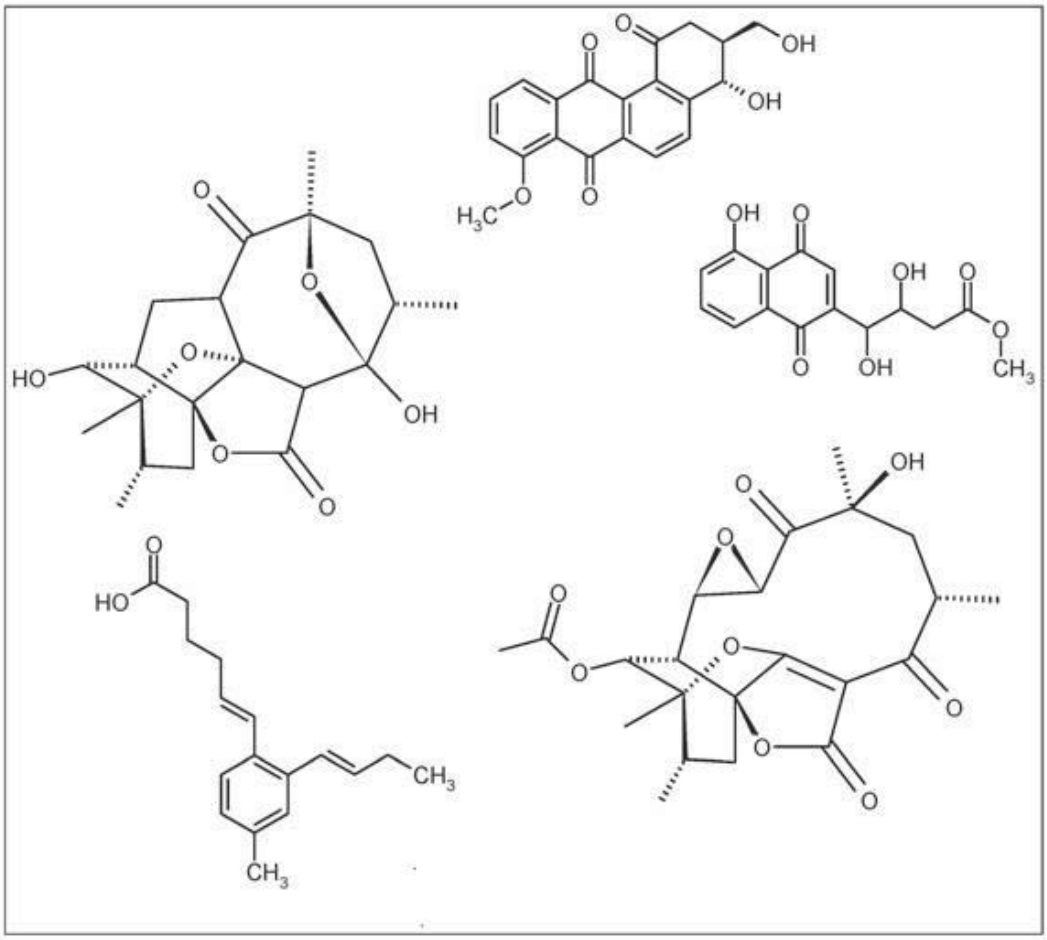
Muna Ali Abdalla Mohamed

ent-Homoabyssomicins A and B, Two New Spirotetronates, Khatmiamycin, a Zoosporicidal Naphthoquinone, and Further New Biologically Active Secondary Metabolites from Marine and Terrestrial *Streptomyces* spp.



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presented by

Muna Ali Abdalla Mohamed

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

Für meine Eltern Ali Al Samahoni und Fathia Ibrahim,

meinen Mann Saad und meine Tochter Abrar

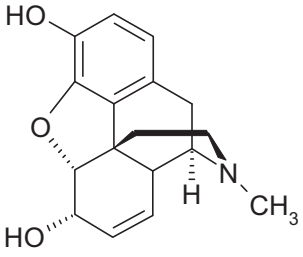
Table of Content

1 Introduction

1.1 Short History of Plant-derived Drugs and Traditional Medicine

In Africa and Asia, the majority of the population lives in rural areas and relies on plant materials to treat their illnesses. The plants are used in different forms such as extracts or decoctions to treat cancer, tuberculosis, HIV, malaria and many other diseases. Accordingly, these drugs, which are referred to as 'traditional herbal medicines' play an important role in improving the health status of the population and preventing endemic and acute diseases.[1] Also in developed countries traditional herbal medicine has attracted great interest,[2] enforced by the green movements and an increasing aversion to synthetic materials. Scientifically, plant metabolites have provided important ideas for discovery and industrial development of therapeutics.[3]

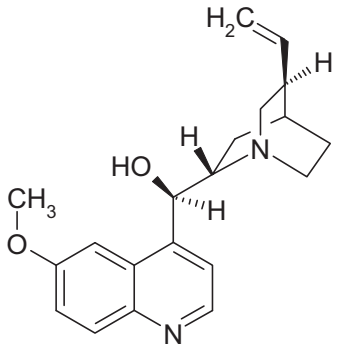
The beginning of this development dates more than 200 years back. The young German pharmacist Friedrich Sertürner (1783-1841) isolated at an age of 21 one of the first pharmacologically active compounds in a pure state: He obtained morphine (**1**) from opium, the latex produced by cutting the seed pods of poppy, *Papaver somniferum.*[4,5] Morphin (**1**) was the first commercially produced plant product with a guarantee for it's purity. Since then the idea to isolate pure compounds from plants began a rapid development, and the plant-focussed traditional medicine was set on a scientific basis.



1

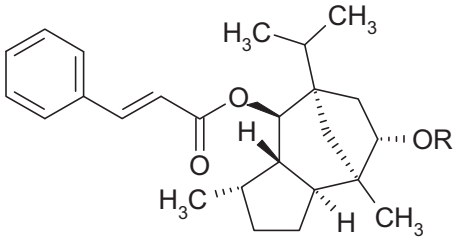
Quinine (**2**) from the *Cinchona* tree[6] was the first effective antimalarial drug used until the 1940s. In the 17th century, Jesuit Missionaries in the Andes (western South America) discovered that the extract of the *Cinchona* tree can treat fever, and the tree was known as "Jesuits' bark" at that time. In 1820 the French chemist Pierre-Joseph Pelletier (1788-1842) together with Joseph-Bienaime Caventou (1795-1877) succeeded to isolate quinine (**2**) from *Cinchona* bark. The structure was confirmed

later by the American organic chemist Robert Burns Woodward (1917-1989) by synthesis.[7]



2

Englerin A (**3a**) and englerin B (**3b**) have been isolated from the stem bark of *Phyllanthus engleri* (Euphorbiaceae) on the basis of ethnomedical reports about their toxicity. Englerin A (**3a**), the major compound of this species, showed indeed a 1000-fold higher selectivity against six renal cancer cell lines, with GI_{50} values ranging from 1–87 nM compared with taxol.[8]

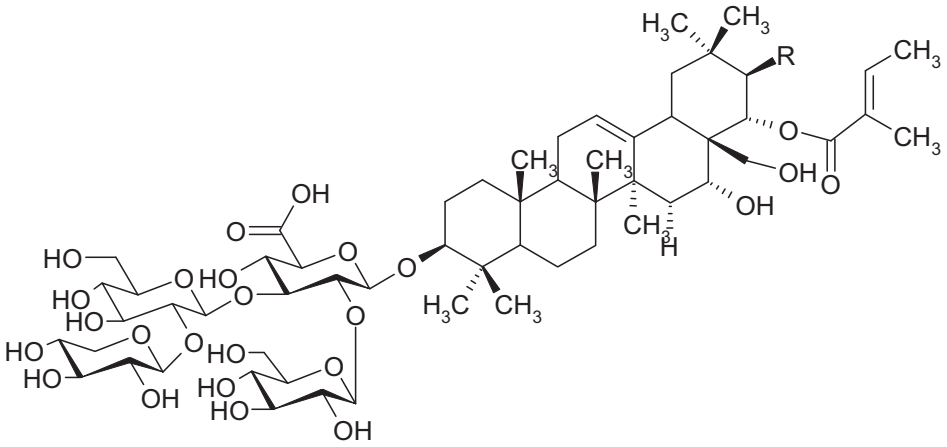


3a: R = $COCH_2OH$

3b: R = H

Recently the methanolic extract of the stem bark of the medicinal plant *Antonia ovata* afforded four highly cytotoxic triterpenoid saponins: Compounds **4a** and **4b** exhibited the most potent cytotoxic activity of all isolated and tested metabolites.[9]

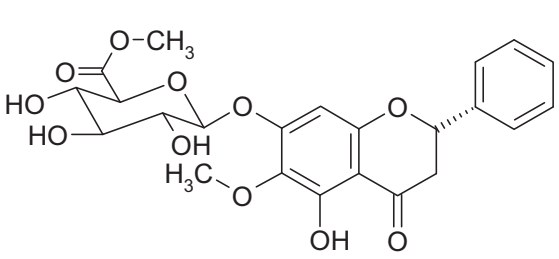
Flavonoids are a group of naturally occurring compounds present in fruits and vegetables. Recent studies indicated that flavonoids play a role in the prevention of cancer. The mechanism is not yet clear, but there are many hypotheses suggesting that these compounds may act as antioxidants or as phytoestrogens and interfere with estrogen metabolism. Another suggestion is that these compounds act by affecting the cell signalling or the regulation of genes involved with carcinogenesis.[10]



4a: R = H

4b: R = $OCOCH_3$

New flavonoid glycosides **5** and **6** were isolated recently from the stem bark of the medicinal plant *Oroxylum indicum* and tested for their ulcer protective effects against various gastric ulceritis inducing models in rats. Compound **5** with a free phenyl ring (ring B) together with a glucuronide linkage afforded the most potent gastroprotective activities.[3]



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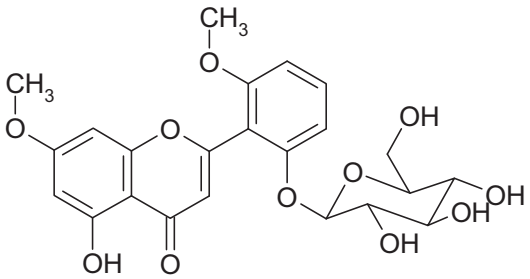
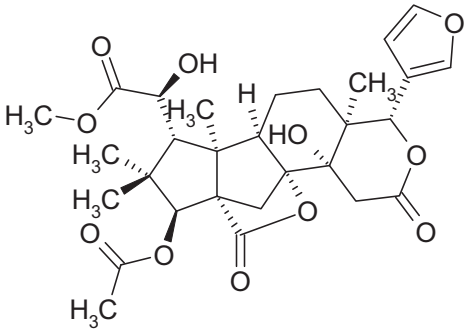


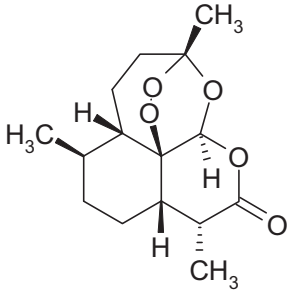
6

Plants are still a source of novel and interesting structures: A recent example is the new limonoid grandifotane A (**7**), which has been isolated from the stem bark of *Khaya grandifoliola* (Meliaceae). The structure was confirmed by X-ray crystallography. This plant has been used traditionally in Africa as antimalarial remedy.[11]



7

The most important example of the recent development in plant therapeutics is certainly artemisinin (**8**) from *Artemisia annua*.[12] It has been found to be up to 97% effective against malaria and is globally distributed, especially in Africa where one child dies from malaria every 30 seconds.[13]

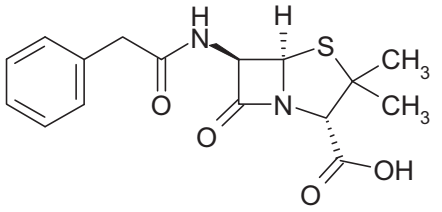


8

1.2 Natural Products in Modern Medicine

While the traditional medicine is mainly based on plants (and to a lesser extend on animals), the "modern" nature-derived medicine relies additionally on microbial metabolites: In 1928, the biologist and pharmacologist Alexander Fleming (1881-1955) discovered that colonies of *Staphylococcus aureus* could be destroyed by the mould *Penicillium notatum* proving the existence of antibacterial agents produced by other microorganisms and assessing the principle that certain drugs could kill pathogenic bacteria inside the body.[14,15] In the 1940s, the use of penicillin (**9**) as an antibiotic was initiated by the contributions of Howard Florey (1898-1968) and Ernst Chain (1906-1979), who purified and characterized the effective ingredient, penicillin.[16] The discovery of penicillin paved the road for the birth of β-lactam antibiotics such as the many modified penicillins and cephalosporins (cephalosporin C, **10**).[17,18] After the Second World War pharmaceutical research was intensified, and the success of penicillins encouraged the scientists to search for further antibiotics from microorganisms. In 1990, nearly 80% of the newly discovered drugs were derived from natural

products. These natural drugs included antibiotics such as tetracycline (from *Streptomyces rimosus*),[19] erythromycin (**11**) (from *Saccharopolyspora erythrea*)[20] as well as antiparasitic drugs like avermectin (avermectin B1a, **12**) (from *Actinomyces* sp.).[21]



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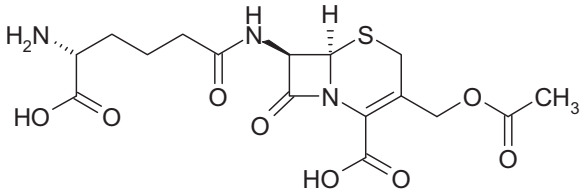

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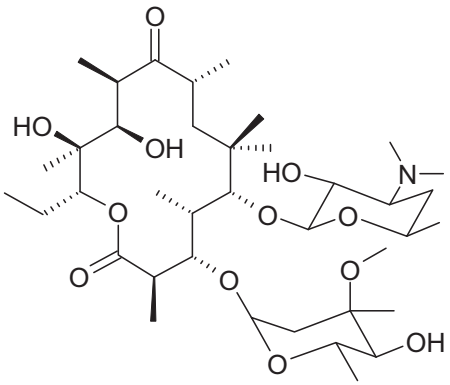

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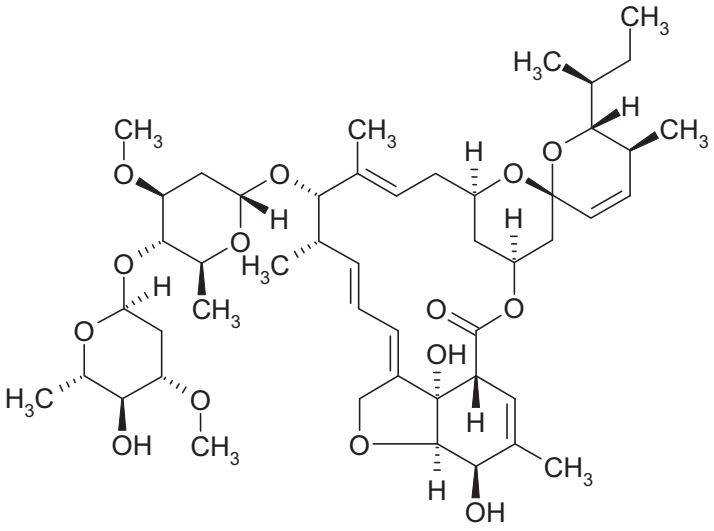

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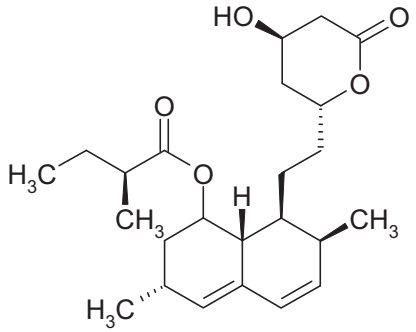
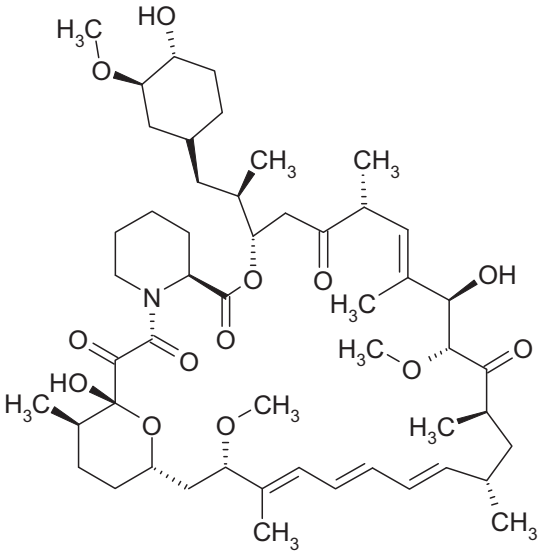


13

Additionally, lipid control agents like lovastatin (**13**) (from the fungus *Aspergillus terreus*),[22] immunosuppressants for organ transplants like cyclosporine,[23,24] (from the fungus *Beauveria nivea*) and rapamycin (**14**)[25] (from *Streptomyces hygroscopicus*) and anticancer drugs such as doxorubicin[26] (from *Streptomyces peucetius*) were discovered. These drugs revolutionised the medicinal industry during the late fifty years in the 20th century till today. Medicinal chemistry developed increasingly new drugs on the bases of natural products.[27]

Stimulated by the successes in medicine, natural products were also applied in agriculture: An important success was the discovery of leading fungicides like strobilurin and synthetic derivatives (e.g. azoxystrobin, *β*-methoxyacrylate) and the insecticide spinosad (a tetracyclic macrolide).[28]

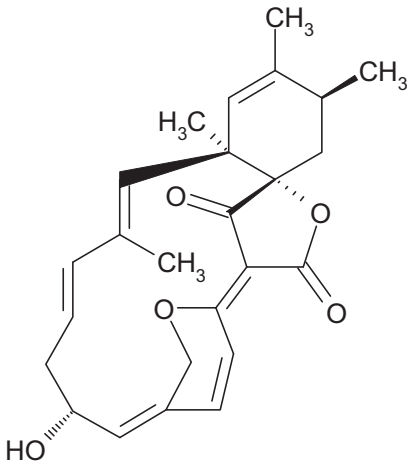


14

1.3 Recently Discovered Metabolites from *Streptomyces* spp.

Streptomycetes are gram-positive bacteria with a high GC content[29] and are widely distributed in soil; over 500 species of these bacteria have been identified.[30,31] *Streptomyces* is the most important genus of the family Streptomycetaceae, belongs to the order Actinomycetales and the phylum Actinobacteria. They produce more than half of the world's antibiotics.[32] Antibiotics like streptomycin or the tetracyclines, neomycin and chloramphenicol have played a vital role in drug evolution.[33,34]

Spirotetronate metabolites have demonstrated potent antitumor activity. Recently, the spirohexenolides A (**15**) and B (**16**) have been discovered in *Streptomyces platensis* and were elucidated by detailed X-ray crystallography, 1D and 2D NMR studies; the tetrocarcins are currently under clinical trials.[35]



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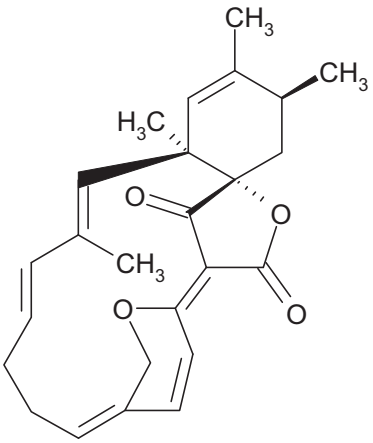
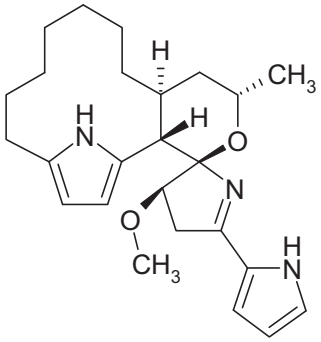


16

Two spiroaminals marineosin A (**17**) and B (**18**), which selectively inhibited HCT-116 cells, were isolated from a *Streptomyces*-related actinomycete from marine sediment. Compound **17** exhibited higher activity than compound **18** and showed selective cytotoxicity against melanoma and leukaemia cell lines.[36]

Streptomyces spp. are known as a source of novel and bioactive drugs, among them angucyclinones such as rabelomycin (**19a**), hatomarubigin (**19b**) and tetrangomycin (**19c**), which have been isolated from various *Streptomyces* strains and exhibited different pharmacological properties, such as antiviral, antifungal, antitumor and enzyme inhibitor activity. The angucyclines/angucyclinones are a third class of clinically useful antibiotics after the tetracyclines and anthracyclines.[37]



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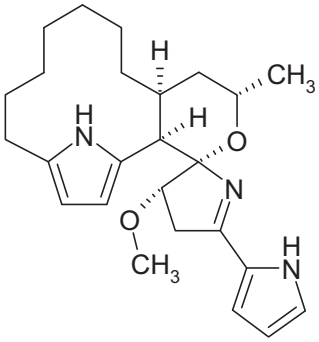

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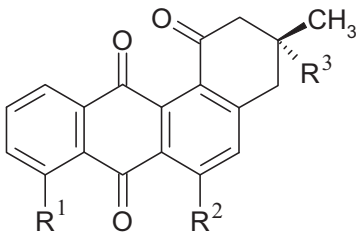
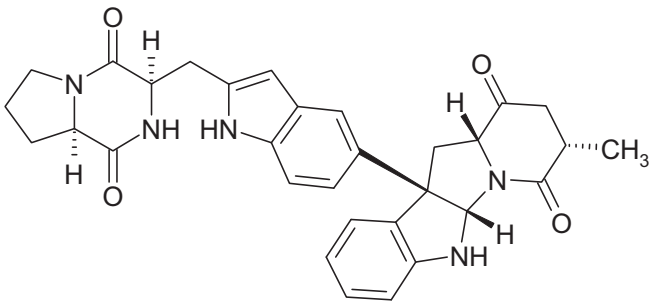


19a: R^1 = OH, R^2 = OH, R^3 = OH

19b: R^1 = OMe, R^2 = OH, R^3 = H

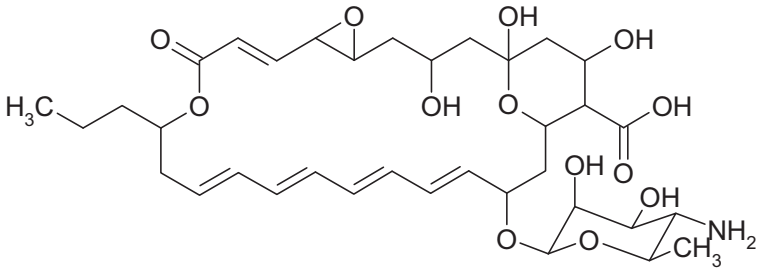
19c: R^1 = OH, R^2 = H, R^3 = OH

Natural products with novel structures can provide new ideas and stimulate synthetic approaches for improving and supporting the drug discovery development. Naseseazine A (**20**), a metabolite of a marine-derived *Streptomyces* species, has a novel dimeric diketopiperazine framework with an unprecedented dimeric structure.[38]



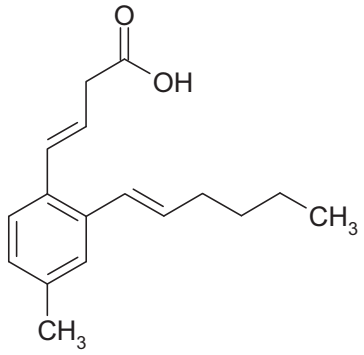
20

The pimaricin analogue JBIR-13 (**21**) was isolated from *Streptomyces bicolor* NBRC 12746 and exhibited like the former one a high antifungal activity. The MIC value of compound **21** against *C. albicans* was 3.13 μg/ml, while that of pimaricin was 1.56 μg/ml.[39]



21

Lorneic acids A (**22**) and B (**23**) were isolated from a marine-derived *Streptomyces* strain (NPS554). Compound **22** exhibited remarkable activity in phosphodiesterase (PDE) inhibition experiments at 10 μM.[40]



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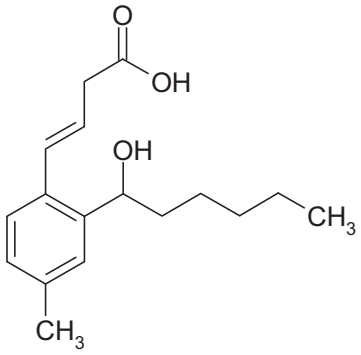
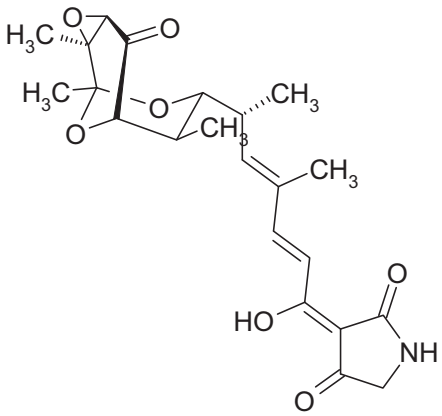


23

The marine environmental isolate *Streptomyces* sp. 307-9, which afforded previously tirandamycins A (**24**) and B (**25**), recently delivered further dienoyl tetramic acids identified as tirandamycin C (**26**) and D (**27**). These compounds demonstrated antibacterial activity against vancomycin-resistant *Enterococcus faecalis*.[41]



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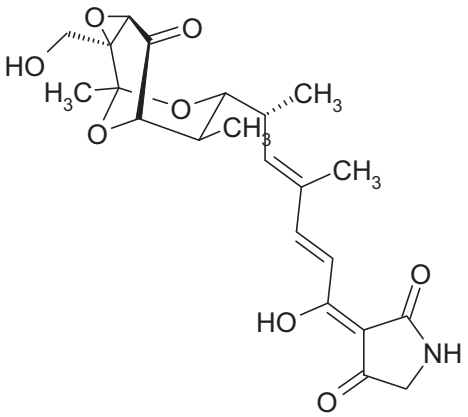

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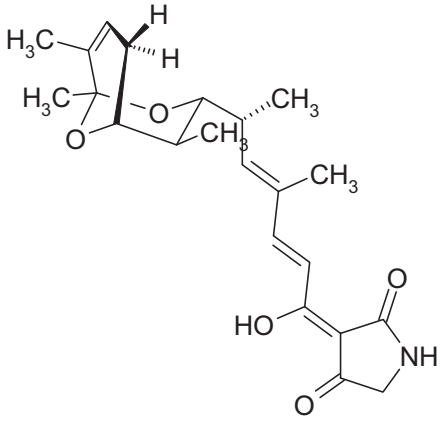

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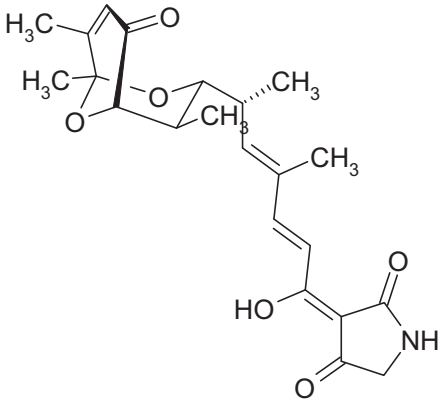
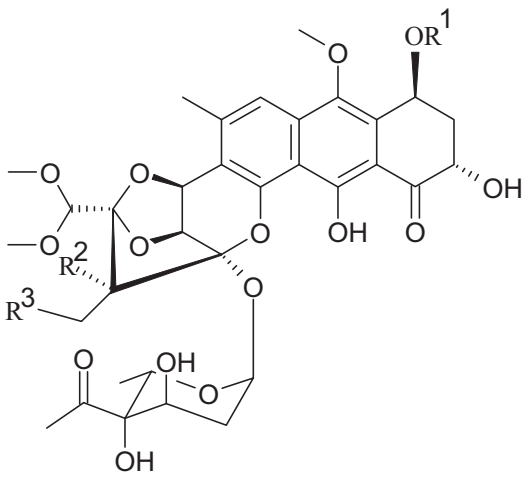
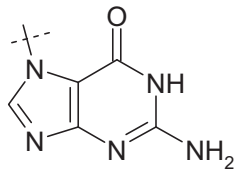


27

An amazing trioxacarcin antibiotic gutingimycin (**28**c) was isolated in our group from the marine derived *Streptomyces* sp. B8652 along with trioxacarcins A (**28a**) to D (**28d**). Gutingimycin (**28c**) as well as trioxacarcins **28a** to **28d** possess antitumor as well as antibacterial activities.[42]





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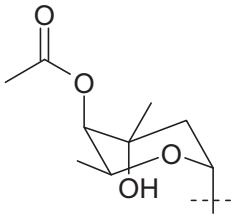


A

28a: R^1 = A, R^2 - R^3 = O

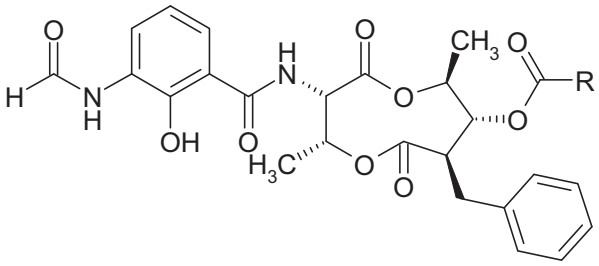
28b: R^1 = A, R^2 = R^3 = OH

28c: R^1 = A, R^2 = OH, R^3 = B

28d: R^1 = H, R^2 = OH, R^3 = B

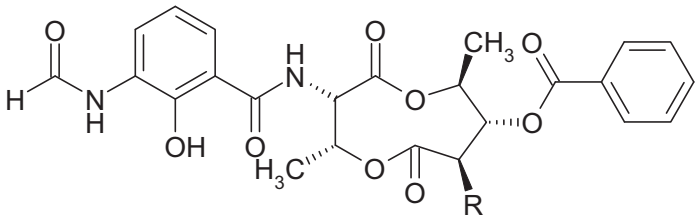
Splenocins (**29a**-**30**) were isolated from *Streptomyces* strain CNQ431 and classified as potent inhibitors of pro-inflammatory cytokine production, characteristic of allergic asthma.[43]

Ammosamides A (**31**) and B (**32**) were recently isolated from *Streptomyces* strain CNR-698 by the group of William Fenical. Both compounds showed significant *in vitro* cytotoxicity against HCT-116 colon carcinoma, each with IC_{50} = 320 nM. Moreover, they exhibited pronounced selectivity in a number of cancer cell lines with values ranging from 20 nM to 1 μM.[44,45]



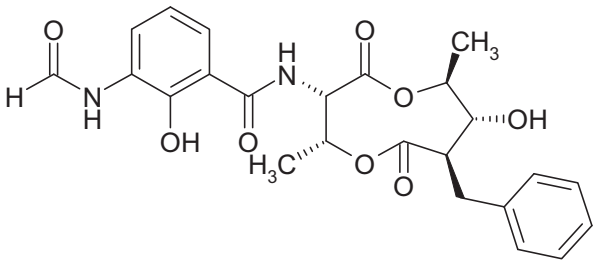
29a: R = CH_3

29b: R = $CH(CH_3)_2$



29c: R = $CH_2(CH_2)_2CH_3$

29d: R = $CH_2(CH_2)_3CH_3$



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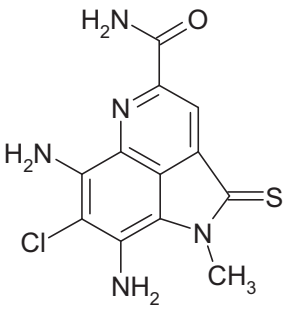

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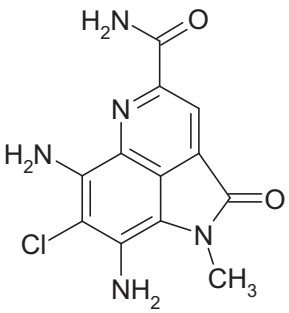
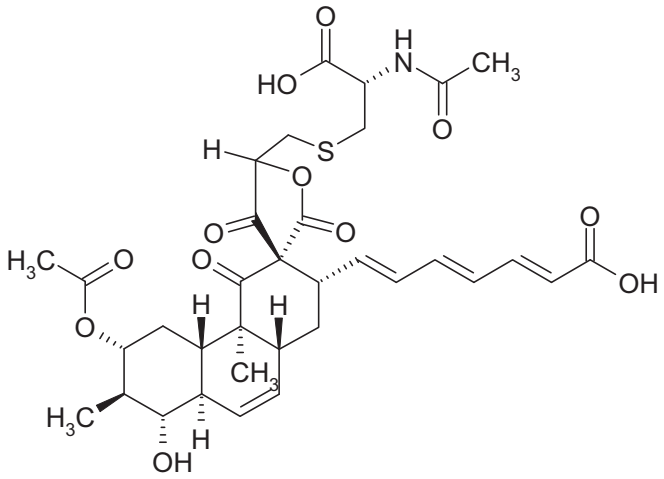


32

Lucensimycins E (**33**) and F (**34**) were isolated recently from *Streptomyces lucensis* MA7349. Compound **33** exhibited the best activity and showed MIC values of 32 μg/ml against *Staphylococcus aureus* and 8 μg/ml against *Streptococcus pneumoniae*.[46]

Cytotoxic (HCT-116) activity has been found for cyclic hexadepsipeptides piperazimycins (**35a**-**35c**), isolated from the fermentation broth of a *Streptomyces* sp.[47] Piperazimycins were also isolated recently in our group. Piperazimycin A (**35**) exhibited cytotoxic activity against range of human tumor cell lines with an overall potency of 0.13 µg/ml (mean IC_{50} value of 36 tumor cell lines tested).[48]



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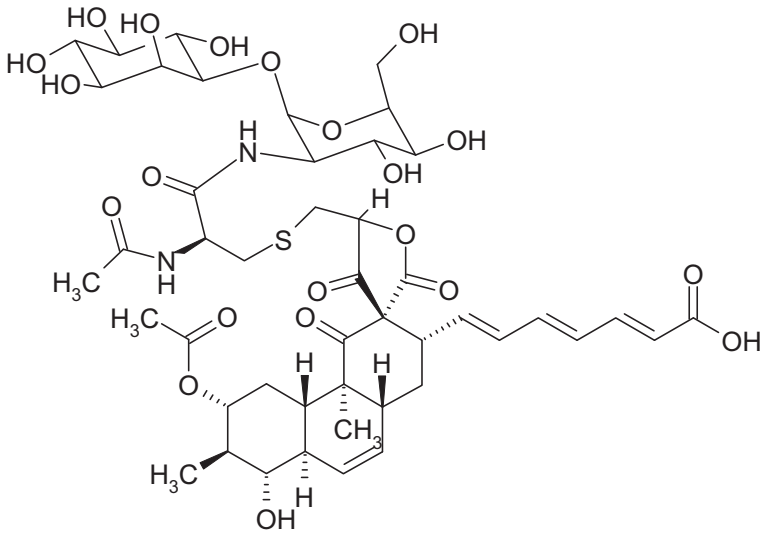

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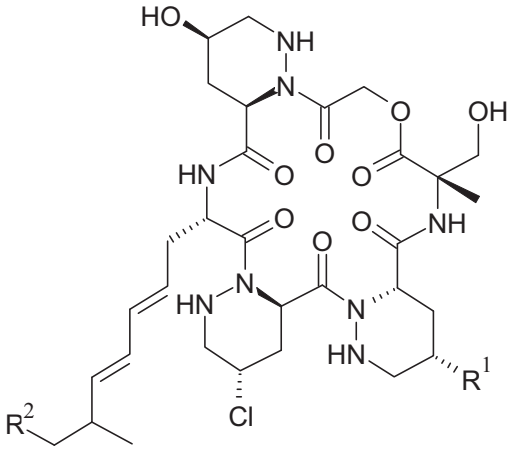


35a: R^1 = OH, R^2 = H

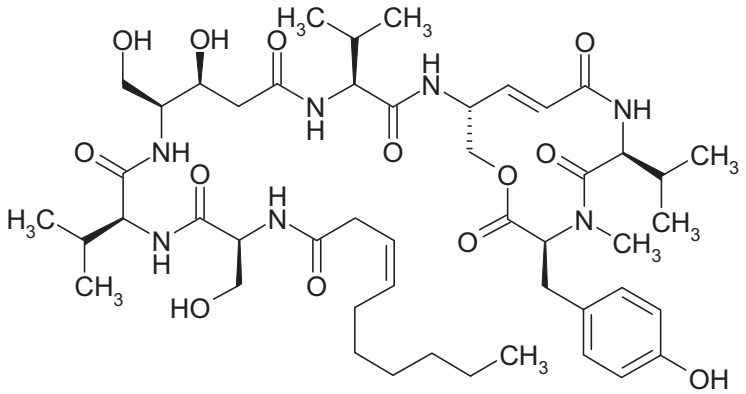
35b: R^1 = R^2 = H

35c: R^1 = OH, R^2 = Me

Immunosuppressive activity in an interleukin-5 production inhibition assay has been found for the bacterial cyclic octapeptides, thalassospiramides A (**36**) and B (**37**), which were isolated from a culture of a new marine α-proteobacterium of the *Thalassospira* genus.[49]

Currently antibacterial activity of Indonesian *Streptomyces* sp. ICBB8198 guided to the isolation of new modified phenazine derivatives **38** and **39**. Compound **38** exhibited antibacterial activity against *S. aureus*, while compound **39** had no antimicrobial activity.[50]

A new bafilomycin F (**40**) was recently isolated from laboratory cultures of two *Streptomyces* spp. and found to be an inhibitor of EGFP (enhanced green fluorescent protein) production showing inhibition of autophagic protein degradation.[51]



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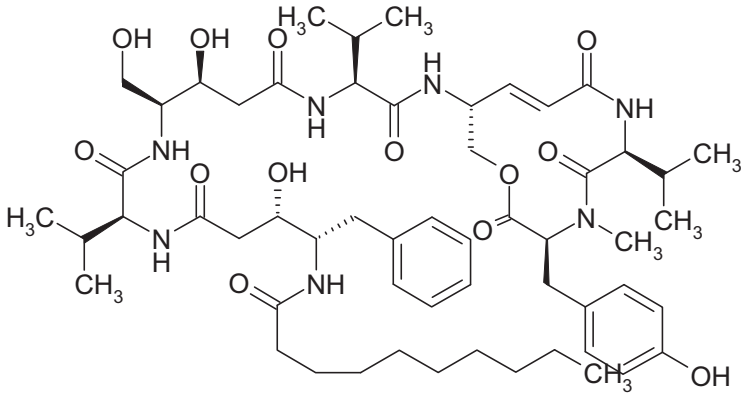

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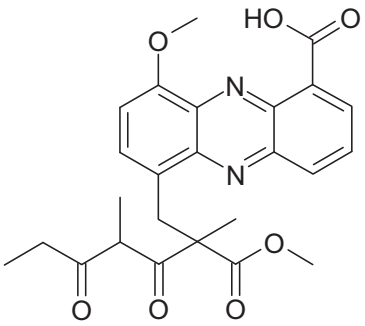

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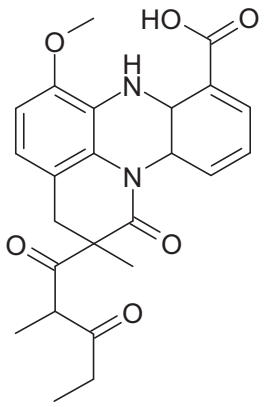

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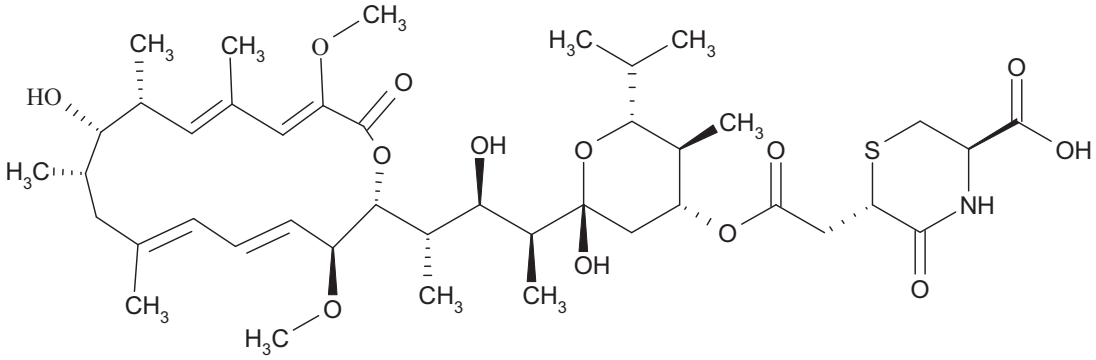
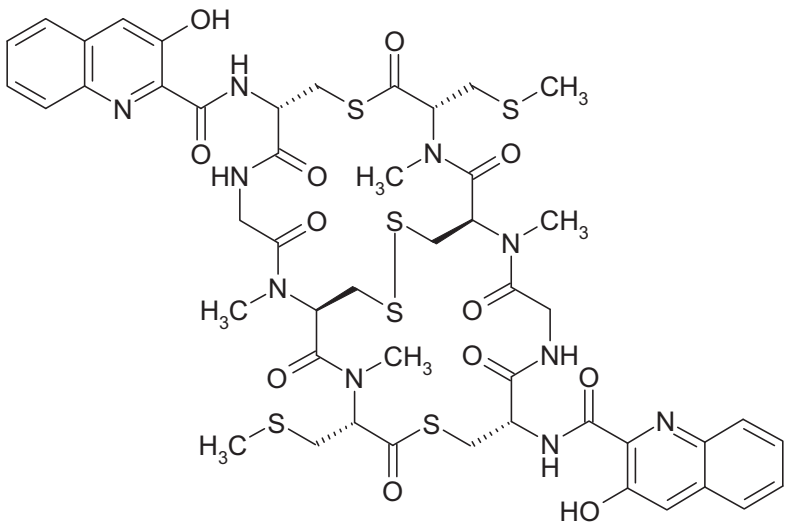


40

The antitumor thiocoraline (**41**) from an actinomycete had potential therapeutic applications like echinomycin. These types of compounds are inhibitors of hypoxia inducible factor-1 that controls genes important for tumor progressing.[52]

Thiopeptides or thiazolylpeptides are a group of naturally occurring antibiotics, known for their complex structures. Philipimycin (**42**) is an example isolated from *Actinoplanes philippinensis* MA7347. It showed strong antibacterial activities against

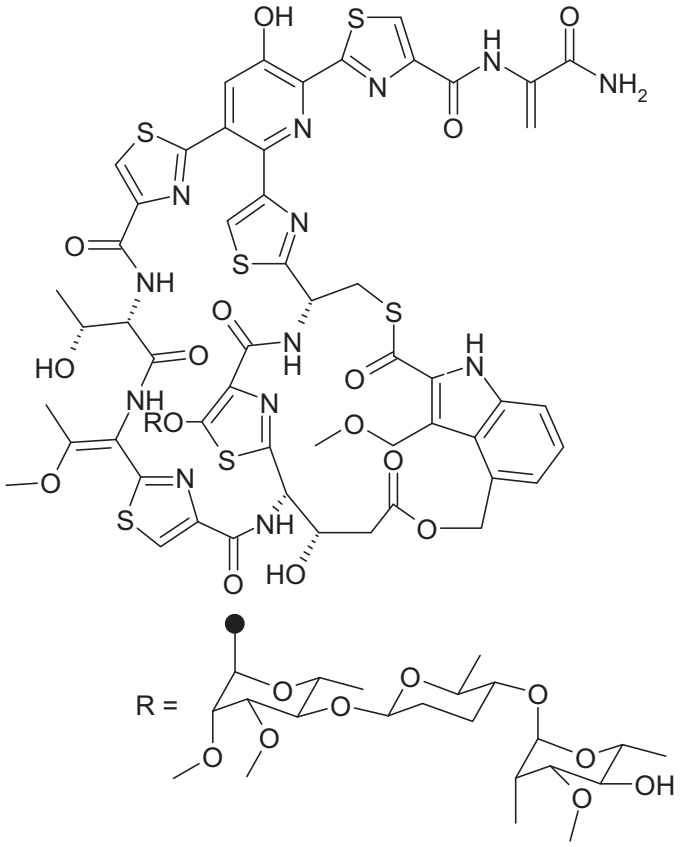
Gram-positive bacteria including MRSA and exhibited MIC values ranging from 0.015 to 1 μg/ml.[53]



41

Siomycin A (**43**) is another example of thiopeptides isolated from *Streptomyces sioyaensis* ATCC 13989, which also exhibited remarkable antibacterial and anticancer activity.[54]

The thiopeptide antibiotic Val-geninthiocin (**44**) was recently isolated in our group from a terrestrial *Streptomyces* sp. RSF18.[55] These types of peptides are known for their antibacterial activity against Gram-positive bacteria and are growth inhibitors of *Plasmodium falciparum*.[56,57]



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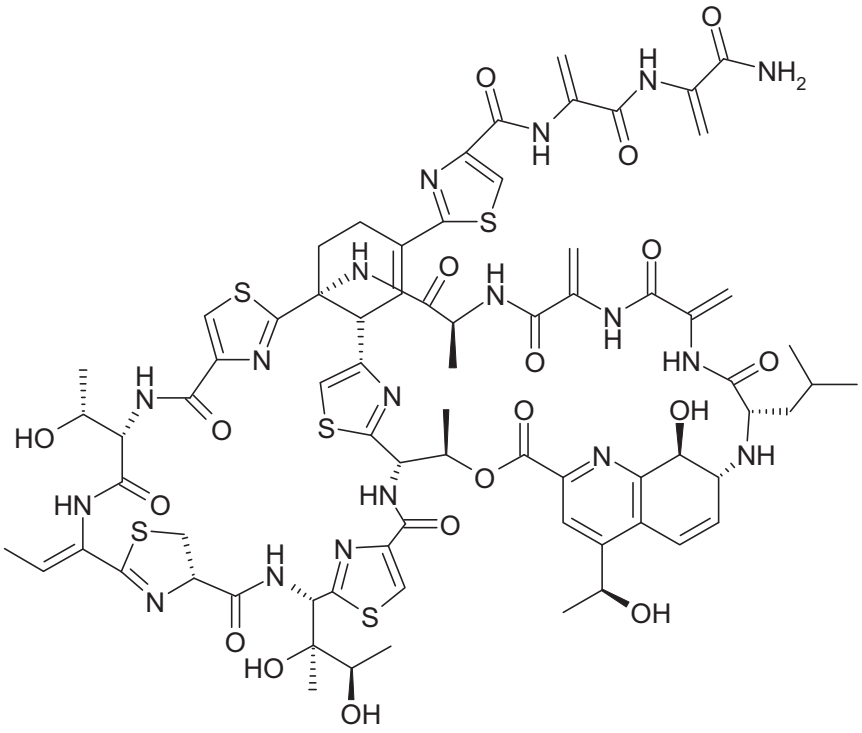

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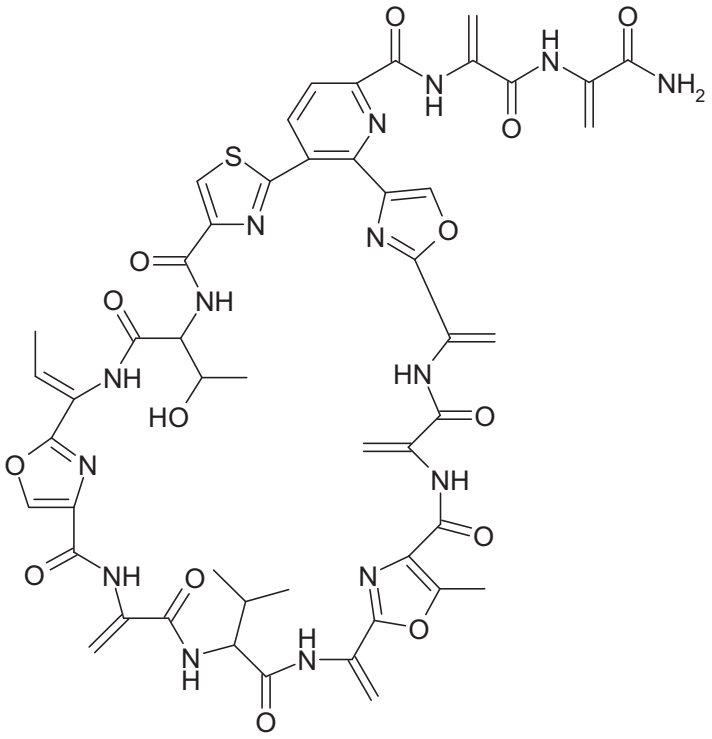
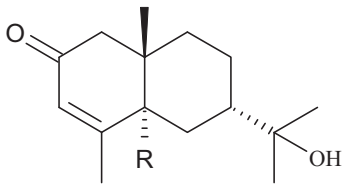


44

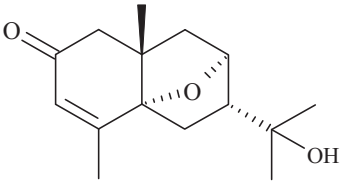
1.4 Natural Products from Endophytes

Surprisingly, the previously clear separation between plant-derived metabolites and microbial natural products is more and more becoming blurred: An increasing number of plant metabolites have been isolated from microbial sources. Recent examples are the isopterocarpolone sesquiterpenoids, which are known plant metabolites. Interestingly four new bacterioisopterocarpolone derivatives **45**-**48** were isolated in our group from a marine derived *Streptomyces* sp. B 7857. It was the first report of bacteria producing this type of sesquiterpenes.[58]



45 R = H

46 R = OH



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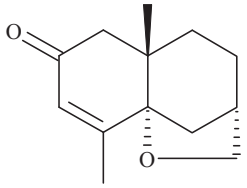
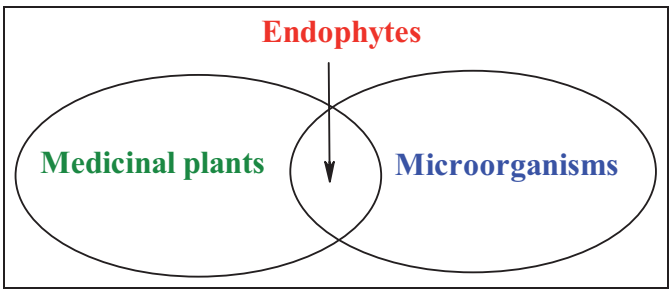
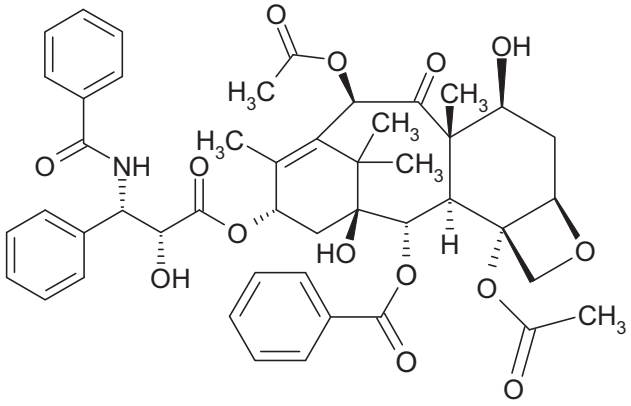


48

Endophytes are microorganisms, which live in the intercellular spaces of host plants without causing any destructive diseases.[59] All higher plants are known to host one or more endophytic microbes. And indeed, some of the metabolites isolated from plant sources trace their origin back to endophytic microbes within the plants.

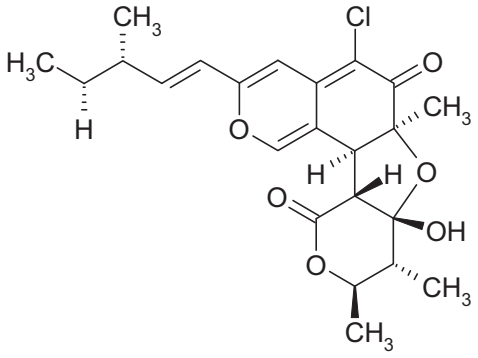


The most famous endophytic product is certainly the anticancer drug, paclitaxel (Taxol, **49**)[60,61] from *Pestalotiopsis microspora*, that colonizes the Himalayan yew tree *Taxus wallichiana* as symptomless parasitic fungus. This discovery has highlighted the term 'endophytes' and paid attention to the interesting metabolites that are produced by these organisms.[62,63]



49

Recently a novel cytotoxic chlorinated azaphilone derivative chaetomugilin D (**50**) was isolated in our group from an endophytic fungus *Chaetomium globosum* following activity-guided fractionation. Chaetomugilin D exhibited growth inhibitory activity against the brine shrimp (*Artemia salina*) and *Mucor miehei*.[64]



50

These facts demonstrated again that the taxonomic kingdoms in nature are not independent from each other, but are having complex ecological interactions on the basis of chemistry. This leads to a new understanding of natural products as words in the language of nature, where ecology is the grammar.[65]

1.5 Scopes of the Present Research Work

The purpose of natural products, i.e. the benefit for the producing organism, has been discussed from many aspects, but is still uncertain. While previously an anthropogenic view was favoured where secondary metabolites were believed to be part of a defence mechanism, natural products seem to be preferably vocabularies in a communication system with ecology as grammar: It can be assumed that all natural products are having any essential function in the respective habitat. But still no logical approach is available to predict this activity, and a random screening is the only way.

To follow ethnomedical applications of plants and plant products is a logical approach to identify bioactive compounds. The rural population in Sudan has a rich tradition in the use of local plants, but in many cases, the chemical background is still completely unknown. Part one in the present investigation was focussed therefore on the identification and chemical characterisation of constituents from ethnomedicinally used plants from Sudan. Four plants, namely *Xanthium brasilicum* Vell, *Albizia zygia*, *Tephrosia apollinea,* and *Aristolochia bracteolata* Lam, were selected on the advice of ethnobotanical information and traditional healers. Sufficient amounts of extracts were available at the beginning of this investigation. The idea behind this work was to rationalize the medicinal activity of these plants scientifically, so that the local community can be advised to use the plants with the best benefit.

As plants and microorganisms are forming close communities and as there is an increasing overlap between metabolites from microbes and plants, it was a logical approach also to investigate selected bacteria and (endophytic) fungi. Depending on the outcome of the plant project in part 1, it was planned also

- ✓ to highlight attention on new structures from marine and terrestrial *Streptomyces* sp. by using modern techniques for separation and structure elucidation,
- ✓ to find new applications of these metabolites in medicine and agriculture, to search for metabolites with activities against e.g. cancer, bacteria, fungi, algae and especially against oomycetes by applying a test for zoosporicidal activity to make a contribution for pharmaceutical industry and drug discovery in the future.

2 Plant Metabolites

2.1 *Xanthium brasilicum* Vell (Asteraceae)

The genus *Xanthium* have been described by a limited number of species, which are distributed in all parts of the world.[66] *X. brasilicum* Vell has been classified as an agricultural and naturalized weed.[67-69] Ethnomedicinal uses have been reported in many parts of the world. In Sudan *X. brasilicum* is used in Sudanese folk medicine for treating endemic diseases.

The aerial parts of *Xanthium brasilicum* were collected from Shambat area, Khartoum, Sudan in June 2006. The plant material was authenticated by Mr. Wail El Sadig (National Research Centre, Sudan) based on comparison with voucher specimen deposited in the herbarium of the Institute of the Medicinal and Aromatic Plants, Khartoum, Sudan.

Various essential oils, terpenes, and sesquiterpenes were previously isolated from related species such as *X. strumarium*.[66] *X. spinosum* L is rich in xanthanolide sesquiterpene lactones.[70] Glucose, sucrose, β-sitosterol, β-sitosterol-D-glucoside, stigmasterol, campesterol, taraxerol, taraxeryl acetate, xanthanodiene and traces of *n*-heptacosane have been reported from the roots of *X. italicum*.[71] Sesquiterpene lactones have been isolated from *Xanthium canadense* Mill.[72] Borneol, fenchone and isobornyl acetate have been obtained from the aerial part of *Xanthium brasilicum* as the major constituents.[66] Studies on the *in vitro* immunomodulating properties of selected Sudanese medicinal plants showed that the extract of the aerial parts of *X. brasilicum* has moderate inhibitory activity (52.2%).[73] In addition to these observations, *X. brasilicum* extract has shown anti-*Helicobacter pylori* activities.[74] Recently an antimalarial activity of the crude extract of *X. brasilicum in vitro* and *in vivo* has been reported.[75]

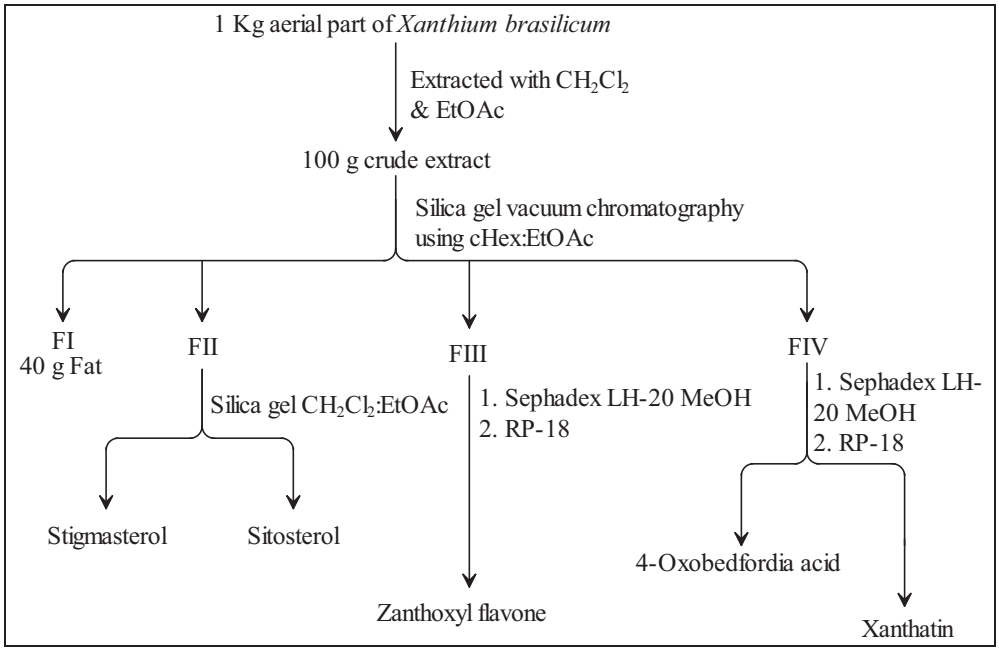
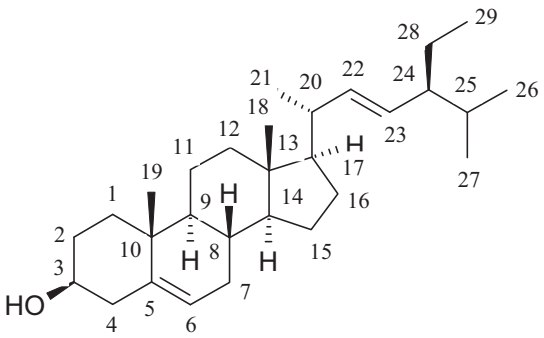


Figure 1: Work up scheme of *X. brasilicum*

2.1.1 Stigmasterol and *β*-Sitosterol

Stigmasterol (**51**) and *β*-sitosterol (**52**) were isolated in mixture as a white powder, which showed an UV absorbing band and turned to violet with anisaldehyde/sulphuric acid. The molecular weights were assigned by EIMS as 412 and 414, respectively, and the molecular formulas were obtained by HRESIMS as $C_{29}H_{48}O$ and $C_{29}H_{50}O$, respectively. The ^{1}H NMR spectrum (Figure 137) showed two olefinic protons at δ 5.36: one proton for **51** (H-6) and the second for **52** (H-6), in addition to two olefinic protons appearing as doublets of doublet at δ 5.16 and 5.02 (H-22) and (H-23), respectively, for **51**. Moreover, a multiplet at δ 3.53 (H-3) is a typical signal for steroids with an oxygenated position 3. ^{13}C NMR spectrum (Figure 138) revealed four olefinic carbons.



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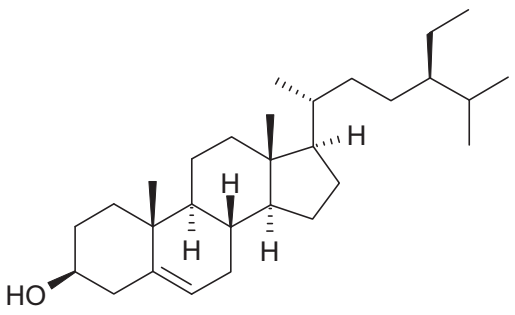
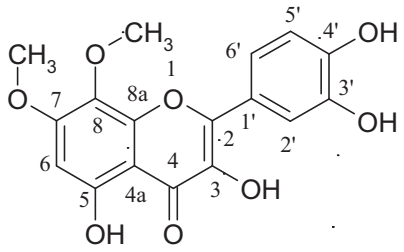


52

In addition, an oxymethine carbon was observed at δ 71.8. Several methine, methylene and methyl carbon signals appeared. The structures were confirmed by comparing the ^{1}H, ^{13}C NMR and MS spectra with the literature data.[76]

2.1.2 Zanthoxyl flavone

Compound **53** was isolated as a yellow solid, which gave a strongly UV absorbing band on TLC at 254 nm and turned to yellow with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **53** displayed four aromatic proton signals at δ 7.60 (H-2'), 7.51 (H-6'), 6.87 (H-5') and a singlet at δ 6.45 (H-6). Additionally two methoxy signals appeared at δ 3.76 (CH_3O-7) and 3.86 (CH_3O-8).



53

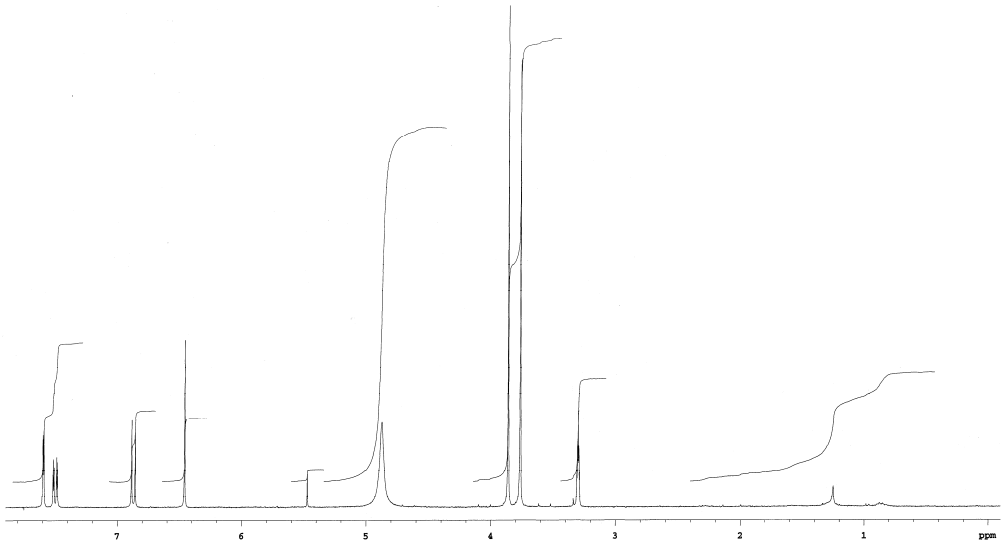


Figure 2: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of compound **53**

In the ^{13}C NMR spectrum 17 signals were visible, among them eleven quaternary carbons between δ 182.8 and 108.8; additionally, four aromatic methine signals and two methoxy carbons at δ 63.5 (CH_3O-8) and 63.0 (CH_3O-7) were observed. ESIMS afforded a *pseudo*molecular ion peak at 369, which gave the molecular weight 346 and the molecular formula $C_{17}H_{14}O_8$ by HRESI MS.

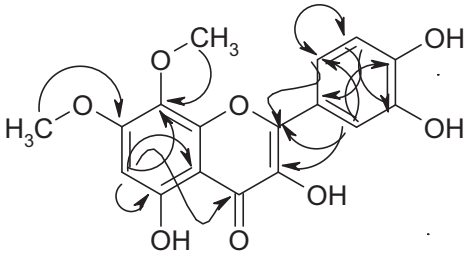


Figure 3: Selected H,H COSY (⟷) and HMBC (⟶) correlations of compound **53**

1D and 2D data obtained for compound **53** showed typical signals for flavones as well as correlations confirming the flavone skeleton. H,H COSY correlations and the signal pattern indicated the presence of a 1,3,4-trisubstituted benzene ring B; oxygen in positions C-3',4' of ring B was confirmed by the chemical shifts and HMBC correlations (Figure 3). Ring A is bearing one hydroxy and two methoxy groups. Their position can be distinguished by the following arguments: The proton in ring A shows a weak ^4J correlation with the carbonyl at C-4, pointing to its position at C-6 or C-8. All remaining positions in ring A are oxygenated, and a proton in position 6 was correlated with oxygenated carbons at C-5 and C-8. Correspondingly, the signal at δ 141.7 must be that of C-7; it is connected with the methoxy group at δ 3.76. This was confirmed by a proton spectrum in DMSO, which show a chelated OH signal at (δ 12.8), thus assighned an OH group at C-5. The second methoxy group is attached to a highfield-shifted carbon (δ 135.1), which is therefore in position C-8. This results in structure **53**.

A literature search in the Dictionary of Natural Products[77] and the Chemical Abstracts afforded the zanthoxyl flavone (**53**), which was known from the seeds of *Zanthoxylum alatum* Roxb. (Rutaceae)[78] and isolated for the first time from *X. brasilicum*. Compound **53** possessed novel motility inhibitory and zoosporicidal activity[79] against the downy mildew pathogen *P. viticola* in a concentration of 5 μg/ml as presented in Table 2.

Table 1: ^{13}C and ^{1}H NMR shifts (125 MHz, 300 MHz) for **53** in CD_3OD

Position	δ_C	C type	δ_H (mult.; J in [Hz])
2	160.6	C_q	
3	156.24	C_q	
4	182.8	C_q	
4a	108.8	C_q	
5	161.3	C_q	
6	97.5	CH	6.45 (s)
7	141.7	C_q	
8	135.1	C_q	
8a	156.16	C_q	
1'	125.5	C_q	
2'	119.0[a]	CH	7.60 (d, $^3J = 2.2$)
3'	149.0	C_q	
4'	152.5	C_q	
5'	118.9[a]	CH	6.87 (d, $^3J = 8.5$)
6'	124.9	CH	7.51 (dd, $^3J = 8.5, 2.2$)
7-OCH_3	63.0	CH_3	3.76 (s)
8-OCH_3	63.5	CH_3	3.86 (s)

[a] signals may be exchanged

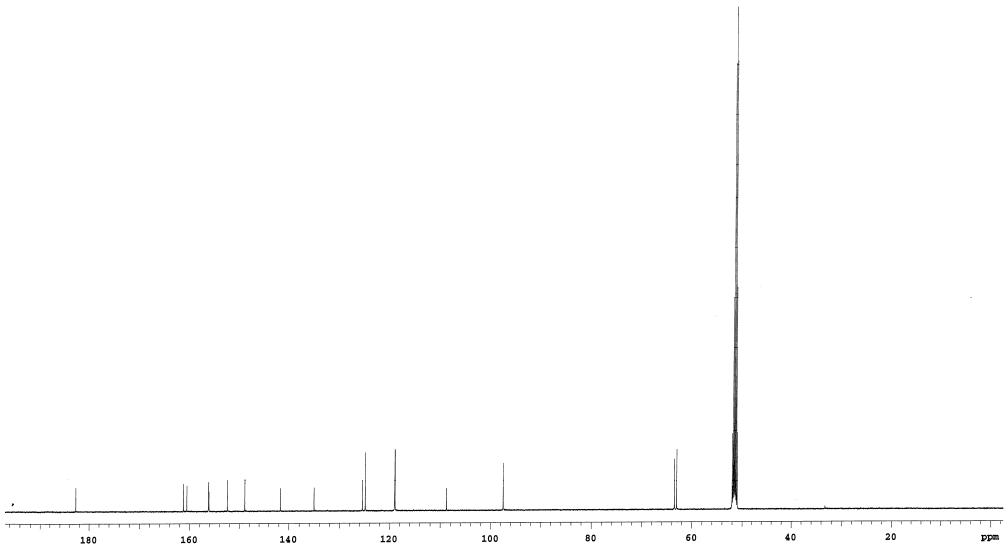


Figure 4: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **53**

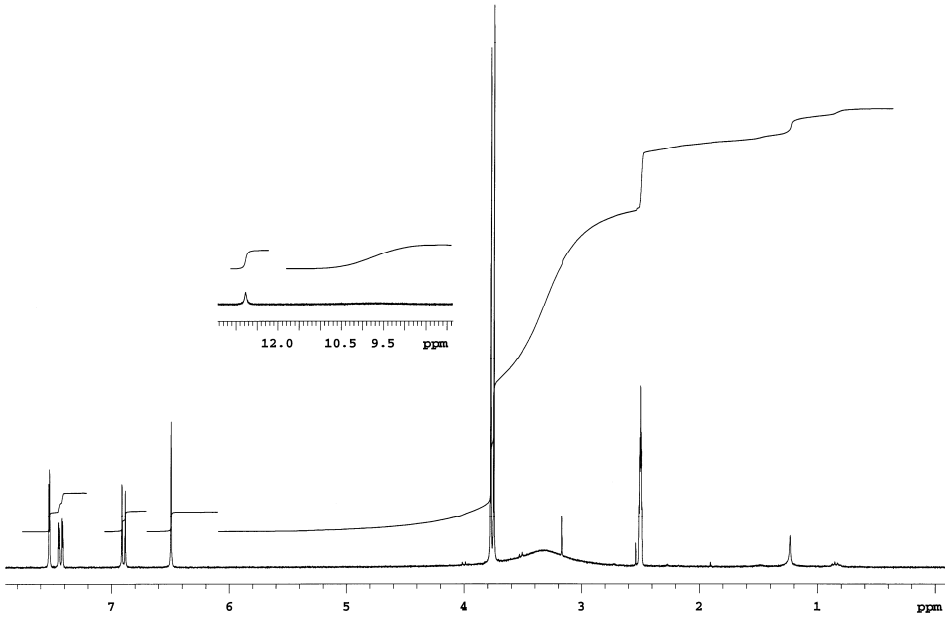
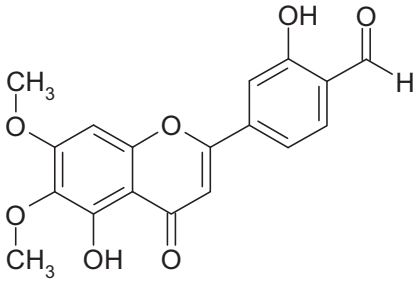


Figure 5: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of compound **53**

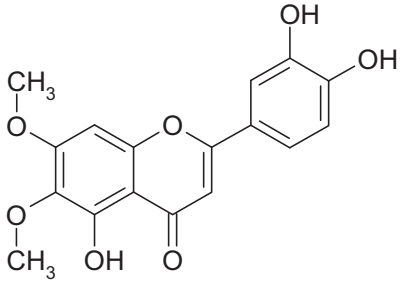
Table 2: Motility inhibition and lytic activity of compound **53** against the downy mildew pathogen *Plasmopara viticola*

Motility halting and zoosporicidal activity (%±SE)					
	Dose (μg/mL)	15 min		30 min	
		Halted	Burst	Halted	Burst
Compound **53**	5	6 ± 0	0 ± 0	100 ± 0	100 ± 0
	10	100 ± 0	100 ± 0	nt	nt

In a recent investigation on the activity of flavonoids it was found that the two related flavones eupatorin (**54**) and cirsiliol (**55**), which had been isolated from the leaf extract of *Lantana montevidensis* Briq. (Verbenaceae), enhanced CYP1 enzyme activity in a concentration-dependent manner in MCF7 human breast adenocarcinoma cells. In the range of 0.1-2.5 μM, compound **55** caused a dose-dependent increase in CYP1B1 mRNA levels and an increase in CYP1A1 mRNA, while eupatorin (**54**) caused an increase in CYP1A1 and CYP1B1 mRNA at higher doses (~5 μM). Cirsiliol (**55**) induces CYP1 enzyme expression in cancer cells and is subsequently converted by CYP1B1 or CYP1A1 into an antiproliferative agent.[10]



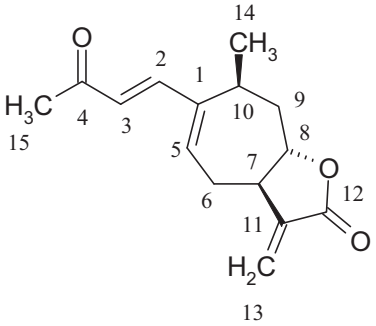
54



55

2.1.3 Xanthatin

Xanthatin (**56**) was isolated as oily substance after purification of fraction FIV. On TLC, it gave a UV absorbing band, which turned to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum (Figure 141) of **56** displayed five olefinic protons in addition to an oxymethine at δ 4.65 (H-8). Moreover, two methine protons at δ 3.42 (H-7) and 2.84 (H-10) and two methylene groups at δ 2.58/2.48 (2H-6) and 2.18/1.91 (2H-9) were observed. In addition two methyl groups appeared, one as a doublet at δ 1.19 (Me-14) and the second as a singlet at δ 2.30 (Me-15).



56

The ^{13}C NMR spectrum delivered 15 signals, which were confirmed by the HR mass spectrum ($C_{15}H_{18}O_3$). This gave an idea that the structure could be a sequiterpenoid, which should follow the isoprene rule. Two carbonyl signals appeared, that of a ketone at δ 198.3 (C-4) and that of a lactone at δ 169.5 (C-12).

Two quaternary carbon signals at δ 142.6 (C-1) and 137.9 (C-11) were obtained in addition to four olefinic carbons. An oxymethine carbon at δ 78.0 (CH-8) was observed along with two methine carbons at δ 31.5 (CH-10) and 41.0 (CH-7), two methylene carbons at δ 36.1 (CH_2-9) and 26.8 (CH_2-6) and two methyl carbons at δ 27.5 (C-15) and 21.3 (C-14). The molecular weight was assigned by ESIMS as *m/z* 246.

H,H COSY correlations from H-14 to H-5 and HMBC correlations from H-10 to C-1 and H-5 to C-1 supported the occurrence of a seven-membered ring. The correlations from H-13 to C-12, C-11 and C-7 and from the oxymethine at δ 4.65 (H-8) to C-12 confirmed the presence of a lactone ring. The value of the carbonyl signal of C-12 (169.5) agrees with the value of a lactone carbonyl. Additionally, olefinic signals at δ 6.99 and 6.14 displayed strong HMBC correlations with a ketone carbonyl at δ 198.3, indicating an α,β-unsaturated system. The connectivity of structure **56** was established by further correlations from H-2 and H-3 to C-1, and of a methyl singlet at δ 2.30 showing correlation with the carbonyl at δ 198.3 (H,H COSY and HMBC spectra see Figure 142 and Figure 143). Further correlations are drawn in Figure 7. A search in the Dictionary of Natural Products[77] and comparing the spectroscopic data with the literature confirmed the structure as xanthatin (**56**). Compound **56** was isolated previously from other species of the genus *Xanthium.*[80] To our knowledge this is first time that xanthatin (**56**) was isolated from *X. brasilicum*.

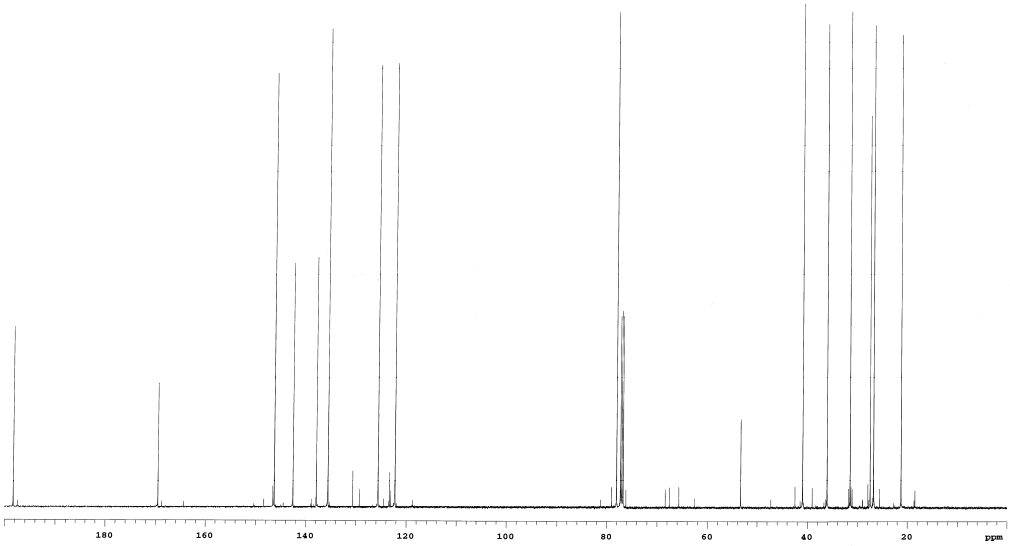


Figure 6: ^{13}C NMR spectrum (125 MHz) of xanthatin (**56**) in $CDCl_3$

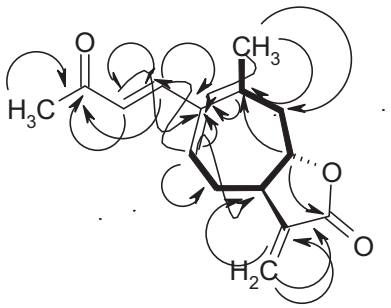


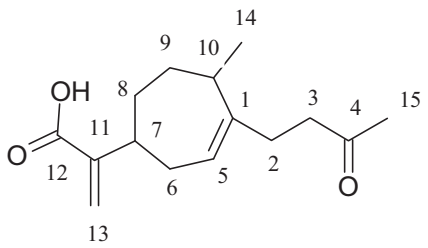
Figure 7: Selected $^{1}H,^{1}H$ COSY and HMBC correlations of xanthatin (**56**)

Table 3: ^{13}C and ^{1}H NMR chemical shifts (125, 300 MHz) of xanthatin (**56**) in $CDCl_3$

Position	δ_C	δ_H (mult.; *J* in [Hz])
1	142.6	
2	146.3	6.99 (d, *J* = 16.3)
3	125.6	6.14 (d, *J* = 16.3)
4	198.3	
5	135.6	6.22 (d, *J* = 6.3)
6	26.8	2.58 (m)
		2.48 (m)
7	41.0	3.42 (m)
8	78.0	4.65 (ddd, 12.3, 8.7, 2.3)
9	36.1	2.18 (ddd, *J* = 11.9, 7.1, 2.3)
		1.91 (ddd, *J* = 14.1, 11.9, 1.0)
10	31.5	2.84 (m)
11	137.9	
12	169.5	
13	122.3	6.31 (d, *J* = 3.3)
		5.58 (d, *J* = 3.0)
14	21.3	1.19 (d, *J* = 6.9)
15	27.5	2.30 (s)

2.1.4 4-Oxobedfordia acid

4-Oxobedfordia acid (**57**) was isolated as oily substance from an UV absorbing band, which turned to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum displayed three olefinic protons at δ 6.25/5.62 (CH_2-13) and 5.39 (CH-5), and five methylene groups at δ 2.52 (CH_2-3), 2.24 (CH_2-2), 2.24/2.04 (CH_2-6), 1.78 (CH_2-8) and 1.68/1.61 (CH_2-9). Moreover, two methine protons appeared at δ 2.46 (H-7), and 2.27 (H-10) in addition to two methyl groups at δ 2.14 (CH_3-15) and 1.09 (H_3-14); for better comparison, the numbering system of Marco *et al*.[80] was used.



57

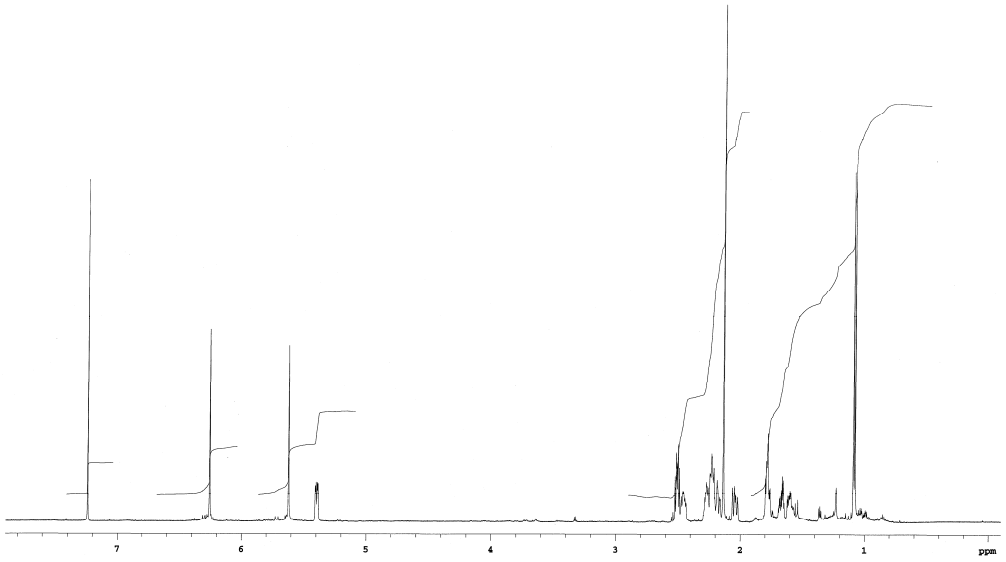


Figure 8: ^{1}H NMR spectrum (300 MHz) of 4-oxobedfordia acid (**57**) in $CDCl_3$

The ^{13}C NMR spectrum (Figure 144) delivered 15 carbons: two carbonyls at δ 209.0 and 171.7 were observed in addition to two quaternary carbons at δ 147.3 (C-1) and 146.2 (C-11) and two olefinic carbons at δ 124.7 (CH_2-13) and 122.6 (CH-5). Moreover, five methylene carbons appeared at δ 42.8 (CH_2-3), 33.9 (CH_2-2), 33.4 (CH_2-6), 32.7 (CH_2-9) and 31.5 (CH_2-8), additionally two methine carbons at δ 39.5 (CH-7) and 37.2 (CH -10) and two methyl carbons at δ 30.0 (CH_3-15) and 16.4 (CH_3-14) were obtained. ESIMS delivered the molecular weight 250. COSY and HMBC correlations in the sequence from H-14 to H-5 in addition to ^{3}J coupling from H-14 to C-1 and from H-6 to C-1 established the seven-membered ring. Additional HMBC correlations confirmed the presence of a methyl ketone (H-15 to C-4) and an acid carbonyl; both methylene protons at H-13 showed HMBC correlation to a carbonyl at C-12. The connectivity of structure **57** was assigned by further HMBC correlations from H-2 and H-3 to C-4 and H-2 to C-1 and C-5, and additionally from H-13 to C-6 and H-7 to C-11 (H,H COSY and HMBC spectra see Figure 145 and Figure 146). Correlations are drawn in Figure 9.

Table 4: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of 4-oxobedfordia acid (**57**) in $CDCl_3$

Position	δ_C	δ_H (mult.; *J* in [Hz])
1	147.3	
2	33.9	2.24 (m)
3	42.8	2.52 (m)
4	209.0	
5	122.6	5.39 (dd, *J* = 9.4, 3.8)
6	33.4	2.04 (dd, *J* = 14.8, 9.3)
		2.24 (m)
7	39.5	2.46 (m)
8	31.5	1.78 (m)
9	32.7	1.68 (m)
		1.61 (m)
10	37.2	2.27 (m)
11	146.2	
12	171.7	
13	124.7	6.25 (s)
		5.62 s
14	16.4	1.09 (d, *J* = 7.3)
15	30.0	2.14 (s)

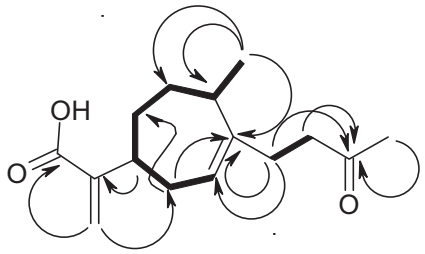


Figure 9: $^{1}H,^{1}H$ COSY (—) and HMBC (⟶) correlations (arrows) of 4-oxobedfordia acid (**57**

A search in the Dictionary of Natural Products[77] and comparing the spectroscopic data with the literature data confirmed compound **57** to be identical with 4-oxobedfordia acid.[80]

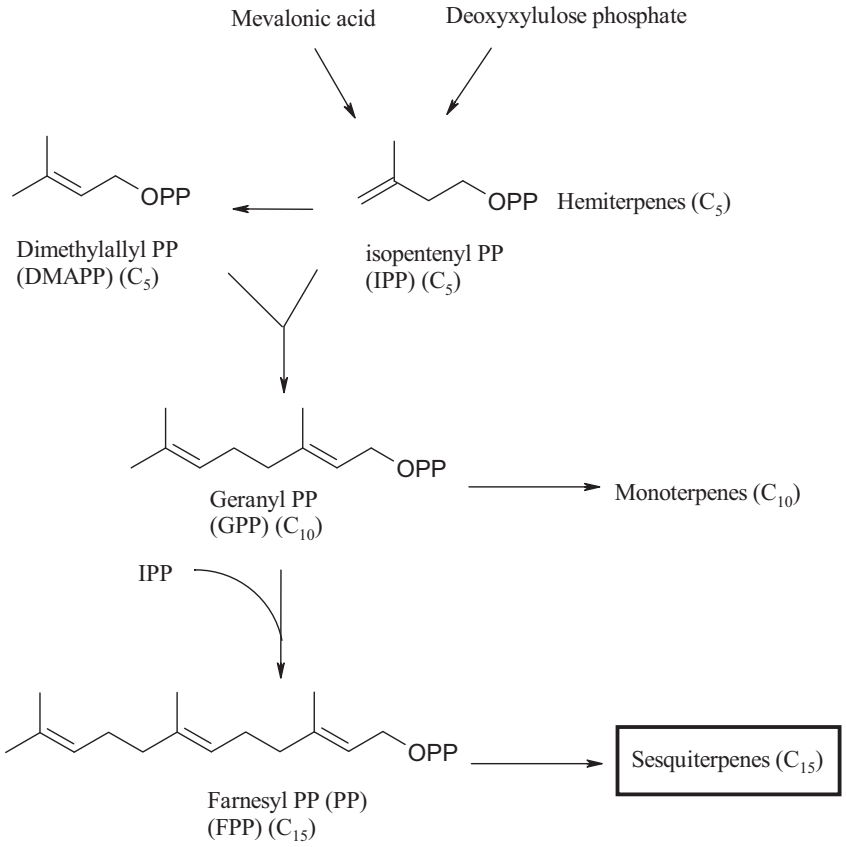


Figure 10: The biosynthetic pathway of the general sesquiterpene precursor, farnesyl diphosphate (FPP)[18]

Compound **57** was isolated previously from other species of the genus *Xanthium*,[80] but it is for the first time isolated here from *X. brasilicum*.

Xanthanolides showed antifungal activity against *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus* and antileishmanial activity against *Leishmania infantum* and *Leishmania mexicana.*[81] The sesquiterpenes **56** and **57** are formed from three C_5 units, where a C_5 isopentenyl pyrophosphate (IPP) unit was added to geranyl PP (GPP) in an extension of the GPP synthase reaction. The biosynthetic pathway of the general sesquiterpene precursor is drawn in Figure 10.

2.2 *Albizia zygia* (Leguminosae, subfamily Mimosoideae)

Albizia zygia D.C. Macbr. (Leguminosae, subfamily Mimosoideae) is a gum producing tree widely found in West Africa.[82] Its bark was collected from Shambat, Sudan in June 2006. The plant material was authenticated by Mr. Wail El Sadig (National Research Centre, Sudan) based on comparison with voucher specimens deposited in the herbarium of the Institute of the Medicinal and Aromatic Plants, Khartoum, Sudan.

In previous phytochemical studies of *A. zygia*, lupen-20(30)-en-3β-ol and its glycosides, stigmast-5-en-3*β*-ol, and 5α-stigmasta-7,22-dien-3*β*-ol were isolated[83] as well as albizziaprenol and phytol.[84] Also the gum of the plant has been widely investigated for its chemical and physical properties (e.g. as thickening agent) in comparison with other mucilages.[85,86,87] In traditional medicine, the powdered bark of *A. zygia* is used alone or as a decoction in southern Sudan as an antimalarial and antiparasitic drug. The methanolic extract of the stem bark exhibited antiprotozoal activity (IC_{50} 1.0 μg/ml) against *Plasmodium falciparum* strain K1, the protozoa responsible for malaria, and *Trypanosoma brucei rhodesiense* (IC_{50} 0.2 μg/ml), which causes African trypanosomiasis.[88]

The genus *Albizia* consists of over 150 species. The occurrence of flavonoids and their glycosides has been previously reported from plants in this genus. Quercetin and kaempferol as well as their glycosides have been isolated from *A. lebbeck*[89] and *A. julibrissin*.[90] In addition, prenylated flavonoids such as sophoflavescenol and kurarinone were also isolated from *A. lebbeck*.[91] The unusual biflavonoids, eucaediflavone and albiproflavone were isolated from *A. procera*.[92] Other flavonoids were reported from *A. amara*[93] and *A. adianthifolia*.[94]

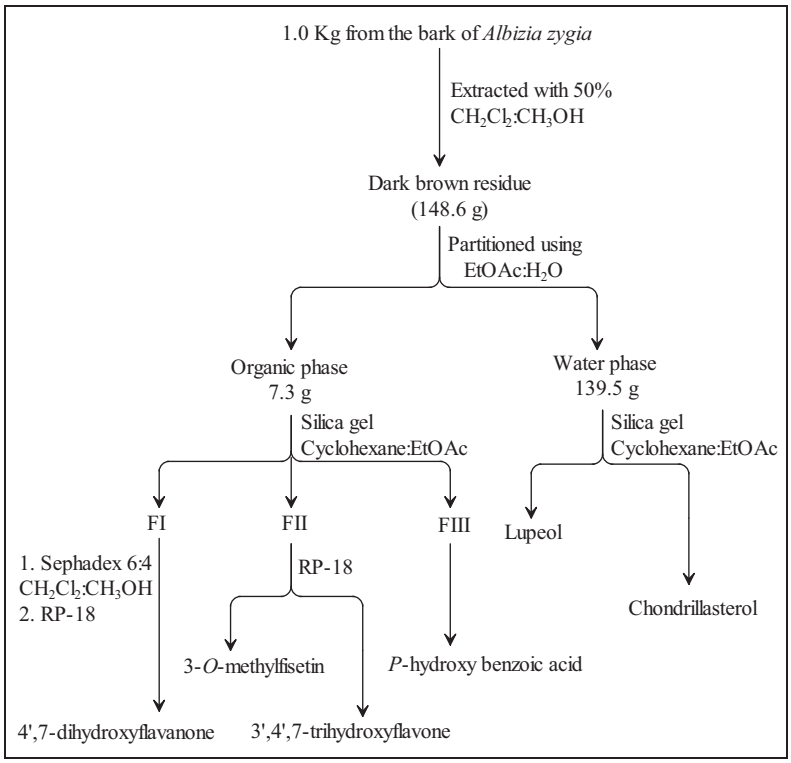
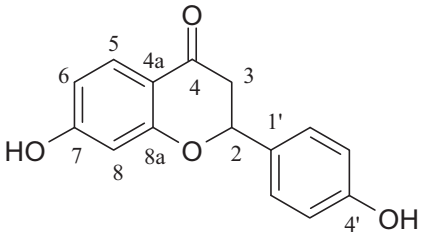


Figure 11: Scheme work up of *Albizia zygia*

2.2.1 4',7-Dihydroxyflavanone

4',7-Dihydroxyflavanone (**58**) was isolated as yellow needles, was UV absorbing on TLC at 254 nm, and turned to yellow with anisaldehyde/sulphuric acid spray reagent. The ^{1}H NMR spectrum (Figure 147) showed seven aromatic proton signals: each two *ortho*-coupled protons of a *p*-disubstituted benzene appeared at δ 7.32 (H-2',6') and 6.81 (H-3',5') in addition to signals of protons in another ring in 1,2,4 position. A methine proton appeared at δ 5.38 (H-2) and methylene protons were observed up-field at δ 3.06/2.68.

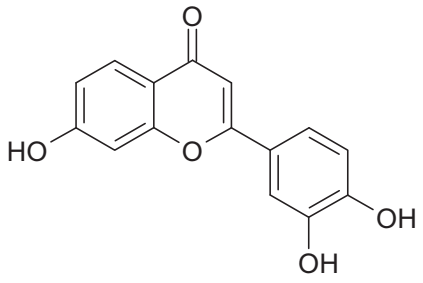


58

ESI MS delivered the molecular weight *m/z* 256, and HRESIMS established the molecular formula $C_{15}H_{12}O_4$. A search in the Chemical Abstracts as well as the Dictionary of Natural Products[77] confirmed the structure as 4',7-Dihydroxyflavanone (**58**) after comparing with literature data.[95]

2.2.2 7,3',4'-Trihydroxyflavone

7,3',4'-Trihydroxyflavone (**59**) was isolated as yellow needles, which were on TLC UV absorbing at 254 nm and turned to yellow with anisaldehyde/sulphuric acid spray reagent. The ^{1}H NMR spectrum (Figure 148) showed the typical pattern of a flavone: seven aromatic protons were observed as four *ortho*-coupled protons at δ 7.95 (H-5) and 6.89 (H-6) and at δ 7.38 (H-6') and 6.88 (H-5') in addition to a *meta*-coupled protons at δ 6.92 (H-8) and 7.38 (H-2'). A singlet for H-3 appeared at δ 6.61.

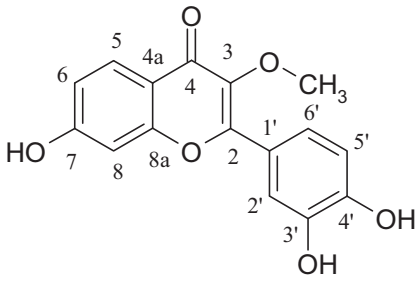


59

ESI MS delivered the molecular weight *m/z* 270 and HRESIMS established the molecular formula $C_{15}H_{10}O_5$. The ^{13}C NMR spectrum revealed 15 carbon signals, eight quaternary carbons and seven aromatic carbons. A search in the Chemical Abstract as well as the Dictionary of Natural Products[77] confirmed the identity after comparison with literature values.[96]

2.2.3 3-*O*-Methylfisetin

3-*O*-Methylfisetin (7,3',4'-trihydroxy-3-methoxyflavone, **60**) was isolated as yellow needles, absorbed UV at 254 nm and turned to yellow with anisaldehyde/sulphuric acid. The ^1H (^1H NMR see Figure 149) and ^{13}C NMR spectra showed similar aromatic patterns like compound **59**, where five aromatic proton and carbon signals established rings A and B. A methoxy singlet was observed at δ 3.77 (3-OCH_3) instead of the signal for H-3.



60

ESIMS of compound **3** afforded the molecular weight *m/z* 300, and HRESIMS delivered the molecular formula $C_{16}H_{12}O_6$. From ^1H,^1H COSY and HMBC spectra, all correlations confirmed the structure of **60** Accordingly, COSY correlations were observed between *ortho*-coupled protons H-5 and H-6 as well as for H-5' and H-6'. The proton H-5 showed a ^3J coupling with a carbonyl at δ 172.9 as well as with C-7 (δ 162.6) and C-8a. Similarly, the proton H-8 showed a ^3J coupling with C-6 and C-4a. HMBC correlations confirmed the correlations on ring B, where the proton at C-2' showed a strong correlation with C-2, C-6' and C-4'. Moreover, proton H-5' showed correlations with C-2 and C-4' as drawn in Figure 13. The data were in agreement with the published values for **60**.[96]

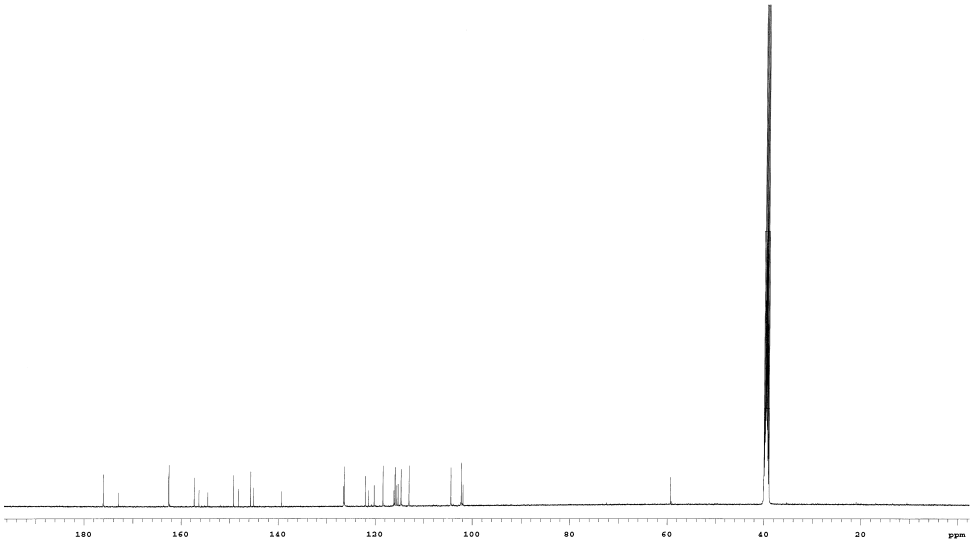


Figure 12: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of 3-*O*-methylfisetin (**60**), minor signals) in mixture with **59** (major signals)

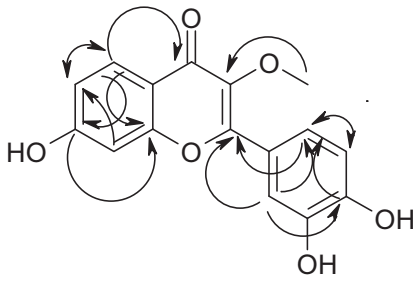


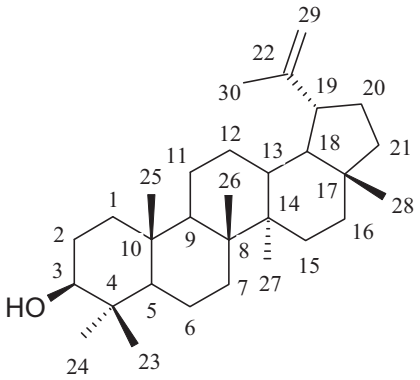
Figure 13: ^{1}H,^{1}H COSY and HMBC correlations of 3-*O*-methylfisetin (**60**)

Compounds **59** and **60** were recently isolated from the heartwood of the traditionally used Taiwanese medicinal plant *Acacia confusa* Merr. (Leguminosae).[96] This is the first report of flavonoids **59** and **60** from the genus *Albizia*, while compound **58** was isolated for the first time from the species *A. zygia*. In our study compounds **58**-**60** were tested against *P. falciparum* K1: compound **59** exhibited high antimalarial activity (IC_{50} 0.078 μg/ml), but unfortunately was rather cytotoxic (IC_{50} 0.405 μg/ml) against L6 strain. Compounds **58** and **60** were inactive.

2.2.4 Lupeol (Lup-20(29)-en-3β-ol)

Lupeol (**61**) was isolated as white amorphous, UV absorbing powder, which turned to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum (see Figure 152) displayed a pattern of a triterpene moiety. Two olefinic protons appeared at δ 4.69 (d) and 4.57 (m), the latter multiplet showed allylic couplings with a proton sig-

nal at δ 2.37 (H-19). A number of methylene groups as well as methyl groups were observed.



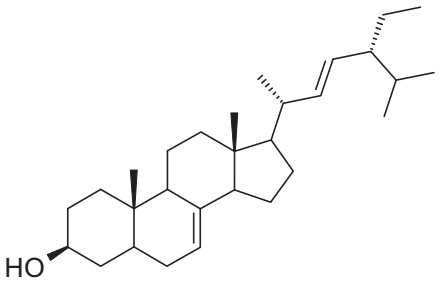
61

EIMS delivered the molecular weight *m/z* 426 and HRESIMS afforded the molecular formula $C_{30}H_{50}O$. A search in Chemical Abstract as well as Dictionary of Natural Products[77] confirmed the structure of **61** after comparing with literature data.[97,98]

Compound **61** was found to be a diet-based triterpene because of it is existence in different kinds of fruits such as mango, olive, strawberry, grapes and figs as well as many medicinal plants. Under *in vivo* conditions compound **61** acts as activator of apoptosis and inhibits the carcinogenesis of human androgen-sensitive CaP cells and decreases the serum prostate-specific antigen levels. Consequently, compound **61** inhibits proliferation of human prostate cancer cells.[99] In a recent report **61** inhibits head and neck cancer in a mouse tumor xenograft model.[100] It is interesting to note that compound **61** has also been found to inhibit the growth of the malaria causing agent *Plasmodium falciparum* by 45% at 25 µg/ml,[101] which could account for the antiprotozoal activity observed for the methanolic extract of *A. zygia*.[88]

2.2.5 Chondrillasterol

A further sterol was isolated as a mixture with fat. The GC/MS spectrum afforded a signal with the molecular weight 412 at a retention time between 31.46-31.56 min, which was tentatively identified by the fragmentation pattern as chondrillasterol (**62**). Compound **62** was isolated previously from several medicinal plants such as *Erigeron acris* L.[102] and aerial parts of *Amaranthus palmeri* S. Wats and was identified by GC/MS.[103]



62

2.3 *Tephrosia apollinea* (Del.) Link (Leguminosae)

Many people in Sudan are used to swallow the intact seeds of *Tephrosia apollinea* as a treatment against malaria (like chloroquine). In addition to an obvious activity, the seeds are available in many areas for free. The plant material was authenticated by Mr. Wail El Sadig (National Research Centre, Sudan) based on comparison with voucher specimen deposited in the herbarium of the Institute of the Medicinal and Aromatic Plants, Khartoum, Sudan.

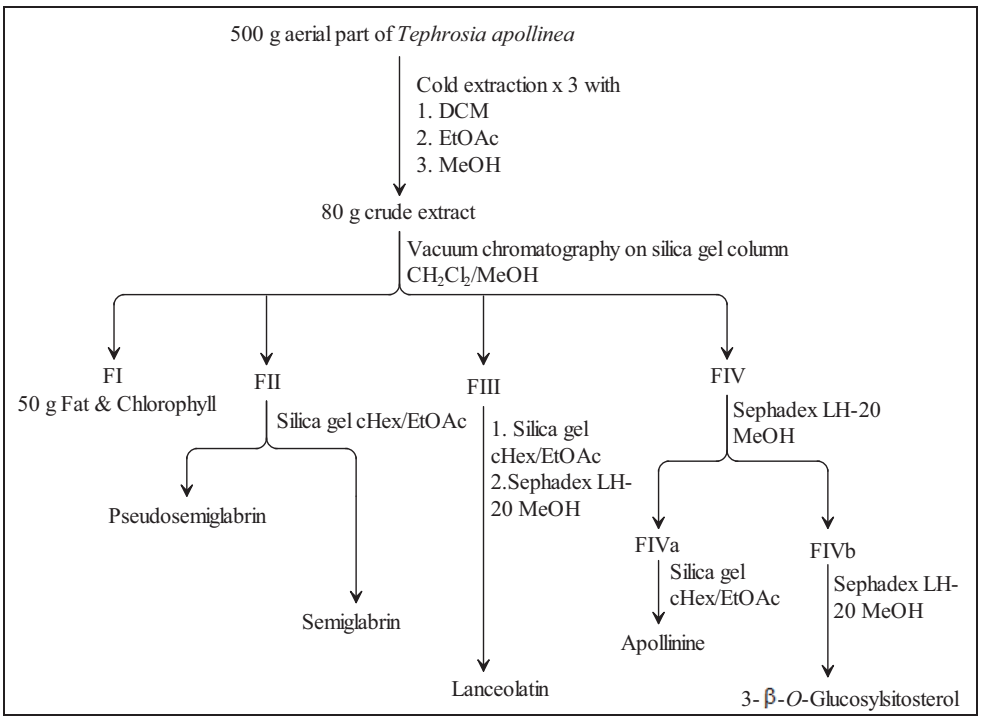


Figure 14: Work up scheme of Sudanese medicinal plant *Tephrosia apollinea*

2.3.1 Pseudosemiglabrin

Pseudosemiglabrin (**63**) was isolated as a white powder from FI after purification by silica gel column chromatography using cyclohexane/EtOAc 0-100 %. It showed a blue fluorescent zone, which gave a blue-green colour with anisaldehyde/sulphuric

acid spray reagent. In the aliphatic region of the ^{1}H NMR spectrum, three methine protons appeared at δ 6.51 (d, H-2"), 5.58 (d, H-3"') and 4.62 (d, H-3"), which is connected to an sp^2 carbon. Methyl signals were observed at δ 1.39 and 1.15 and assigned to H_3-4"' and H_3-5"', respectively. An acetyl signal was observed at δ 1.22. In the aromatic region three aromatic signals with the intensity of five protons appeared as an indication of a mono-substituted benzene ring (ring B). In addition, two *ortho*-coupled aromatic protons appeared at δ 8.12 (d, H-5, ^{3}J = 8.6 Hz) and 6.90 (d, H-6, ^{3}J = 8.6) Hz, and a singlet at δ 6.72.

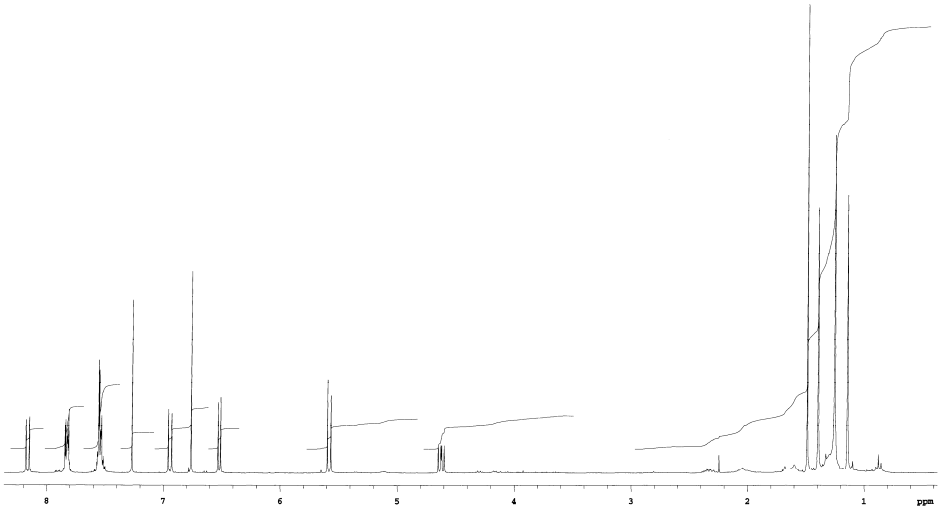
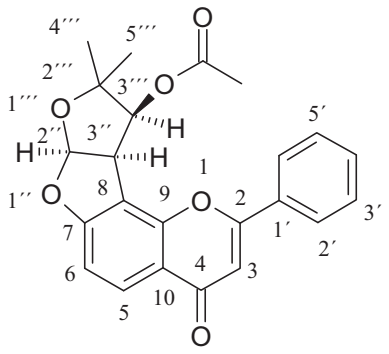


Figure 15: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of pseudosemiglabrin (**63**)



63

The ^{13}C NMR spectrum of compound **63** showed the presence of 23 signals, among them nine quaternary carbons. Additionally, eight sp^2 carbon signals appeared between δ 131.8 - 107.5, which altogether established a flavone building block (rings A, B and C). Moreover three methine carbon signals were assigned to an acetal carbon at δ 111.7 (C-2"), an oxygenated methine at δ 84.6 (C-3"') and another methine carbon at δ 47.9 (C-3"). Three methyl signals were assigned to C-4"' (δ 27.4), C-5"' (δ 23.2), and an acetyl group (δ 20.3). The NMR data are listed in Table 5. ESI MS dis-

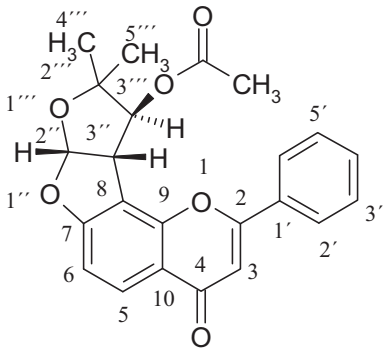
played a molecular weight of 392, and HRESIMS pointed to the molecular formula $C_{23}H_{20}O_6$. These data confirmed pseudosemiglabrin (**63**), a compound which had been isolated previously in our group.[104]

Table 5: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of pseudosemiglabrin (**63**) in $CDCl_3$

Position	δ_C	δ_H (mult.; *J* in [Hz])
2	162.7	
3	107.5	6.78 (s)
4	177.7	
5	128.7	8.18 (d, ^3J = 8.6)
6	109.0	6.96 (d, ^3J = 8.6)
7	164.6	
8	111.4	
9	153.8	
10	118.3	
1'	131.3	
2'	126.2	7.83 (dd, ^3J = 7.9, ^4J = 1.8)
3'	129.1	7.56 (dd, ^3J = 7.9, ^4J = 1.7)
4'	131.8	7.55 (m)
5'	129.1	7.56 (dd, ^3J = 7.9, ^4J = 1.7)
6'	126.2	7.83 (dd, ^3J = 7.9, ^4J = 1.8)
2''	111.7	6.52 (d, ^3J = 6.5)
3''	47.9	4.63 (dd, ^3J = 8.8, ^3J = 6.5)
2'''	84.6	
3'''	77.0	5.58 (d, ^3J = 8.8)
4'''	27.6	1.40 (s)
5'''	23.2	1.15 (s)
$COCH_3$-	169.8	
$COCH_3$-	20.3	1.49 (s)

2.3.2 Semiglabrin

Semiglabrin (**64**) was isolated as white powder from fraction FII after purification by silica gel column chromatography using cyclohexane/EtOAc 0 to 95 %. It showed a blue fluorescent zone, which gave a blue-green colour with anisaldehyde/sulphuric acid. Compound **64** exhibited nearly the same spectroscopic information as **63** and also the same molecular weight and molecular formula. The only differences were in the chemical shift value of the acetyl group and the coupling pattern of H-3'' and H-3'''. Structure **64** was confirmed by comparison of the spectroscopic data with literature values.



64

Table 6: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of semiglabrin (**64**) in $CDCl_3$

Position	δ_C	δ_H (mult.; J in [Hz])
2	162.8	
3	107.7	6.78 (s)
4	177.4	
5	128.8	8.15 (d, ^3J = 8.6)
6	109.0	6.93 (d, ^3J = 8.6)
7	163.7	
8	112.4	
9	153.2	
10	118.7	
1'	131.5	
2'	126.3	7.91 (m)
3'	129.0	7.56 (m)
4'	131.6	7.55 (m)
5'	129.0	7.56 (m)
6'	126.2	7.91 (m)
2''	112.3	6.63 (d, ^3J = 6.5)
3''	52.8	4.30 (d, ^3J = 6.3)
2'''	87.8	
3'''	80.2	5.65 (s)
4'''	27.4	1.33 (s)
5'''	23.1	1.10 (s)
$COCH_3$-	169.6	
$COCH_3$-	20.8	2.24 (s)

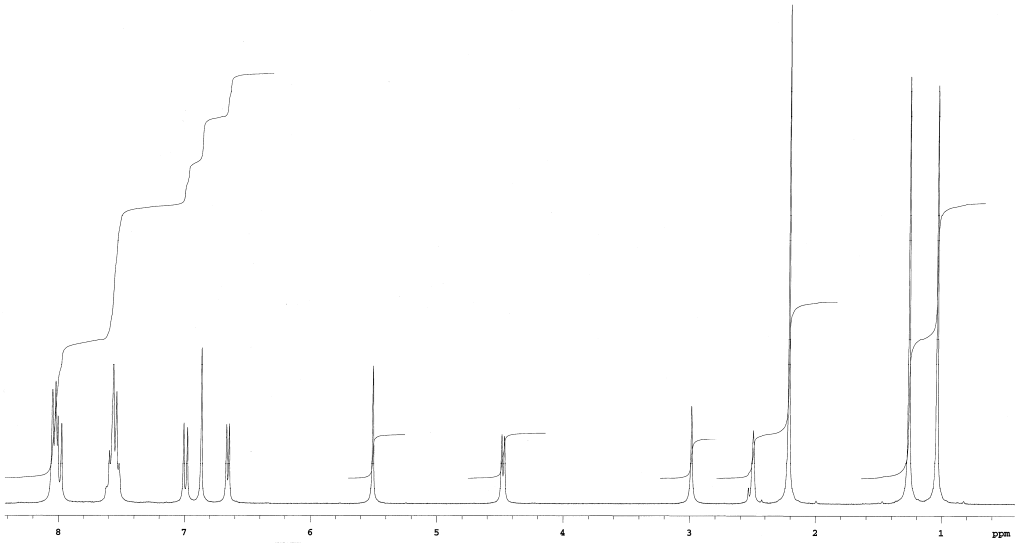
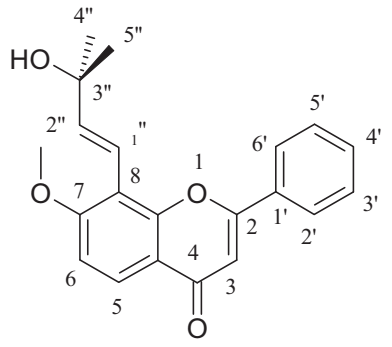


Figure 16: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of semiglabrin (**64**)

2.3.3 Lanceolatin A

Lanceolatin A (**65**) was isolated as white solid from fraction FII (see Figure 14) by silica gel column chromatography using cyclohexane/EtOAc followed by Sephadex LH-20 using MeOH. The ^{1}H NMR spectrum of lanceolatin A (**65**) indicated the presence of eight aromatic protons between δ 8.14-6.79 and two olefinic protons at δ 7.01 and 6.86, in addition to a methoxy at δ 4.00. A further singlet with the intensity of 6 protons appeared at δ 1.53, indicating the presence of two magnetically equivalent methyl groups. The APT/^{13}C NMR spectrum showed eight quaternary carbons, eight aromatic carbons, two olefinic carbons in addition to methoxy and methyl signals. The molecular weight 336 was obtained by ESIMS and the molecular formula $C_{21}H_{20}O_4$ was afforded by ESI HRMS. These data were in close agreement with those previously reported for lanceolatin (**65**), a metabolite from *Tephrosia* spp. including *T. apollinea*.



65

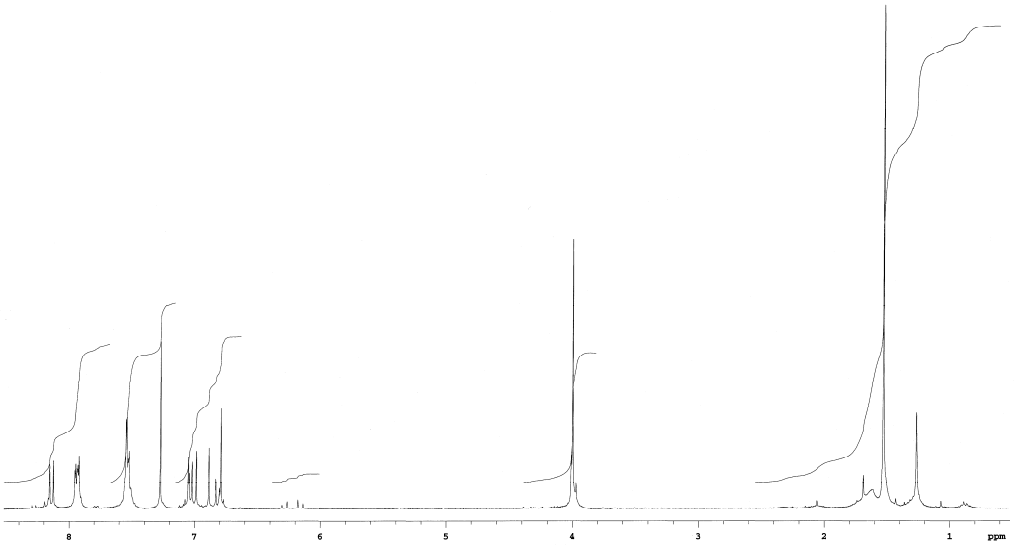


Figure 17: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of lanceolatin A (**65**)

Table 7: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of lanceolatin A (**65**) in $CDCl_3$

Position	δ_C	δ_H (mult.; J in [Hz])
2	163.2	
3	107.0	6.79 (s)
4	178.1	
5	125.4	8.14 (d, ^{3}J = 8.9)
6	109.0	7.03 (d, ^{3}J = 8.9)
7	161.1	
8	114.2	
9	154.4	
10	118.1	
1'	132.1	
2', 6'	126.3	7.93 (m)
3', 5'	128.9	7.52-7.55 (m)
4'	131.4	7.52-7.55 (m)
1"	144.0	7.01 (d, ^{3}J = 16.5)
2"	115.1	6.86 (d, ^{3}J = 16.5)
3"	71.5	
CH_3-4", CH_3-5"	30.1	1.53 (s)
OCH_3-7	56.2	4.00 (s)

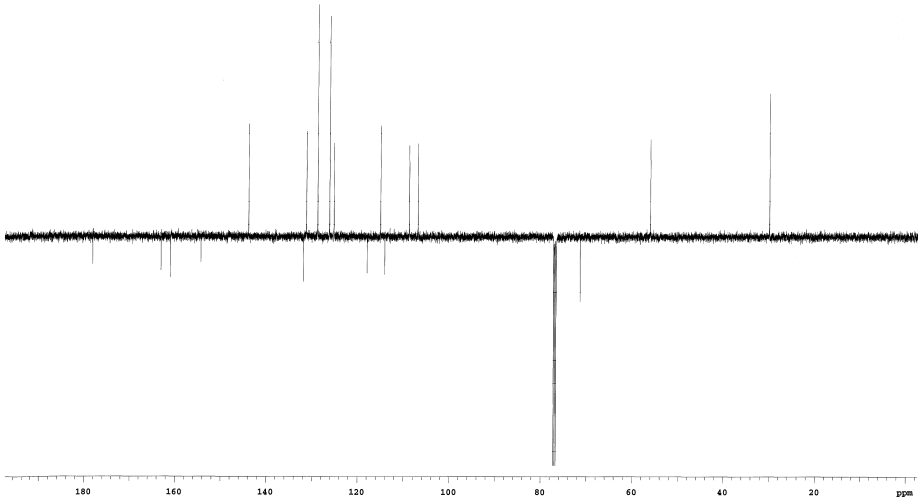
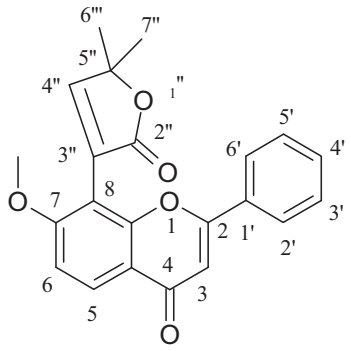


Figure 18: APT/^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of lanceolatin A (**65**)

2.3.4 Apollinine

Apollinine (**66**) was isolated as white powder from fraction FIII after purification on a silica gel column using cyclohexane/EtOAc. The ^{1}H NMR spectrum displayed eight signals for aromatic protons between δ 8.20 – 6.69, in addition to an olefinic proton at δ 7.54. A singlet at δ 3.92 with the intensity of three protons was observed as indication of a 7-methoxyflavone; in addition one singlet at δ 1.64 (6H) with the intensity of 6 protons was due to two methyl groups at CH_3-6" and CH_3-7". The ^{13}C NMR spectrum of apollinine (**66**) pointed 22 signals: eight aromatic carbons between δ 131.7 - 107.1, ten quaternary carbon signals between δ 177.5 - 107.6 and an olefinic carbon at δ 160.0 were observed. A methyl carbon appeared at δ 25.7 for the two methyl groups at CH_3-6" and CH_3-7", in addition to a methoxy carbon at δ 56.5; the detailed NMR data are listed in Table 8. ESI MS delivered the molecular weight 362 and HRESI/MS afforded the molecular formula $C_{22}H_{18}O_5$.



66

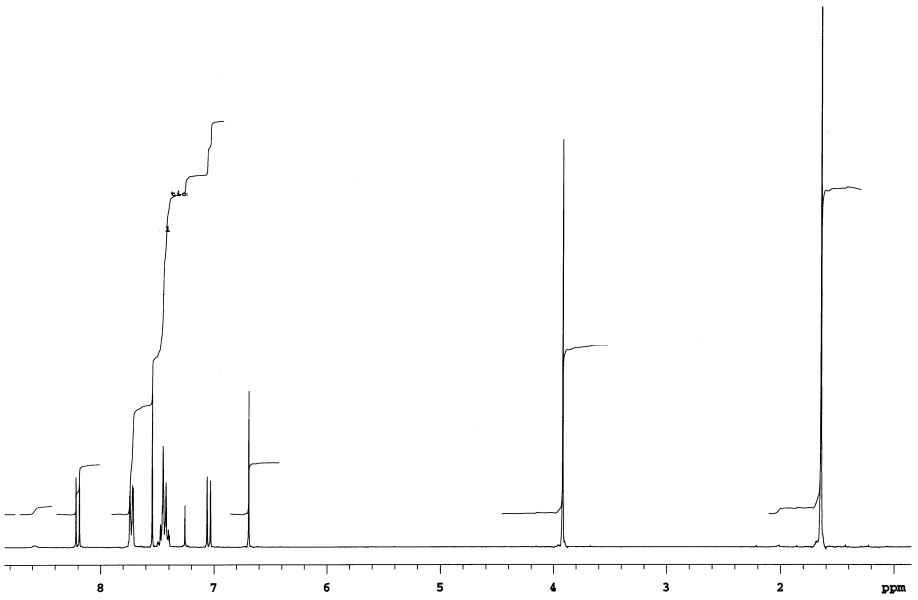


Figure 19: ^{1}H NMR spectrum (300 MHz) of apollinine (**66**) in $CDCl_3$

The H,H COSY, HSQC and HMBC spectra showed strong correlations, confirming a monosubstituted benzene (ring B). Two *ortho*-coupled protons at δ 8.20 (H-5) and 7.02 (H-6) showed ^{3}J couplings with the quaternary carbons at δ 154.7 (C-9) and 107.6 (C-8), respectively; an olefinic proton at δ 7.54 (H-4") showed an HMBC correlation with a carbonyl at δ 170.6. Two methyl groups at δ 1.64 showed HMBC correlation with a quaternary carbon at δ 84.9 (C-5"); correlations are drawn in Figure 20. A search in the Dictionary of Natural Products[77] led to structure of **66**.

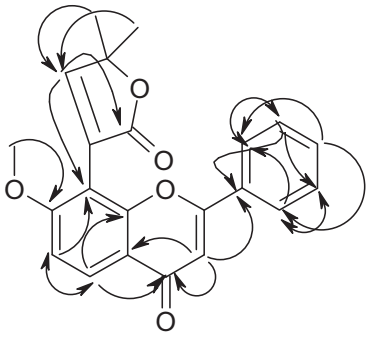


Figure 20: Selected H,H COSY and HMBC correlations of apollinine (**66**)

Table 8: ^{13}C and ^{1}H NMR (125, 300 MHz) spectroscopic data of apollinine (**66**) in $CDCl_3$

Position	δ_C	δ_H (mult.; *J* in [Hz])
2	163.0	
3	107.1	6.69 (s)
4	177.5	
5	127.9	8.20 (d, ^3J = 9.0)
6	109.2	7.05 (d, ^3J = 9.0)
7	161.6	
8	107.6	
9	154.7	
10	117.8	
1'	131.4	
2'	126.1	7.73 (dd, ^3J = 7.9, ^4J = 1.7)
3'	128.9	7.40 (dd, ^3J = 7.6, ^4J = 1.8)
4'	131.7	7.43 (m)
5'	128.9	7.40 (dd, ^3J = 7.6, ^4J = 1.8)
6'	126.1	7.73 (dd, ^3J = 7.9, ^4J = 1.7)
2"	170.6	
3"	124.0	
4"	160.0	7.54 (s)
5"	84.9	
6"	25.7	1.64 (s)
7"	25.7	1.64 (s)
OCH_3-7	56.5	3.92 (s)

The proton spectrum of the apollinine sample showed an additional 2H multiplet at δ 8.55, which had disappeared when ^{13}C and 2D spectra were measured a few days later. Also several other signals indicated another structure, e.g. an additional 1H triplet of a triplet at δ 7.62 and a multiplet at 7.24 (Figure 21).

Obviously, apollinine (**66**) was formed from an un-identified precursor under acidic conditions on standing in chloroform. When we became aware of this fact, the whole sample was already used up or had been rearranged. In the literature, no information about the formation of **66** was found.

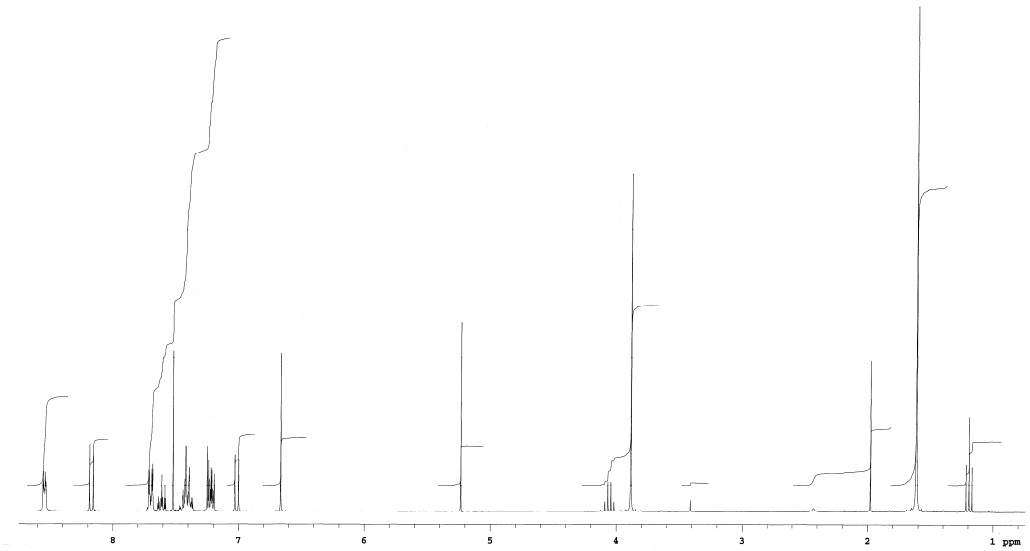
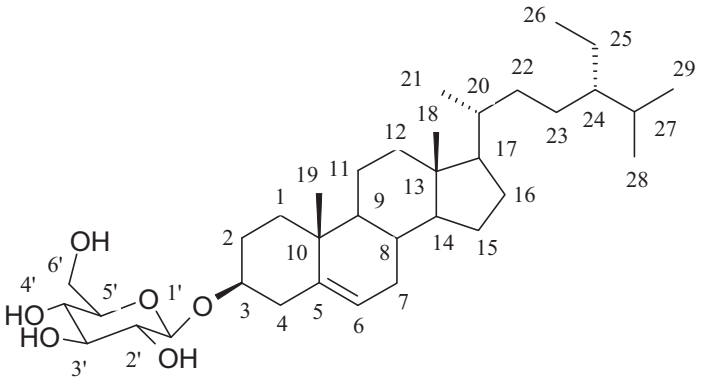


Figure 21: ^{1}H NMR spectrum (300 MHz) of apollinine-precursor in $CDCl_3$

The metabolites pseudosemiglabrin (**63**) and apollinine (**66**) have been isolated previously from the roots and seeds of *Tephrosia apollinea*[105,106] and several other *Tephrosia* species, including *Tephrosia semiglabra*,[107] *Tephrosia nubica*,[108] *Tephrosia pupurea*[109,110] and *T. hokeriana*.[111] We have tested these compounds now for inhibition of human topoisomerase I, a molecular target against cancer.[112] Unfortunately the above mentioned four compounds pseudosemiglabrin (**63**) - apollinine (**66**) were inactive in our test.

2.3.5 3β-*O*-Glucosylsitosterol

A sterol-glucoside was isolated as UV absorbing substance, which turned to black in the chromatogram with anisaldehyde sulphuric acid spray reagent. The ^{1}H NMR spectrum showed the typical pattern of a steroid with many overlapping proton signals in the upfield region. An olefinic proton appeared at δ 5.33, six methyl groups were observed between δ 0.93 - 0.69, in addition to seven methine protons. Six oxymethines of a sugar moiety appeared between δ 4.27- 3.11 along with an oxymethylene at δ 3.49 and 11 methylene protons.



67

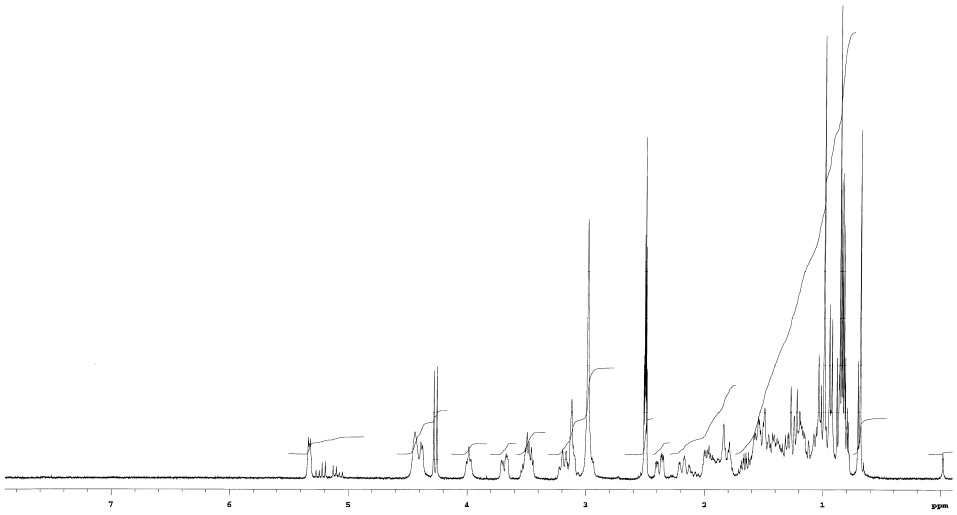


Figure 22: ^{1}H NMR spectrum of 3*β*-*O*-glucosylsitosterol (**67**)

The ^{13}C NMR spectrum delivered 35 carbon signals: three quaternary, one olefinic carbon, seven oxygenated carbons (including the sugar unit), 11 methylene carbons, seven methine and six methyl carbons. The ESI mass spectrum delivered the *pseudo*-molecular ion peak at 599 ($[M + Na]^+$, which gave a molecular weight of 576. The molecular formula $C_{35}H_{60}O_6$ was obtained by HRESI/MS. A search in AntiBase[113] and the Chemical Abstracts led to 3*β*-*O*-glucosylsitosterol (**67**), which showed closely related data.[114]

2.4 *Aristolochia bracteolata* Lam (Aristolochiaceae)

The genus *Aristolochia* in the *f*amily Aristolochiaceae is a large genus of herbs, found in the tropical and temperate regions of the world.[115] Most of the species of the genus *Aristolochia* contain alkaloids, which were reported to be useful in the treatment of snake bites.[116] *Aristolochia bracteolata* is a glabrous trailing perennial herb. Leaves are alternate, flowers are solitary, axillary, subtended by a leafy bract. Fruits

form capsules, sub globose, splitting longitudinally, up to 2.5 cm long, brown. In Sudanese folk medicine the maceration of the roots of *A. bracteolata* is used against malaria as well as anti-parasitic and anti-rheumatic drugs.[117]

The roots of *Aristolochia bracteolata* were collected from Shambat area (Faculty of Agriculture, University of Khartoum, Sudan) in June 2006. The plant material was authenticated by Mr. Wail El Sadig (National Research Centre, Sudan) based on comparison with voucher specimen deposited in the herbarium of the Institute of the Medicinal and Aromatic Plants, Khartoum, Sudan.

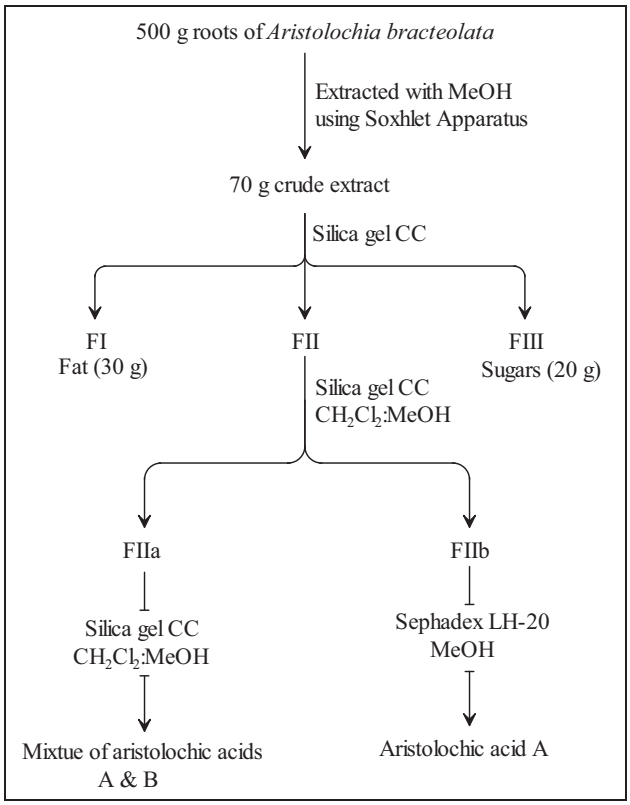


Figure 23: Work up scheme of *A. bracteolata*

2.4.1 Aristolochic acid A

Aristolochic acid A (**68**) was isolated as yellow amorphous powder, which gave a UV absorbing band and a yellow colour with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum revealed an acidic proton signal at δ 13.30, five aromatic protons between δ 8.65 - 7.34, in addition to a singlet of dioxymethylene protons, which appeared downfield at δ 6.50, and a methoxy signal at δ 4.06. The ^{13}C NMR spectrum revealed 17 carbon signals: five aromatic and ten quaternary carbons were observed, in addition to a methoxy and a dioxymethylene carbon.

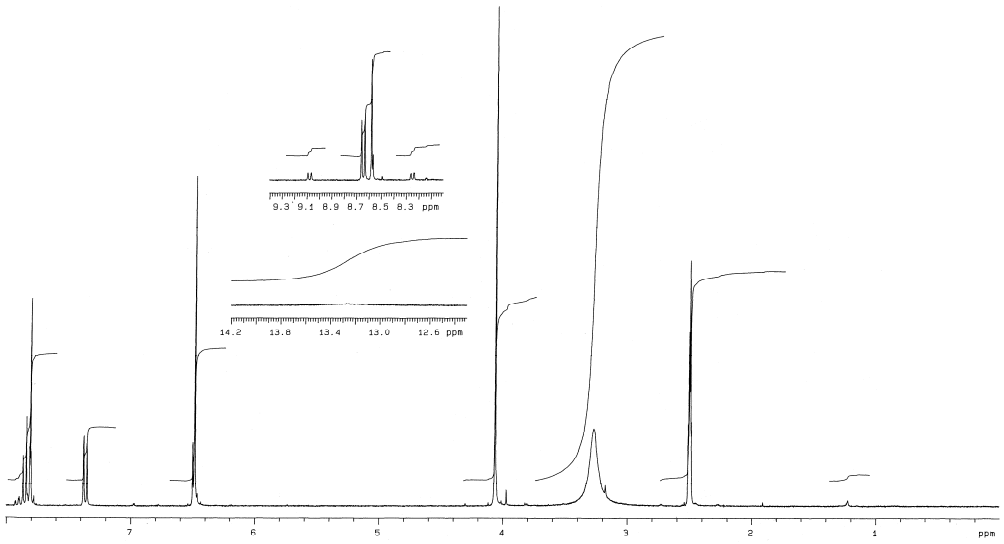
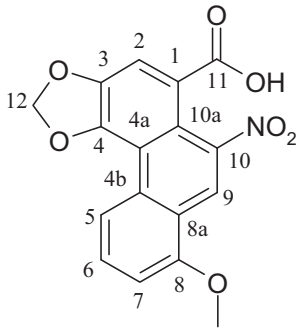


Figure 24: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of aristolochic acid A (**68**)



68

ESI MS delivered the molecular weight 341, while HRESIMS delivered the molecular formula $C_{17}H_{11}NO_7$. The H,H COSY correlations showed an ABC system, where a proton at δ 8.65 (H-5) showed a strong correlation with a signal at δ 7.83 (H-6), which appeared as triplet. The latter showed a correlation with a proton at δ 7.34 (H-7). The HMBC correlations from H-2 to C-4, C-10a and C-11, H-12 to C-3 and C-4, H-5 to C-4a and C-7, H-9 to C-4b, C-8 and C-10a, C-7 to C-8a supported the presence of a phenanthrene ring. A search in the Chemical Abstract as well as the Dictionary of Natural Products[77] and comparing the above spectroscopic information with the literature identified this compound as aristolochic acid A (**68**)

Table 9: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of aristolochic acid A (**68**) in DMSO-d_6

Position	δ_C	δ_H (mult.; J in [Hz])
1	124.0	
2	112.1	7.80 (s)
3	145.86	
4	145.90	
4a	117.2	
4b	129.8	
5	118.3	8.65 (d, J = 8.0)
6	131.4	7.83 (t, J = 8.0)
7	108.8	7.38 (d, J = 8.0)
8	156.2	
8a	118.8	
9	119.3	8.60 (s)
10	145.90	
10a	116.8	
11 CO_2H	167.6	
12 CH_2O_2	102.8	6.50 (s)
8-OCH_3	56.2	4.06 (s)
OH	-	13.30 (sbr)

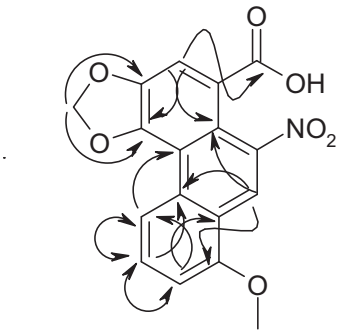
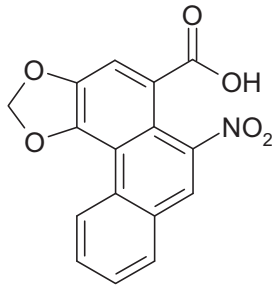


Figure 25: Selected H,H COSY and HMBC correlations of aristolochic acid A (**68**)

Aristolochic acid A (**68**) is known as a major constituent of the family Aristolochiaceae. Several previous reports studied its importance and biological activities, such as antitumor,[118] antimicrobial,[119] and insect chemo-sterilant properties.[120]

2.4.2 Aristolochic acid B

Aristolochic acid B (**69**) was isolated as a yellow mixture with aristolochic acid A (**68**). It appeared as yellow powder, which gave a UV absorbing band and turned yellow with anisaldehyde/sulphuric acid spray reagent. One mg of the mixture was subjected to HPLC/MS, and the chromatogram delivered two peaks as shown in Figure 26. These two peaks were aristolochic acid A (**68**), which is the major component of the root extract of *A. bracteolata*, and another derivativ. The HPLC/MS spectrum (see Figure 27) delivered the molecular weight of this compound as 311, and HRESIMS afforded the molecular formula $C_{16}H_9NO_6$. As only one natural product with this empirical formula is listed in the Dictionary of Natural Products,[77] the identity with aristolochic acid B (**69**) is very likely.



69

Aristolochic acids A (**68**) and B (**69**) are the main bioactive ingredients in most *Aristolochia* plants, which have been used for dietary supplements, slimming pills and traditional Chinese medicine. Recent reports indicated that ingestion of aristolochic acids can cause serious nephropathy and was found to be mutagenic and carcinogenic. Consequently, quality control and quantitative analysis for the medicines that contain aristolochic acids are of serious importance.[121]

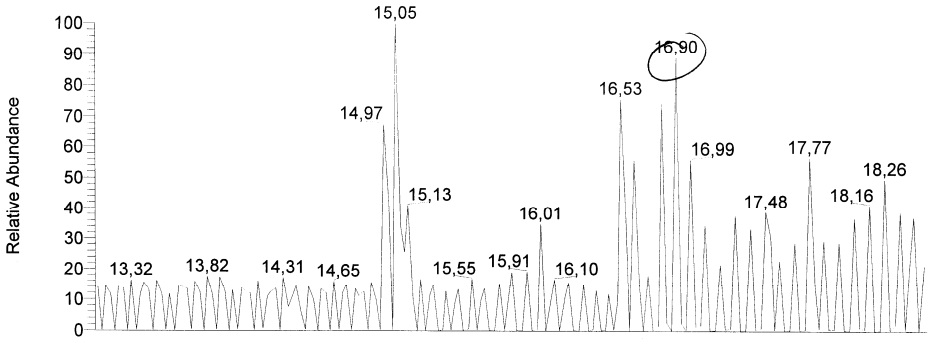


Figure 26: HLPC chromatogram of aristolochic acids A (**68**) and B (**69**) at retention times of 16.90 and 16.80 min, respectively

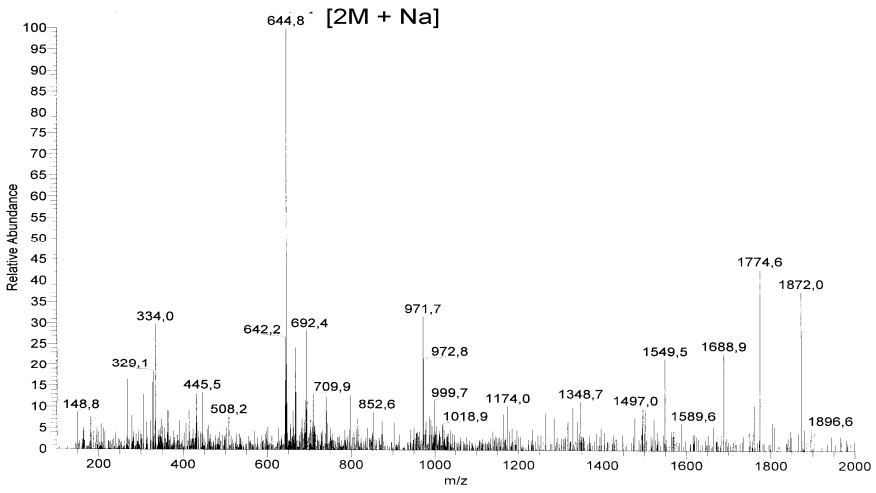


Figure 27: HLPC/mass spectrum of aristolochic acid B (**69**)

3 General Techniques for Streptomycetes

3.1 Collection of Bacterial Strains

Marine and terrestrial bacterial strains were obtained from different collaborations 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 WO) were obtained from Prof. Dr. Wolf (for searching of compounds with activity against plant-pathogenic fungi.
- The terrestrial *Streptomyces* spp. (code beginning with GW) were obtained from the laboratory of Dr. Iris Grün-Wollny, Giessen.
- The terrestrial *Streptomyces* spp. (Red 202 and HO9) were obtained from our partner Prof. Dr. Saisamorn Lumyong, Department of Biology, Faculty of Science, Chiang Mai University, Thialand.
- *Bacillus sp.* strain M10 was obtainedö from Mr. Muaaz Al-Ajlani, Department of Microbiology and Molecular Genetics, Lahore, Pakistan.
- The marine derived *Streptomyces* spp. (code beginning with B) were obtained from Dr. E. Helmke, Alfred-Wegner-Institute for Polar and Marine Research, Bremerhaven, Germany.

3.2 Strategic Procedure for Evaluating Bacterial Strains

For investigating large numbers of bacterial strains, a well-defined strategy to perform a good evaluation of the samples in order to select the most suitable strains is essential. This can be achieved by combining simple biological assays with chemical screening techniques. Once a candidate strain has been chosen, an isolation procedure will be employed for the isolation of the active principles. The applied strategic procedure used for evaluating the bacterial strains to be investigated is summarized in Figure 28.

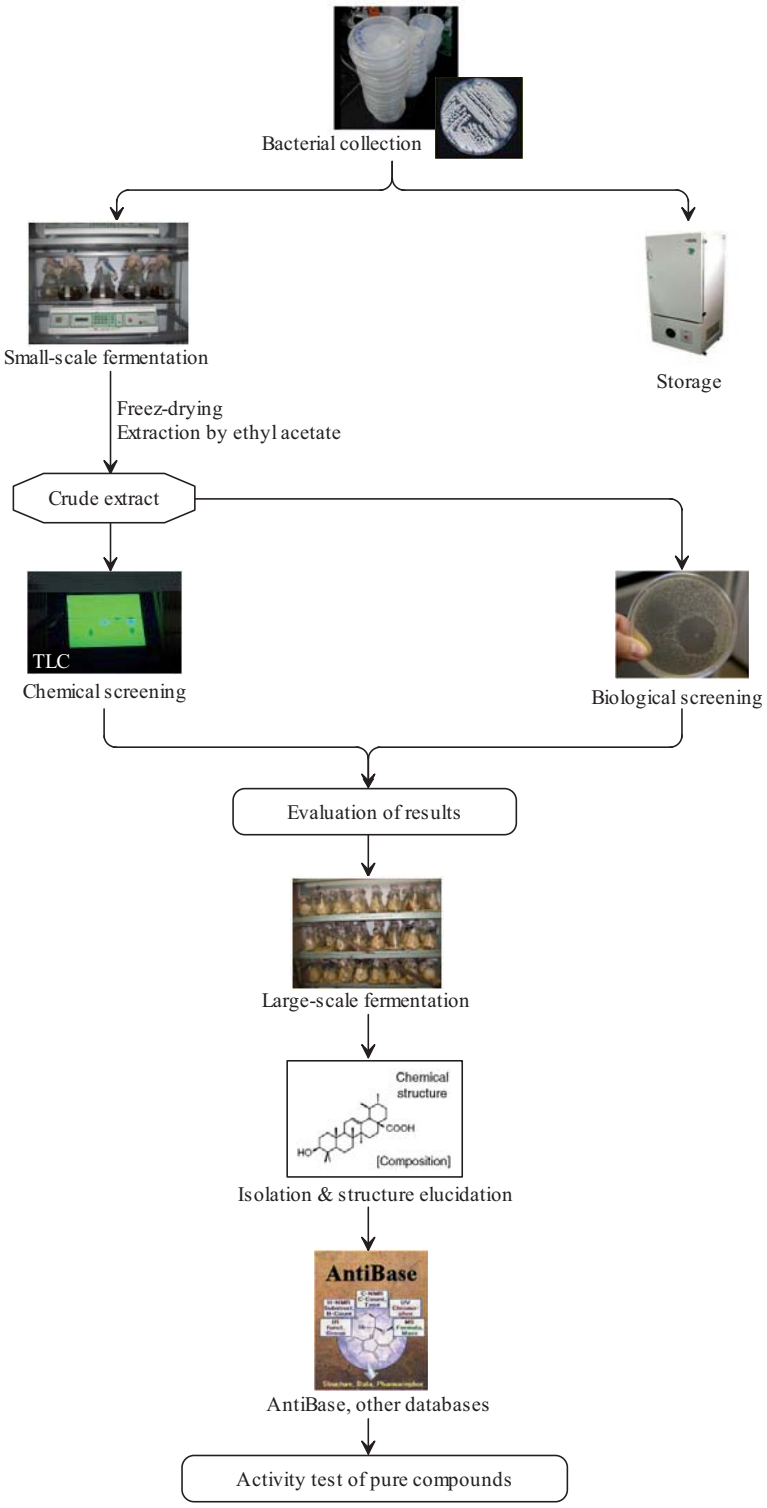


Figure 28: General screening of the selected strains

3.3 Pre-screening

Two different techniques were applied to select promising strains, the biological and chemical screening methods. In this respect, the pre-screening is a initial and

important process to isolate finally biological active and/or chemically interesting compounds.

In a first step, the strains were sub-cultured on agar plates for 3-7 days and microscopically examined for contaminations. Small pieces of the agar culture were then used to inoculate 1 L Erlenmeyer flasks with inflections containing 250 ml of medium, followed by incubation on a rotary shaker at 28 °C. As culture medium, we used routineously M_2^+ medium, which contains malt extract, yeast and glucose.

The culture broth was then lyophilised and the dried residue extracted with ethyl acetate. The obtained crude extract was used for biological, chemical and HPLC-MS tests.

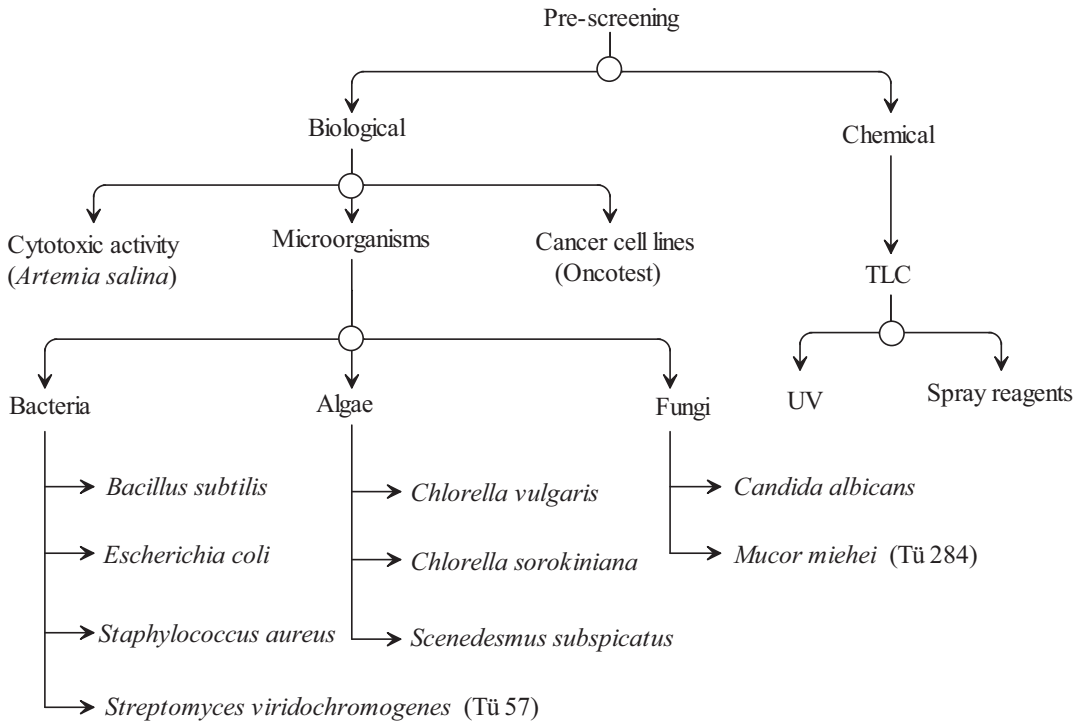


Figure 29: General strategy for pre-screening

3.3.1 Biological Screening

In industrial research, the biological screening is usually performed as a sequence of receptor-based tests with high selectivity and a very high number of samples: In the search for new antibiotics acting on new targets, platensimycin was found by Merck Research Laboratories in a screening of more than 250.000 bacterial extracts during a search for inhibitors of the fatty acid biosynthesis.

In a single cell, more than 10.000 further receptors rsp. Screening targets may exist. It is obvious that in university laboratories these screening concepts cannot perform, due to the missing facilities and infrastructures, costs and manpower. Instead of this detailed "vertical screening", we decided to use a "horizontal screening", were only selected tests are applied, which detect activity ranges instead of specific properties.[122]

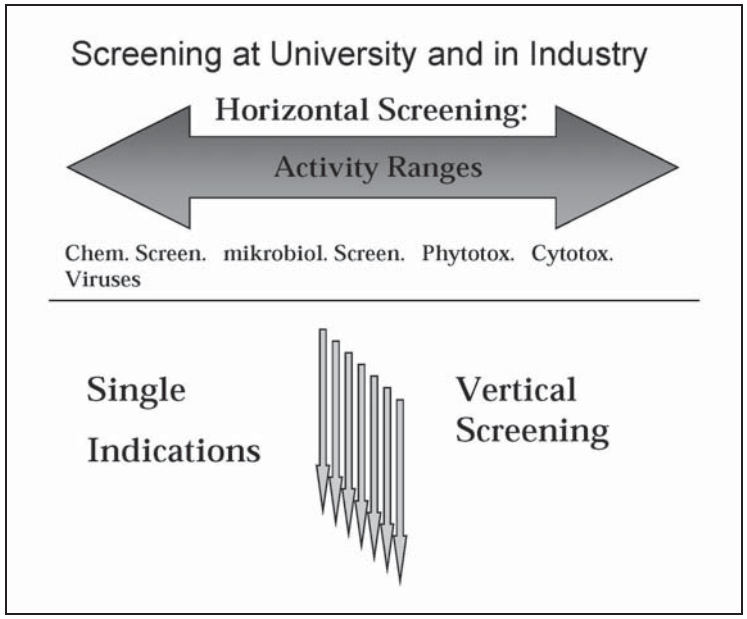


Figure 30: Comparison on the activity screening of bioactive compounds between industry and university

The horizontal screening exhibits low selectivity, however, affords broad results and gives therefore 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 sp. (Gram-positive, Gram-negative), two fungi, microalgae as representatives of plants and brine shrimps (*Artemia salina*) as synonym for higher organisms. The crude extracts are tested against these different microorganisms as mentioned in Figure 29. Additionally the brine shrimp toxicity, which is applied in our group has a strong correlation with cellular cytotoxicity and is therefore a good indicator for potential anticancer activity. The bio-autography on TLC gives simultaneously more information about an unknown bioactive component in the crude extract. This is readily seen for antimicrobial and phytotoxic compounds.

In the case of a positive response, the samples are forwareded to industrial partners for a more detailed vertical screening. The cytotoxicity tests in our group were carried

out at Oncotest GmbH (Freiburg), applications in agriculture are tested by BASF AG (Ludwigshafen), etc.

3.3.2 Chemical Screening

The chemical screening is a method, which allows the detection of potentially interesting compounds at the earliest stages of separation. The TLC (thin layer chromatography) is a simple method used for the detection of bacterial constituents in the crude extracts. Compared with other methods like HPLC it is easy to perform, cheap and sufficiently reproducible. A spot of the crude extract is developed on a TLC plate with a CH_2Cl_2/MeOH solvent system. The zones on the developed TLC plate are visualized under UV light and further localized by exposure to a suitable spray reagent. Many spray reagents are available for the detection, some of them are specific and others are universal. The following spray reagents are 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.

3.4 Culturing Bacteria and Working up

Based on the pre-screening assessment, the interesting and more promising strains were selected for further investigations. Depending on the initial status (active substance level), culturing condition optimisation might be done to improve the microbial yield and in particular of interesting secondary metabolites.

Well-grown agar sub-cultures were monitored and finally selected for performing the subsequent inoculation. A number of usually 100 Erlenmeyer flasks (1 L) each containing 250 ml culturing medium (pH 7.8) were used for the inoculation and propagation of the bacteria on a linear shaker (28 °C). After four to five days, culture

broths were harvested, mixed with Celite (diatomaceous earth as filter aid) and filtered under pressure (filter press). The latter step is necessary to separate the water phase, which was successively adsorbed on Amberlite adsorption resin XAD-16 and the latter finally extracted with methanol. The biomass remaining after filtration was exhaustively extracted with ethyl acetate and acetone. Finally, the crude extracts were evaporated under vacuum and tightly kept for subsequent chromatographic work.

3.5 Isolation Techniques

According to the amount of the obtained crude extracts, a suitable isolation method will be determined, with respect to the polarity of the compounds of interest. Extracts from water phase and cell mass are combined, if the composition is similar; otherwise they are separated independently. For separation of accompanying fats, the crude extracts were initially subjected to a silica gel column, which is normally eluted with a stepwise gradient of dichloromethane/methanol or cyclohexane/ethyl acetate. In some cases, a distribution of the extract between Methanol and cyclohexan may be advisable, prior to the chromatography to separate lipids. Thereafter, the separated fractions are subjected to size exclusion chromatography on Sephadex LH-20. The latter has the advantage of a high recovery rate as well as to minimize the destruction of labile compounds. For further purification of the fractions, the following isolation methods accompanied by TLC monitoring are applicable: PTLC, RP-18 chromatography, HPLC, or just again silica gel or Sephadex LH-20 as summarized in Figure 31.

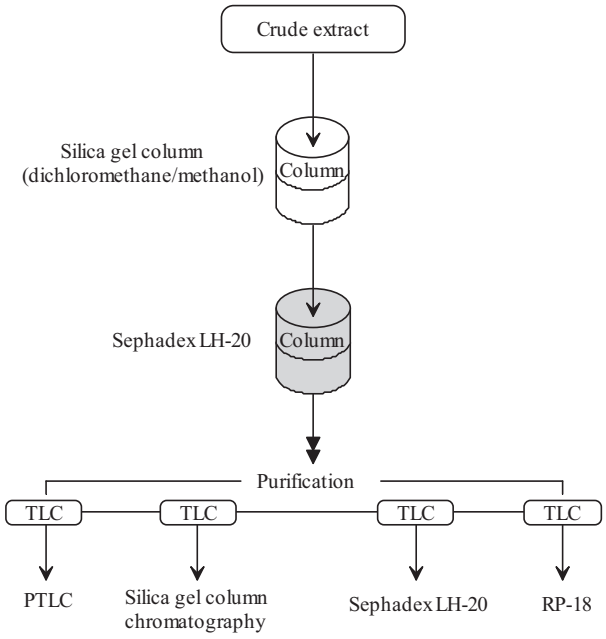


Figure 31: Different types of purification techniques

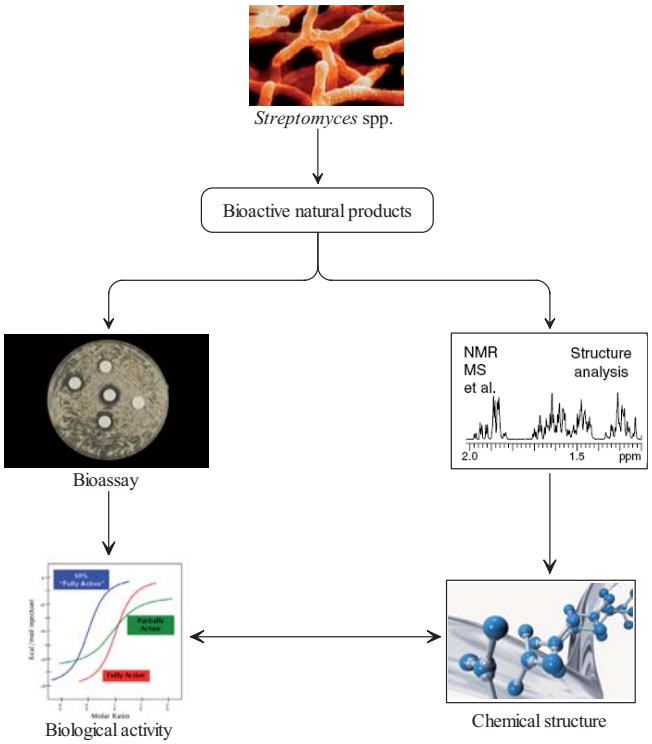


Figure 32: Work up scheme of *Streptomyces* sp. following activity-guided fractionation

The resulting fractions as well as sub-fractions must be tested against different microorganisms in order to isolate the active compounds. Following activity guided fractionation will deliver at the end a pure compound with biological activity as summarized in Figure 32.

3.6 Dereplication

Since the 1950s, which is widely known as *Golden Age of Antibiotics*, natural products chemists have faced the steadily increasing problem of how to maximize the discovery of new compounds and to minimize the reevaluation of metabolites which are already well defined and described in the literature. Up to date, more than 170.000 natural products have been discovered with an average of around 700 new microbial compounds documented annually. A recent comprehensive review by Blunt et al.[123] has indicated more than 21.000 articles currently (over the period 1959–2008) published in the field of natural products with the majority representing new metabolites. Most of these newly discovered compounds were accepted for publication in leading periodicals such as the *Journal of Natural Products* as mentioned in Figure 33.

The term *dereplication* is commonly used in the natural products community for the complementary processes of rapid identification of known compounds.For this pur-

pose, a number of techniques have been adopted. The comparison of UV as well as mass data in conjunction with HPLC retention times of compounds from our own database is an efficient method. The advantage of this method is, that it needs a low amount of samples and can even be applied to crude extracts. The disadvantage is, that an authentic sample must exist for comparison; this is in most cases not given. In addition, also the identification of new compounds can be managed by comparing by MS the molecular weight, the fragmentation pattern as well as the chromophore of the respective compound. A HPLC-UV-ESI-MS/MS database with over 600 compounds has been established in our group for dereplication on the basis of crude extracts. The identification of a given component could be accomplished by fitting of the obtained data with reference spectra and also by comparison with related compounds, which have the same chromophore or aglycone.

In our group we have access to the important leading databases The Dictionary of Natural Products (Chapman & Hall),[77] AntiBase (Chemical Concepts)[113] and the Chemical Abstracts. The Dictionary of Natural Products (DNP) collects nearly all natural products, especially plant metabolites. The disadvantage of DNP is the lower search capability as well as limited spectroscopic information. On the other hand, AntiBase is more practical, efficient and advanced for the dereplication of microbial metabolites. More than 37.000 compounds from microbial sources are available. Wide range of substructure search capabilities is available in our database. The known compounds can be identified by comparison of ^{1}H NMR spectroscopic data in many cases, in other cases the mass or high-resolution mass spectra are needed for the final confirmation, so we can easily identify known compounds. The most important advantage of AntiBase is that it offers an access to the ^{13}C NMR data for nearly all compounds with known structures. Not only these but also thousands of original 1D as well as 2D spectra are available for comparison. The most comprehensive worldwide database is the Chemical Abstracts; normally a search in CA is the final step to confirm the novelty of a compound.

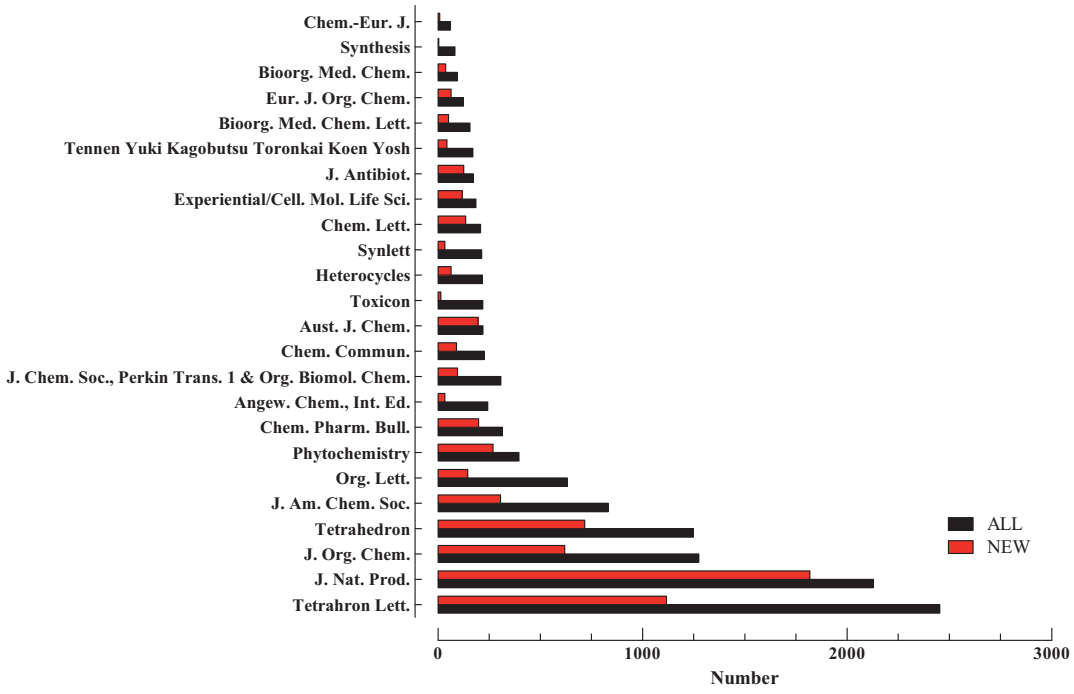


Figure 33: Total number of papers published over the period 1959–2008 (ALL = total number; NEW = new compounds). The figure has been sorted using the ALL data[123]

4 Investigation of the Selected Bacterial Strains

4.1 Terrestrial *Streptomyces* sp. ANK 210

The terrestrial strain *Streptomyces* sp. ANK 210 was selected according to the pre-screening, in which TLC of the crude extract exhibited two interesting UV active bands, which gave yellow colourations with anisaldehyde/sulphuric acid. Additionally it showed anticancer activity against cancer cell lines with IC_{50} and IC_{70} values of 32.0 and 86.7 μg/ml, respectively.

The well-grown colonies of ANK 210 on agar were used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2^+ medium, which were incubated as shake culture at 28 °C for 7 days. The resulting brown culture broth was mixed with *ca*. 1 kg diatomaceous earth (Celite) and pressed through a filter press to afford the filtrate and a mycelial fraction. The filtrate was extracted by Amberlite XAD-16 resin and the latter eluted using MeOH. The mycelium was extracted (3×) with EtOAc followed by acetone (1×). The EtOAc and acetone phases were evaporated to dryness. The MeOH fraction was concentrated to the aqueous residue, which was extracted using EtOAc and the latter evaporated to dryness. On TLC the three crude extracts showed no differences, and accordingly they were combined to obtain 5.8 g of a yellowish brown crude extract. This extract was dissolved in methanol, defatted by extraction with cyclohexane and subjected to silica gel column chromatography. Fraction FII was purified on Sephadex LH-20 using MeOH followed by RP-18 using a MeOH/H_2O gradient (10 to 50 % MeOH) to deliver *ent*-homoabyssomicins A (**70**) and B (**72**). Fraction FIII was purified on Sephadex LH-20 using MeOH followed by silica gel using cyclohexane/EtOAc (gradient 0 to 100 % EtOAc) to afford indole-3-carboxylic acid, 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**79**) and Niax (**81**). Fraction FIV was purified on RP-18 using MeOH/H_2O (4:6) to afford benadrostin (**80**).

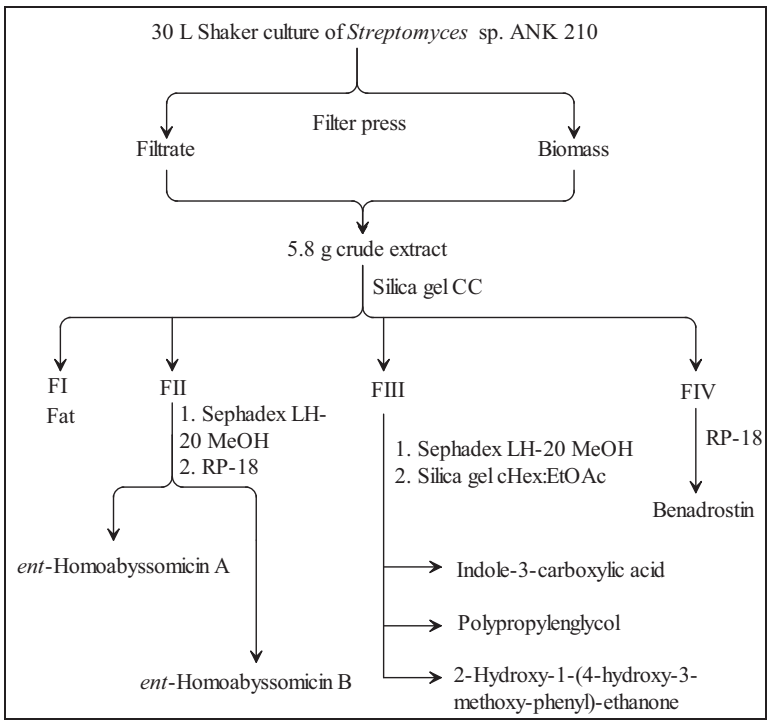


Figure 34: Work up scheme of terrestrial *Streptomyces* sp. ANK 210

4.1.1 *ent*-Homoabyssomicin A

Compound **70** was isolated as white crystals after purification of fraction FII on Sephadex LH-20 followed by reverse phase RP-18 using MeOH/H_2O (3:7). Compound **70** was obtained as UV absorbing substance, which turned to yellow with anisaldehyde/sulphuric acid. In the complex ^{1}H NMR spectrum, one methine appeared as doublet of doublet, another two methine signals appeared at δ 3.51 and 3.46, which may be connected to oxygen or carbonyl groups. Additionally, three methine signals were recognized in a multiplet at δ 2.37, 2.38 and 2.41. Moreover, three methylene and four methyl signals were observed.

^{13}C NMR and HSQC experiments indicated the presence of two carbonyl groups, one ketal carbon, one oxymethine, four quaternary oxygenated carbons, two tertiary and two secondary methyl groups, five sp^3 methine and three sp^3 methylenes. The NMR spectroscopic data are listed in Table 13. The ESI mass spectrum displayed a *pseudo*molecular ion at *m/z* 379 $[M + H]^+$, which gave the molecular weight as 378 Dalton, while HRESIMS established the molecular formula as $C_{20}H_{26}O_7$.

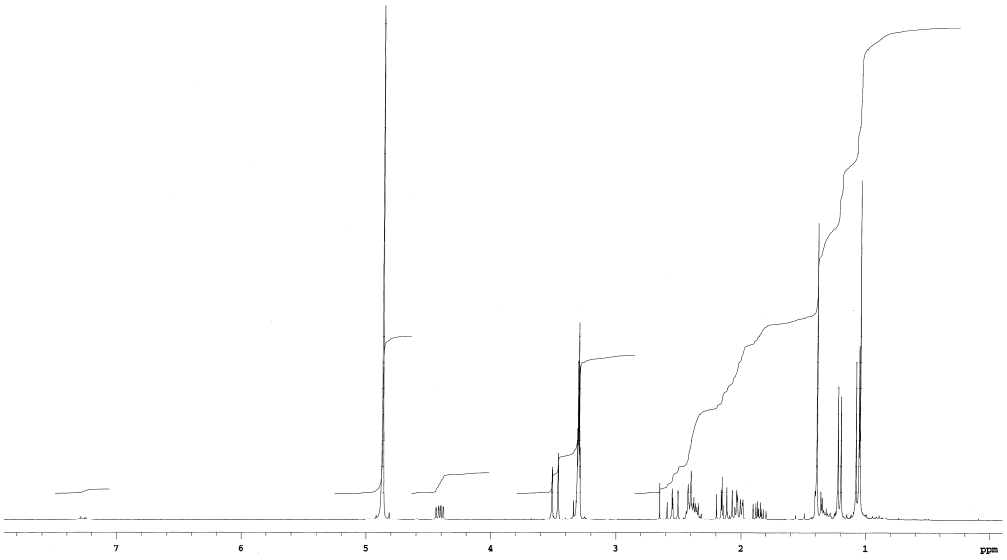


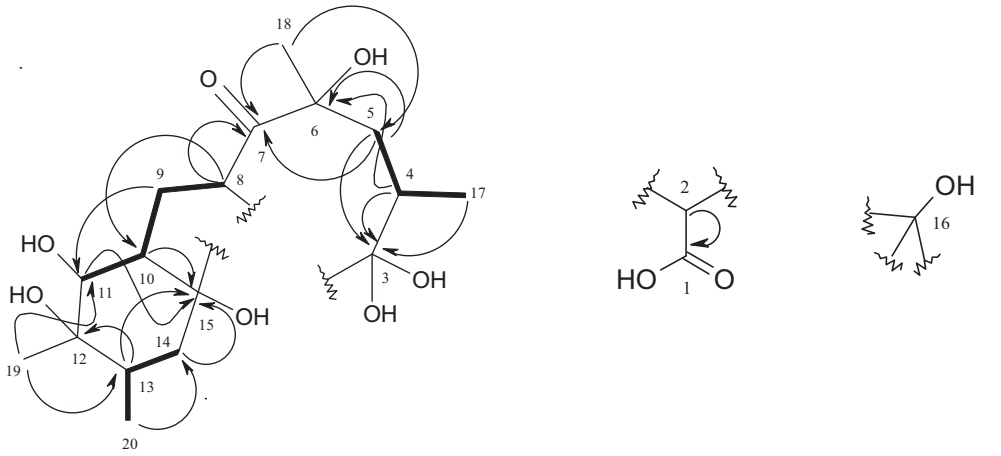
Figure 35: ^{1}H NMR spectrum (CD_3OD, 600 MHz) of *ent*-homoabyssomicin A (**70**)

The complex structure was elucidated with the help of 2D NMR measurements. Analysis of ^{1}H,^{1}H COSY as well as TOCSY spectra revealed the presence of three spin systems (Figure 36) among protons H-4, H-5, CH_3-17, from H-8 to H-11 and CH_3-20, H-13, H-14 (bold lines in Figure 36). $^{2,3}J$ HMBC correlations of CH_3-17 with C-3, from CH_3-18 with C-5, from H-5 to C-7, C-6 and C-3 supported the carbon network from C-3 to C-7. Further HMBC along with COSY correlations confirmed the partial structure I as drawn in Figure 36. A second two carbons chain (fragment II) and one quaternary oxygenated carbon substituent (fragment III) are left, so that alltogether five bonds are open. The logical consequence is that fragments II and III must be connected and can be joined at C-3, C-8 and C-15 in three diffferent ways: According to the HMBC correlation of H-2 with C-16, C-8, C-3, the connection of C-2 with C-3 is the most plausible alternative; C-16 is connected with C-8 and C-15, resulting in substructure IV (Figure 37).

According to the empirical formula, compound **70** has eight double bond equivalents: The presence of two carbonyl groups and the absence of double bonds suggested the presence of six rings. In substructure IV, three rings are therefore still to be formed. One of them is due to a lactone, as the carbonyl at δ 177.5 indicated. The remaining two must contain oxygen as bridge atom, giving rise to a spiroketal skeleton as assigned in structure **70**. After searching in AntiBase[113] it was found that spectroscopic data for the spiroketal part of the molecule showed close similarity with those of spirotetronate metabolites and also of abyssomicins, especially abyssomicin D (**71**).

Table 10: ^{13}C and ^{1}H NMR shifts (125, 600 MHz) of *ent*-homoabyssomicin A (**70**) in CD_3OD

Position	δ_C	δ_H (mult.; *J* in Hz)
1	177.5	
2	51.5	3.51 (s, 2-CH)
3	110.1	
4	45.0	2.38 (m, 4-CH)
5a	40.4	2.15 (ABX, *J* = 13.4, 11.4, 5a-CH_2)
5b		2.03 (ABX, *J* = 13.4, 10.6 Hz, 5b-CH_2)
6	87.0	
7	212.8	
8	53.2	4.41 (dd, *J* = 11.1, 6.0, 8-CH)
9a	27.8	1.85 (ddd, *J* = 13.5, 11.2, 6.8, 9a-CH_2)
9b		2.01 (m, 9b-CH_2)
10	52.1	2.41 (d, *J* = 6.7, 10- CH)
11	85.8	3.46 (s, 11-CH)
12	77.7	
13	29.0	2.37 (m, 13-CH)
14a	32.0	2.54 (ABX, *J* = 14.1, 12.2, 14a-CH_2)
14b		1.37 (ABX, *J* = 14.1, 3.3, 14b-CH_2)
15	89.3	
16	86.4	
17	14.5	1.21 (d, *J* = 7.1 Hz, 17-CH_3)
18	24.8	1.39 (s, 18-CH_3)
19	21.0	1.05 (s, 19-CH_3)
20	16.7	1.07 (d, *J* = 7.2, 20-CH_3)



I **II** **III**

Figure 36: $^{1}H,^{1}H$ COSY and HMBC correlations of *ent*-homoabyssomicin A (**70**) observed for fragments **I**, **II** and **III**

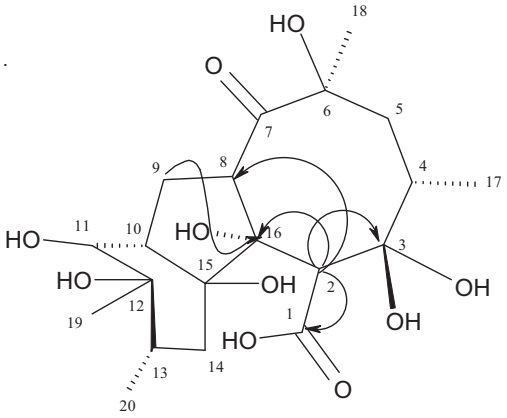
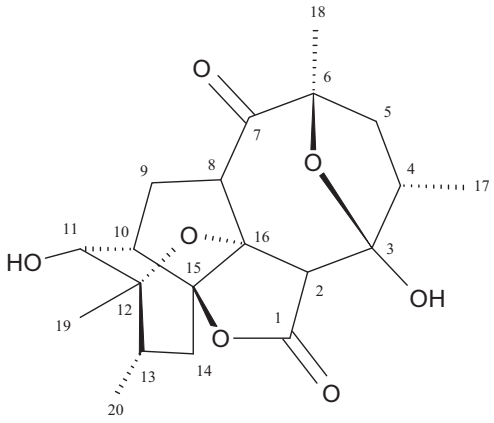
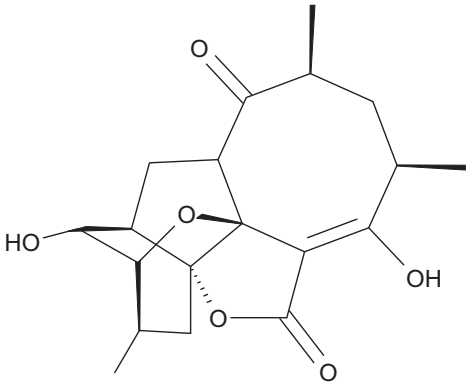


Figure 37: HMBC correlations of *ent*-homoabyssomicin A (**70**) observed for substructure IV



70



71

The structure was finally confirmed by single crystal X-ray diffraction experiments. The absolute stereochemistry of the molecule was assigned as (2*S*,3*R*,4*S*,6*S*,8*S*, 10*S*,11*S*,12*S*,13*S*,15*S*,16*R*). The relative configuration of abyssomicins such as abyssomicin D (**71**) was assigned by X-ray data, and the absolute stereochemistry was determined by both the Mosher and Helmchen methods. Both methods confirmed the *R* configuration at C15, C13, C12, C11 and C4, which is opposite to that in compound **70**, so that the new derivative is based on the *ent*-abyssomycin skeleton.

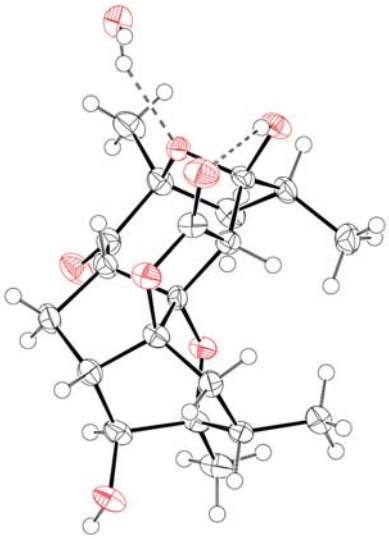


Figure 38: ORTEP plot of *ent*-homoabyssomicin A (**70**)

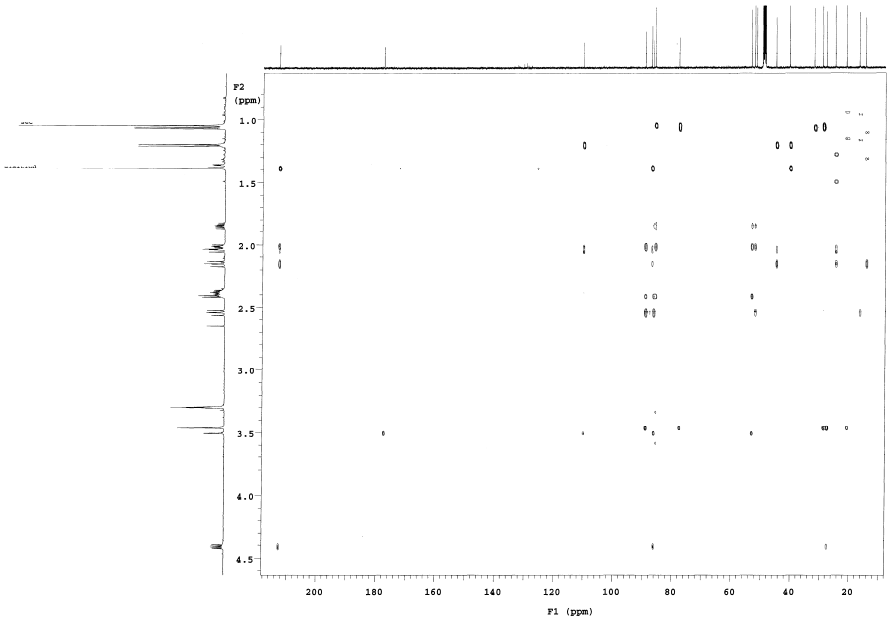


Figure 39: HMBC spectrum (CD_3OD, 600 MHz) of *ent*-homoabyssomicin A (**70**)

The name *ent*-homoabyssomicin A has been assigned based on its similarities with the abyssomicin skeleton, due to the excess carbon atom and the opposit stereochemistry. The oxygen bridge as well as the skeletal methyl group present in **70** were, however, absent in compound **71**.

4.1.2 *ent*-Homoabyssomicin B

ent-Homoabyssomicin B (**72**) was isolated as white solid after purification of fraction FII on Sephadex LH-20 using MeOH followed by reverse phase chromatography. Compound **72** was UV absorbing and turned to yellow with anisaldehyde/sulphuric

acid. The ESI mass spectrum displayed a *pseudo*molecular ion at *m/z* 457 $[M + Na]^+$, and HRESIMS established the molecular formula as $C_{22}H_{26}O_9$, i.e. a difference of C_2O_2 was observed in comparison with *ent*-homoabyssomicin A (**70**).

In the ^1H NMR spectrum of *ent*-homoabyssomicin B (**72**), four methine, two methylene and additionally five methyl signals were observed. The ^{13}C NMR spectrum pointed to nine quaternary carbon signals, four sp^2 signals along with two methylene carbons, two methine and five methyl signals (Table 15).

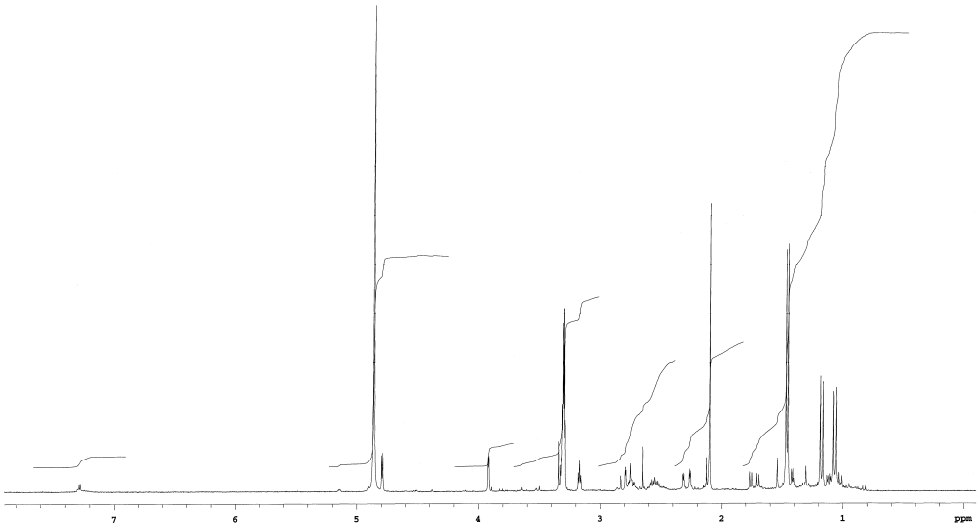
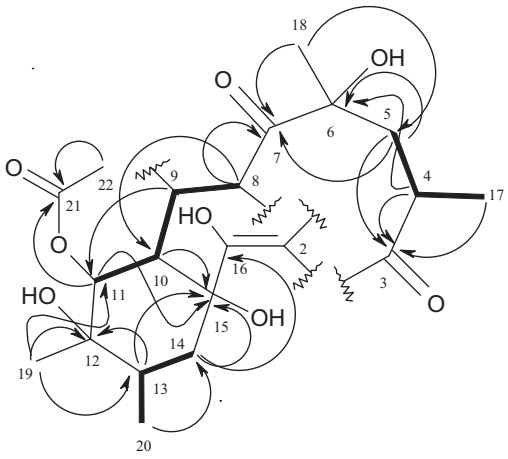
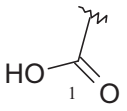


Figure 40: ^1H NMR spectrum (CD_3OD, 600 MHz) of *ent*-homoabyssomicin B (**72**)

Analysis of the $^1H,^1H$ COSY and TOCSY spectra revealed the presence of three spin systems (Figure 41) among protons H-5, H-4, CH_3-17, from H-8 to H-11 and CH_3-20, H-13, H-14, similar to that of compound **70**. Key $^{2,3}J$ HMBC correlations as represented in Figure 41 confirmed the fragment from C-3 to C-16. ^3J HMBC correlations from H-14 to C-16 suggested a double bond between C-16/C-2 or alternatively, CO at C-16 and an acetal at C-2. HMBC correlations along with COSY correlations confirmed the partial structure **I** (Figure 41) for compound **72**. ^{13}C NMR and HSQC data allowed the complete carbon assignment and localized the additional two carbon atoms in an acetyl residue. Analysis of the ^{13}C, HSQC and HMBC data confirmed that one carbon chain (fragment **II**) established a spirotetronic acid moiety attached at position C-2 of fragment I. The resulting structure of **72** was similar to the basic skeleton of abyssomicin C (**73**). The Michael acceptor of **73** has been epoxidised in *ent*-homoabyssomicin B (**72**).

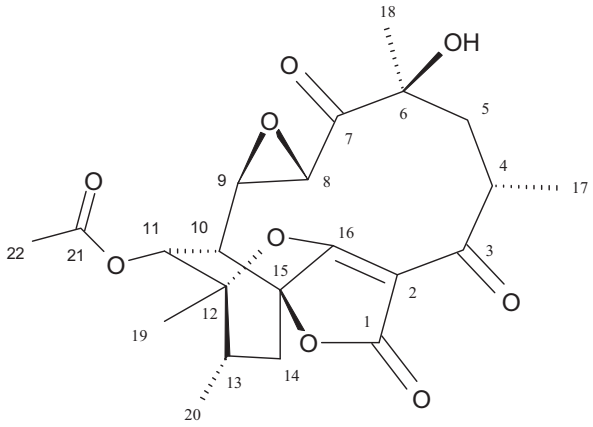


I

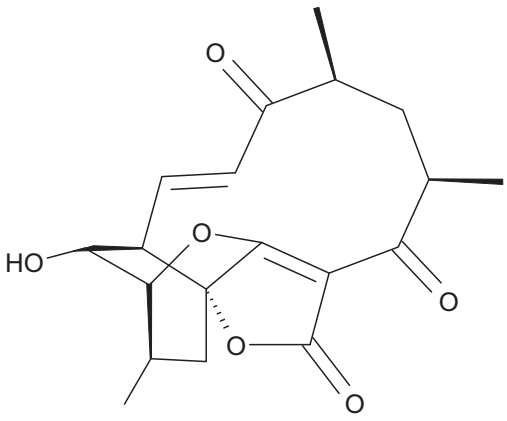


II

Figure 41: $^{1}H,^{1}H$ COSY and HMBC correlations of *ent*-homoabyssomicin B (**72**) observed for fragments **I** and **II**



72



73

The relative stereochemistry of *ent*-homoabyssomicin B (**72**) as determined from the key NOESY correlations was in agreement with the configuration of **70**. As both compounds were found in the same strain, identical absolute configurations can be assumed, so that also **72** is also an *ent*-homoabyssomycin.

Abyssomicin C (**73**) is a potent inhibitor of *p*ABA/tetrahydrofolate biosynthesis.[124] The activities of *ent*-homoabyssomicins A (**70**) and B (**72**) have not been tested so far.

Table 11: ^{13}C and ^{1}H NMR shifts (125, 600 MHz) of *ent*-homoabyssomicin B (**72**) in CD_3OD

No.	δ_C	δ_H (mult.; *J* in Hz)
1	170.5	
2	103.0	
3	200.5	
4	42.5	2.74 (m, 4-CH)
5a	41.9	2.28 (ABX, *J* = 16.2, 2.4, 5a-CH_2)
5b		1.73 (ABX, *J* = 16.2, 5.4, 5b-CH_2)
6	81.2	
7	207.9	
8	49.8	3.92 (d, *J* = 1.8, 8-CH)
9	56.8	3.31 (dd, *J* = 3.4, 2.0, 9-CH)
10	46.1	3.17 (t, *J* = 3.0, 10-CH)
11	69.3	4.79 (d, *J* = 2.7, 11-CH)
12	87.7	
13	31.0	2.55 (m, 13-CH)
14a	36.3	2.79 (ABX, *J* = 12.8, 11.0, 14a-CH)
14b		1.45 (ABX, *J* = 12.8, 3.3, 14b-CH)
15	79.1	
16	179.9	
17	17.8	1.17 (d, *J* = 6.7, 17-CH_3)
18	26.4	1.45 (s, 18-CH_3)
19	19.1	1.47 (s, 19-CH_3)
20	16.4	1.07 (d, *J* = 7.2, 20-CH_3)
21	170.0	
22	20.8	2.10 (s, 22-CH_3)

Abyssomicins are complex polycyclic compounds. In 2004 the group of Fiedler and Süssmuth in Tübingen (Germany) discovered the first abyssomicins B (**74**), C (**73**) and D (**71**) in a rare actinomycete *Verrucosispora* strain, which was isolated from a sediment sample collected in the Japanese Sea at a depth of 289 m.[125] Only abyssomicin C (**73**) exhibited antibiotic activity against Gram-positive bacteria including pathogenic *Staphylococcus aureus*. Later other derivatives of abyssomicins have been investigated. Atrop-abyssomicin C (**75**) was synthesized firstly from abyssomicin C and converted to abyssomicin D (**71**). It inhibits *S. aureus* N315 (MRSA).[126]

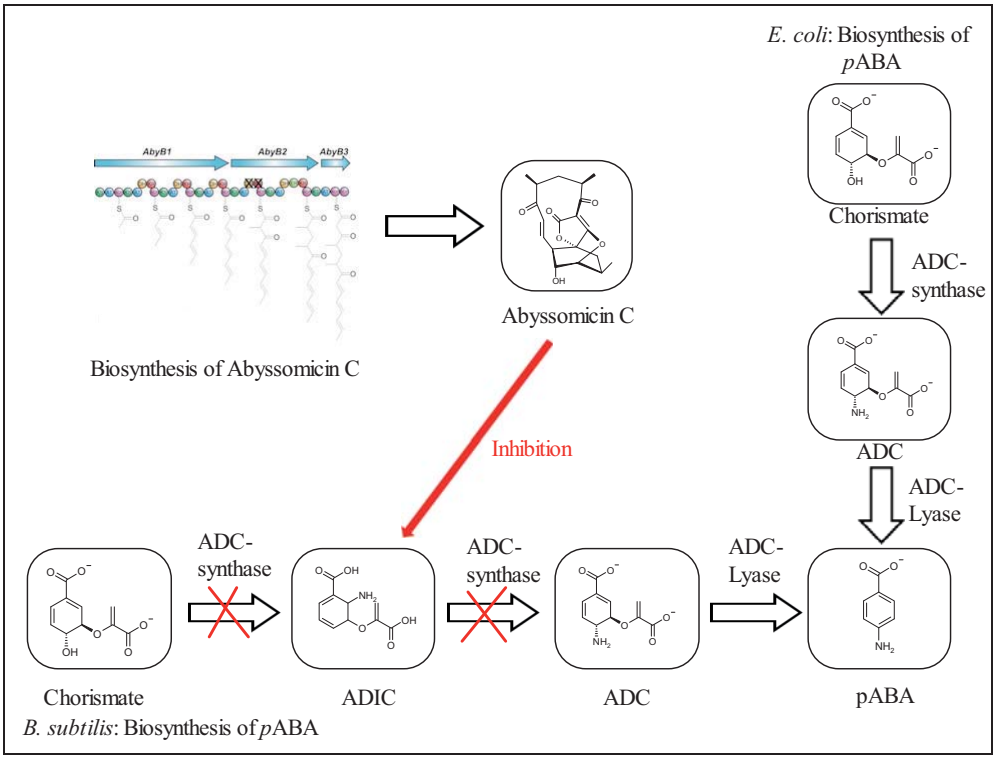
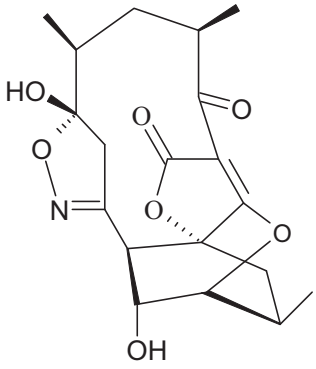
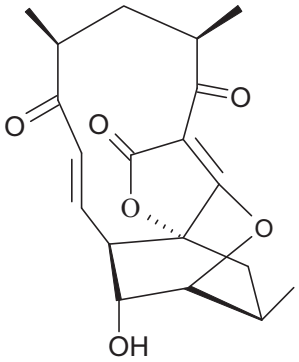


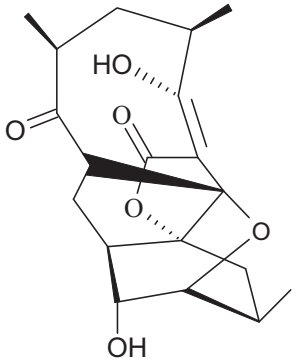
Figure 42: The biosynthetic pathway of abyssomicin C (**73**) and the inhibition of the *p*ABA/tetrahydrofolate pathway.[127]



74



73



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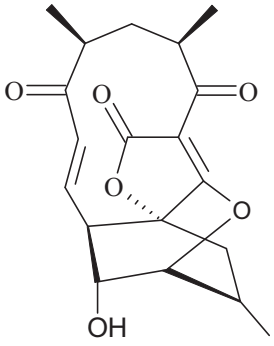
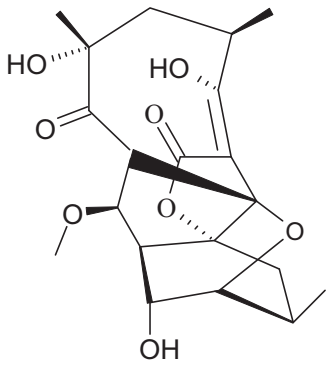
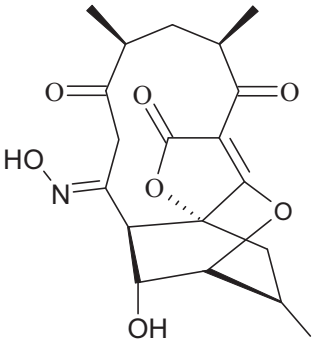


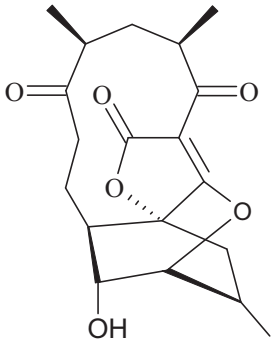
75



76



77



78

In 2007 abyssomicin E (**76**)[128] was isolated from *Streptomyces* sp. HKI0381 in Jena (Germany) and fully elucidated by X-ray crystallography, NMR and MS data. In the same year the group of Fiedler and Süssmuth again were successful and found atrop-abyssomicin C (**75**) also in nature, along with abyssomicins G (**77**) and H (**78**); the latter three derivatives were isolated from the same strain as the previous abyssomicins.[129]

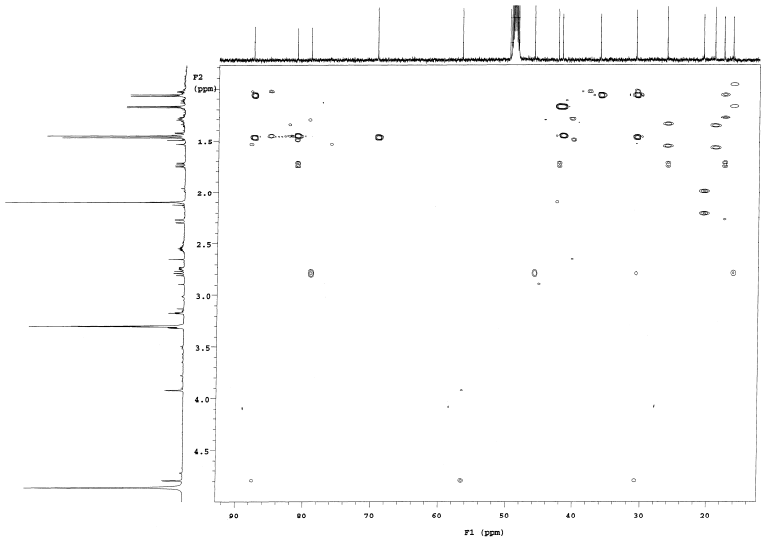
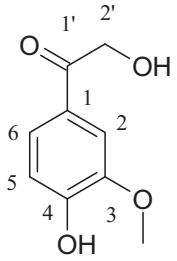


Figure 43: HMBC spectrum (CD_3OD, 600 MHz) of *ent*-homoabyssomicin B (**72**)

4.1.3 2-Hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone

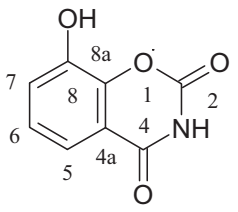
2-Hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**79**) was isolated as colourless solid, which absorbed at 254 nm in the UV range and turned to greenish-blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum in CD_3OD exhibited two *ortho*-coupled protons at δ 7.50 and 6.86. A *meta*-coupled proton appeared at δ 7.52 (J = 2.0 Hz) resulting in a 1,2,4-tri-subistituted benzene ring. In the upfield region, a 3H singlet for a methoxy group was observed at δ 3.90 together with an oxymethylene group at δ 4.83. The ESI mass spectrum indicated a molecular weight of 182, and ESI HRMS confirmed the molecular formula as $C_9H_{10}O_4$ entailing five double bond equivalents. A search in AntiBase[113] using the spectroscopic information led to the structure of **79**, which was confirmed by comparing with the literature data.[130]



79

4.1.4 Benadrostin

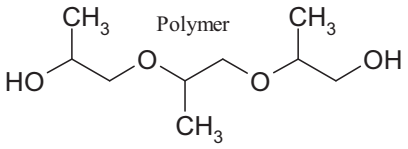
Benadrostin (**80**) was isolated as colourless solid. In the ^{1}H NMR spectrum no aliphatic proton signals were obtained, while in the aromatic region two doublets of doublets appeared at δ 7.46 (H-5) and 7.21 (H-7), in addition to a triplet at δ 7.17 (H-6) indicating three adjacent aromatic protons. The molecular weight of was obtained by the ESI/MS as 179, and HRESIMS established the corresponding molecular formula $C_8H_5NO_4$. A search in AntiBase[113] resulted in benadrostin (**80**), which was isolated previously in our group.[131] Compound **80** is known as inhibitor of poly(ADP-ribose) synthetase.[132,133]



80

4.1.5 Polypropylenglycol

Polypropylenglycol (**81**) was isolated as oil from a colourless UV inactive zone, which turned to pink with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum displayed a methyl doublet at δ 1.10 along with a 3H multiplet at δ 3.51, which could be explained by an oxymethine and additional oxymethylene groups. A search in AntiBase[113] using the mentioned spectroscopic information assigned the isolated compound as **81**. Polypropylenglycol (**81**) is often used as antifoaming agent in fermentor cultures, but later it was isolated in our group also as natural product.



81

4.2 Terrestrial *Streptomyces* sp. ANK 313

The terrestrial *Streptomyces* sp. ANK 313 was selected according to the prescreening, in which the crude extract exhibited three interesting UV active bands, which gave yellow colourations with anisaldehyde/sulphuric acid. Additionally, the extract showed zoosporicidal activity against *Plasmopara viticola* at a concentration of 100 µg/ml.

Well-developed agar plates of ANK313 were used for the inoculation of 100 of 1 L Erlenmeyer flasks each containing 300 ml of M_2^+ medium. The culture was incubated on a linear shaker at 28 °C for 7 days. Consequently, the culture broth was pressed through a pressure filter to afford the water phase and biomass. The water phase was passed through XAD-16 resin and the latter extracted with MeOH; the biomass was extracted with ethyl acetate followed by acetone. Both organic phases were evaporated to dryness. The combined extracts were chromatographed on silica gel using a CH_2Cl_2/MeOH gradient to obtain three fractions. On TLC, fraction F1 showed two yellow components, which were further purified on Sephadex LH-20 followed by PTLC and again Sephadex LH-20 to get three compounds. Fraction I delivered the pure components khatmiamycin (**82**), GTRI-02 (**84**) and 4-ethyl-5-methyl-heptanamide. Fraction II was subjected to Sephadex LH-20 using MeOH followed by RP-18 to deliver compounds aloesaponarin II (**85)** and LL-C10037α (**86**). Moreover, purification of FIII on RP-18 followed by PTLC revealed compounds LL-C10037β (**87**) and omdurmycin (**88**). Compound **82** and **88** are new compounds and the remaining five compounds were known.

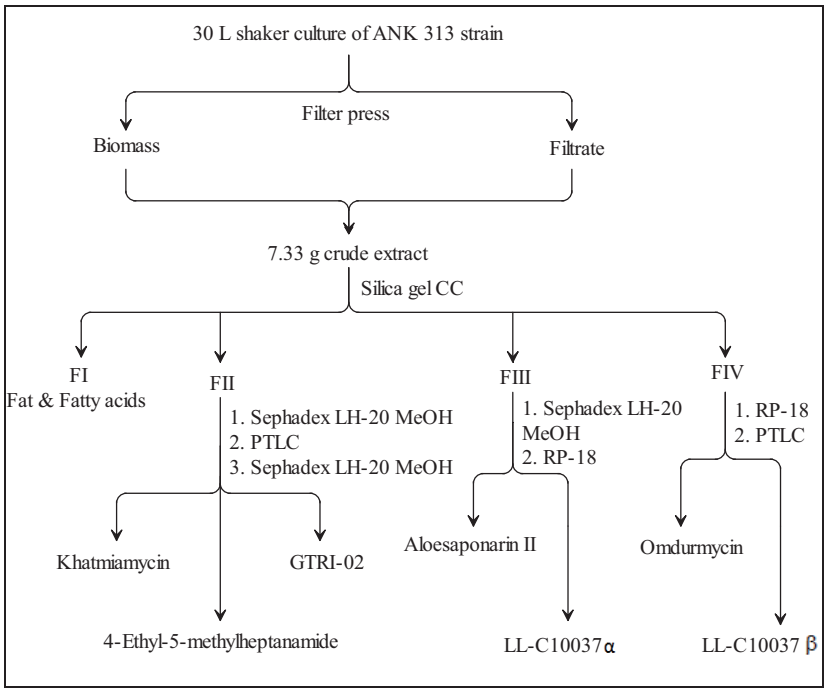


Figure 44: Work up scheme of terrestrial *Streptomyces* ANK 313

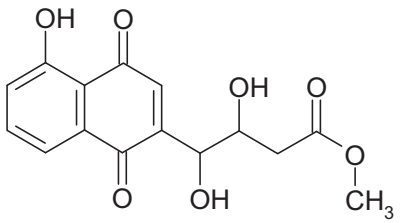
Table 12: Antimicrobial activity of the three fractions from *Streptomyces* sp. ANK 313

Tested Microorganisms	Fraction (FI)	Fraction (FII)	Fraction (FIII)
Escherichia coli			22
Bacillus subtilis	13	14	18
Staphylococcus aureus	26	30	28
S. viridochromogenes (Tü57)	25	26	40
Candida albicans	11	11	11
Mucor miehei (Tü 284)		13	11

4.2.1 Khatmiamycin

Khatmiamycin (**82**) was isolated as yellow solid; it showed UV absorption at 254 nm, turned to black with anisaldehyde/sulphuric acid and became blue with 2N NaOH on TLC, indicating a *peri*-hydroxyquinone.

The ^{1}H NMR spectrum of khatmiamycin (**82**) displayed an aromatic ABC system for three adjacent protons. In addition, there was a further doublet at δ 7.04. In the aliphatic region, the spectrum showed two oxygen-bound protons by signals at δ 4.21 and 4.79, a methoxy signal at δ 3.69 and diastereotopic methylene protons at δ 2.78 and 2.66. In the ^{13}C NMR and HSQC spectra, there were 15 carbon signals as indicated by the HR ESIMS-derived formula $C_{15}H_{14}O_7$. Two ketone carbonyl groups at δ 190.3 and 184.5, one carbonyl of an acid or ester at δ 173.1, eight sp^2 carbons (four quaternary and four methine carbons) and four sp^3 carbons (two oxygenated CH, one OCH_3 and one CH_2) completed the spectrum.



82

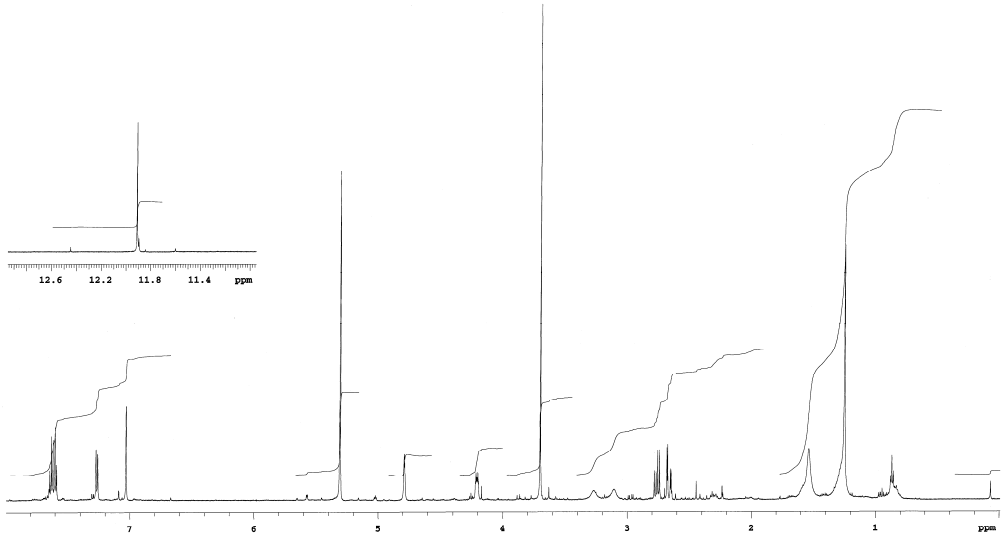
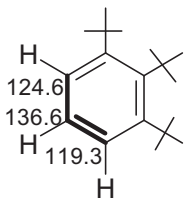
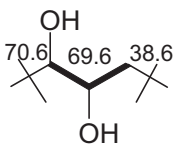


Figure 45: ^{1}H NMR spectrum (CD_2Cl_2, 300 MHz) of khatmiamycin (**82**)

By interpretation of the COSY spectrum, the two substructures **I** and **II** were deduced.





The HMBC spectrum showed correlations from H-7 to C-5 and C-8a, the former one (C-5) could be linked to oxygen, from H-8 to C-1 (184.5, CO), C-6 and C-4a, from H-6 to C-8 and C-4a. Furthermore, H-3 showed couplings with C-1 (CO), C-2, C-4a and C-9 of fragment **II**. H-11 and the methoxy group showed correlations with the carbonyl of an ester at δ 173.1 (C-12). According to the above spectroscopic data, the following partial structure with 7 double bond equivalents can be drawn:

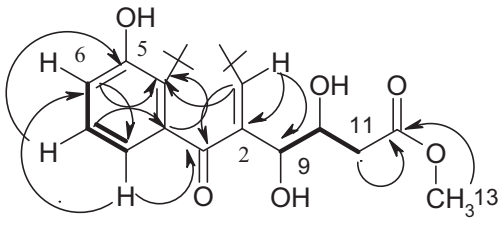


Table 13: ^{13}C (125 MHz) and ^{1}H NMR data (300 MHz) of khatmiamycin (**82**) in CD_2Cl_2

No.	δ_C	δ_H (mult.; J in [Hz])	Selected HMBC correlations
1	184.5		C-3, C-8
2	150.9		C-3
3	135.4	7.04 (d, J = 1.42)	C-1, C-2, C-4a, C-9
4	190.3		
4a	115.2		C-3, C-8, C-6
5	161.5		C-7
6	124.6	7.27 (dd, J = 7.7, 1.9)	C-4a, C-8
7	136.6	7.64 (t, J = 7.9)	C-5, C-8a
8	119.3	7.62 (dd, J = 7.9, 2.5)	C-1, C-4a, C-6, C-8a
8a	132.4		C-7
9	70.6	4.79 (s, br)	C-3
10	69.6	4.21 (m)	C-11
11	38.6	2.78 (ABX, J = 16.6, 8.4) 2.66 (1H, ABX, J = 16.6, 3.9)	C-10, C-12
12	173.1		C-11, OMe
OMe	52.3	3.69 (s)	C-12, (11)
OH	-	11.82 (s)	C-4a, 5, 6, (7)

The remaining ketone carbonyl at δ 190.3 showed no correlations. Placing this carbonyl at C-4 and closing the ring for the remaining double bond equivalent completes the structure.

According to previous reports, numerous naphthoquinone derivatives have been isolated from *Streptomyces* spp. and fungi. Most of them are having an additional annellated ring, forming anthraquinones or naphthopyranequinones, whereas compound **82** bears an open chain.

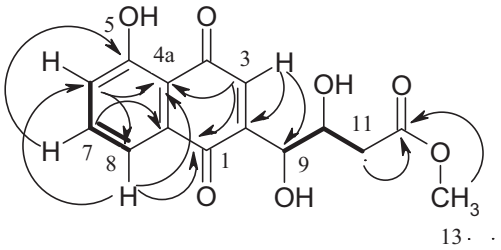
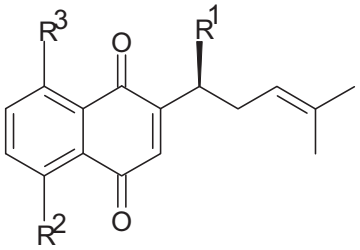


Figure 46: Selected $^{1}H,^{1}H$ COSY and HMBC correlations of khatmiamycin (**82**)

Quinones are usually acetogenins and are formed by cyclisation of a respective polyketide. In the case of **82**, a heptaketide precursor can be assumed. In most other cases, such oligoketides are forming anthraquinones, anthracyclinones, angucyclinones etc. As the strain produced additionally anthraquinones, compound **82** is probably formed on the same biosynthetic acetate pathway as drawn in Figure 47. Quinones with partially cyclized ketide chain as in **82** are less common: 8-*O*-methyl-2-hydroxyjavanicin, novarubin, 8-*O*-methylsolaniol, lapachol, lamatiol, streptocarpone, arnebin-5, trichione, 2-dodecyl-naphthoquinone, 3,5,8-trihydroxy-6-methoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone, 6,7-dimethoxylindbladione and juglomycin E are microbial examples of the alkyl-naphthoquinone series listed in AntiBase. Recently similar new naphthoquinones, 5-*O*-methyl-11-deoxyalkannin (**83a**) and 8-*O*-methyl-11-deoxyalkannin (**83b**) were isolated from the roots of *Alkanna cappadocica*. They exhibited cytotoxicity against 12 human cancer cell lines.[134]



	R^1	R^2	R^3
83a	H	OCH_3	OH
83b	H	OH	OCH_3

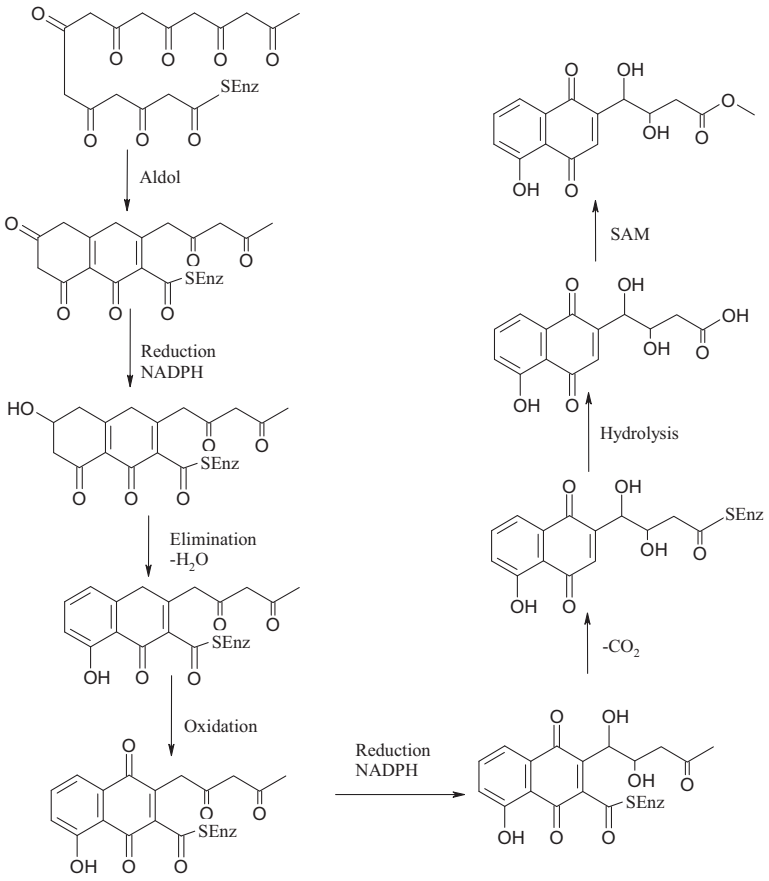


Figure 47: Suggested biosynthetic pathway for khatmiamycin (**82**)

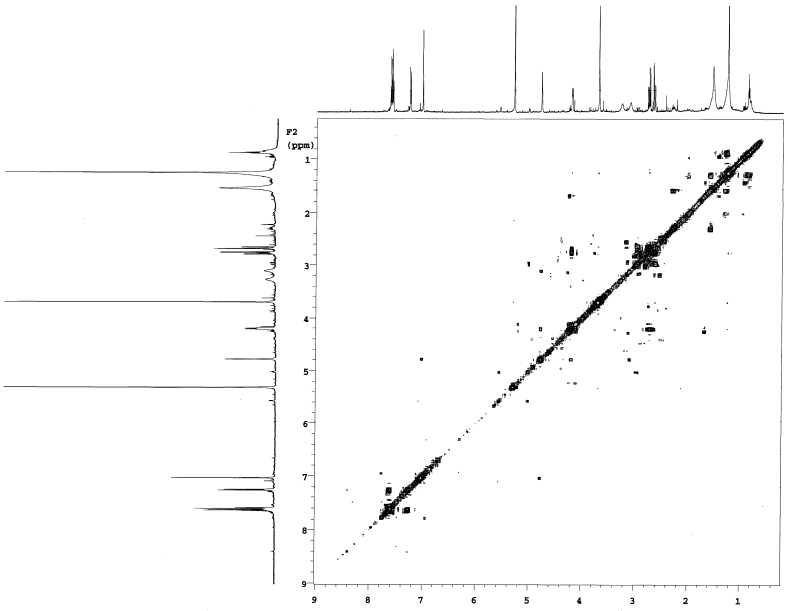


Figure 48: $^{1}H,^{1}H$ COSY spectrum (CD_2Cl_2, 300 MHz) of khatmiamycin (**82**)

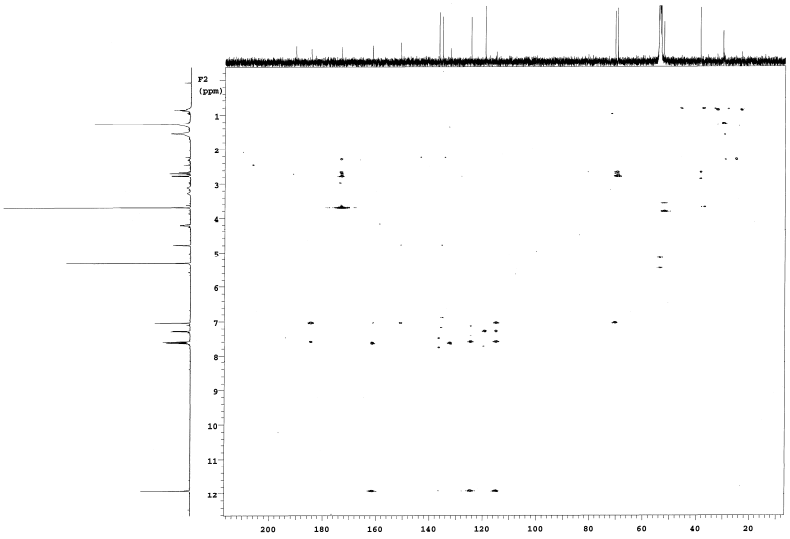


Figure 49: HMBC spectrum (CD_2Cl_2, 300 MHz) of khatmiamycin (**82**)

4.2.2 GTRI-02

GTRI-02 (**84**) was isolated from fraction FI as white powder. The ^{1}H NMR spectrum revealed an aromatic proton appearing as singlet at δ 6.62 along with an oxymethine at δ 4.23, two methylene signals between δ 2.62 and 3.16 and two methyl groups at δ 2.33 and 2.29. The ^{13}C NMR spectrum delivered 13 signals, among them seven quaternary carbons between δ 124.8-208.1, an oxymethine at δ 66.7, two methylene carbons at δ 48.5 and 40.5 and two methyl carbons at δ 32.6 and 19.2.

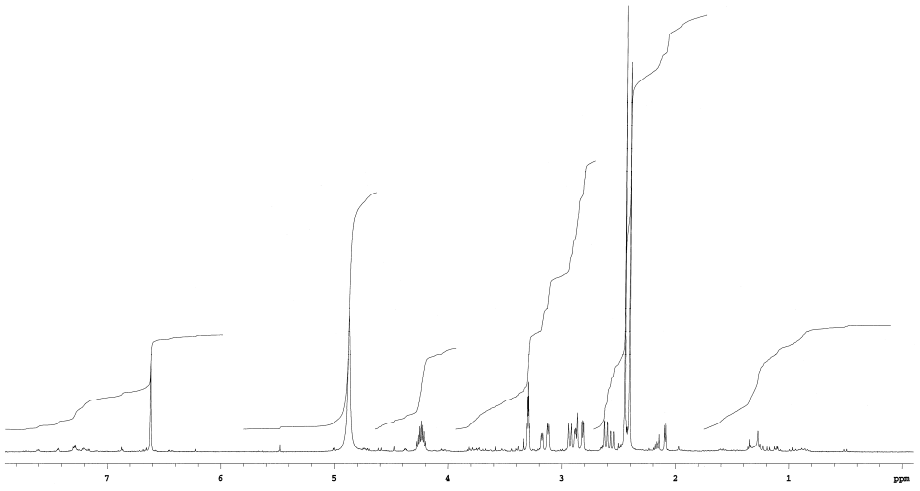
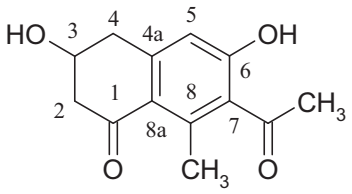


Figure 50: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of GTRI-02 (**84**)



84

The CI as well as EI mass spectra delivered a molecular weight of 234 Dalton. COSY correlations from H-2 to H-4 together with HMBC correlations from both H-3 and H-2 to C-1 and H-4 to C-8a supported a six-membered ring. The connectivity of compound **84** was confirmed by further HMBC correlations from H-5 to C-4, C-7 and both carbonyl groups at δ 208.1 and 199.1. Additional correlations were found from the methyl signal at δ 2.39 to C-7, C-8 and C-8a and from the methyl signal at δ 2.43 to the carbonyl at δ 208.1.

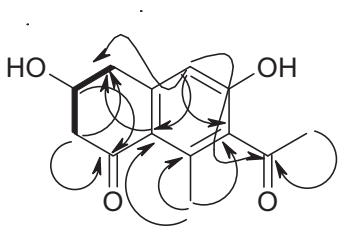


Figure 51: Selected ^{1}H,^{1}H COSY and HMBC correlations of GTRI-02 (**84**)

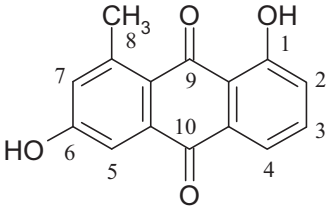
A search in AntiBase[113] led directly to the structure **84**, and the spectroscopic values above were in agreement with the reference data. Compound **84** is known as lipid peroxidation inhibitor.[135]

Table 14: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of GTRI-02 (**84**) in CD_3OD

No.	δ_C	δ_H (mult.; *J* in Hz)
1	199.1	
2	48.5	2.83 (ABX, *J* = 16.0, 5.0, 2-H_a) 2. 62 (ABX, *J* = 16.0, 7.6, 2-H_b)
3	66.7	4.23 (m)
4	40.5	2.93 (ABX, *J* = 16.0, 7.4, 4-H_a) 3.16 (ABX, *J* = 16.0, 3.7, 4-H_b)
4a	146.9	
5	114.6	6.62 (s)
6	158.7	
7	132.2	
8	140.2	
8a	124.8	
8-CH_3	19.2	2.39 (s)
7-*CO*CH_3	208.1	
7-*CH*$_3$CO	32.6	2.43 (s)

4.2.3 Aloesaponarin II

Aloesaponarin II (**85**) was isolated as a yellow, under UV orange fluorescent solid, which turned to violet with NaOH indicating a *peri*-hydroxyquinone. In the ^{1}H NMR spectrum a chelated OH signal was observed at δ 12.99 together with three signals at δ 7.72 (t), 7.66 (dd) and 7.35 (dd), indicating three adjacent aromatic protons. Two aromatic *meta*-coupled doublets were observed at δ 7.47 and 7.05. In the upfield region, a methyl singlet appeared at δ 2.73. The ESI mass spectrum revealed the molecular weight 254 and HRESIMS afforded the molecular formula $C_{15}H_{10}O_4$. A search in AntiBase[113] led to aloesaponarin II (**85**), which has been isolated first from *Aloe saponaria* Haw[136] and later also from a number of streptomycetes.[137,138]



85

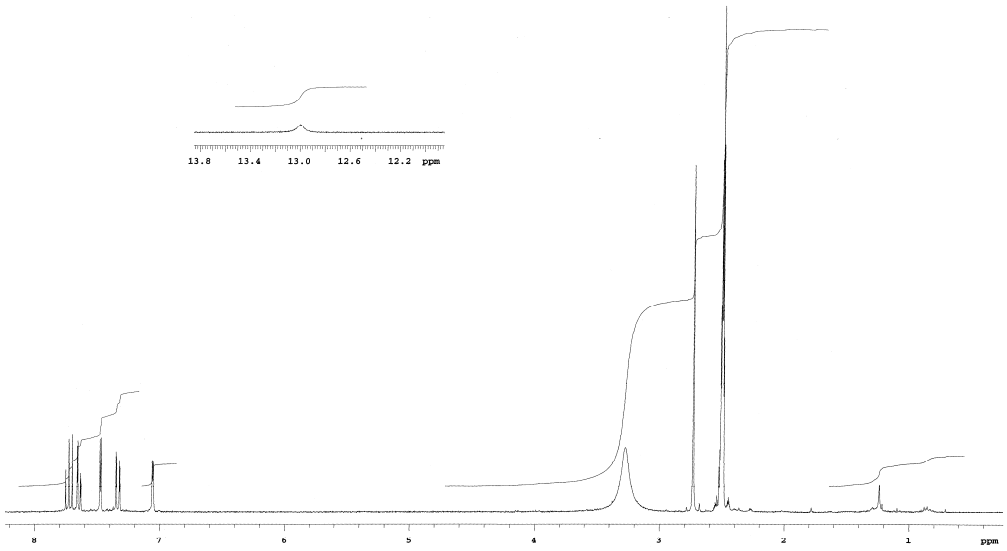


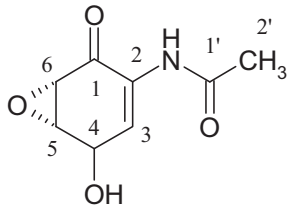
Figure 52: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of aloesaponarin II (**85**)

Table 15: ^{1}H NMR shifts (300 MHz) of aloesaponarin II (**85**) in DMSO-d_6

Position	δ_H (mult.; J in Hz)
1	
2	7.66 (dd, J = 7.5, 1.3)
3	7.72 (t, J = 8.1, 7.5)
4	7.35 (dd, J = 8.1, 1.3)
5	7.47 (d, J = 2.6)
6	
7	7.05 (d, J = 2.6)
8	
8-CH_3	2.73 (s)
OH	12.99 (s br)

4.2.4 LL-C10037α

LL-C10037α (**86**) was isolated from fraction FII as white needles. The ^{1}H NMR spectrum was simple, with an aromatic singlet at δ 7.19 in addition to three oxymethine signals at δ 3.80, 3.50, 3.33 and a methyl singlet at δ. 2.08.



86

Table 16: ^{13}C and ^{1}H NMR data (125, 300 MHz) of LL-C10037α (**86**) in CD_3OD

No.	δ_C	C type	δ_H (mult.; *J* in Hz)
1	190.2	C_q	
2	129.7	C_q	
3	128.6	CH	7.19 (t, *J* = 5.4, 2.6)
4	65.4	CH	4.80 (d, *J* = 4.0)
5	55.0	CH	3.80 (dd, *J* = 4.1, 2.6)
6	53.5	CH	3.50 (d, *J* = 4.1)
1'	172.3	C_q	
2'	23.8	CH_3	2.08 (s)

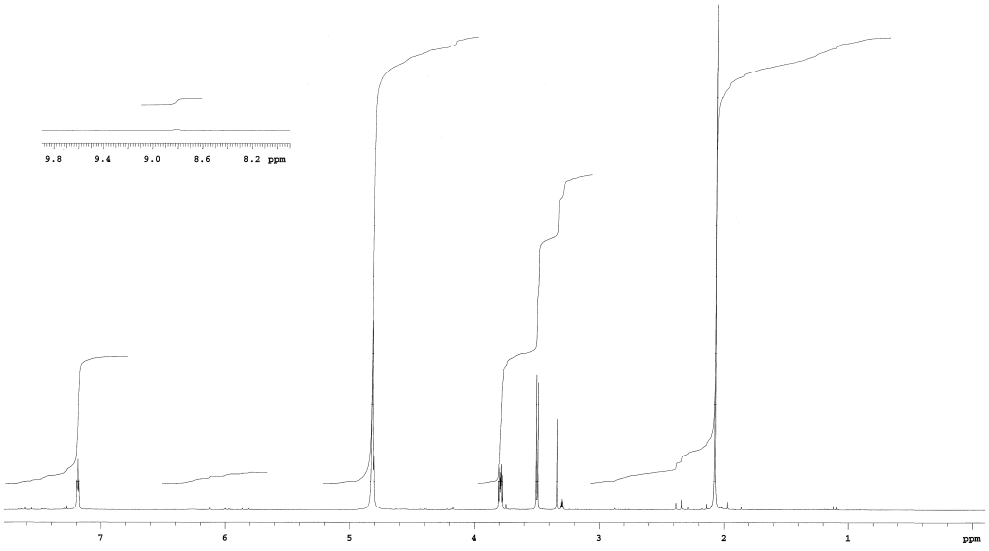


Figure 53: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of LL-C10037α (**86**)

The ^{13}C NMR spectrum revealed eight signals of three quaternary, one olefinic, three oxymethine carbons and one methyl group. ESIMS afforded the *pseudo*molecular ion at 206 $[M + Na]^+$, which gave a molecular weight of 183 Dalton; HRESIMS revealed the molecular formula as $C_8H_9NO_4$. COSY as well as HSQC and HMBC spectra showed strong correlations as drawn in Figure 54, and a search in AntiBase[113] with the COSY correlation from H-3 to H-6 together with HMBC couplings from H-6 to C-2 and C-3, from H-5 to C-3 and H-3 to C-1 and H-2' to C-1' led to LL-C10037α (**86**). Surprising is the strong W coupling between H-3 and H-5.

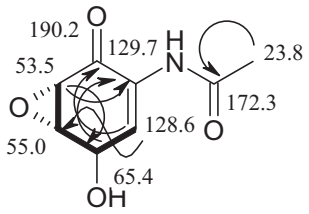


Figure 54: Selected $^{1}H,^{1}H$ COSY and HMBC correlations of LL-C10037α (**86**)

LL-C10037α (**86**) has been isolated in 1984; the biosynthesis is derived from 3-hydroxyanthranilic acid *via* the shikimic acid pathway. Compound **86** was reported to exhibit high antitumor activity.[139,140]

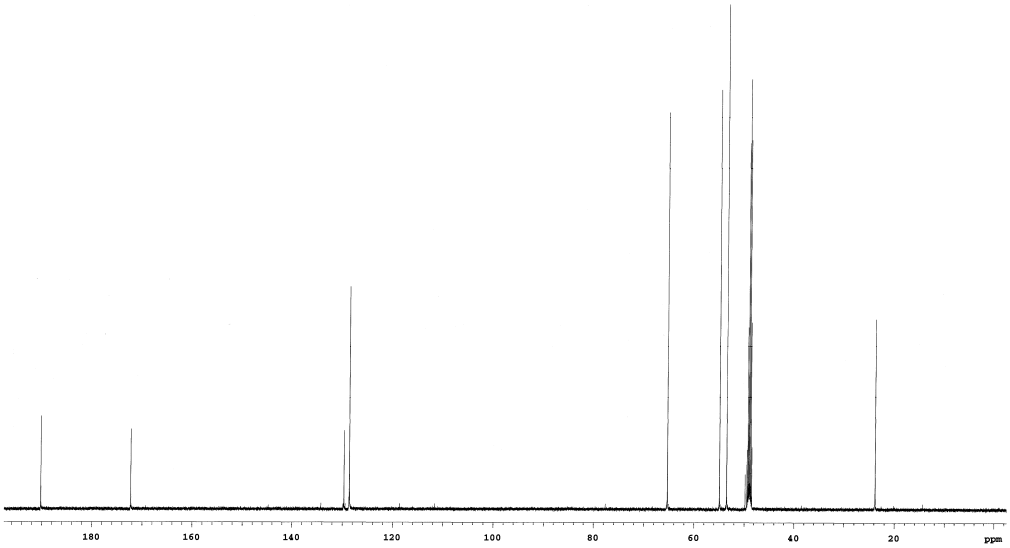
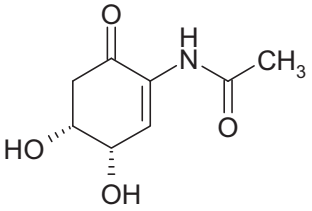


Figure 55: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of LL-C10037α (**86**)

4.2.5 LL-C10037β

Compound **87** was isolated from fraction FIII as white solid. The ^{1}H NMR spectrum showed similarity to compound **86**, but a signal of methylene protons at δ 2.80 (H-6) appeared instead of a methine signal in **86**. ESI MS afforded a *pseudo*molecular ion at 208 $[M + Na]^+$, which delivered the molecular weight as 185 Dalton. HRESI/MS revealed the molecular formula $C_8H_{11}NO_4$. A search in AntiBase[113] confirmed the identity with LL-C10037β (**87**), which was isolated previously from *Streptomyces* LL-C10037, the same strain, which produced compound **86**. Regarding the strong antitumor activity of **86** and **87**, many derivatives from both compounds have been synthesized.[140]



87

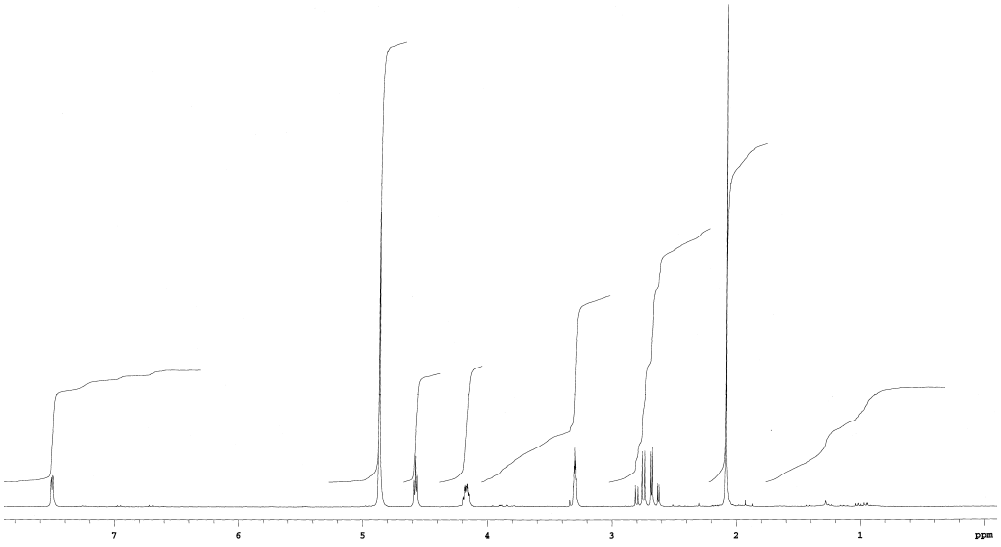


Figure 56: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of LL-C10037β (**87**)

All compounds isolated from strain ANK 313 were tested against different micro-organisms, but only compounds **82**, **84** and **86** displayed antibacterial activity (Table 17).

Table 17: Antimicrobial activity of pure compounds isolated from terrestrial *Streptomyces* sp. ANK 313 (40 µg/paper disc, diameter of inhibition zones in mm)

Compound Name	*Bacillus subtilis*	*Staphylococcus aureus*	*Streptomyces viridochromogenes* (Tü57)	*Escherichia coli*
82	0	11	14	0
84	0	0	0	11
86	11	13	0	11

4.2.6 Zoosporicidal Activity of the Isolated Compounds

Peronosporomycetes are distinct from fungi and are phylogenetic relatives of brown algae and diatoms.[79] They cause many destructive diseases in plants, animals, fishes and humans. One of the most notorious members of peronosporomycetes, *Plasmopara viticola,* is a serious pathogen of grapevine worldwide. Many fungicides are ineffective against this phytopathogen, and hence, bioactive compounds with new mode of action are needed to combat this economically important pest. Under favourable environmental conditions, the fungus *P. viticola* infects grapevine leaves by means of characteristic biflagellated motile zoospores released from airborne sporangia coming from other infected plants. The zoospores aggregate to stomata of the grapevine leaf by swimming through water films and then rapidly encyst to become round cystospores by shedding their flagella.[141,142] The cystospores then rapidly germinate to form germ tubes and penetrate host tissue through the stomata. Interruption of any of these asexual stages eliminates the potential for pathogenesis.[143] The success of any zoosporic pathogen can be attributed in part to the speed of asexual differentiation to generate bi-flagellated motile zoospores and their ability to find host through chemotaxis.[144] Therefore, compounds that can interfere with normal swimming behaviour and early development of *P. viticola* are supposed to be important as lead compounds in the management of this phytopathogen.[142] The crude extract of the terrestrial *Streptomyces* sp ANK 313 as well as isolated compounds were tested for the zoosporicidal activity.

Microscopic observation revealed that the crude extracts and the isolated metabolites showed zoosporicidal activity against *P. viticola,* which were due to the compounds described here: Khatmiamycin (**82**) exhibited the strongest motility inhibitory

and subsequent lytic activities against the zoospores at 10 µg/ml followed by compounds **84, 85**, **86** and **87**. Compound **86** displayed 2-fold higher zoospores motility inhibitory activity than compound **87**. Initially, the zoospores were halted and the cellular materials gradually fragmented and dispersed into the surrounding water medium by bursting cell membranes. The motility inhibitory and zoosporicidal activities of the isolated compounds from *Streptomyces* sp. ANK 313 against downy mildew pathogen *P. viticola* are presented in Figure 57 and Table 18.

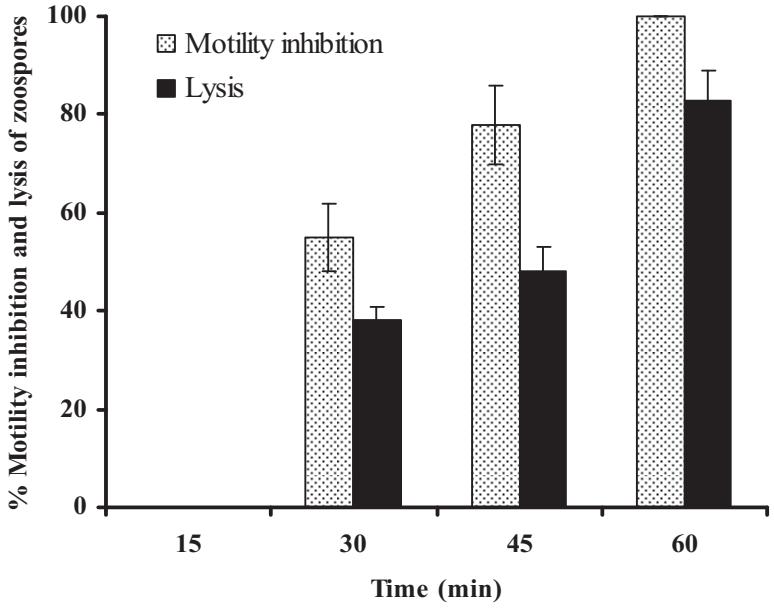


Figure 57: Motility inhibition and lytic activity of khatmiamycin (**82**) at 10 µg/ml (ca. 30 µM) against zoospores of the downy mildew pathogen *Plasmopara viticola*.

Table 18: Motility inhibitory and lytic activities of compounds isolated from *Streptomyces* sp. ANK 313 against the zoospores of the grapevine downy mildew pathogen *Plasmopara viticola*

Compound	Dose (µg/ml)	Motility inhibitory and lytic activity (% ± SE)[a] against *Plasmopara viticola* zoospores							
		15 min		30 min		45 min		60 min	
		Motility inhibition	Lysis	Motility inhibition	Lysis	Motility inhibition	Lysis	Motility inhibition	Lysis
82	5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	10	0 ± 0	0 ± 0	55 ± 7	38 ± 3	78 ± 8	48 ± 5	100 ± 0	83 ± 7
	25	73 ± 7	47 ± 6	97 ± 2	77 ± 5	100 ± 0	87 ± 8	100 ± 0	99 ± 1
	50	100 ± 0	100 ± 0	nt	nt	nt	nt	nt	nt
84	10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	25	0 ± 0	0 ± 0	15 ± 4	0 ± 0	35 ± 3	0 ± 0	45 ± 7	0 ± 0
	50	47 ± 5	30 ± 6	100 ± 0	100 ± 0	nt	nt	nt	nt
85	10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	25	41 ± 7	0 ± 0	90 ± 8	51 ± 5	93 ± 6	69 ± 7	100 ± 0	88 ± 5
	50	88 ± 5	31 ± 7	100 ± 0	95 ± 3	100 ± 0	98 ± 3	100 ± 0	100 ± 0
86	10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	25	0 ± 0	0 ± 0	0 ± 0	0 ± 0	19 ± 5	0 ± 0	52 ± 6	0 ± 0
	50	49 ± 5	21 ± 3	100 ± 0	100 ± 0	nt	nt	nt	nt
87	25	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	50	30 ± 3	0 ± 0	59 ± 7	33 ± 2	78 ± 9	49 ± 5	81 ± 8	51 ± 3

[a] Data presented here are average value ± SE of at least three replications in each dose of test compound.

4.2.7 Omdurmycin

Omdurmycin (**88**) was isolated as colourless solid from a UV absorbing zone, which turned to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of (**88**) displayed quite a number of signals in the aliphatic region and two olefinic proton signals at δ 6.28 and 5.35. Several oxymethine protons appeared between δ 4.57-3.24, and eight methine protons were observed between δ 3.31-1.41 along with three methylene groups between δ 2.02-0.65 and five methyl groups between δ 1.20 and 0.84.

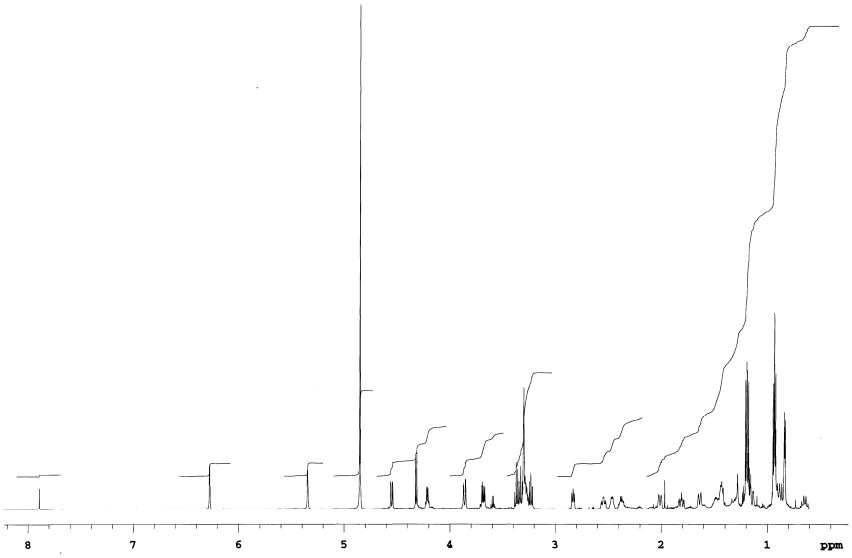
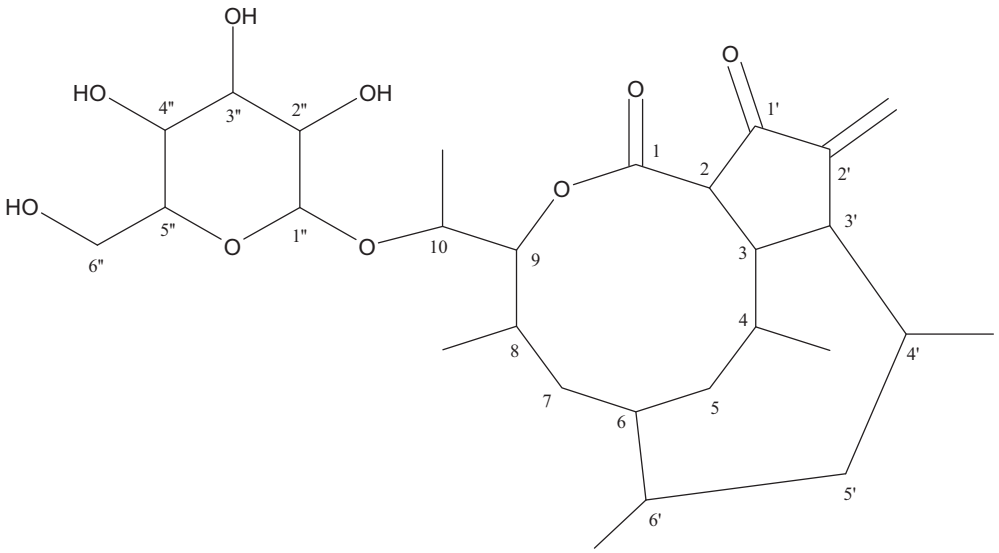


Figure 58: ^{1}H NMR spectrum (300 MHz) of omdurmycin (**88**) in CD_3OD

In the ^{13}C NMR spectrum twenty-eight carbons were observed; three quaternary carbons appeared at δ 203.1, 179.2 and 141.4 together with an olefinic CH_2 at δ 123.0. In addition to an anomeric carbon at δ 102.3, six oxymethine carbons and one oxymethylene carbon were observed. Further signals are listed in Table 19. The ESI mass spectrum afforded the *pseudo*molecular ion at 547 $[M + Na]^+$, which gave the molecular weight at 524 Dalton and HRESIMS revealed the molecular formula $C_{28}H_{44}O_9$.



Proposed structure of omdurmycin (**88**)

The presence of an anomeric proton at δ 4.32 and oxygenated protons between δ 3.87-3.24 pointed to a sugar moiety, which was further confirmed by means of the 2D spectroscopic information, especially by COSY and HMBC correlations between three oxymethine protons, the anomeric proton and the oxymethylene group. The anomeric proton H-1" (δ 4.32) showed COSY correlations to H-2" as well as TOCSY correlations to H-3" and H-5". The proton H-3" showed HMBC correlations to C-5", and H-4" to C-3" in addition to COSY correlations from H-5" to H-6" (Figure 59):

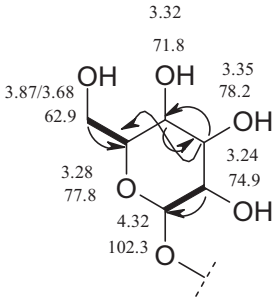


Figure 59: Selected H,H COSY and HMBC correlations of the sugar in omdurmycin (**88**)

The MS/MS measurements afforded *quasi*molecular ion at 547 $[M + Na]^+$ in addition to two fragments at 385 and 367. The fragment at 367 indicated the loss of hexose unit after the cleavage of the glycosidic bond between the C-1" and C-10. With respect to the coupling constant of the anomeric proton (J = 7.7 Hz), the sugar is β-connected and should have (D)-configuration, according to the Klyne rule[145]. Hydrolysis experiments to distinguish between the eight stereoisomeric hexoses by sily-

lation and GC/MS similarly as with stemphol galactoside (**127**)[146] are still to be done.

Table 19: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of omdurmycin (**88**) in CD_3OD

Position	δ_C	δ_H (mult.; *J* in [Hz])
1	179.2	
2	41.2	1.41 (m)
3	51.9	2.84 (dd, 8.4, 7.4)
4	43.0	1.43 (m)
4-CH_3	19.8	0.84 (d, 5.9)
5	45.0	0.65 (q, 11.3, 12-H_a) 1.64 (dd, 13.0. 2.0, 12-H_b)
6	37.0	2.48 (m)
7	39.4	1.12 (m, 10-H_a) 1.82 (ddd, 11.5, 7.4, 3.6, 10-H_b)
8	39.0	2.37 (dd, 10.4, 6.6)
8-CH_3	19.0	0.93 (d, 6.7)
9	87.6	4.57 (dd, *J* = 10.4, 1.7)
10	72.5	4.22 (dd, *J* = 6.4, 1.7)
10-CH_3	16.7	1.20 (d, 6.3 Hz)
1'	203.1	
2'	141.4	
2'-CH_2	123.0	5.35 (s) 6.28 (s)
3'	48.3	3.31 (m)
4'	47.0	2.56 (m)
4'-CH_3	25.0	1.19 (d, 7.5)
5'	35.7	0.90 (dd, *J* = 8.3, 6.0, 13-H_a) 2.02 (m, 13-H_b)
6'	31.9	1.46 (m)
6'-CH_3	22.9	0.94 (d, 6.4)
1"	102.3	4.32 (d, *J* = 7.7)
2"	74.9	3.24 (dd, *J* = 8.9, 7.8)
3"	78.2	3.35 (m)
4"	71.8	3.32 (m)
5"	77.8	3.28 (m)
6"	62.9	3.87 (dd, *J* = 11.5, 2.0) 3.68 (dd, *J* = 11.5, 5.1)

The anomeric proton showed a strong HMBC correlation to an oxymethine signal at δ 72.5/4.22, and *vice versa*. The latter proton H-10 showed COSY correlations with the methyl proton at δ 16.7/1.20. COSY and HMBC correlations indicated that this attachment point was part of a butanediol system, resulting in partial structure A (Figure 60).

The HMBC correlation of H-9 with a carbonyl at 179.2 can be explained best by an ester bond, in spite of the abnormal CO value. COSY correlations from H-8 (δ 2.37) to 8-CH_3 (δ 0.93), H-7 and H-9 as well as HMBC correlations from H-9 to H-7 and 8-CH_3 were observed (Figure 60). From further correlations, an exomethylene-cyclopentanone unit was constructed, which forms with the ester a 1,3-dicarbonyl system (fragment B). The sp^2 methylene proton at lower field did not give a HSQC signal, which made the assignment difficult. There was, however, in the HMBC spectrum a ^1J correlation of this proton with the *ipso*-carbon 2'-CH_2.

In addition to HMBC correlations from 2'-CH_2 (δ 6.28, 5.35) to C-1' and C-2', further correlations from both protons at δ 2.84 (H-3) and δ 3.31 (H-3') to C-2' and 2'-CH_2 were observed indicating the presence of a five-membered ring. HMBC correlations from H-2, H-3 and H-3' to the carbonyl at δ 179.2 supported fragment B in Figure 60:

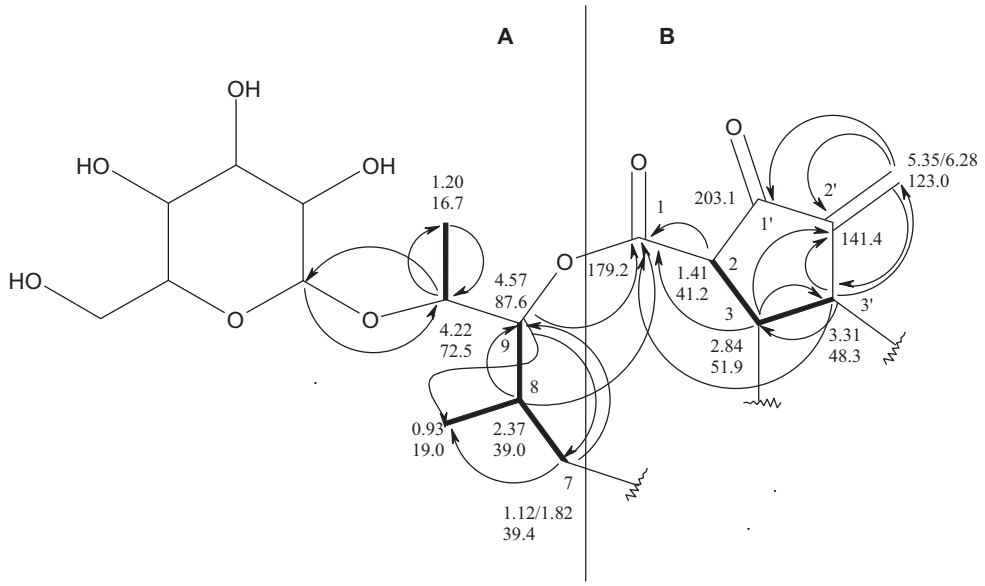


Figure 60: Selected H,H COSY and HMBC correlations of fragments A and B for omdurmycin (**88**)

Further analysis of COSY, HMBC and TOCSY spectra supported fragment C, where COSY correlations from H-3 to 4-CH_3 (δ 0.84) were observed.

From H-4' to 4'-CH_3 (δ 1.19) and from H-6' to 6'-CH_3 (δ 0.94), COSY correlations were observed. Fragment C did not show further COSY couplings, but the TOCSY spectra listed all signals of the respective spin system. Their connection was again derived from HMBC couplings (Figure 61).

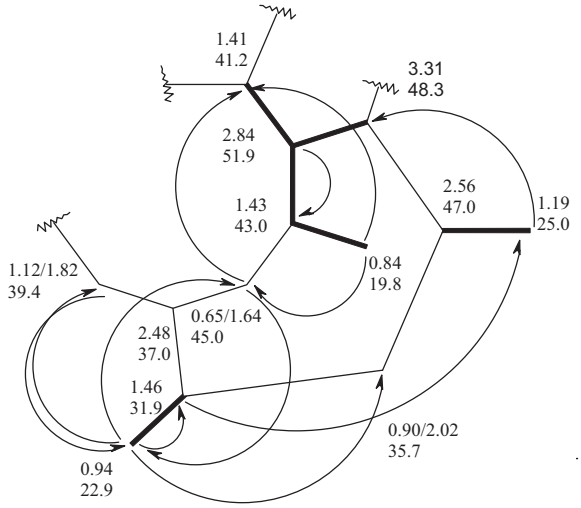


Figure 61: Selected H,H COSY and HMBC correlations of fragment C for omdurmycin (**88**)

The three substructures were combined by analysis of further HMBC and TOCSY correlations, resulting in the proposed structure (**88**) of omdurmycin. A search in AntiBase[113] as well as the Chemical Abstracts by using substructures afforded no hits pointing to a new skeleton. Due to the bridging atoms C-4' - C-6', the molecule adopts a ball-shaped structure, which may also be responsible for some of the abnormal shift values (e.g. 203.1 for C-1', 179.2 for C-1, 87.6 for C-9 and 41.1 for C-2) and the missing COSY correlations. The configuration at the 10 stereo centres of the aglycone remains still open; only an α-orientation at C-3' and C-6 can be assumed, due to the steric hindrance of a hypothetical *trans* connection.

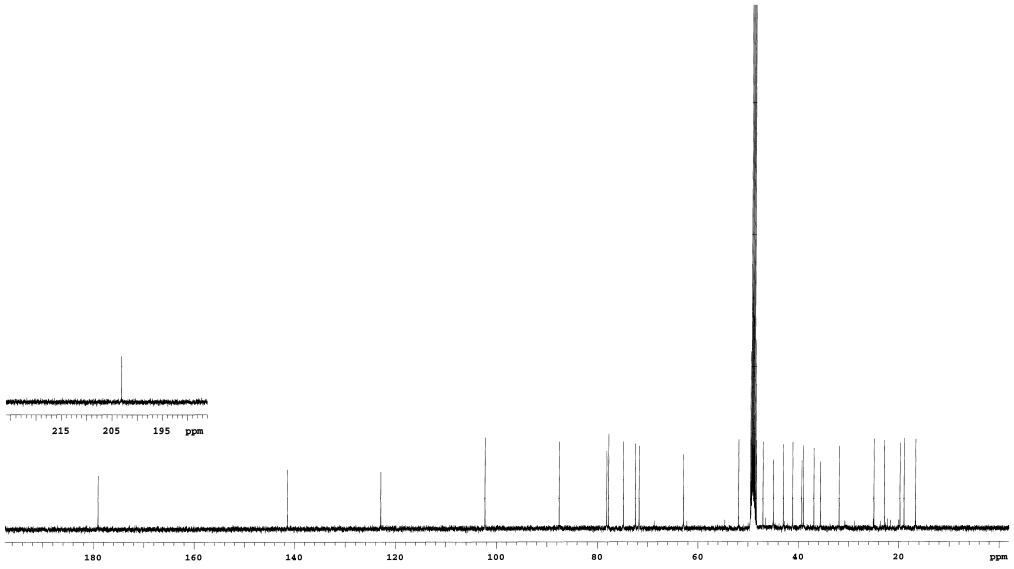


Figure 62: ^{13}C NMR spectrum (125 MHz) of omdurmycin (**88**) in CD_3OD

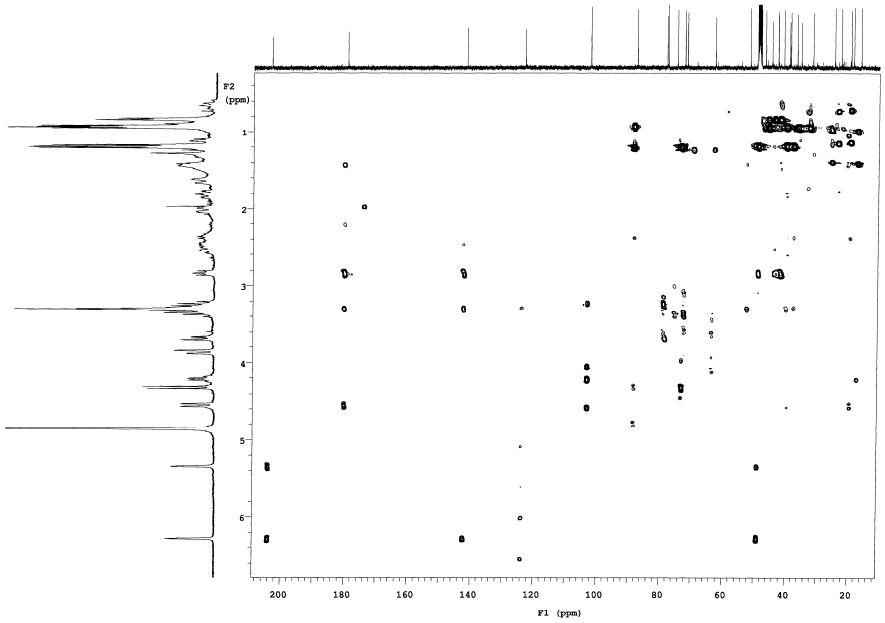


Figure 63: HMBC spectrum (CD_3OD, 300 MHz) of omdurmycin (**88**)

4.3 Terrestrial *Streptomyces* sp. GW08/253

The terrestrial *Streptomyces* sp. GW08/253 was selected according to the chemical and biological screening. On TLC the crude extract showed two UV absorbing bands of middle polarity, which turned to blue colour with anisaldehyde/sulphuric acid. The crude extract showed good biological activities against different microorganisms as mentioned in Figure 64.

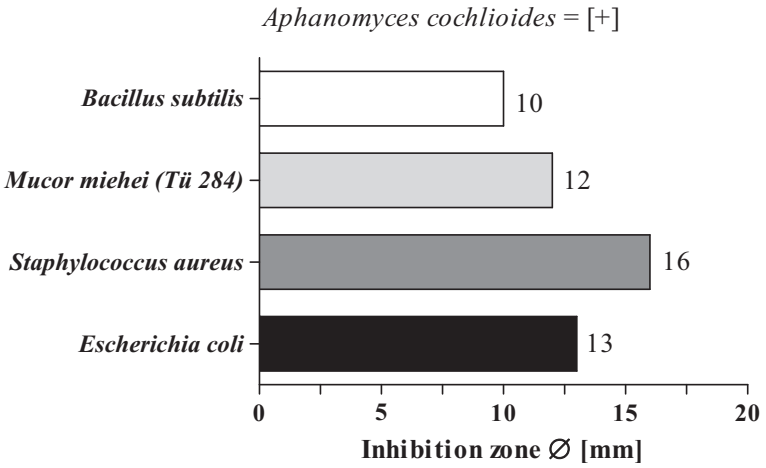


Figure 64: Antimicrobial activity of the crude extract of terrestrial *Streptomyces sp.* GW08/253

An agar plate of the terrestrial *Streptomyces* GW 08/253 was used to inoculate 100 of 1 L Erlenmeyer flasks each containing 300 ml of M_2^+ medium; the culture was incubated for 8 days at 28 °C on a linear shaker. The culture broth was filtered using a filter press to deliver the mycelium and the water phase. The mycelium and the filtrate were extracted separately by using ethyl acetate. The organic phases were evaporated under reduced pressure and combined to yield 7.10 g of a dark brown oily crude extract. The latter was subjected to column chromatography using a CH_2Cl_2/MeOH gradient and separated into three fractions. Further purification has been done of the three fractions on Sephadex LH-20, PTLC, silica gel and RP-18 to afford six known compounds and one new metabolite, namely cyclooctatin (**89**), phenazine-1-carboxamide (**90**), phenazine-1-carboxylic acid (**91**), 6-[2-(1-hydroxy-butyl)-4-methyl-phenyl]-hex-5-enoic acid (**92**), the new 6-(2-but-1-enyl-4-methyl-pheny)-hex-5-enoic acid (**93**), 4-hydroxy-benzoic acid methyl ester (**94**), and 3-(hydroxyacetyl)-indole (**95**).

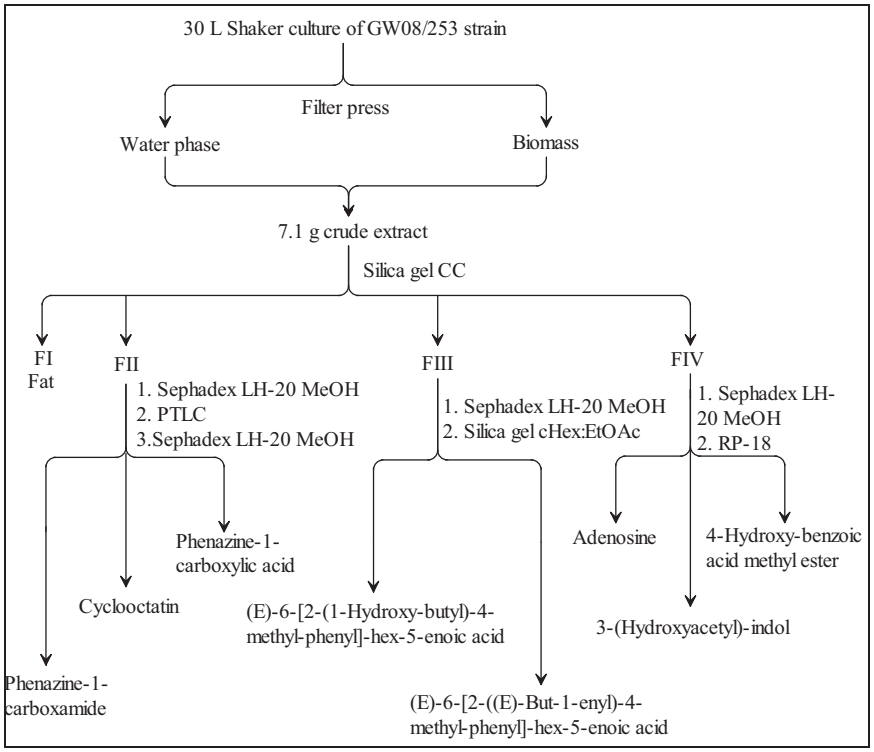
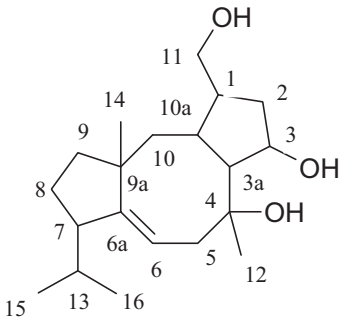


Figure 65: Work up scheme of terrestrial *Streptomyces* sp. GW08/253

4.3.1 Cyclooctatin

Cyclooctatin (**89**) was obtained from fraction FII as white powdery UV absorbing substance, which turned to black with anisaldehyde/sulphuric acid spray reagent. In the ^{1}H NMR spectrum quite a number of proton signals appeared in the aliphatic region, where no aromatic signals were detected. An olefinic proton was observed at δ

5.28, along with an oxymethine signal at δ 4.44, oxymethylene at δ 3.66, five methine, five methylene and three methyl signals.



89

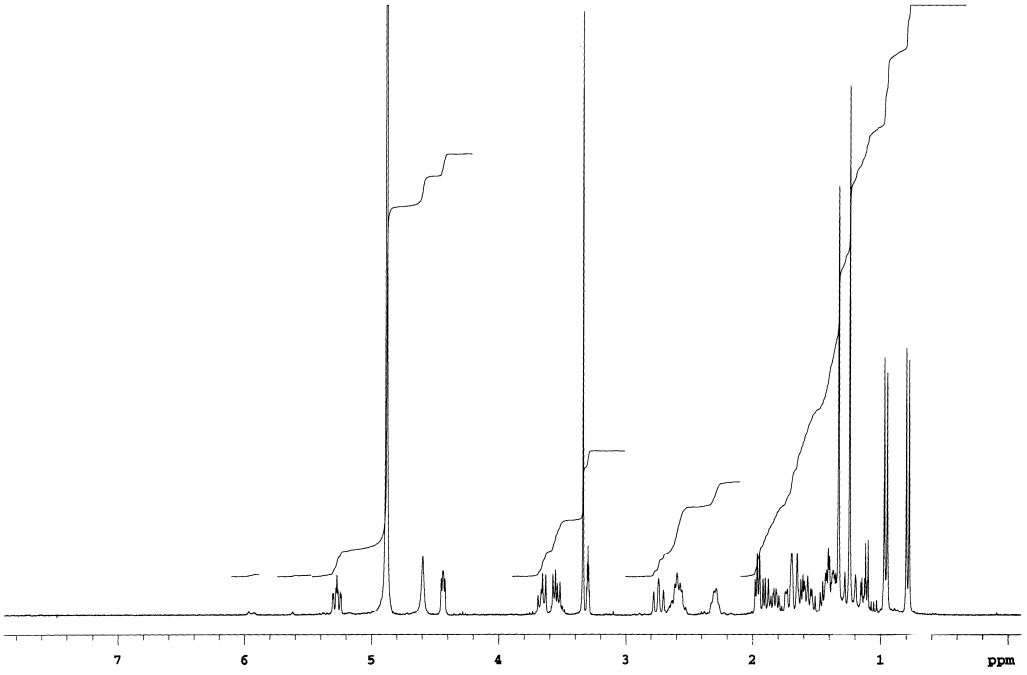
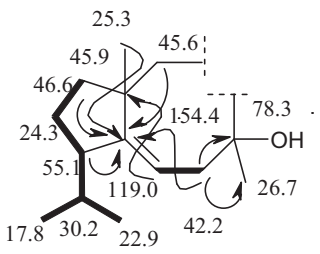


Figure 66: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of cyclooctatin (**89**)

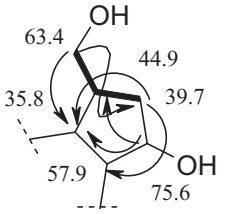
The ^{13}C NMR spectrum of cyclooctatin (**89**) presented twenty signals, three quaternary carbon signals at δ 154.5, 78.4 and 45.9 were observed, in addition to an olefinic carbon at δ 119.1. Moreover an oxymethylene carbon at δ 75.7, oxymethine carbon at δ 63.4 were recognized along with five methine carbons, five methylene carbons and four methyl carbons between δ 17.8 and 26.7; the ^{1}H and ^{13}C NMR values are listed in Table 20 in comparison with the literature values.

The ESI mass spectrum gave the *pseud*omolecular ion peak at *m/z* 345 $[M + Na]^+$, which fixed the molecular weight to 322 Dalton. The HRESI mass spectrum delivered the molecular formula $C_{20}H_{34}O_3$. COSY, HSQC and HMBC correlations supported

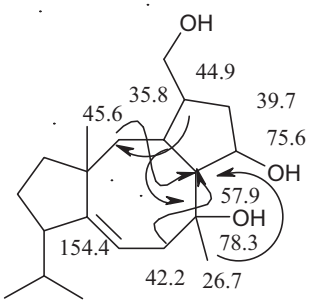
the presence of both five and eight membered rings. Signals at chemical shifts of δ 2.72 (H-5) 2.31 (H-7), 1.59 (H-9), 1.24 (H-14) and 1.67 (H-10) showed correlations with a quaternary carbon at δ 154.4 (C-6a). An olefinic carbon at δ 5.28 (H-6) displayed a ^{3}J coupling with a quaternary carbon at δ 45.9 (9a). The methylene signal at δ 2.72 (H-5) exhibited COSY correlations with an olefinic proton at δ 5.28 (H-6) as well as HMBC correlations with a quaternary carbon at δ 78.3 (C-4) and a methyl carbon at δ 26.7 (CH_3-12). An eight membered ring was confirmed by correlations from both resonances at δ 2.72 (H-5) and 1.67 (H-10) to a carbon at δ 57.9 (CH-3a) as well as from methylene proton at δ 1.67 (H-10) to the methine carbon at δ 35.8 (CH-10a). Analysis of H,H COSY correlations from H-16 and H-15 to H-13, from H-13 to H-7 and from H-7 till H-9 supported the existence of a five membered ring as drawn in Figure 67. Additional 2D spectroscopic data were in agreement with a second five membered ring, where H,H COSY correlations from H-1 to H-11 as well as HMBC correlations from H-2 to C-11, C-10a and C-3a and ^{3}J coupling from H-3 to C-1 and C-10a were evident.



Fragment **a**



Fragment **b**



Fragment **c**

Figure 67: Selected H,H COSY (▬) and HMBC (→)correlations of cyclooctatin (**89**)

Table 20: Comparison of ^{13}C and ^{1}H NMR chemical shifts (125 and 300 MHz) of cyclooctatin (**89**) in CD_3OD, with reference data.[147]

Position	δ_C [147]	δ_H (*J* in Hz) [147]	δ_C	δ_H (*J* in Hz) (**89**)
1	44.9	2.61 (m)	44.9	2.60 (m)
2	39.7	1.38 (dt, 12.6, 3.4) 1.71 (br dd, 12.6, 5.0)	39.7	1.38 (dt, 11.5, 3.8) 1.72 (br dd, 11.5, 5.1)
3	75.7	4.44 (br dd, 3.4, 5.0)	75.6	4.44 (dd, 5.0, 3.3)
3a	58.0	1.97 (t, 5.0)	57.9	1.97 (t, 3.9)
4	78.4		78.3	
5	42.2	1.91 (dd, 12.8, 7.4) 2.74 (br t, 11.6)	42.2	1.90 (dd, 12.8 ,7.4) 2.72 (br t, 11.5)
6	119.1	5.28 (ddd, 10.8, 7.4, 2.2)	119.0	5.28 (ddd, 12.4, 7.9, 2.3)
6a	154.5		154.4	
7	55.1	2.30 (m)	55.1	2.31 (m)
8	24.3	1.38 (m) 1.56 (m)	24.3	1.39 (m) 1.57 (m)
9	46.6	1.42 (m) 1.59 (m)	46.6	1.45 (m) 1.59 (m)
9a	45.9		45.9	
10	45.6	1.20 (t, 12.8) 1.68 (br d, 12.8)	45.6	1.20 (m) 1.67 (br d, 12.4)
10a	35.8	2.56 (m)	35.8	2.57 (m)
11	63.4	3.55 (dd, 10.8, 6.8) 3.66 (dd, 10.8, 7.4)	63.4	3.54 (dd, 10.3, 6.1) 3.67 (dd, 10.5, 7.7)
12	26.7	1.33 (br s)	26.7	1.33 (br s)
13	30.2	1.83 (m)	30.2	1.86 (m)
14	25.2	1.25 (s)	25.3	1.24 (s)
15	17.8	0.79 (d, 6.6)	17.8	0.79 (d, 6.7)
16	22.5	0.96 (d, 6.6)	22.6	0.96 (d, 6.8)

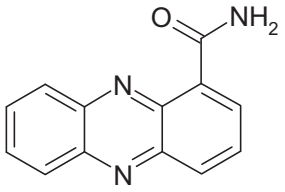
A search in AntiBase[113] by using detailed spectroscopic data and 2D analysis confirmed the identity with cyclooctatin (**89**) by comparing the spectroscopic data with the literature.[147] Compound **89** was found to be an inhibitor of lysophospholipase (Lyso-PL).

Cyclooctatin (**89**) is related to group of compounds known as ophiobolins (A-H)[148] and fusicoccin A.[149] While these compounds belong to the class of sesterterpenoids, cyclooctatin (**89**) is a diterpenoid, that is produced by actinomyetes, while the mentioned related compounds are produced by fungi.[147] Compound **89** was tested in our lab against microorganisms and *Artemia salina*: Interestingly it exhibited a very fascinating activity against *Artemia salina* (100 % at 10 μg/ml), and after one hour all

of them were dead. It showed weak antibacterial activity against *Bacillus subtilis* and *Escherichia coli* by causing inhibition zones of 9.5 mm at 40 μg/disk.

4.3.2 Phenazine-1-carboxamide

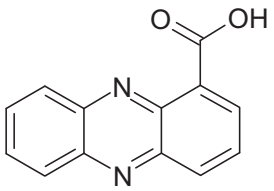
Phenazine-1-carboxamide (**90**) was isolated from fraction FII as pale yellow solid. In the ^{1}H NMR spectrum of **90**, seven aromatic proton signals were observed; three were adjacent in a first spin system, and four in a second disubstituted benzene ring. Signals in the aliphatic region were absent. The ESI mass spectrum delivered a *pseudo*molecular ion peak at *m/z* 246 for $[M + Na]^+$, which gave a molecular weight of 223 Dalton. The HRESI mass spectrum established the molecular formula $C_{13}H_9N_3O$. A search in AntiBase[113] by comparing the spectroscopic information led to phenazine-1-carboxamide (**90**).



90

4.3.3 Phenazine-1-carboxylic acid

Phenazine-1-carboxylic acid (**91**) was isolated from fraction FII as yellow needles. The ^{1}H NMR spectrum showed the same pattern as compound **91**. The acidic proton was observed as broad singlet at δ 15.58. The structure was confirmed by comparing the ^{1}H NMR spectrum of **91** with an authentic spectrum of phenazine-1-carboxylic acid in AntiBase.[113] Phenazine-1-carboxylic acid was known as antibiotic that inhibits *Gaeumannomyces graminis* var. *tritici* and other fungal root pathogens *in vitro* at less than 1 μg/ml.[150]



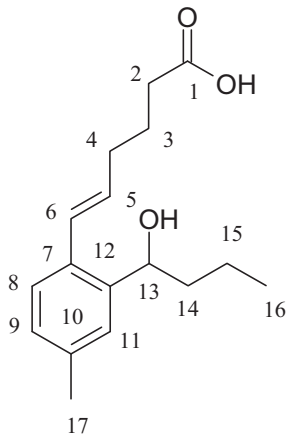
91

Most phenazine alkaloids are yellow to orange coloured unpolar antibiotics. Several bacterial species produce one or more of the 50 known phenazine compounds. These species include *Pseudomonas*, *Streptomyces*, *Nocardia*, *Sorangium*, *Brevibacterium*, and *Burkholderia*. All compounds of the phenazine class exhibit a variety of

antibacterial properties especially against plant pathogenic Gram-positive bacteria and fungi. Phenazine carboxamide (**90**) and phenazine-1-carboxylic acid (**91**) were found to be active against *Bacillus cereus* (MIC by disk assay < 0.5 μg/ml); phenazine-1-carboxylic acid (**91**) is more potent than phenazine carboxamide (**90**). Both compounds were less active against *M. luteus* and *S. aureus* (MIC > 5 μg/ml).[151] Phenazine-1-carboxylic acid (**91**) was additionally tested in our lab against *Candida albicans* and caused inhibition zones of 11 mm at 40 μg/disk.

4.3.4 6-[2-(1-Hydroxy-butyl)-4-methyl-phenyl]-hex-5-enoic acid

6-[2-(1-Hydroxy-butyl)-4-methyl-phenyl]-hex-5-enoic acid (**92**) was isolated from fraction FIII as colourless solid. On TLC it gave a UV absorbing band, which turned to blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum indicated five olefinic protons, five methylene protons and two methyl groups. The ^{13}C NMR spectrum revealed four quaternary carbons at δ 176.0, 143.0, 137.7 and 133.9 and five olefinic carbons. Moreover five methylene signals were observed in addition to two methyl carbons. ESIMS delivered a molecular weight of 276 Dalton, and HRESIMS afforded the molecular formula $C_{17}H_{24}O_3$. Analysis of H,H COSY as well as HSQC and HMBC correlations supported the structure **92** as drawn in Figure 68. A search in Chemical Abstracts gave one hit indicating that compound **92** is a known compound isolated previously from *Streptomyces* sp. X-537.[152]



92

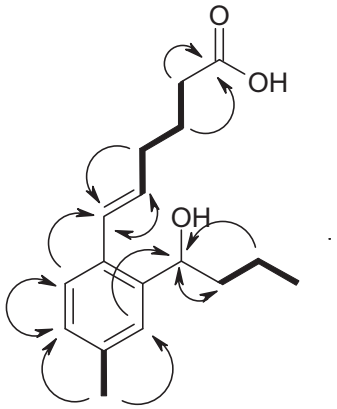


Figure 68: Selected $^{1}H,^{1}H$ COSY and HMBC correlations of **92**

Table 21: ^{13} C and ^{1}H NMR shifts (125 and 600 MHz) of compound **92** in CD_3OD

Position	δ_C	δ_H (mult.; *J* in Hz)
1	176.0	
2	34.9	2.33 (t, 7.4)
3	26.1	1.78 (quint, 7.4)
4	33.7	2.25 (m)
5	132.1	5.98 (dd, 15.5, 7.0)
6	129.3	6.68 (d, 15.6)
7	133.9	
8	126.9	7.26 (d, 7.9)
9	128.9	6.98 (m)
10	137.7	
11	127.1	7.24 (d, 1.4)
12	143.0	
13	70.8	4.93 (dd, 7.7, 5.3)
14	42.0	1.63 (m)
15	20.2	1.32 (m), 1.41 (m)
16	14.4	0.92 (t, 7.4)
17	21.4	2.30 (s)

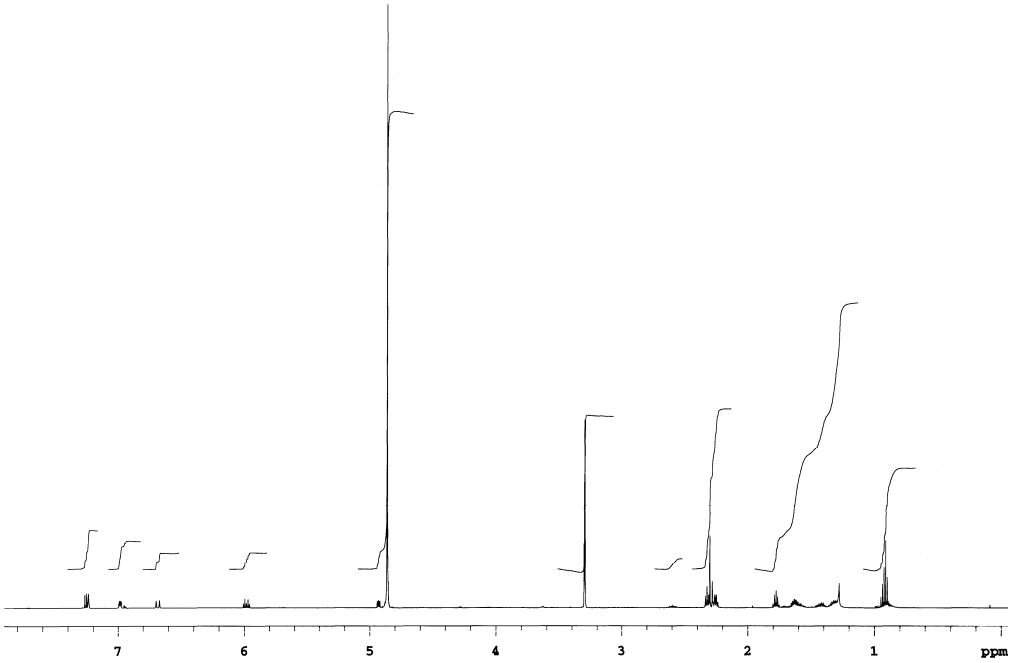


Figure 69: ^{1}H NMR spectrum (CD_3OD, 600 MHz) of compound **92**

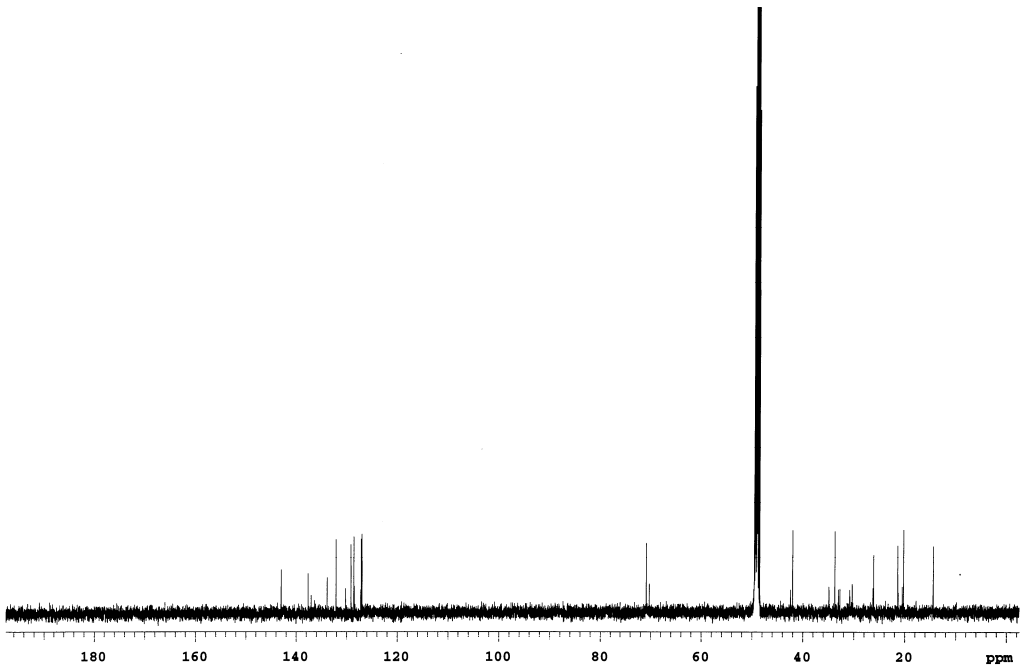
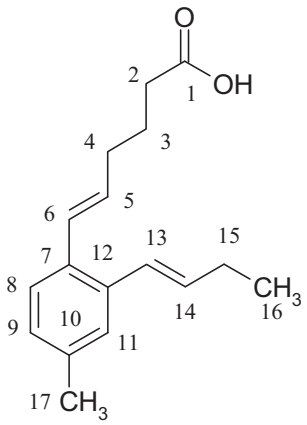


Figure 70: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **92**

4.3.5 6-(2-But-1-enyl-4-methyl-pheny)-hex-5-enoic acid

6-(2-But-1-enyl-4-methyl-pheny)-hex-5-enoic acid (**93**) was isolated from fraction FIII as colourless solid. In the chromatogram, it gave a UV absorbing band, which turned to blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed two

aromatic *ortho*-coupled protons in addition to one *meta*-coupled signal. Moreover four olefinic protons were observed along with four methylene protons and two methyl signals; one of the latter appeared as singlet, the other one as doublet. The ^{13}C NMR spectrum revealed 17 signals, one carbonyl, four quaternary carbons (three of them within the benzene ring), seven olefinic and aromatic carbons, four methylene and two methyl carbons.



93

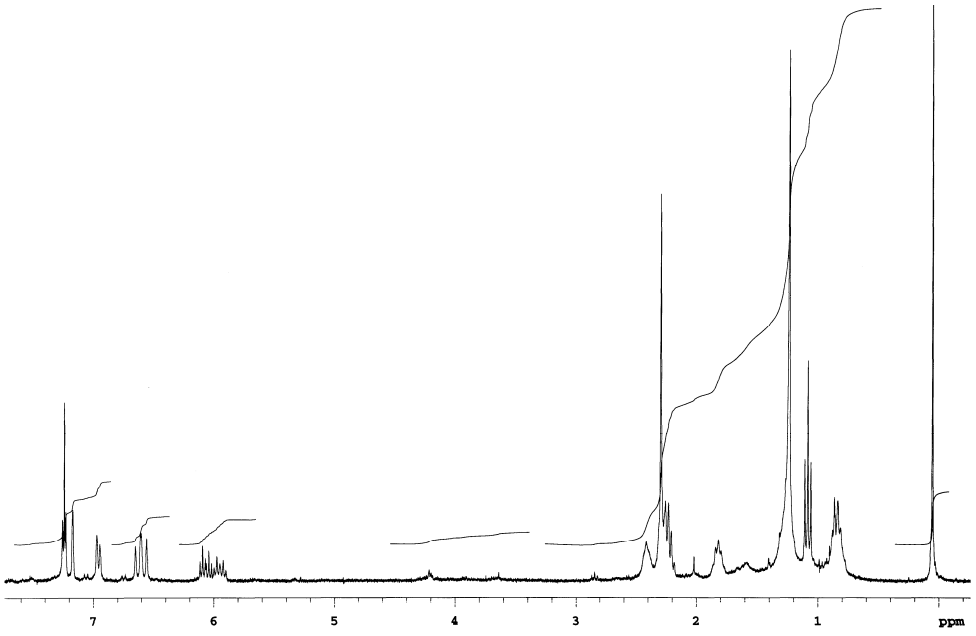


Figure 71: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of compound **93**

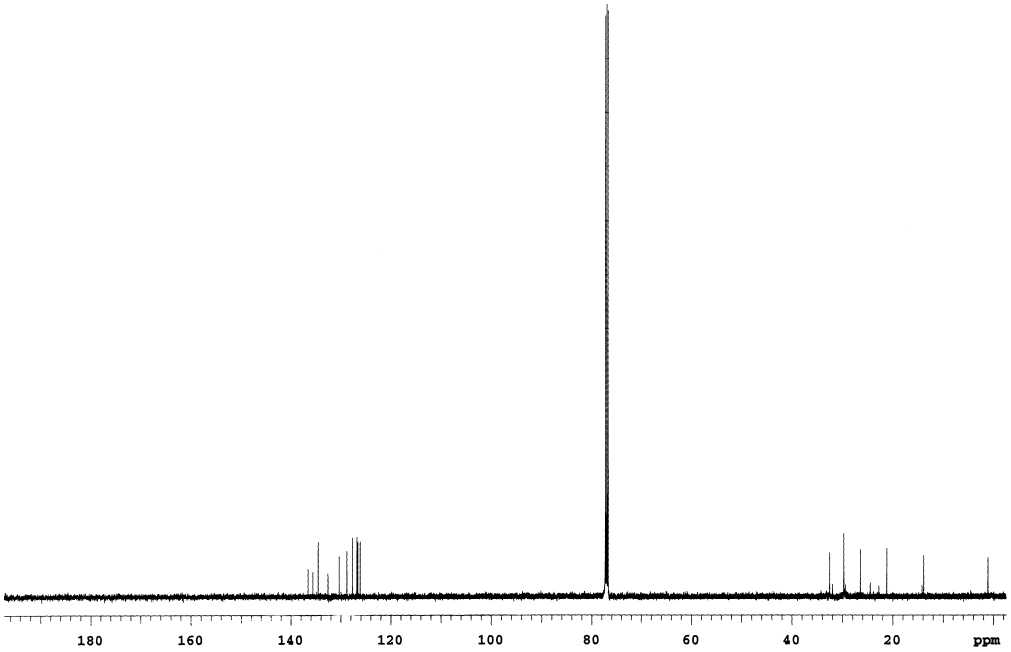


Figure 72: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound **93**

Further 2D NMR analysis confirmed the structure **93** by two spin systems, i. e. by $^{1}H,^{1}H$ COSY correlations from H-2 (δ 2.42) to H-6 (δ 6.63) and from H-13 (δ 6.07) to H-16 (δ 1.02). The methyl signal at H-17 (δ 2.30) showed ^{3}J HMBC couplings with both carbons C-11 (δ 126.8) and C-9 (δ 127.6). The presence of the benzene ring was confirmed by strong COSY correlations of proton H-9 (δ 6.96) with H-8 (*ortho*-coupling), as well as three bond correlations with C-11 and the quaternary carbon C-7. On the other hand proton at H-8 (δ 7.24) showed ^{3}J coupling with olefinic carbon C-6 as well as quaternary carbon C-12. The proton at H-11 (δ 7.17) showed three bond correlations with the olefinic proton H-13. Further correlations are depicted in Figure 73

The ESI mass spectrum afforded a *pseud*omolecular ion peak at 259 $[M + H]^+$, and the HRESI/MS delivered the molecular formula $C_{17}H_{22}O_2$. A search in Anti-Base[113] as well as Chemical Abstracts showed no hits and confirmed that compound **93** was a new compound.

Table 22: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of **93** in $CDCl_3$

Position	δ_C	δ_H (mult.; *J* in Hz)
1	178.0	
2	33.0	2.42 (t, 7.4)
3	24.4	1.82 (q, 7.2)
4	32.5	2.29 (m)
5	130.3	5.95 (dt, 15.7, 7.1)
6	128.8	6.63 (d, 15.6)
7	132.6	
8	126.1	7.24 (d, 7.8)
9	127.6	6.96 (d br, 7.0)
10	136.5	
11	126.8	7.17 (s br)
12	135.6	
13	126.6	6.58 (d br, 15.6)
14	134.5	6.06 (dt, 15.6, 6.6)
15	26.4	2.21 (m)
16	13.9	1.08 (t, 7.4)
17	21.2	2.30 (s)

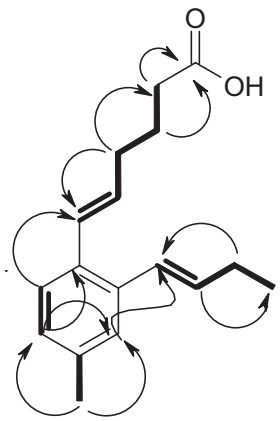


Figure 73: Selected ^{1}H,^{1}H COSY and HMBC correlations of **93**

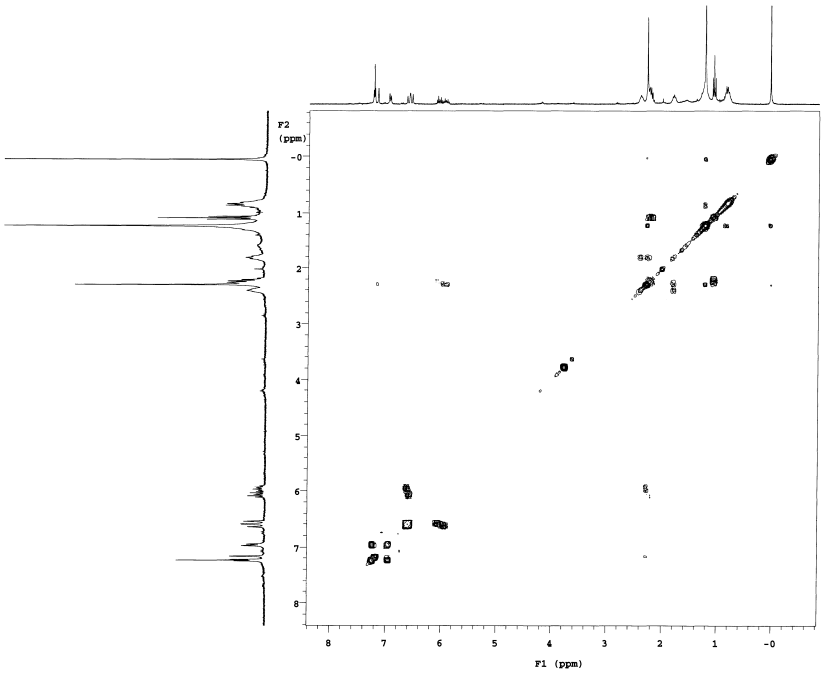


Figure 74: $^{1}H,^{1}H$ COSY spectrum ($CDCl_3$, 300 MHz) of **93**

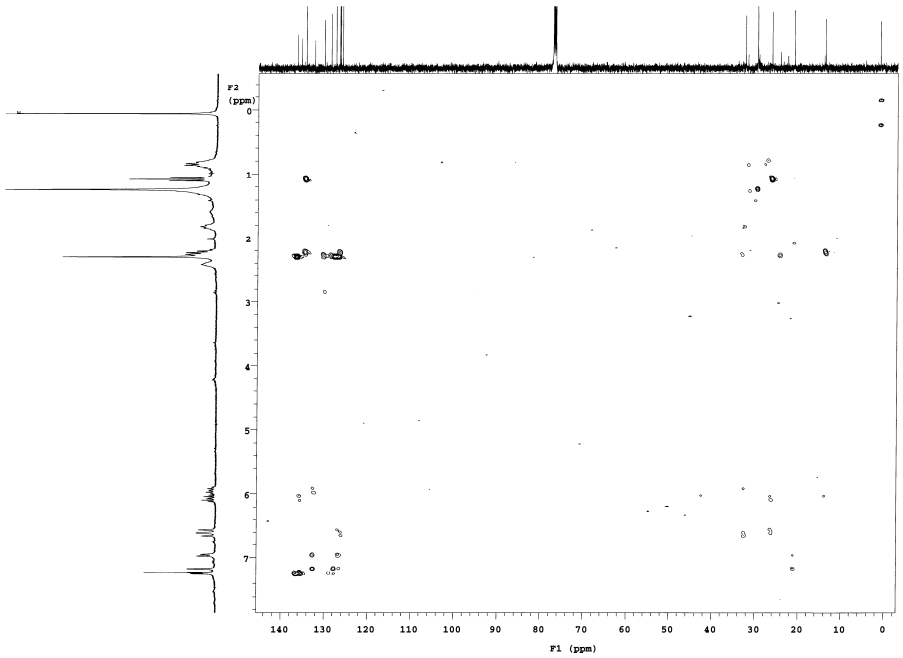
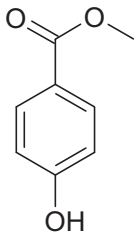


Figure 75: HMBC spectrum ($CDCl_3$, 300 MHz) of **93**

4.3.6 4-Hydroxy-benzoic acid methyl ester

4-Hydroxy-benzoic acid methyl ester (**94**) was isolated as colourless solid, which gave a white spot on a pink background in the chromatogram with anisaldehyde/sulphuric acid spray reagent. The ^{1}H NMR spectrum displayed two aromatic

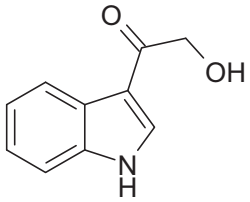
A_2B_2 signals with intensity of four at δ 7.94 and 6.85 as indication of a 1,4-disubstituted benzene ring. Moreover a broad signal of an exchangeable proton was observed at δ 5.70. In the aliphatic region a methoxy singlet was obtained at δ 3.87. The EI mass spectrum revealed the molecular weight as 152 Dalton. HRESIMS delivered the molecular formula $C_8H_8O_3$. A search in AntiBase[113] led to 4-hydroxybenzoic acid methyl ester (**94**).



94

4.3.7 3-(Hydroxyacetyl)-indole

3-(Hydroxyacetyl)-indole (**95**) was isolated as yellow solid from a UV absorbing zone, which turned to green yellowish with anisaldehyde/sulphuric acid spray reagent. The ^{1}H NMR spectrum showed an indole pattern consisting of five proton signals at δ 8.26, 7.90, 7.44 and 7.32 (2H). A further oxymethylene singlet was observed at 4.77. The ESI mass spectrum delivered the *pseud*omolecular ion peak at *m/z* 198 [M + Na]$^+$. A search in AntiBase[113] and comparing the ^{1}H NMR spectrum with authentic data led to compound **95**.



95

4.4 Terrestrial *Streptomyces* sp. Red 202

The crude extract of the terrestrial *Streptomyces* sp. Red 202 showed activity against *Escherichia coli*. In the chemical screening three UV absorbing zones were observed on TLC, which turned to red and black with anisaldehyde/sulphuric acid spray reagent.

Twenty-five litres of M_2^+ medium were inoculated and incubated at 28 °C on a linear shaker for seven days. Consequently the culture broth was filtered using a filter

press. The water phase was passed over an XAD-16 column, the resin was washed with distilled water and eluted with methanol. The mycelium was extracted with ethyl acetate followed by acetone. Both organic phases were evaporated to dryness to obtain two crude extracts. The extract obtained from the filtrate was chromatographed on silica gel by column chromatography using a CH_2Cl_2/MeOH gradient to obtain two fractions, namely FI-FII. FII was purified on Sephadex LH-20 using MeOH to deliver nocardamine (**96**) and diastovaricin I (**97**). The extract obtained from the biomass was chromatographed on silica gel by column chromatography using cyclohexane/EtOAc gradient 0 to 100 to obtain two fractions namely MI-MII. MI contained fat; MII was subjected to Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O to afford 6-hydroxy-5-methoxyisatine (**98**) and the metabolites thymidine and xanthosine (**99**).

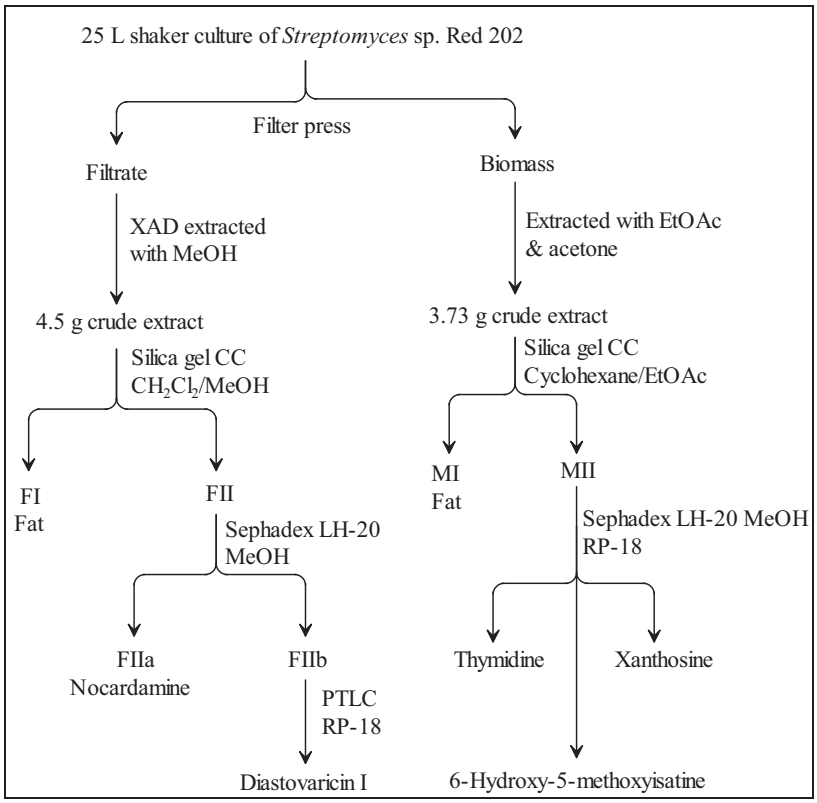


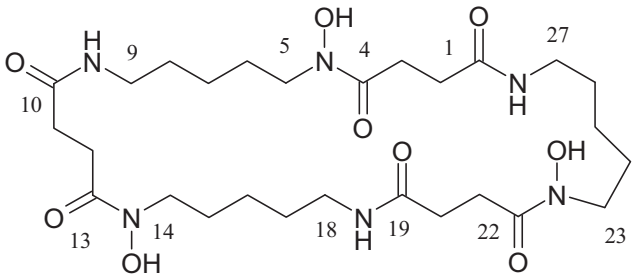
Figure 76: Scheme work up of terrestrial *Streptomyces* sp. Red 202

4.4.1 Nocardamine

Norcardamine (**96**) was isolated as white UV inactive amorphous solid from fraction FII. On TLC, it turned to pale violet with anisaldehyde/sulphuric acid. In the ^1H NMR spectrum seven methylene signals were observed, in addition to an OH signal at δ 9.55 and an NH signal at δ 7.68. The ^{13}C NMR spectrum displayed only nine carbon signals, two carbonyl groups of amide or acid derivatives at δ 171.8 and 171.4 and

seven methylene carbon signals. ESIMS afforded the *pseudo*molecular ions at *m/z* 601 $[M + H]^+$ and 623 $[M + Na]^+$, or 599 $[M - Na]^-$ in the negative mode, which gave a molecular weight of 600 Dalton; the HRESI mass spectrum established the molecular formula $C_{27}H_{48}N_6O_9$. A search in AntiBase[113] led to the known nocardamine (**96**), which has a characteristic 3-fold symmetry. It had been isolated previously in our group, the structure was confirmed by comparing the above spectroscopic data with the literature.

Nocardamine (**96**) had been isolated previously from various bacterial strains such as *Nocardia* sp., *Pseudomonas stutzeri*, and *Streptomyces hygroscopicus* var. *geldanus*.[153] It is related to the ferrichrome antibiotics and shows a high binding affinity to ferric ions.[154,155] According to previous reports it displays antibacterial activity against mycobacteria.[156] In a genomic analysis of *Streptomyces avermitilis*, the putative biosynthetic gene cluster for nocardamine has been found (Figure 79).



96

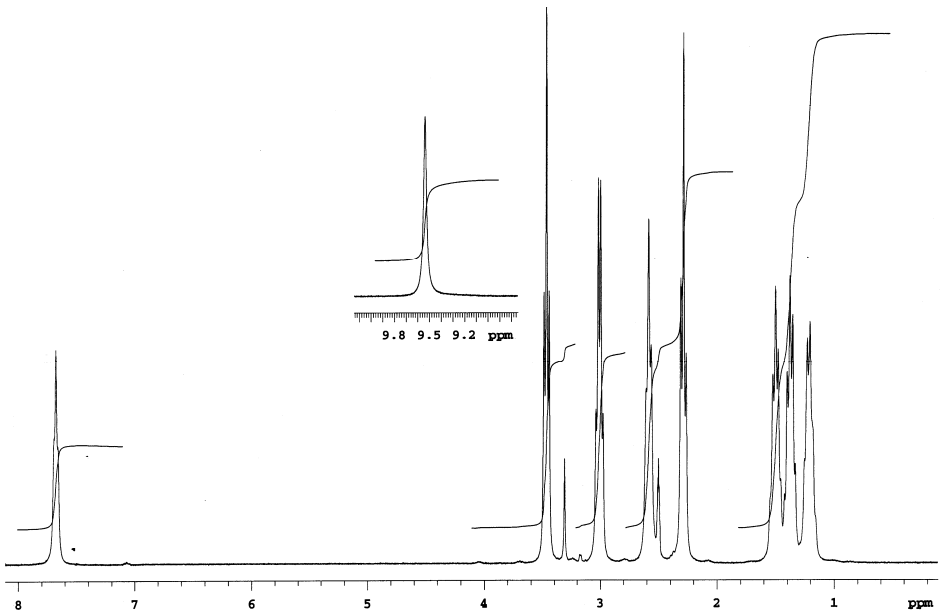


Figure 77: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of norcardamine (**96**)

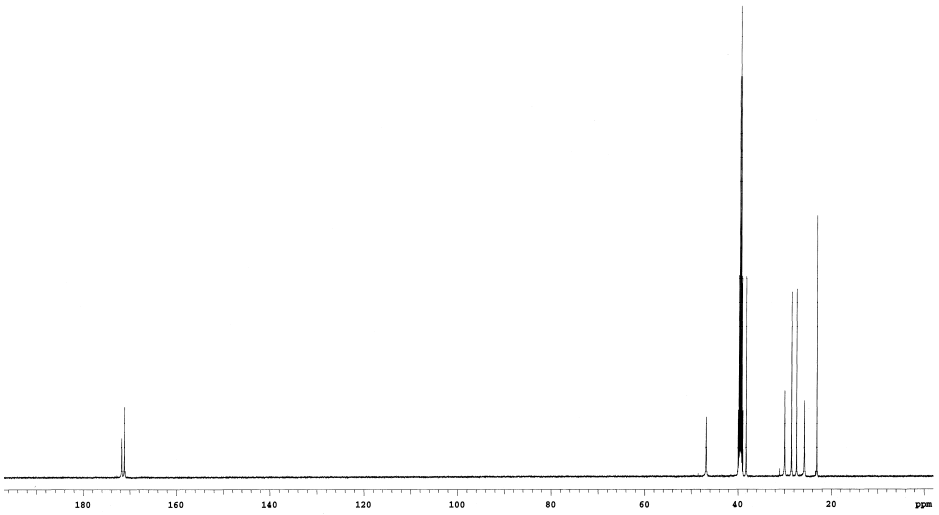


Figure 78: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of norcardamine (**96**)

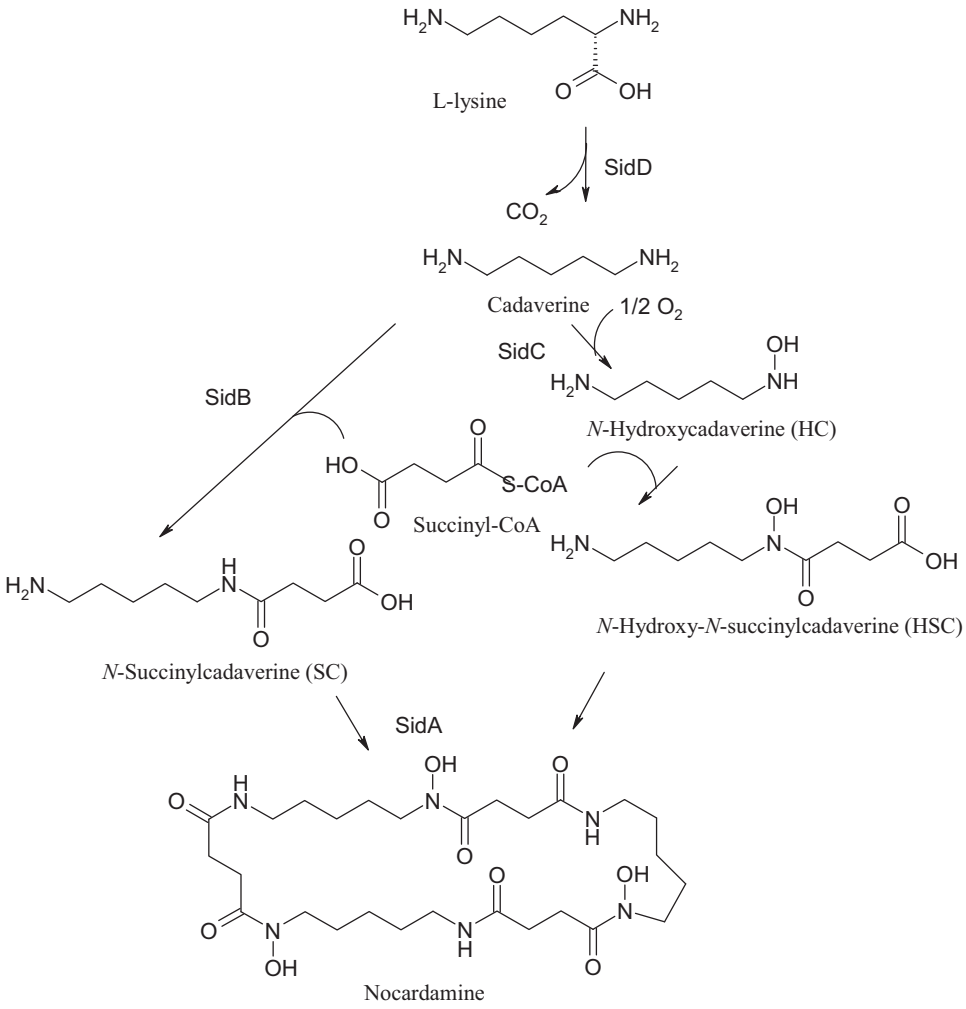
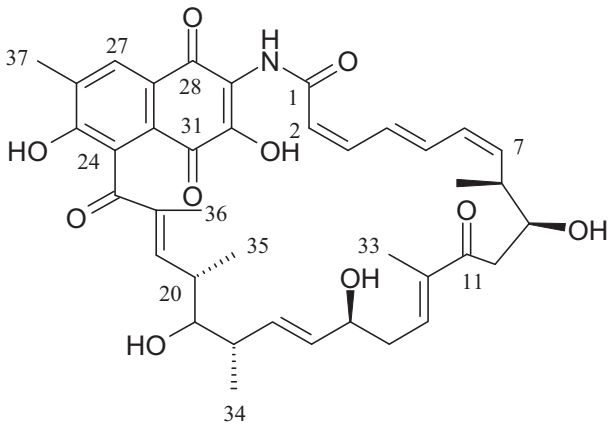


Figure 79: Plausible biosynthetic pathway of nocardamine (**96**) based on the predicted biosynthetic functions in *Streptomyces avermitilis*[157]

4.4.2 Diastovaricin I

Diastovaricin I (**97**) was isolated as reddish needles from a UV absorbing zone, which turned to black with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum pointed to a complex structure: many olefinic protons appeared between δ 7.89 – 5.50, together with three oxymethine signals at δ 4.05, 3.59 and 3.14 and two methylene protons, three methine protons and six methyl signals. The ^{13}C NMR spectrum of compound **97** displayed signals of fourteen quaternary carbons between δ 203.7 and 118.0 and eleven olefinic signals between δ 145.8 – 117.2, in addition to three oxymethine at δ 75.5, 73.8 and 70.9 and two methine carbon signals at δ 45.1 and 41.8. Additionally, two methylene carbons at δ 40.3 and 37.5 and six methyl signals between δ 17.6 and 10.5 were observed.



97

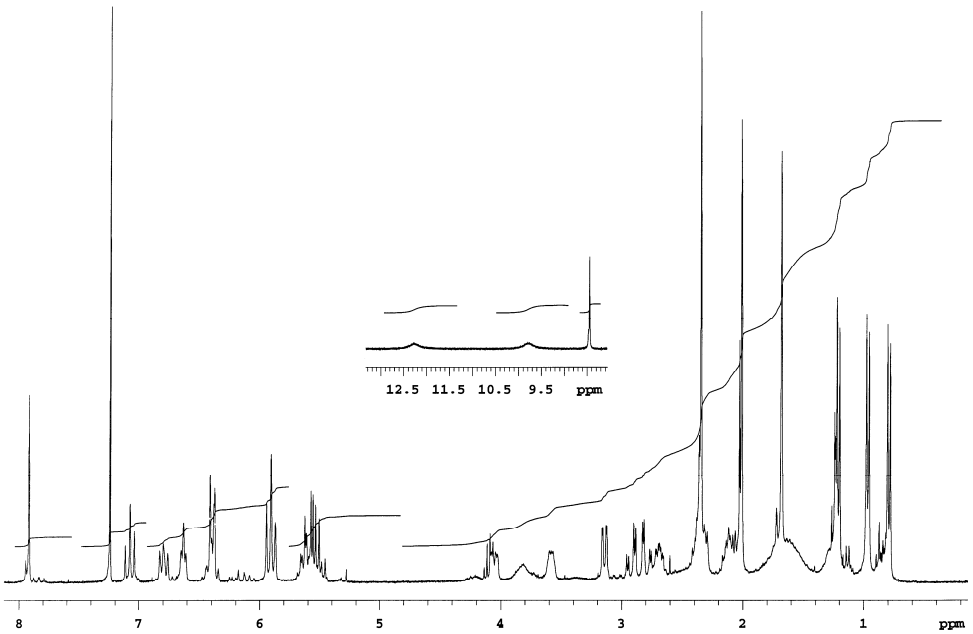


Figure 80: ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of diastovaricin I (**97**)

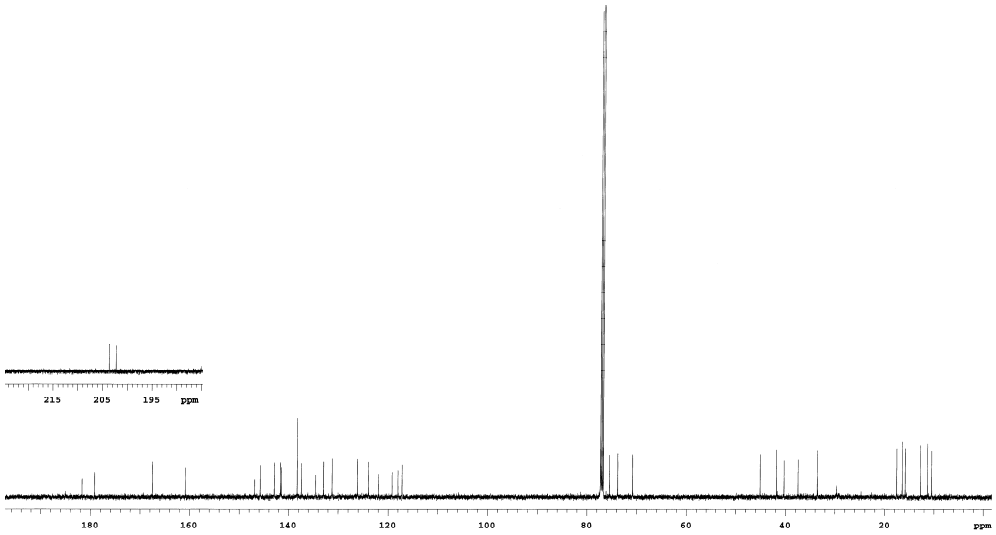


Figure 81: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of diastovaricin I (**97**)

The ESI mass spectrum revealed a *pseudo*molecular ion at *m/z* 710 $[M + Na]^+$, which delivered the molecular weight of 687 Dalton. HRESIMS established the molecular formula $C_{39}H_{45}NO_{10}$. A database search pointed to diastovaricin I (**97**) or the stereoisomeric actamycin. Diastovaricin I (**97**) was isolated previously from *Streptomyces diastochromogenes* subsp. *variabilicolor* and is a member of the ansamycin antibiotics.[158]

The HMBC spectrum, along with ^{13}C shifts at δ 181.7 and 179.2, represented carbonyl groups of a quinone moiety. A proton down field at δ 7.89 (CH-27) showed HMBC correlations with C-28 (δ 181.7), C-31a (δ 134.5), C-25 (δ 160.8) and a methyl carbon at C-37 (δ 16.4).

A hydroxynaphthoquinone moiety was supported by ^{13}C shifts of quaternary carbons at δ 160.8 (C-25) (C-30, Figure 82, fragment a). The OH group was obviously not in a *peri*-position, as the closely related CO shifts indicated; and OH signal was in MeOD not visible. Interpretation of the HMBC, HSQC and COSY couplings delivered an additional fragment b, where an olefinic proton at δ 5.90 (CH-21) exhibited HMBC correlations with C-35 (δ 10.5), C-23 (δ 202.3) and C-36 (δ 12.8), in addition to a COSY correlation with H-20 (δ 2.68).

Table 23: ^{13}C and ^{1}H NMR shifts (125, 600 MHz) of diastovaricin I (**97**) in $CDCl_3$ with reference data[159]

	diastovaricin I (**97**)		diastovaricin I (**97**) [159]	
Position	δ_C	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)
1	167.5		167.6	
2	117.2	5.93 (d, 11.1)	116.2	5.85 (d, 11.0)
3	141.5	7.08 (t, 11.2)	147.4	6.73 (m)
4	124.0	6.80 (dd, 11.0, 10.1)	129.1	7.31 (dd, 14.5, 11.0)
5	138.3[a]	6.37 (dd, 13.3, 11.5)	139.1	6.74 (dd)
6	126.2	6.41 (dd, 13.3, 11.6)	129.2	6.27 (dd, 11.0, 11.0)
7	142.9	5.60 (dd, 10.4, 10.4)	141.3	5.46 (dd, 11.0, 11.0)
8	45.1	2.37 (m)	38.3	2.73 (m)
9	73.8	3.59 (q, 4.6)	72.8	3.58 (m)
10	40.3	2.81 (ABX, 17.4, 3.6)	40.4	2.80 (ABX, 17.5, 6.5)
		2.90 (ABX, 17.4, 5.0)		2.94 (ABX, 17.5, 3.5)
11	203.7		202.4	
12	138.3[a]		137.9	
13	141.7	6.63 (t, 5.6)	142.2	6.70 (m)
14	37.5	2.34 (m), 2.07 (m)	36.7	2.20 (m)
15	70.9	4.05 (dd, 11.2, 5.6)	71.3	4.07 (m)
16	137.4	5.58 (dd, 15.3, 5.6)	137.3	6.60 (dd, 15.0, 7.0)
17	132.94	5.50 (dd, 15.3, 9.3)	133.7	5.49 (dd, 15.0, 9.0)
18	41.8	2.12 (m)	41.5	2.10 (m)
19	75.5	3.14 (dd, 9.9, 2.3)	75.8	3.12 (dd, 15.0, 2.0)
20	33.6	2.68 (m)	33.6	2.66 (m)
21	145.8	5.90 (dd, 10.5, 1.0)	146.0	5.86 (dd, 10.5, 1.0)
22	138.3[a]		138.2	
23	202.3		203.5	
24	122.0		122.4	
25	160.8		160.7	
26	119.2		119.5	
27	131.2	7.89 (d, 0.8)	131.3	7.92 (d, 0.5)
27a	132.91		132.7	
28	181.7		181.3	
29	118.0		117.8	
30	146.9		146.7	
31	179.2		179.1	
31a	134.5		133.9	
32	17.6	1.20 (d, 6.5)	17.4	1.18 (d, 6.5)
33	11.3	1.67 (s)	11.1	1.63 (s)
34	15.9	0.96 (d, 6.6)	15.7	0.96 (d, 6.5)
35	10.5	0.79 (d, 6.7)	10.4	0.80 (d, 6.5)
36	12.8	2.00 (s)	12.7	2.01 (d, 1.0)
37	16.4	2.32 (s)	16.5	2.32 (d, 0.5)

[a] signal with the intensity of three carbons

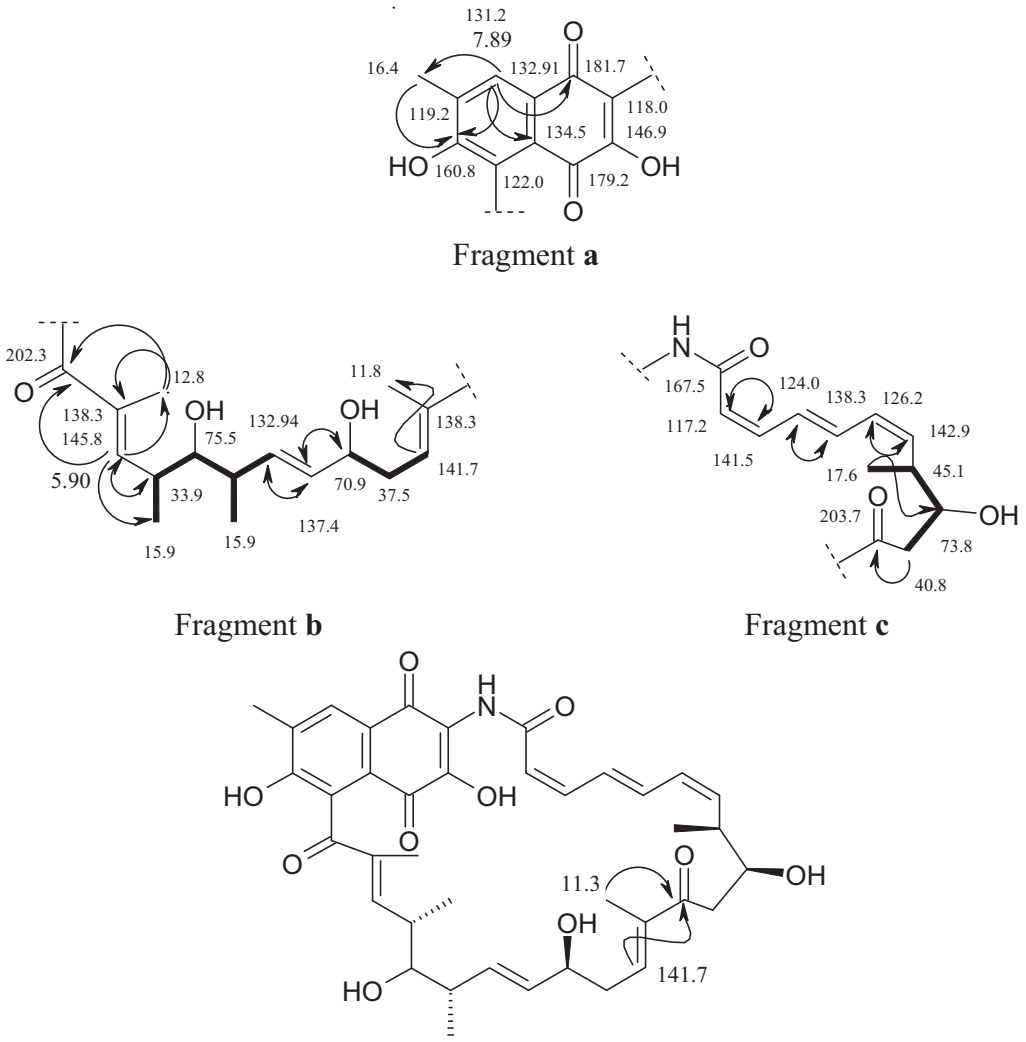


Figure 82: Substructures and selected H,H COSY and HMBC correlations of diastovaricin I (**97**)

Further COSY correlations were observed from the methyl group C-35 (δ 0.79) to H-20 (δ 2.68) and from the latter to the proton H-13 (δ 6.63). The latter olefinic proton exhibited HMBC correlations with a methyl carbon C-33 (δ 11.3), additionally confirming fragment b. A number of COSY correlations supported the connectivity of the three double bonds as drawn for fragment c. The presence of an amide at C-1 (167.5) was supported by the ^{13}C shifts of C-2 (δ 117.2) and C-3 (δ 141.5). COSY correlations were found from a methyl group H-32 (δ 1.20/17.6) to H-8 (δ 2.37/45.1) and from the latter proton to H-9 (δ 3.59/73.8) and to H-10 (δ 2.81/40.3). Methylene protons at C-10 showed HMBC correlations with the ketone carbonyl C-11 (δ 203.7). HMBC correlations from the olefinic proton at H-13 (δ 6.63/141.7) and methyl proton at H-33 (δ 1.67/11.3) supported the connectivity of fragments b and c as drawn in Figure 82.

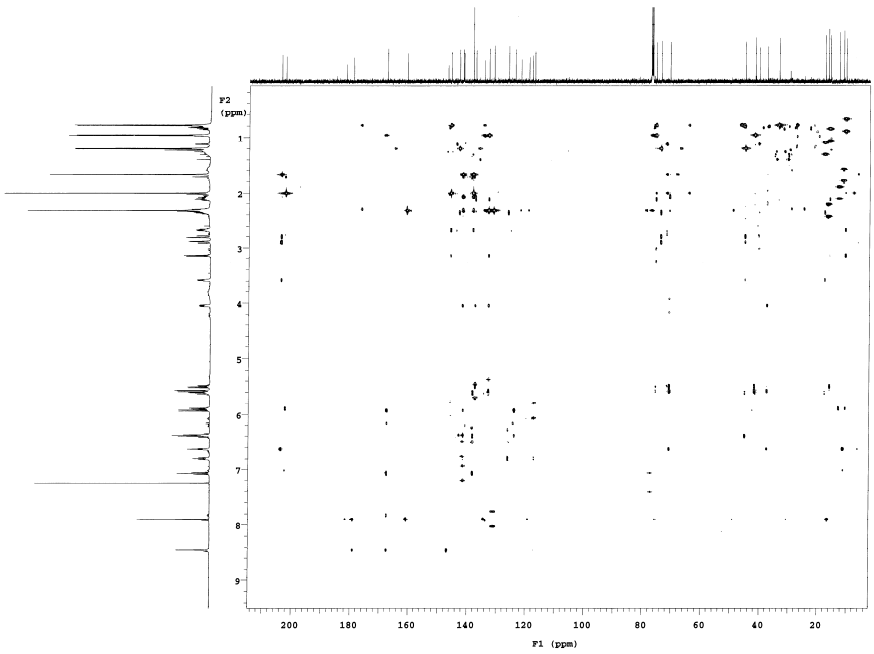
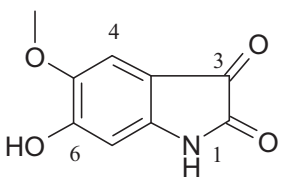


Figure 83: HMBC spectrum (CD_3OD, 600 MHz) of diastovaricin I (**97**)

The ^{1}H and ^{13}C NMR data were similar to published data.[159] A decision between diastovaricin I (**97**) and actamycin was, however, not possible due to insufficient data in the literature of actamycin.

4.4.3 6-Hydroxy-5-methoxyisatine

6-Hydroxy-5-methoxyisatine (**98**) was isolated as reddish solid from a UV absorbing orange zone, which turned to red with anisaldehyde/sulphuric acid. In the ^{1}H NMR spectrum only three signals were observed, of which two aromatic protons appeared as singlets at δ 6.70 (H-4) and 5.83 (H-7). A methoxy singlet was found at δ 3.69. The ^{13}C NMR spectrum delivered nine carbon signals: six were quaternary and appeared between δ 178.9-103.5; two CH signals appeared at relatively high field at δ 105.5 and 102.7, and a methoxy group was found at δ 55.8. The ESI mass spectrum revealed the *pseudo*molecular ion peak at 216 $[M + Na]^{+}$; HRESIMS afforded the molecular formula $C_9H_7NO_4$.



98

Analysis of HMBC and HSQC spectra supported the presence of two aromatic protons located at *para* positions; one at C-4 (δ 6.70) displayed correlations to the carbonyl C-3 (δ 178.9) as well as further strong three-bond correlations to C-5 (δ 150.0) and C-7a (δ 154.0). The proton at H-7 (δ 5.83) displayed strong three bond correlations to both quaternary carbons C-3a (δ 103.5) and C-5 (δ 150.0) as well as small correlations to the quaternary carbon C-7a (δ 154.0). The methoxy proton at δ 3.69 showed only one correlation with the quaternary atom C-5 (δ 150.0). No correlations were observed to the carbonyl at δ 168.0.

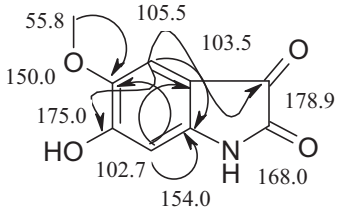


Figure 84: Selected HMBC correlations of 6-hydroxy-5-methoxyisatine (**98**)

A search in AntiBase[113] confirmed the structure of compound **98**, which had been synthesized previously[160] and was also isolated last year in our group. The cytotoxic activity of isatin derivatives against a human monocyte like histiocytic lymphoma (U937) cell line was reported.[161]

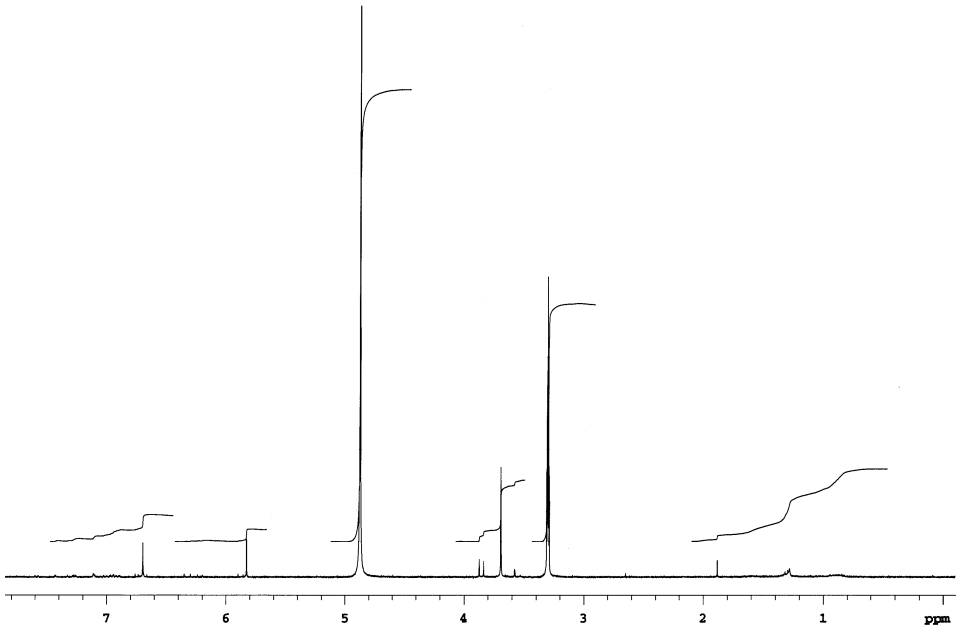
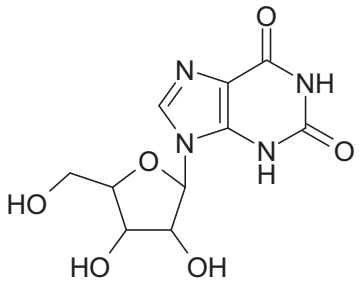


Figure 85: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 6-hydroxy-5-methoxyisatine (**98**)

4.4.4 Xanthosine

Xanthosine (**99**) was UV absorbing, colourless oil, which turned to blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed a pattern like adenosine and other primary metabolites. One singlet was observed in the aromatic region along with six oxygenated protons in sugar region. The ESI/MS revealed the *pseudo*molecular ion peak at 307 $[M + Na]^+$; HRESIMS afforded the molecular formula $C_{10}H_{12}N_4O_6$. A search in AntiBase[113] confirmed the structure after comparing with the authentic spectrum.



99

4.5 Terrestrial *Streptomyces* sp. WO 1004

For the pre-screening, the terrestrial *Streptomyce*s sp. WO 1004 was cultivated in Erlenmeyer flasks in M_2^+ medium at 28 °C for five days. After work-up, the resulting crude extract showed on TLC two UV absorbing bands, which gave a black colour with anisaldehyde/sulphuric acid.

A culture on agar was used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2^+ medium (25 L): After incubation at 28 °C on a linear shaker for seven days, the dark brown culture broth was filtered under vacuum. The biomass was extracted with ethyl acetate followed by acetone. The filtrate was subjected to XAD-16 column; the resin has been washed with distilled water. The combined extracts were concentrated under vacuum to obtain a brown oily crude extract (7.3 g), which was subjected to silica gel column chromatography (CC) eluting with CH_2Cl_2, followed by stepwise addition of CH_3OH to yield five fractions FI to FV. FII was subjected to Sephadex LH-20 eluted with CH_3OH followed by reverse phase RP-18 to afford isomacrolactinic acid (**100**). FIII was purified on silica gel to yield tyrosol and uridine. Moreover FIV was purified on Sephadex LH-20 and delivered macrolactin A (**101**), while FV was purified on RP-18 to deliver dihydromacrolactin F (**102**).

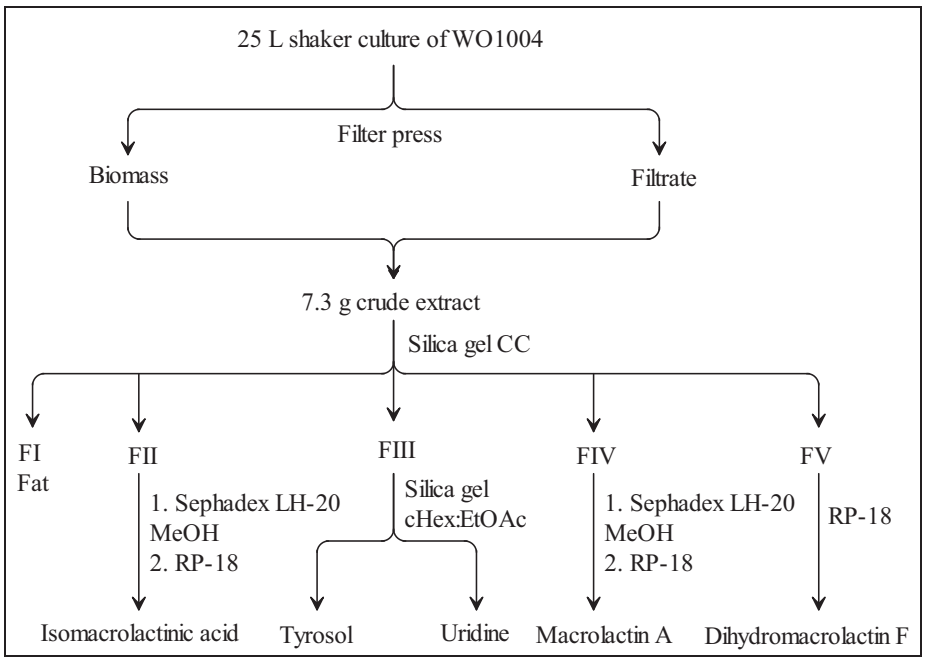
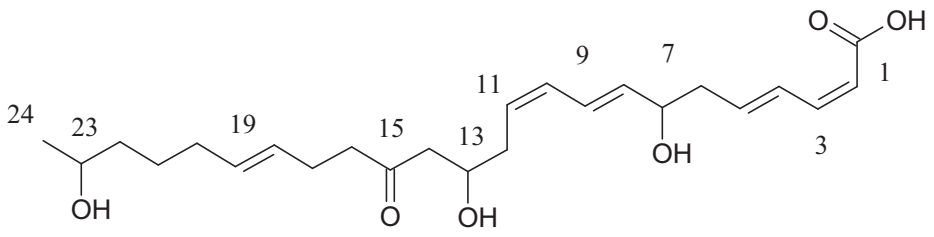


Figure 86: Work up scheme of the terrestrial *Streptomyces* sp. WO 1004

4.5.1 Isomacrolactinic acid

Isomacrolactinic acid (**100**) was isolated from fraction FII, after initial separation on Sephadex LH-20 using MeOH and reverse phase RP-18 eluting with MeOH/water (20/80%). Compound **100** was isolated as yellow oil, absorbed UV at 254 nm, and turned to black with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **100** displayed ten olefinic proton signals between δ 7.40 – 5.42, three oxymethine signals at δ 4.27, 4.10 and 3.71, in addition to eight methylene signals and one methyl doublet.



100

The ^{13}C NMR spectrum of **100** showed 2 carbonyl signals at δ 211.8 and 170.2 indicating ketone and acid derivatives, respectively, along with 10 olefinic carbon signals between δ 145.9 – 117.8, which were assigned to five double bonds.

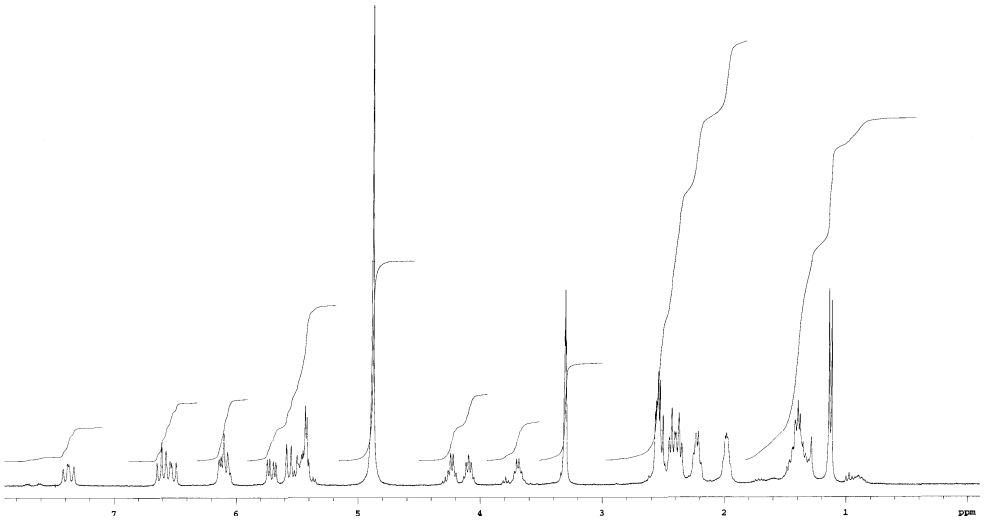


Figure 87: ^{1}HNMR spectrum (CD_3OD, 300 MHz) of isomacrolactinic acid (**100**)

In addition eight methylene carbon signals between δ 50.3 – 26.8, three oxymetine carbons at δ 72.6, 68.8 and 68.5 and one methyl carbon signal at δ 23.5 were observed. The molecular weight was deduced by CIMS as *m/z* 419 $[M - H]^-$ and HRESIMS afforded the molecular formula $C_{24}H_{36}O_6$. Further H,H COSY as well as HMBC correlations were observed as shown in Figure 89. A proton signal at δ 4.27 (H-7) showed a strong COSY correlation with a signal at 5.74 (H-8) as well as HMBC correlation with C-9. Moreover, a signal at δ 2.55 (H-16) showed a COSY correlation with a proton at δ 2.21 and HMBC correlations with a carbon C-18 as drawn in Figure 89.

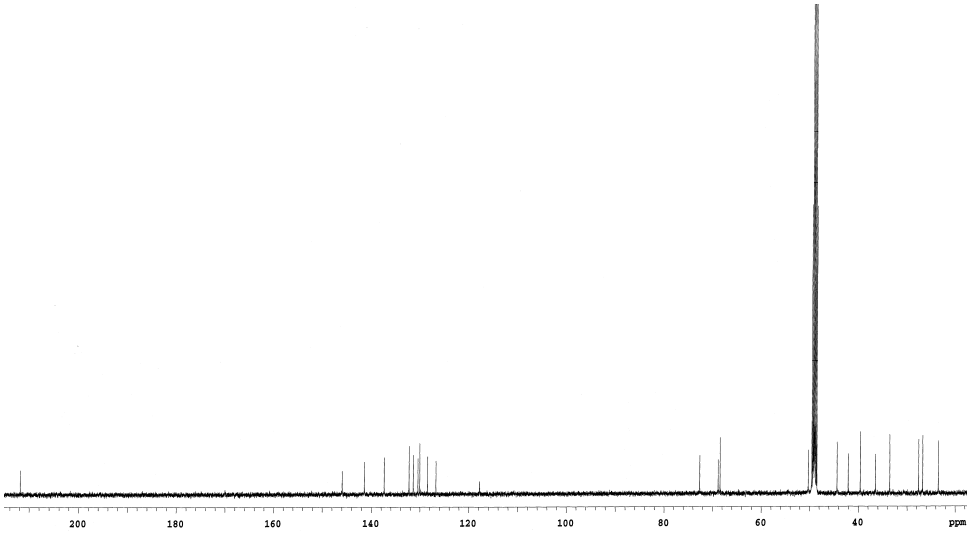


Figure 88: ^{13}CNMR spectrum (CD_3OD, 125 MHz) of isomacrolactinic acid (**100**)

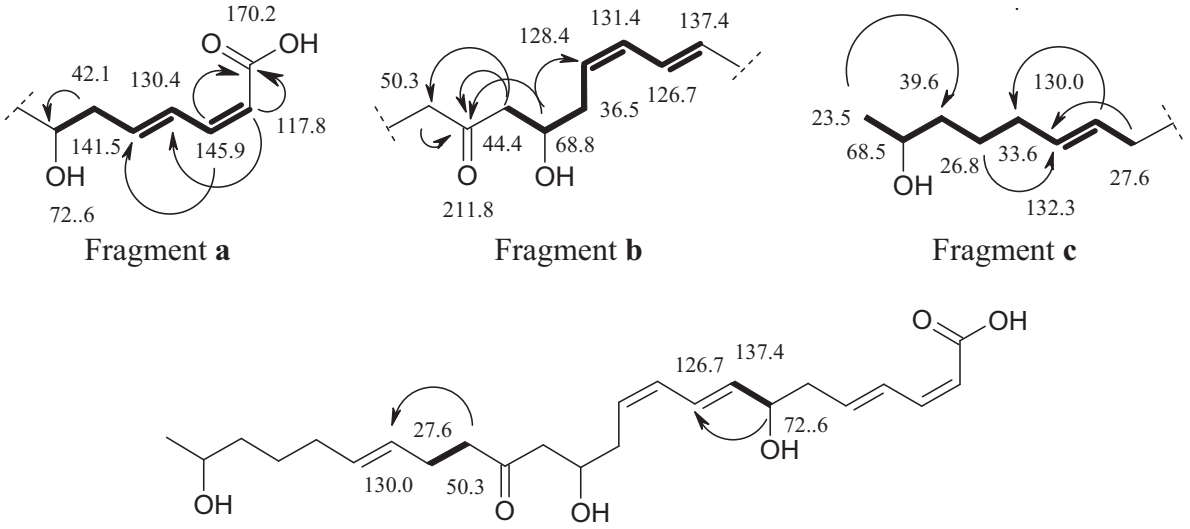


Figure 89: Selected H,H COSY and HMBC correlations of isomacrolactinic acid (**100**)

A search in AntiBase[113] using above spectroscopic data confirmed the structure as isomacrolactinic acid (**100**).[162]

Table 24: ^{13}C and ^{1}H NMR spectroscopic data (125, 300 MHz) for isomacrolactinic acid (**100**) in CD_3OD

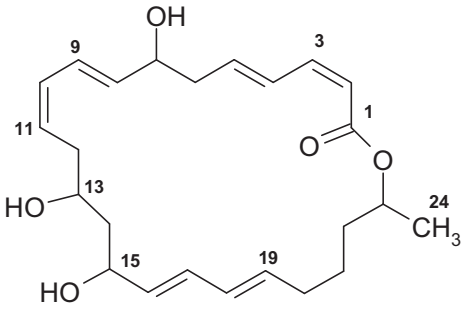
Position	δ_C	δ_H (mult.; *J* in Hz)
1	170.2*	
2	117.8	5.57 (d, 11.4)
3	145.9	6.63 (dd, 11.3, 10.5)
4	130.4	7.40 (dd, 15.4, 11.3)
5	141.5	6.12 (dd, 11.7, 6.5)
6	42.1	2.43 (m)
7	72.6	4.27 (dt, 6.6, 6.4)
8	137.4	5.74 (dd, 15.1, 6.3)
9	126.7	6.51 (dd, 15.1, 11.1)
10	131.4	6.10 (dd, 16.1, 7.0)
11	128.4	5.47 (dt, 12.0, 7.3)
12	36.5	2.39
13	68.8	4.10 (m)
14	44.4	2.50 (m)
15	211.8	
16	50.3	2.55 (m)
17	27.6	2.21 (m)
18	130.0	5.43 (m)
19	132.3	5.42 (m)
20	33.6	1.98 (m)
21	26.8	1.39 (m)
22	39.6	1.43 (m)
23	68.5	3.71 (m)
24	23.5	1.13 (d, 6.2)

* visible only in the HMBC spectrum

4.5.2 Macrolactin A

Macrolactin A (**101**) was isolating as oily substance from fraction FIV after purification on Sephadex LH-20 followed by RP18. On TLC it gave a UV absorbing band at 254 nm, which stained to black with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed twelve olefinic protons between δ 7.23-5.53. Additionally four oxymethine protons were observed at δ 5.01, 4.29, 4.23 and 3.84. In the upfield region of the spectrum six methylene groups between δ 2.48-1.49 and a methyl doublet at δ 1.24 were observed. The ^{13}C NMR spectrum revealed 24 carbon signals: a carbonyl at δ 168.0 for amide or lactone, 12 olefinic carbons between δ 145.0-118.0, four oxymethine carbons at δ 72.3, 72.2, 69.8, and 69.2. Further on six methylene signals between δ 43.9-25.7 and a methyl carbon at δ 20.1 were observed. The molecular weight

402 was obtained by ESIMS and the molecular formula $C_{24}H_{34}O_5$ was established by HRESIMS.



101

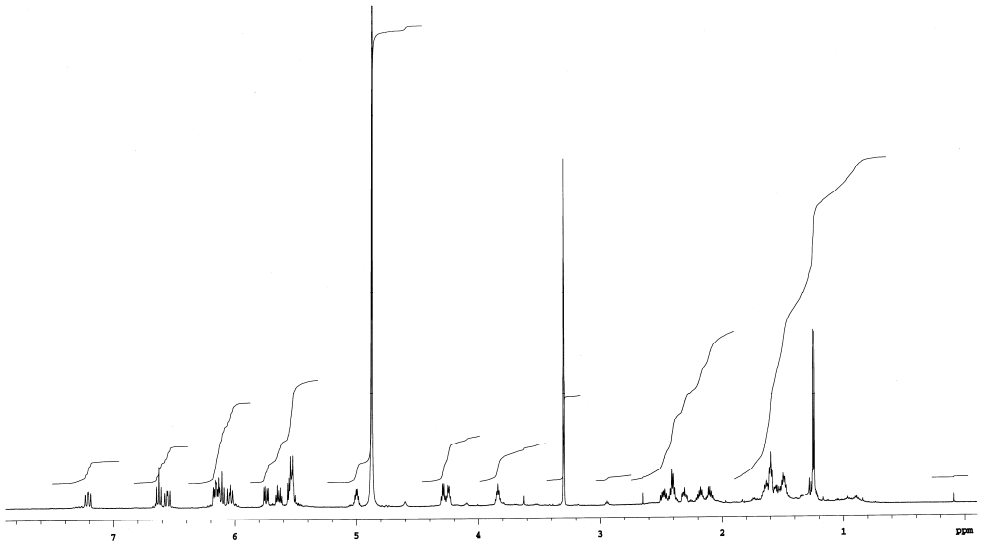


Figure 90: ^{1}HNMR spectrum (CD_3OD, 300 MHz) of macrolactin A (**101**)

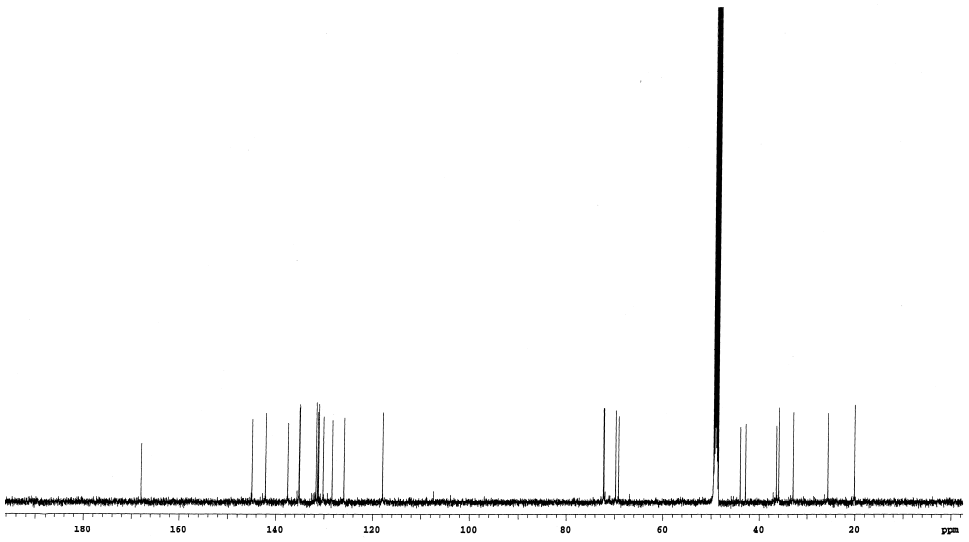


Figure 91: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of macrolactin A (**101**)

The H,H COSY spectrum showed strong correlations among the olefinic protons as well as methylene and oxymethine protons from H-2 to H-12 as drawn in fragment a, and from H-13 to H-24 as drawn in fragment b. The HMBC correlations confirmed both fragments, where strong ^{3}J couplings were found as drawn in Figure 92. Both fragments were connected on the basis of H,H and HMBC correlations, where a proton at δ 2.48 (H-12) showed H,H correlation with the proton at δ 3.84 (H-13) and HMBC correlation with a methylene carbon at δ 43.9. The oxymethine proton at δ 3.84 (H-13) displayed ^{3}J coupling with the olefinic carbon at δ 128.4 (C-11). On the other hand both olefinic protons at δ 6.64 (H-3) and 5.53 (H-2) displayed HMBC correlations with the lactone carbonyl at δ 168.0 (C-1) giving a 24-membered ring.

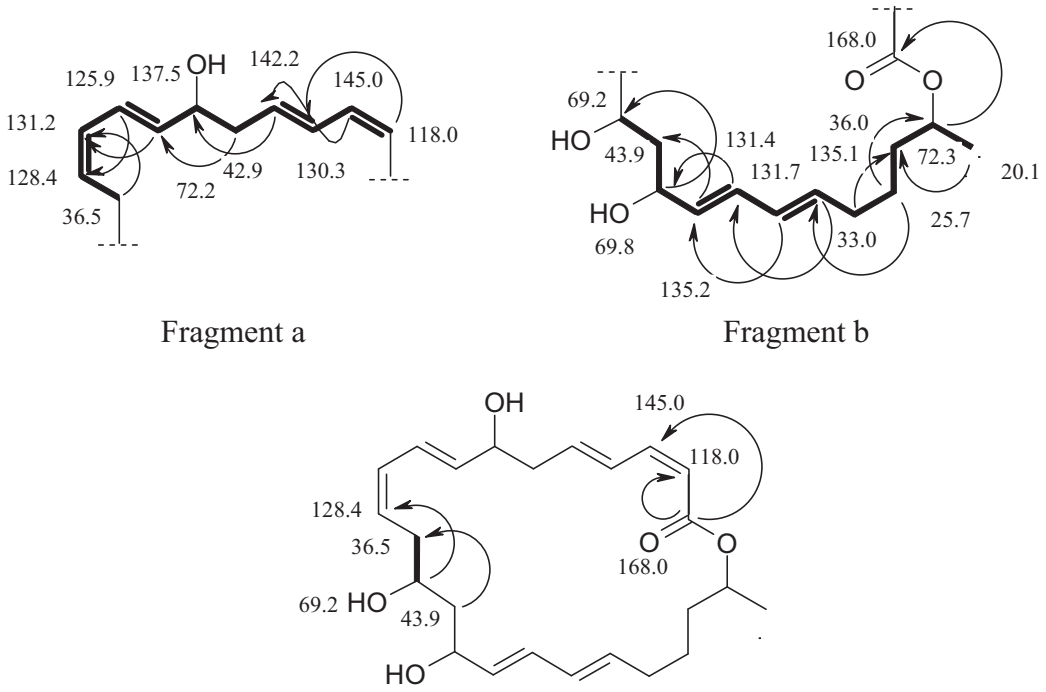


Figure 92: Selected H,H COSY and HMBC correlations of macrolactin A (**101**)

A search in AntiBase[113] and comparing the spectroscopic data of compound **101** confirmed the structure as macrolactin A (**101**), which was isolated by W. Fenical's group in 1989. Till now 18 macrolactins have been isolated.[163]

Macrolactin A (**101**) exhibited selective antibacterial activity as well as cytotoxicity against B16-F-10 murine melanoma cancer cells, and antiviral activity against Herpes simplex and HIV. In previous reports compound **101** was isolated from a culture broth of *Actinomadura* sp. as a neuronal-cell-protecting substance[164] as well as from a *Bacillus* sp. sunhua of the soil of a potato-cultivating area.[165] In addition to these results, macrolactin A (**101**) and macrolactin F (**112**) were produced by a soil

Streptomyces species and found to be inhibitors of squalene synthase.[166] It is important to mention that the amazing biological activities of **101** encouraged scientists to understand its mode of action. The biosynthetic genes of compound **101** have not been sequenced so far, however its structure suggests that it can be assembled by a modular polyketide synthase (PKS system) typical for macrolides. The PKS system is a primary and an important as well as essential element of the biosynthetis of macrolides.[167]

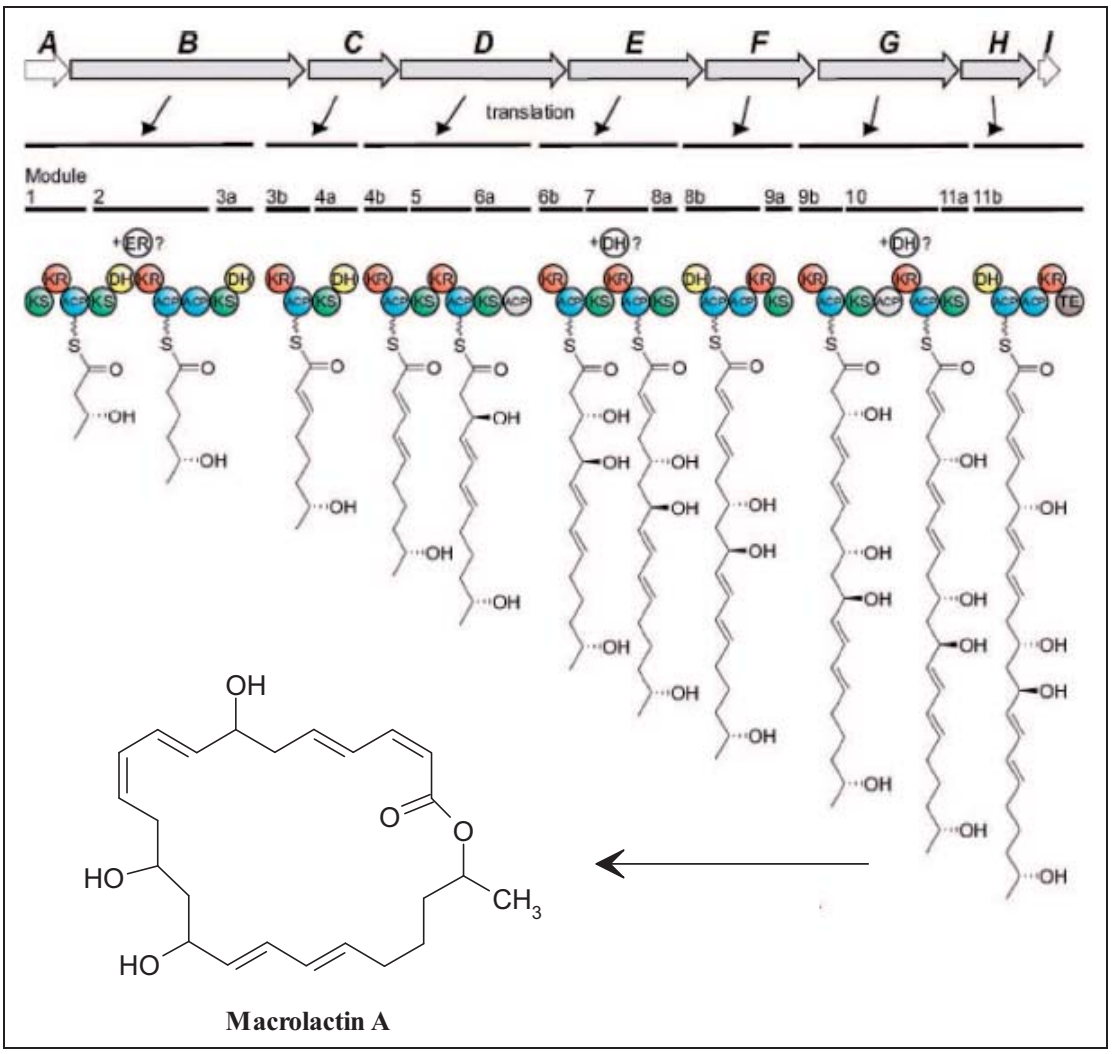
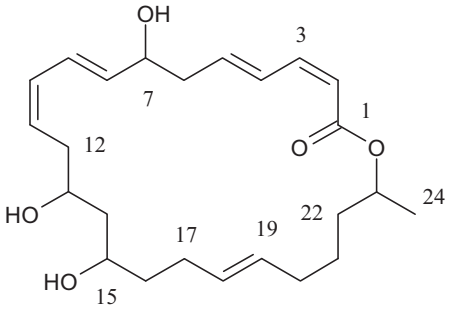


Figure 93: Suggested biosynthetic pathway of macrolactin A (**101**) as product of the *pks*2 cluster of *B. amyloliquefaciens*.[168]

4.5.3 Dihydromacrolactin F

Dihydromacrolactin F (**102**) was isolated as yellow oil, showed UV absorbance at 254 nm, and turned to brown with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum displayed ten olefinic signals between δ 7.23 – 5.53 as well as eight methylene signals between δ 2.43 – 1.50, four oxymethine signals at δ 4.98, 4.26, 3.91 and 3.81

and a methyl doublet at δ 1.23. In the ^{13}C NMR spectrum a carbonyl at δ 168.0 was observed in addition to ten olefinic carbons between δ 145.1 – 118 Moreover, eight methylene carbon signals appeared between δ 43.2 – 26.1 as well as oxymethine carbons at δ 72.3, 72.1, 69.9 and 68.8 and a methyl carbon at δ 20.2. ESIMS delivered the molecular weight 404.



102

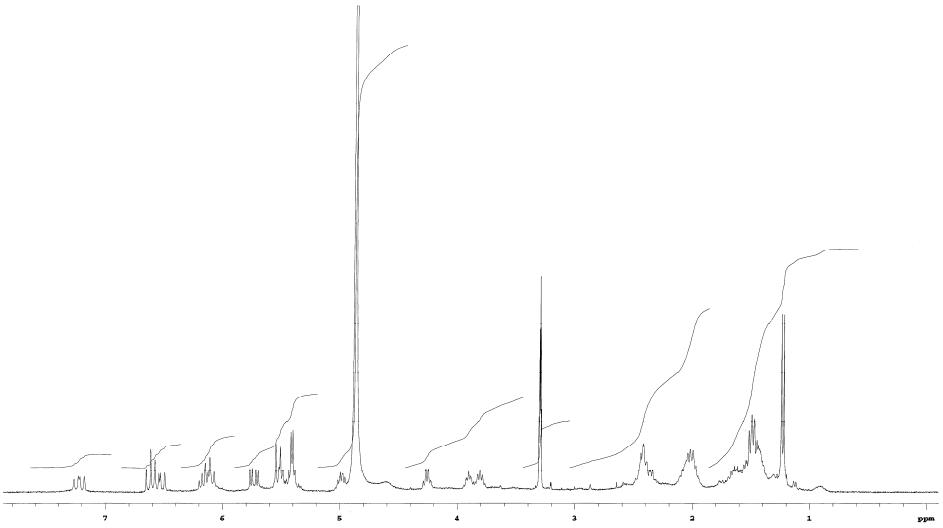


Figure 94: ^{1}HNMR spectrum (CD_3OD, 300 MHz) of dihydromacrolactin F (**102**)

The H,H COSY spectrum established parts of the structure, where a proton at δ 5.53 showed correlation with the neighbouring proton at δ 6.62, the latter showed correlation with the other neighbouring proton at δ 7.23 as it is shown in fragment a. On the other hand HMBC correlations confirmed the locations of further carbons: a proton at δ 6.62 showed a ^{3}J correlations with a carbonyl at δ 168.0 and with C-5; the proton at δ 6.54 (H-9) showed 2- and 3-*J* coupling with C-10 and C-11, as drawn in fragment a. In fragment b (Figure 96) connectivity was confirmed by further H,H COSY and HMBC correlations.

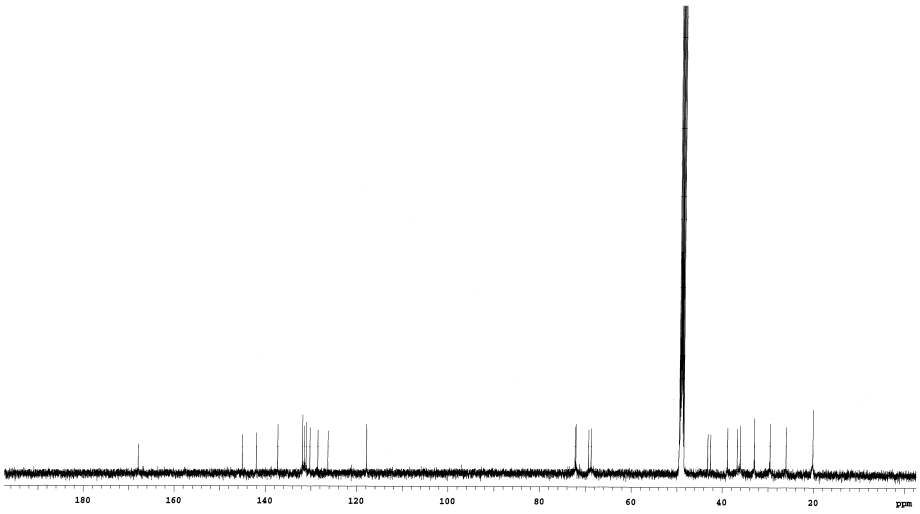
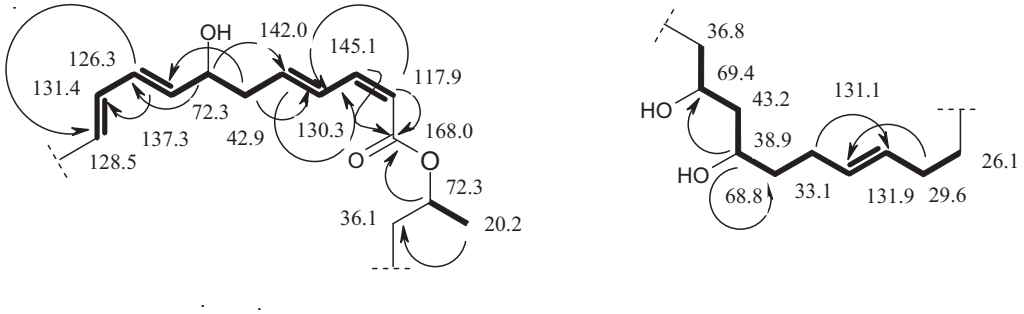


Figure 95: ^{13}CNMR spectrum (CD_3OD, 125 MHz) of dihydromacrolactin F (**102**)



Fragment a Fragment b

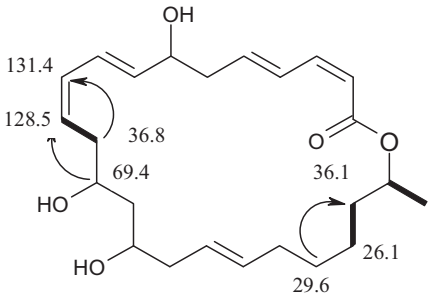


Figure 96: H,H COSY and HMBC correlations of dihydromacrolactin F (**102**)

The structure was assigned as dihydromacrolactin F (**102**) after the connection of the two fragments a and b, whereas methylene proton at δ 2.43 (H-6) showed H,H COSY correlation with the olefinic proton at δ 6.14, and HMBC correlation with an olefinic carbon at δ 130.3. Another methylene proton at δ 1.67 (H-22) showed H,H COSY correlations with a methylene proton at δ 1.43 (H-21) and a ^3J correlations with a methylene carbon at δ 29.6 (C-20).

Compound **102** was synthesized by the group of William Fenical by reduction of **112** using sodium borohydride.[169] In our test compound **102** showed very good activity in a concentration of 10 μg/ml against oomycetes. Oomycetes are phylogenetic relatives of brown algae that cause many destructive diseases of plants, and are responsible for several animal and human diseases.[170]

Table 25: ^{13}C and ^{1}H NMR chemical shifts (125, 300 MHz) for dihydromacrolactin F (**102**) in CD_3OD

Position	δ_C	δ_H (mult.; *J* in Hz)
1	168.0	
2	117.9	5.53 (d, 11.3)
3	145.1	6.62 (t, 11.5)
4	130.3	7.23 (dd, 15.2, 11.4)
5	142.0	6.14 (m)
6	42.6	2.43 (m)
7	72.3	4.26 (quint, 5.9)
8	137.3	5.74 (dd, 15.1, 5.7)
9	126.3	6.54 (dd, 15.2, 11.0)
10	131.4	6.06 (m)
11	128.5	5.51 (m)
12	36.8	2.35 (m) and 2.44 (m)
13	69.4	3.91 (m)
14	43.2	1.48 (m)
15	68.8	3.81 (m)
16	38.9	1.50 (m)
17	33.1	2.00 (m)
18	131.1	5.40 (m)
19	131.9	5.40 (m)
20	29.6	2.05 (m)
21	26.1	1.43 (m)
22	36.1	1.67 (m) and 1.54 (m)
23	72.1	4.98 (m)
24	20.2	1.23 (d, 6.3)

4.6 Terrestrial *Streptomyces* sp. HO9

The crude extract of the terrestrial *Streptomyces* sp. HO9 did not show any biological activities in our test system, while on TLC the extract gave many different UV absorbing bands, which turned to violet and red colours with anisaldehyde/sulphuric acid.

Well-grown agar plates of the terrestrial *Streptomyces* sp. HO9 were used to inoculate 100 of 1L Erlenmeyer flasks each containing 250 ml M_2^+ medium. The cul-

ture was incubated at 28 °C for 8 days using a linear shaker. The dark brown culture broth was filtered under vacuum using a filter press. The biomass was extracted three times with ethyl acetate followed by acetone. The filtrate was subjected to XAD-16 column, the resin washed with distilled water and extracted with MeOH. The combined extracts were concentrated under vacuum till dryness to obtain 3.5 g of a crude extract. This was subjected to silica gel column chromatography eluting with CH_2Cl_2 followed by stepwise addition of CH_3OH (gradient 0 to 20 %) to yield four fractions. Fraction II was subjected to silica gel column chromatography and eluted with cyclohexane/EtOAc gradient to afford *β*-sitosterol and stigmasterol. On the other hand FIII was purified by Sephadex LH-20 column chromatography and eluted with CH_3OH followed by reverse phase RP-18 (MeOH/water gradient) to afford indole-3-carboxylic acid and 1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**103**). FIV was chromatographed on Sephadex LH-20 using MeOH followed by PTLC to obtain anthranilic acid and 2,6-dimethyl-oxepan-4-one (**104**). Moreover, FV was purified on Sephadex LH-20 using MeOH to afford indole-3-carboxylic acid methyl ester (**106**).

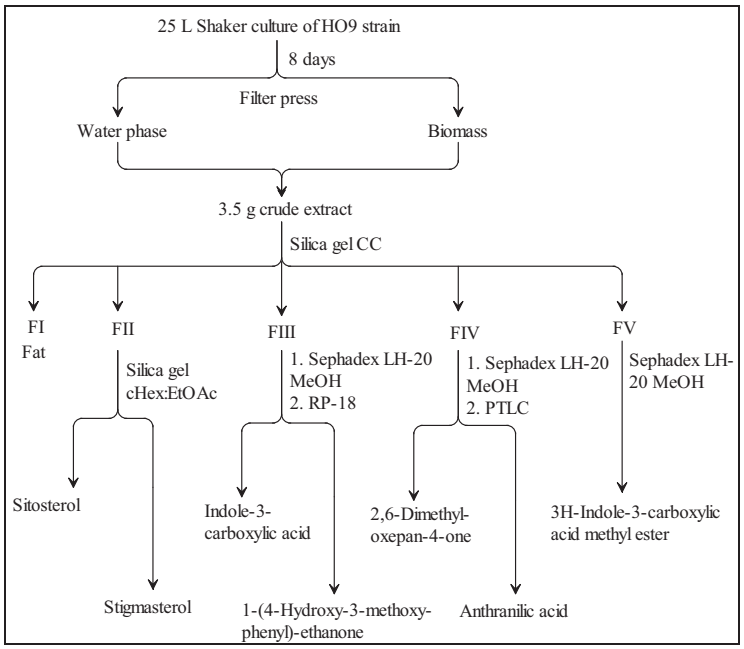
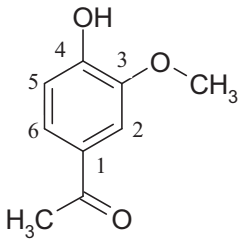


Figure 97: Work up scheme of *Streptomyces* sp. HO9

4.6.1 1-(4-Hydroxy-3-methoxy-phenyl)-ethanone

1-(4-Hydroxy-3-methoxy-phenyl)-ethanone (**103**) was obtained from fraction FIII as UV absorbing white solid. The ^{1}H NMR spectrum showed three aromatic proton signals, where the proton H-6 showed *ortho* and *meta*-coupling with protons H-5 and H-2, respectively. In addition, two 3H singlets were found at δ 2.49 and 3.85, which could be methyl and methoxy groups, respectively. The ^{13}C NMR spectrum showed

nine signals, four quaternary carbons were at δ 200.0, 158.7, 152.9 and 131.7, and three signals of aromatic carbons were at δ 128.7, 119.4 and 113.7. Moreover, carbon signals of a methyl and a methoxy appeared at δ 28.5 and 58.6, respectively; the ^{1}H and ^{13}C NMR data are shown in Table 26. The molecular weight of compound **103** was obtained by EIMS as 166, while HRESI/MS delivered the molecular formula $C_9H_{10}O_3$.



103

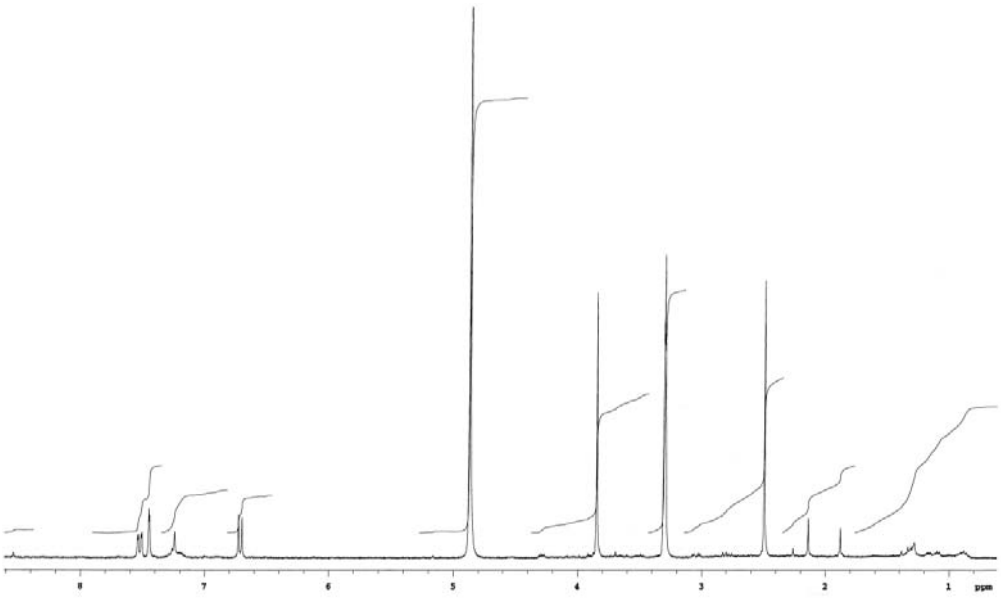


Figure 98: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**103**)

Due to the small sample size, not all signals were visible in the ^{13}C NMR spectrum. The proton H-6 (δ 7.52) showed COSY correlation with proton H-5 (δ 6.71) as well as HMBC couplings with C-4 (δ 131.9), C-2 (δ 113.7) and a carbonyl at δ 200.0. Proton H-5 (δ 6.71) supported the presence of both quaternary carbons C-4 (δ 131.9) and C-3 (δ 152.9) by displaying HMBC correlations. Proton H-2 (δ 7.24) displayed HMBC coupling with C-4 (δ 131.9) and a carbonyl at δ 200.0. A methyl singlet at δ 2.49 showed correlation with a carbonyl at δ 200.0 and the methoxy protons at δ 3.85

showed correlation with a quaternary carbon at C-3 (δ 152.9). These correlations were in agreement with structure **103**.

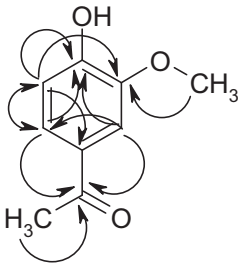


Figure 99: Selected H,H COSY and HMBC correlations of 1-(4-hydroxy-3-methoxy-phenyl)-ethanone (acetovanillone, **103**)

A search in AntiBase[113] using the spectroscopic information led to compound **103**, which known from synthesis and was isolated previously from plants and Arctic ice bacteria. It is known as acetovanillone and apocynin and was reported to have many activities: it acts as NADPH oxidase inhibitor for the management of atrial fibrillation,[171,172] it was reported also to be released from wood and to enrich the sensorial characteristics of the product.[173]

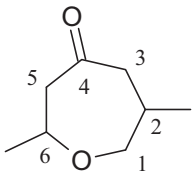
Table 26: ^{13}C and ^{1}H NMR data (125 MHz, 300 MHz) of acetovanillone (**103**) in CD_3OD

Position	δ_C	δ_H (mult.; *J* in Hz)
1	158.7	
2	113.9	7.44 (d, 2.0)
3	152.9	
4	131.7	
5	119.4	6.71 (d, 8.3)
6	128.7	7.52 (dd, 8.2, 2.0)
1-CO	200.0	
1- CH_3	28.5	2.49 (s)
3 -OCH_3	58.6	3.85 (s)

4.6.2 2,6-Dimethyl-oxepan-4-one

2,6-Dimethyl-oxepan-4-one (**104**) was isolated from fraction FII as colourless solid UV absorbing compound. The ^{1}H NMR spectrum showed no aromatic proton signals, but several aliphatic proton signals appeared, where the signal δ 4.20 may be due to an oxygenated methine; signals at δ 3.41 and 3.35 could be attributed to an oxygenated methylene group. Two further methylene signals appeared between δ 2.24

and 2.60 and one methine was at δ 2.15. Two doublets at δ 1.18 and 0.90 could be attributed to two methyl groups. The ^{13}C NMR spectrum showed eight carbon signals, where a quaternary carbon was at δ 211.7, three methylene groups at δ 67.7, 52.9 and 48.2. A methine carbon was found at δ 33.0 and two methyl groups gave signals at δ 23.5 and 17.1. CIMS afforded the molecular weight 142, while HRESI/MS delivered the molecular formula $C_8H_{14}O_2$.



104

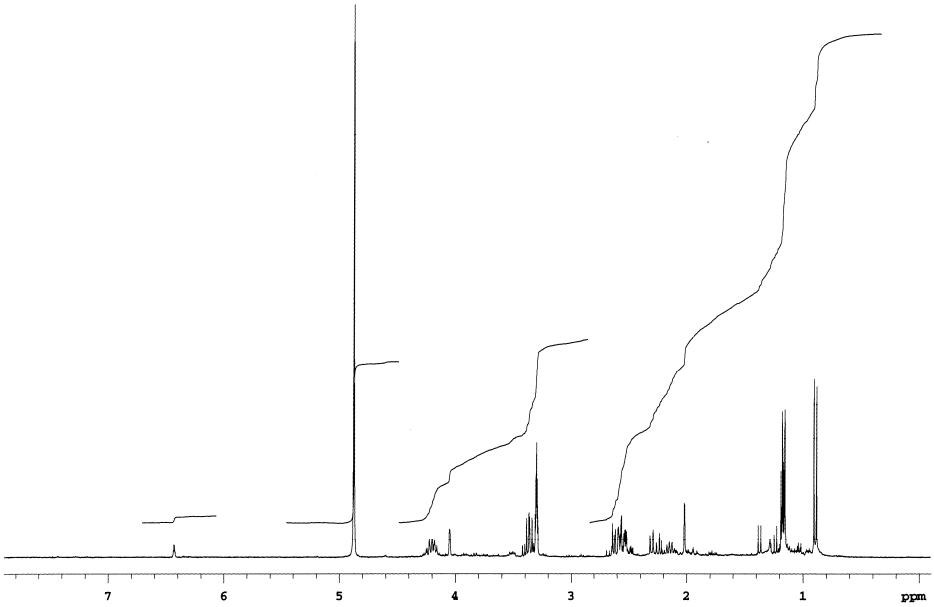


Figure 100: ^1H NMR spectrum (CD_3OD, 300 MHz) of 2,6-dimethyl-oxepan-4-one (**104**)

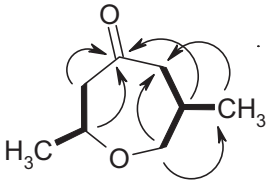
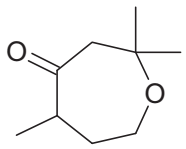


Figure 101: Selected H,H COSY and HMBC correlations of 2,6-dimethyl-oxepan-4-one (**104**)

Analysis of $^1H,^1H$ COSY, HSQC and HMBC spectra supported the structure **104**, where methylene protons at δ ~2.6 as well as both methine protons at δ 2.15 (H-2) and

4.20 (H-6) showed correlations to C-4; moreover COSY correlations were found from H-1 to H-3, H-6 to H-5, and both CH_3-6 and CH_3-2 groups coupled to H-6 and H-2, respectively (Figure 101). A search in AntiBase[113] with the spectroscopic data above confirmed that compound **104** is new compound. The related 2,2,5-trimethyl-4-oxepanone (**105**) has been synthesized by treating citronellol with fluorosulphonic acid at -78 °C.[174,175]



105

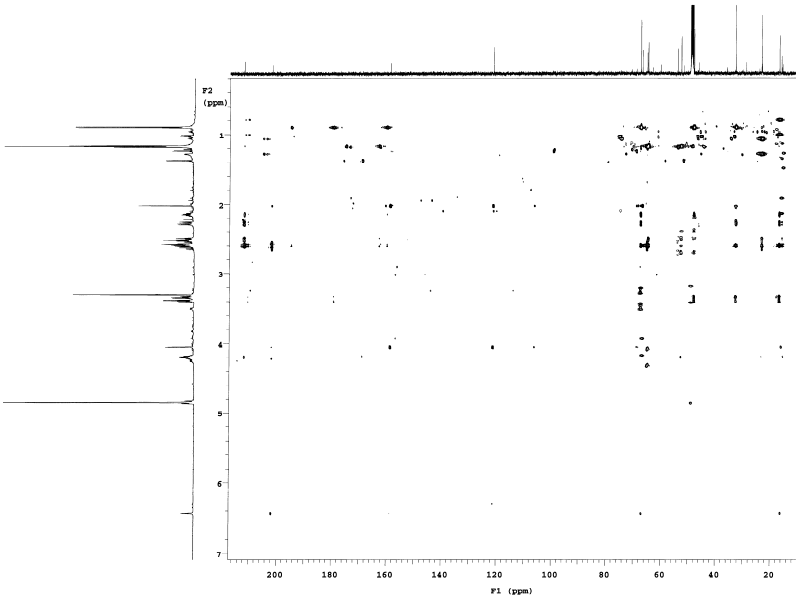
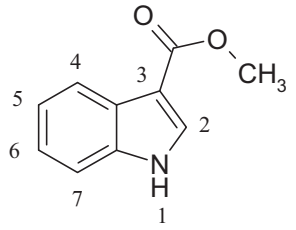


Figure 102: HMBC spectrum (CD_3OD, 300 MHz) of 2,6-dimethyl-oxepan-4-one (**104**)

4.6.3 Indole-3-carboxylic acid methyl ester

Indole-3-carboxylic acid methyl ester (**106**) was isolated as solid, which turned to orange with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum displayed five aromatic protons: two appeared as two doublets at δ 7.54 (H-4) and 7.34 (H-7) and further two protons appeared as triplets at δ 7.08 (H-5) and 7.02 (H-6), which gave the pattern of an ortho-disubstituted benzene ring. A singlet at δ 7.18 (H-2) was typical for the indole system, and a methoxy singlet was observed at δ 3.65. ESIMS delivered a molecular weight *m/z* 175. A search in AntiBase[113] showed identity with indole-3-carboxylic acid methyl ester (**106**).



106

4.7 *Bacillus* sp. M10

A culture of *Bacillus* sp. M10 was collected from Northern Pakistan and identified by Muaaz AL-AJLANI. It was cultivated on LB-medium in a 15 L shake culture to obtain 8.64 g crude extract. In the chemical screening the crude extract showed on TLC two UV absorbing spots, which gave a black colour with anisaldehyde/sulphuric acid.

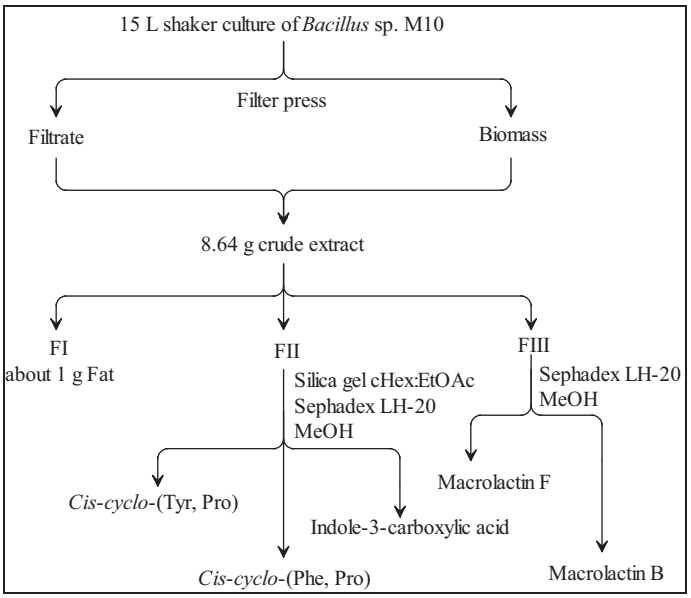


Figure 103: Work up scheme of *Bacillus* sp. M10

4.7.1 *Cis-cyclo*-(Tyr,Pro) and *cis-cyclo*-(Phe,Pro)

Cis-cyclo-(Tyr,Pro) (**107**) was isolated as a mixture with *cis-cyclo*(Phe,Pro) (**109**). The ^{1}H NMR of **107** showed two aromatic A_2B_2 signals at δ 7.02 and 6.70 indicating a 1,4-disubstituted benzene ring. Further two methine signals and four methylene proton signals were observed in the aliphatic region. The molecular weight 260 was afforded by (-)-ESIMS. A search in AntiBase[113] led to the two isomers *trans-cyclo*-(Tyr,Pro) (**108**) and *cis-cyclo*-(Tyr,Pro) (**107**). After comparison of the chemical shifts of H-3 and CH_2-10 with the two isomers and comparing the spectroscopic data with the literature, the structure was assigned as **107**.[151]

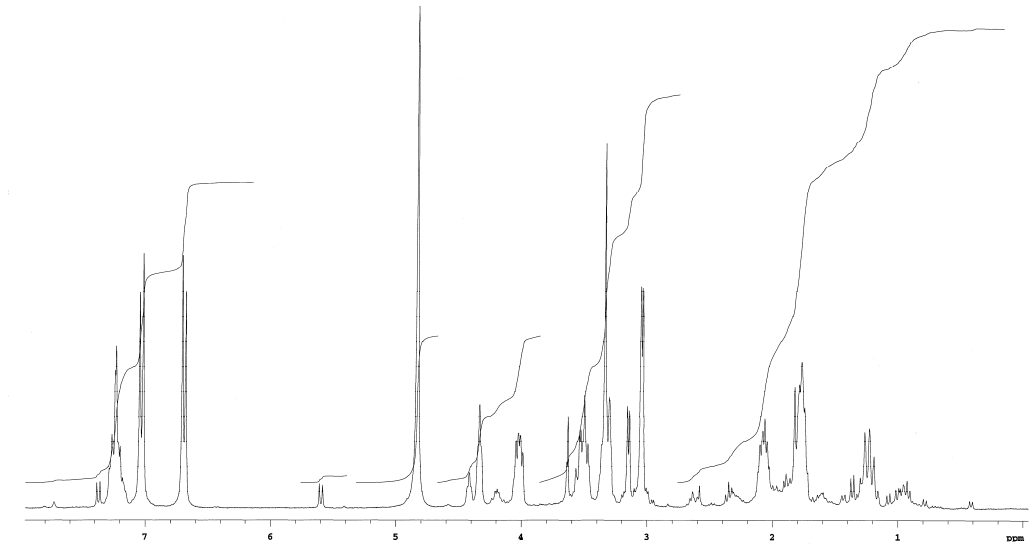
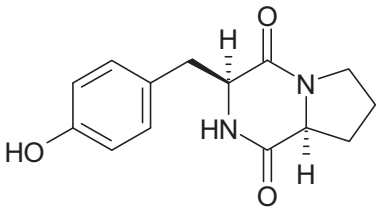
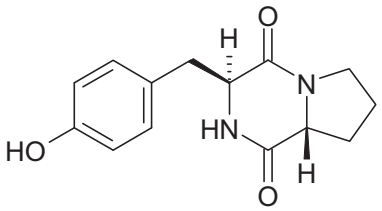


Figure 104: ^{1}H NMR spectrum of mixtures of compounds **107** and **108**

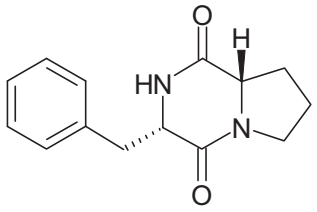


107

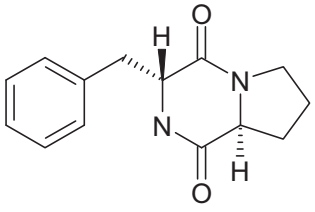


108

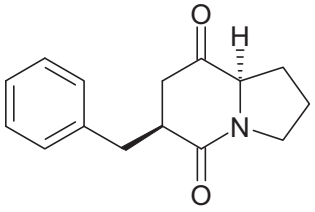
By means of the (+)-ESI mass spectrum (MW 244) and the NMR data, the minor component in the **107** mixture was identified as *cis-cyclo*(Phe,Pro) (**109**). Three isomers had previously described, *cis-cyclo*(Phe,Pro) (**109**)[176], *trans-cyclo*(Phe,Pro) (**110**),[177] and *cyclo*(D-Phe,D-Pro) (**111**).[178] The fourth stereoisomer is still unknown from nature.



109



110



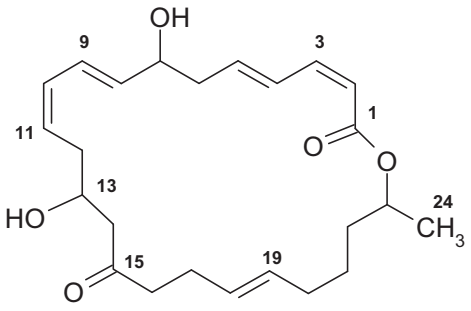
111

Compounds of this type are isolated regularly in our group as trivial contaminats, and so no further measurements were applied to assign the configuration.

4.7.2 Macrolactin F

Macrolactin F **(112)** was isolated as yellow oily UV absorbing compound, which turned to dark brown with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **112**

revealed ten olefinic proton signals between δ 7.3 and 5.4, moreover three oxymethines appeared at δ 4.98, 4.24 and 4.11, eight methylene proton signals were displayed between δ 1.4 and 2.6 and a methyl doublet was seen at δ 1.23. The molecular weight *m/z* 402 was determined by ESI MS, and ESI HRMS afforded the molecular formula $C_{24}H_{34}O_5$. A search in AntiBase[113] led to macrolactins A (**101**), F (**112**) and K. By comparison of the spectroscopic data, especially ^{1}H NMR spectra and the coupling constants with the literature,[131] the structure was confirmed as **112**. Macrolactin F was also isolated from deep see bacteria and identified in 1989.[162]



112

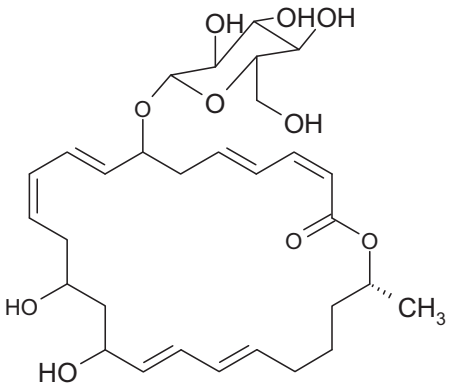
Table 27: ^{1}H NMR shifts (300 MHz) of macrolactin F (**112**) in CD_3OD

Position	δ_H (mult.; *J* in Hz)
1	
2	5.55 (d, 11.4)
3	6.64 (t, 11.4)
4	7.26 (dd, 15.8, 11.2)
5	6.18 (dd, 14.9, 8.3)
6	2.45 (t, 7.2)
7	4.24 (qn, 6.7)
8	5.74 (dd, 15.3, 6.0)
9	6.46 (dd, 15.2, 10.7)
10	6.09 (t, 11.2)
11	5.49 (d, 11.0)
12	2.38 (m)
13	4.11 (qn, 6.3)
14	2.56 (dd, 6.2, 4.1)
15	
16	2.47 (d, 7.4)
17	2.20 (m)
18	5.40 (m)
19	5.40 (m)
20	2.20 (m), 1.97 (m)
21	1.40 (m)
22	1.62 (m), 1.55 (m)
23	4.98 (m)
24	1.23 (d, 6.3)

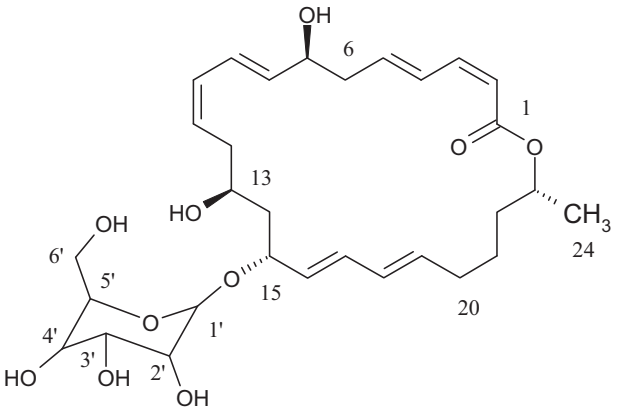
4.7.3 Macrolactin B

Macrolactin B (**113**) was isolated as colourless solid, was UV absorbing and turned to black with anisaldehyde/sulphuric acid spray reagent. The ^{1}H NMR spectrum of **113** revealed 12 olefinic proton signals between δ 7.2 - 5.5 as well as six methylene proton signals. A signal interpreted as an anomeric proton was observed at δ 4.32 (d, ^{3}J = 7.6 Hz) along with four oxymethine protons between δ 3.33 - 3.22, which established a sugar unit together with oxymethylene protons at δ 3.87 and 3.66. Moreover four oxymethine proton signals appeared between δ 5.01 - 3.92. In the upfield region, a methyl doublet was observed at δ 1.24 (CH_3-24). The ESI mass spectrum delivered the *pseudo*molecular ion peak at 587 $[M + Na]^+$. A search in AntiBase[113] using the obtained spectroscopic information led to the structure of macrolactins B (**113**) and C (**114)** which are positional isomers. Both compounds are having macrolactin A (**101**) as aglycon, but differ in the position of the sugar unit: Com-

pound **113** is connected with the sugar moiety at C-7, while compound **114** is connected at C-15. The experimental proton data were closer to those of macrolactin B (**113**) than those of macrolactin C (**114**), and accordingly the structure was assigned as **113**. The sugar moiety was reported to be connected as β-glucoside.[162] As the sample decomposed and no carbon spectra could be measured, a certain doubt may remain.



113



114

Table 28: ^{1}H NMR data (300 MHz) of macrolactin B (**113**) in CD_3OD

Position	δ_H (mult.; *J* in Hz)
1	
2	5.54 (d, 11.7)
3	6.63 (dd, 11.6, 11.2)
4	7.19 (dd, 14.5, 11.3)
5	6.18 (ddd, 14.8, 8.3, 5.8)
6	2.53 m, 2.59 m
7	4.49 (dd, 7.5, 5.2)
8	5.62 (dd, 15.0, 7.1)
9	6.72 (dd, 15.1, 11.0)
10	6.14 (dd, 15.0, 10.8)
11	5.59 (dd, 11.0, 6.7)
12	2.44 m
13	3.92 m
14	1.61 m
15	4.48 m
16	5.56 m
17	6.19 (dd, 14.3, 10.7)
18	6.02 (dd 14.7, 10.6)
19	5.58 m
20	2.13 m
21	1.49 m
22	1.58 m
23	5.01 m
24	1.24 (d, 6.3)
1'	4.32 (d, 7.6)
2'	3.24 (dd, 7.7, 8.8)
3'	3.33 (dd, 8.8, 1.2)
4'	3.30 (q, 1.6)
5'	3.22 m
6'	3.87 (dd, 11.9, 1.8)
	3.66 (dd, 11.8, 5.7)

4.8 Terrestrial *Streptomyces* sp. WO 521

In the pre-screening, the crude extract of the terrestrial *Streptomyce*s sp. WO 521 exhibited high activity against *Mucor miehei*, the plant pathogenic fungus *Aphanomyces cochlioides* and cytotoxicity against *Artemia salina*.

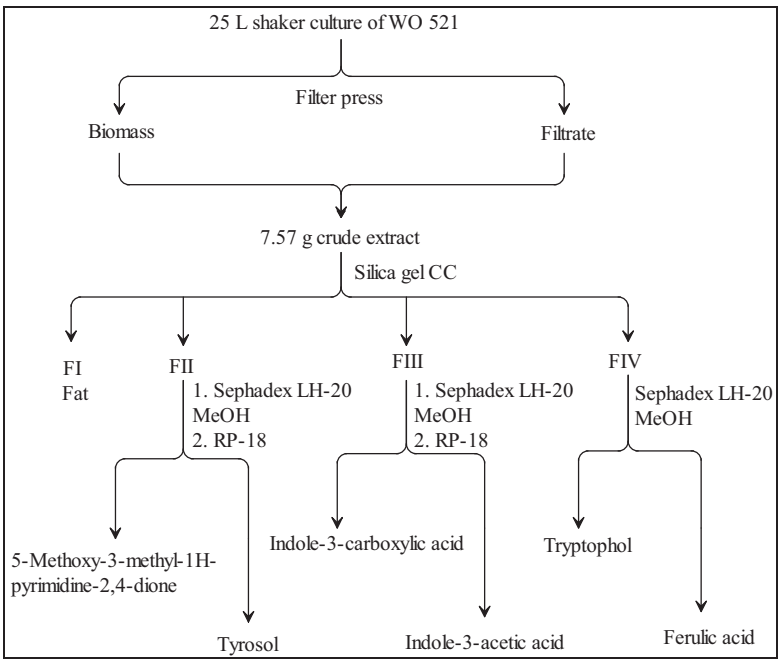
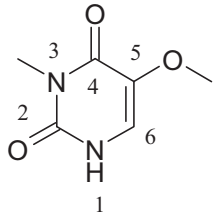


Figure 105: Work up scheme of *Streptomyces* sp. WO 521

4.8.1 5-Methoxy-3-methyl-1H-pyrimidine-2,4-dione

Compound **115** was isolated from a UV absorbing (254 nm) zone, which turned to pale brown with anisaldehyde/sulphuric acid. In the ^{1}H NMR spectrum, a 1H singlet at δ 7.92 was observed along with 3H singlets at δ 3.79 and 2.39. The EI mass spectrum revealed a molecular weight of 156, and the HRESI mass spectrum established the molecular formula $C_6H_8N_2O_3$. The ^{13}C NMR spectrum pointed three quaternary carbon signals, one olefinic, methyl and methoxy carbon signals.



115

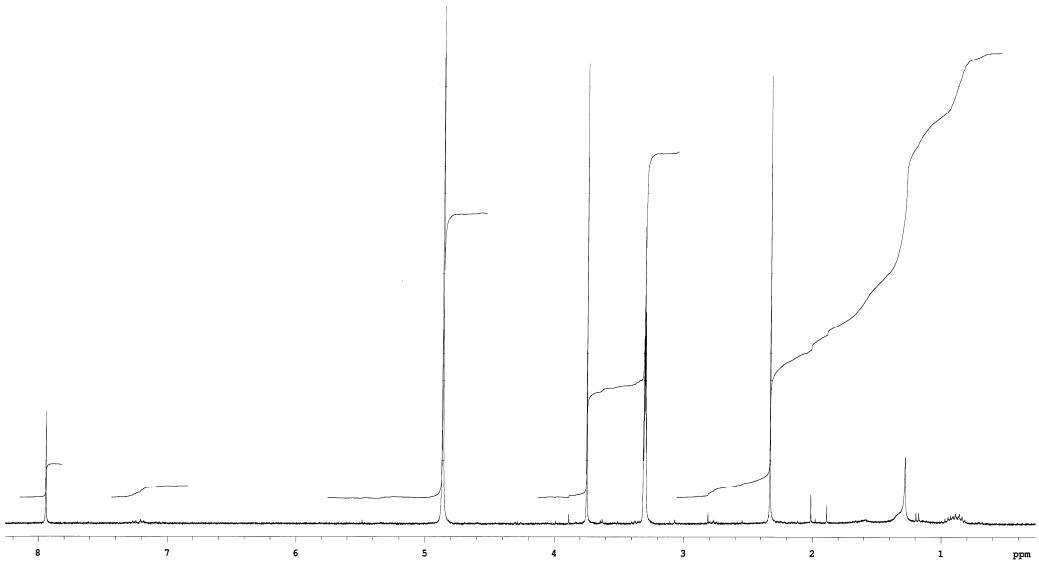


Figure 106: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of compound **115**

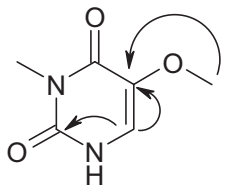


Figure 107: Selected HMBC correlations of compound **115**

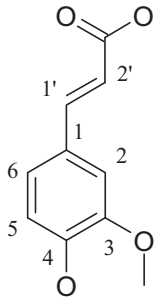
Analysis of HMBC spectrum revealed ^{3}J correlations from proton at δ 7.92 to carbon at δ 148.2 and carbonyl of amide at δ 152.0. A methoxy singlet at 3.79 showed HMBC correlations with quaternary carbon at δ 148.2.

A search in Chemical Abstract led to the structure of compound **115**, which is a new natural compound but known from synthesis.[179]

4.8.2 Ferulic acid

Ferulic acid (**116**) was isolated as colourless oil from a UV absorbing zone, which turned to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum indicated two 1H doublets at δ 7.55 and 6.23 with a coupling constant of 15.8 Hz indicating the presence of an α,β-unsaturated carbonyl fragment. A doublet of doublet was observed at δ 7.04 (H-6) in addition to further two doublets at δ 7.09 (H-2), 6.80 (H-5) and. The proton H-6 displayed *meta*-coupling with H-2 and *ortho*-coupling with H-5. One methoxy singlet was observed at δ 3.92. The EI mass spectrum delivered the molecular weight of 194 Dalton. A search in AntiBase[113] led to **116**, which was isolated

frequently in our group. Compound **116** has been reported recently as an strong agent in prevention of hypercholesterolemia.[180]



116

4.9 The Marine Derived *Streptomyces* sp. B6219

The crude extract of the marine derived *Streptomyces* sp. strain B6219 showed high antitumor activity against cancer cell lines with IC_{50} and IC_{70} values of 17.0 and 36.0 μg/ml, respectively. The chemical screening indicated an orange fluoresent band, which turned to violet with 2N NaOH, indicating the presence of a *peri*-hydroxyquinone. Additionally on TLC two middle and less polar UV absorbing zones were seen, which turned to yellow with anisaldehyde/sulphuric acid.

Agar plates of the strain B6219 were used to inoculate 30 L of M_2^+ medium (with 50% sea water) and the culture incubated on a linear shaker at 28 °C for 8 days. The culture broth was pressed through a pressure filter to afford biomass and filtrate. The filtrate was passed through XAD-16 resin and extracted with MeOH, while the biomass was extracted with EtOAc three times followed by acetone. The organic phases were brought to dryness. The combined crude extract (6.43 g) was defatted by cyclohexane and chromatographed on a silica gel column using a CH_2Cl_2/MeOH gradient to deliver four fractions FI-FIV. FII was purified on Sephadex LH-20 using MeOH to afford two sub-fractions. FIIa was later purified using PTLC and again Sephadex LH-20 using MeOH to afford a new bioactive angucyclinone, fujianmycin C (**119**), while FIIb after purification on Sephadex LH-20 afforded fujianmycin A (**118**) and ochromycinone (**120**). Separation of FIII on silica gel and Sephadex LH-20 delivered two sub-fractions: FIIIa afforded on PTLC fujianmycin B (**117**), while FIIIb was purified on Sephadex LH-20 to deliver ochromycinone methyl ether (**121**). FIV delivered in a similar way tetrangulol methyl ether (**122**).

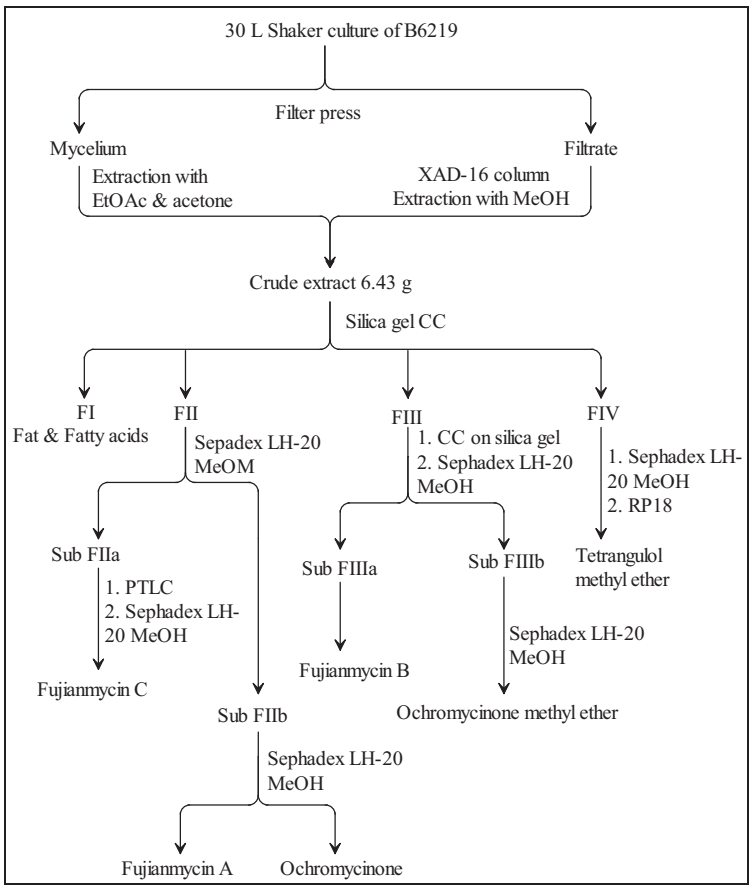
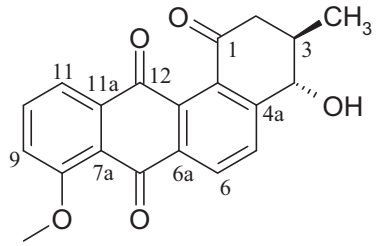


Figure 108: Work up scheme of *Streptomyces* sp. B6219

4.9.1 Fujianmycin B

Fujianmycin B (**117**) was isolated as yellow needles from fraction FIII using silica gel, Sephadex LH-20 and PTLC; it was UV absorbing at 254 nm. In the ^{1}H NMR spectrum, five proton signals were observed in the aromatic region: three adjacent protons of a trisubstituted benzene ring, and an AB system of two further protons at δ 8.22 (H-6) and 8.02 (H-5) in *ortho* position in another benzene ring. A methoxy signal appeared at δ 3.94l. The full ^{1}H and ^{13}C NMR data are listed in Table 29.

The ESIMS afforded the molecular weight 336 and HRESI/MS established the molecular formula $C_{20}H_{16}O_5$. A search in AntiBase with these data resulted in fujianmycin B (**117**): this suggestion was confirmed by comparing the spectroscopic information with literature data.[184]



117

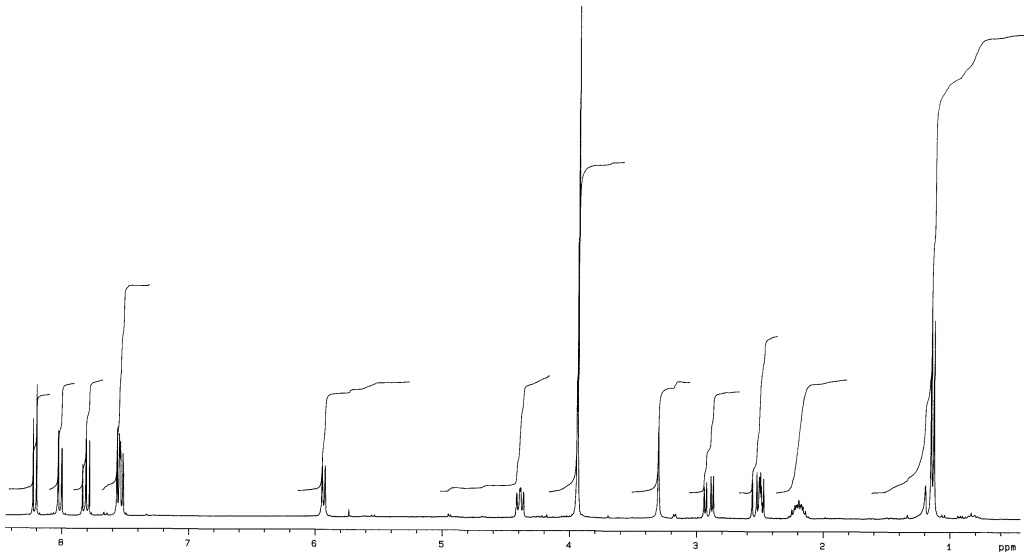


Figure 109: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of fujianmycin B (**117**)

Table 29: NMR shifts (125, 300 MHz) of fujianmycin B (**117**) in DMSO-d_6

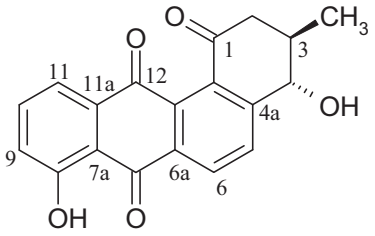
Position	δ_C		δ_H (mult.; J in Hz)
1	196.8	C	
2	44.4	CH_2	2.93, 2.55 (ABX, J_{AB} = 16.3, J_{AX} = 5.7, J_{BX} = 10.6)
3	37.7	CH	2.20 m
3-Me	18.1	CH_3	1.14 (d, ^3J = 6.6)
4	71.5	CH	4.39 (dd, ^3J = 6.8, 9.3)
4a	152.4	C_q	
5	131.1	CH	8.02 (d, ^3J = 8.1)
6	128.9	CH	8.22 (d, ^3J = 8.2)
6a	133.58[a]	C	
7	180.1	C	
7a	119.9	C	
8	159.5	C	
8-OMe	56.3		3.94 s
9	118.36[b]	CH	7.54 (d, ^3J = 8.5)
10	135.6	CH	7.81 (t, ^3J = 8.2, ^3J = 7.7)
11	118.33[b]	CH	7.56 (d, ^3J = 7.5)
11a	136.8	C	
12	183.6	C	
12b	134.7	C	
12a	133.56[a]	C	
4-OH	-		5.94 (d, ^3J = 6.8)

a, b: may be exchanged pairwise

4.9.2 Fujianmycin A

Fujianmycin A (**118**) was isolated as yellow needles after repeated purification of fraction FII on Sephadex LH-20. It was UV absorbing at 254 nm and changed to violet with 2 N NaOH indicating a *peri*-hydroxyquinone. As the isolated amount was very small, the purity was less than that of **117**. The ^{1}H NMR spectrum of **118** showed, however, the typical pattern of **117**, so that another fujianmycin derivative was expected. The pattern in the aliphatic region was very similar as well, however, the methoxy group was substituted by an OH group.

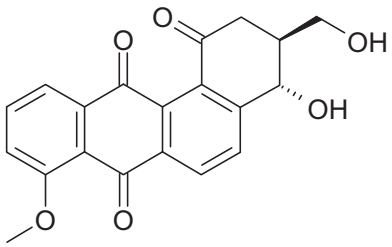
ESI MS delivered the molecular weight 322 and HRESIMS established the molecular formula as $C_{19}H_{14}O_5$. A search in AntiBase[113] using the above spectroscopic data led to fujianmycin A (**118**), which was confirmed by comparing with the literature data.[184,181]



118

4.9.3 Fujianmycin C

Fujianmycin C (**119**) was isolated from fraction FII (Figure 108) as yellow needles, was UV absorbing on TLC and turned to dark yellow with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **119** showed a similar spectroscopic pattern as for **117** and **118**, where the same five aromatic proton signals indicated the ABC system in the first ring and an AB system in the other benzene ring.



119

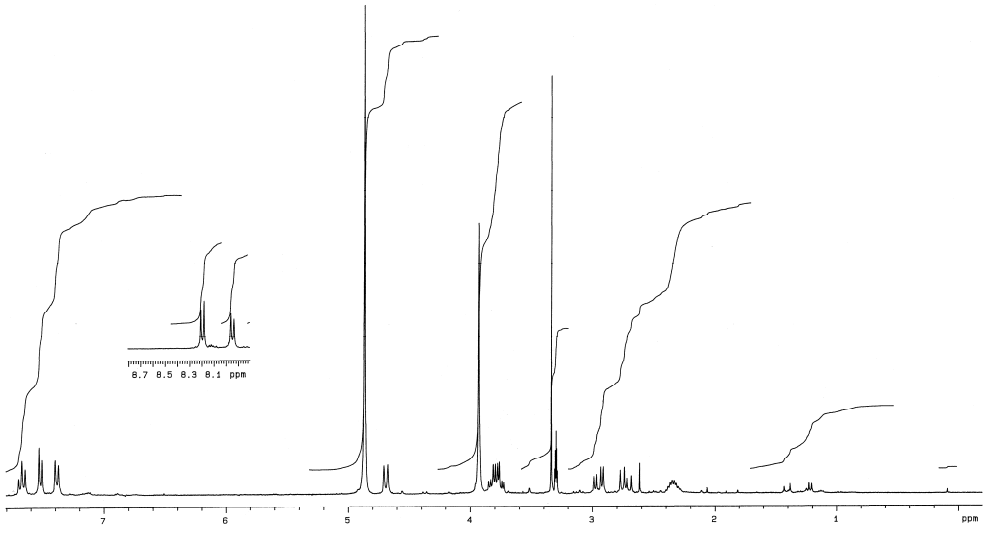


Figure 110: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of fujianmycin C (**119**)

In the aliphatic region there were a methoxy singlet at δ 3.95, two methylene groups at δ 3.82/3.75 (CH_2-13) and 2.99/2.77 (CH_2-2) and two methine signals at δ 4.75 (H–4) and δ 2.37 (H–3). In the ^{13}C NMR spectrum, 20 carbon signals were

visible: one ketone carbonyl, two carbonyl signals of a quinone or acid derivatives, twelve sp^2 and five sp^3 carbon signals.

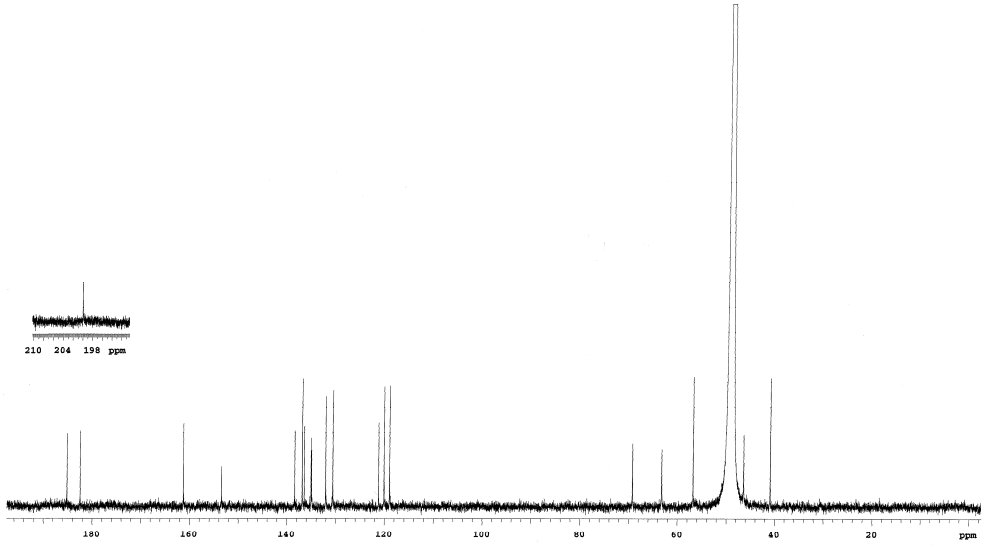
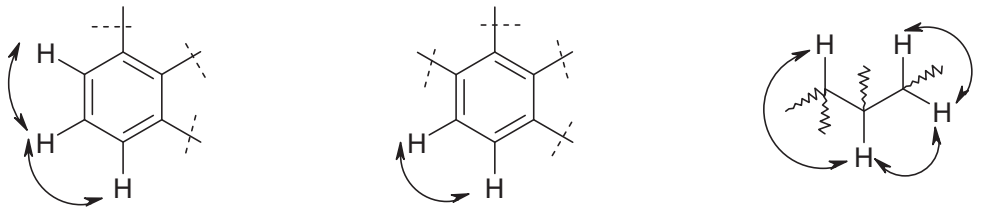


Figure 111: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of fujianmycin C (**119**)

The detailed NMR spectroscopic data were listed in Table 30. The CI mass spectrum pointed to a *pseudo*molecular ion at 353 $[M + H]^+$, which gave the molecular weight of 352 Dalton. HRESI/MS of (**119**) delivered the formula $C_{20}H_{16}O_6$. As a search in AntiBase delivered no hits, 2D spectra were measured.

The ^1H-^1H COSY spectrum confirmed the benzene ring with three adjacent protons as in fragment a (Figure 112) and the *ortho*-coupled protons in another benzene ring as in fragment b. Beside one methylene there were two adjacent methine groups present in an aliphatic moiety as drawn in fragment c.



Fragment **a** Fragment **b** Fragment **c**

Figure 112: ^1H,^1H COSY correlations of fujianmycin C (**119**)

A quinone nucleus was predicted on the basis of colour on TLC and the presence of carbonyl signals at δ 182.7 and 185.4. From HMBC correlations, the proton at car-

bon 10 showed correlations with C-8 and 11a. The deep-field proton signal at δ 7.66 showed a correlation with a quinone carbonyl and must be in *peri*-position with respect to C-12. Further correlations confirmed the methoxy group in the same ring. Proton H-6 correlated with the second quinone carbonyl (C-7) and must be in the opposite *peri* position of a second ring.

Further HMBC and COSY correlations completed the angucyclinone skeleton as drawn in Figure 113. The relative configuration was supposed to be the same *trans* orientation as for fujianmycins A (**118**) and B **(117**): Forcefield calculations resulted in a bis-equatorial orientation of the substituents at C-3 and C-4, which afforded a coupling constant of $J_{H\text{-}3,4} \sim 10$ Hz. This agrees well with the experimental value of 9.3 Hz for H-4. The small NOE between 3-H and 4-H is also observed for fujianmycin B **(117**) and was neglected. As the observed optical rotation of **119** is nearly the same as for **117** and as their structures are closely related, also their absolute configurations should be the same The new fujianmycin derivative **119** is the methyl ether of YM-181741[182,183] and the second natural hydroxymethyl-angucyclinone.

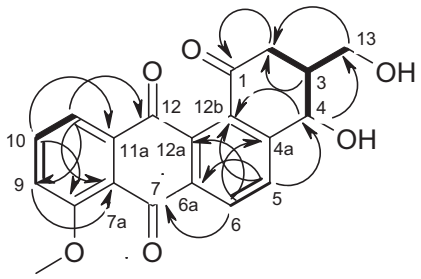


Figure 113: Selected H,H COSY and HMBC correlations of fujianmycin C (**119**)

Fujianmycins are members of the angucyclinone family. Currently, more than 40 derivatives are known; most are found as free aglyca, but all of them are oxygenated at C-4. While the related linear anthracyclinones are usually having low pharmaceutical activities, the angucyclinones show pronounced antibacterial properties.[184] Fujianmycin C (**119**) was tested in our lab and exhibited antibacterial activity against *Streptomyces viridochromogenes* Tü57 by causing inhibition zones of 12 mm at 40 µg/disk.

Table 30: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of fujianmycin C (**119**) in CD_3OD

Position	δ_C		δ_H (mult.; J in Hz)
1	200.0	C	
2	41.0	CH_2	2.99, 2.77 (ABX, J_{AB} = 16.2, J_{AX} = 6.3, J_{BX} = 10.4)
3	46.4	CH	2.37, m
4	69.4	CH	4.75 (d, ^3J = 9.3)
4a	153.6	C_q	
5	132.3	CH	8.04 (d, ^3J = 8.2)
6	130.7	CH	8.33 (d, ^3J = 8.2)
6a	135.1	C	
7	182.7	C	
7a	121.5	C	
8	161.5	C	
8-OMe	56.9		3.95 s
9	119.1	CH	7.51 (d, ^3J = 8.5)
10	137.0	CH	7.79 (t, ^3J = 8.4, ^3J = 7.6)
11	120.2	CH	7.66 (d, ^3J = 7.6)
11a	138.6	C	
12	185.4	C	
12a	135.3	C	
12b	136.7	C	
13	63.3	CH_2	3.82, 3.75 (ABX, J_{AB} = 10.9, J_{AX} = 5.5, J_{BX} = 4.6)

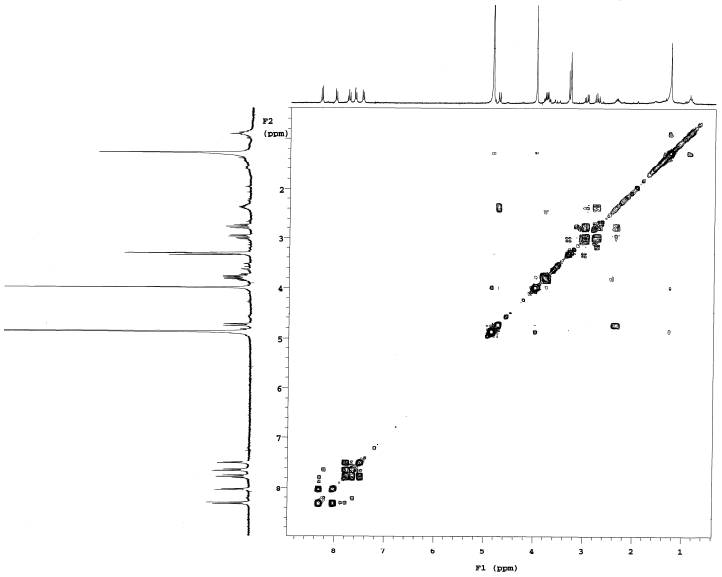


Figure 114: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) of fujianmycin C (**119**)

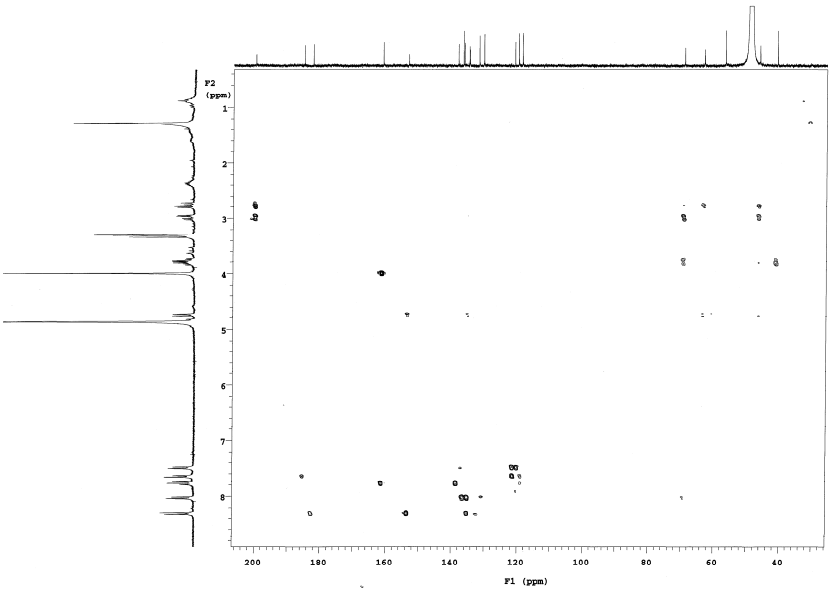
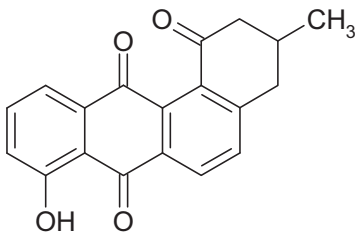


Figure 115: HMBC spectrum (CD_3OD, 300 MHz) of fujianmycin C (**119**)

4.9.4 Ochromycinone

Ochromycinone (**120**) was isolated from fraction FII after repeated purification on Sephadex LH-20 using MeOH. Compound **120** reacted with 2N NaOH and changed to violet indicating a *peri* hydroxyquinone. It was obtained as yellow needles, which showed an orange fluorescence under UV light. The ^{1}H NMR spectrum of **120** showed the same aromatic pattern as the pervious quinones, while in the aliphatic region the hydroxy group on C-4, which was observed in compounds **119**, **118** and **117**, was absent. [185]

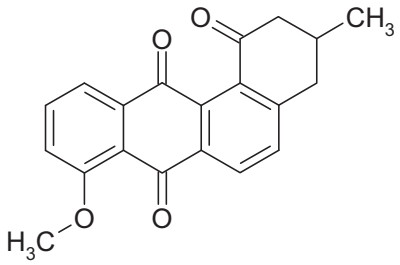


120

4.9.5 Ochromycinone methyl ether

Ochromycinone methyl ether (**121**) was isolated as yellow needles from fraction FIV after repeated separation on silica gel followed Sephadex LH-20. It was UV absorbing and turned to yellow with anisaldehyde/sulphuric acid, but did not react with sodium hydroxide. The ^{1}H NMR spectrum revealed a similar spectroscopic pattern as for **121**, but gave in addition a methoxy singlet instead of a hydroxy group at C-8.

ESIMS revealed the molecular weight 320, and HRESIMS established the molecular formula $C_{20}H_{16}O_4$. Structure **121** was confirmed by searching in AntiBase[113] and comparing the spectroscopic information with literature data.[185]

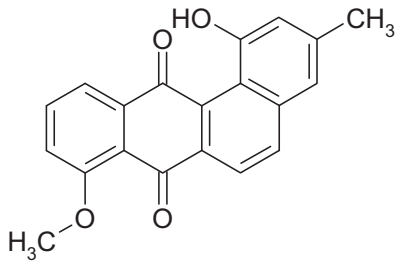


121

The fujianmycins and the ochromycinone derivatives represent a third class of antibiotics after the tetracyclines and anthracyclines.[37] The angucyclines/angucylinones and their derivatives are known for their antitumor activity.[186]

4.9.6 Tetrangulol methyl ether

Tetrangulol methyl ether (**122**) was isolated from fraction FV after purification on Sephadex LH -20 followed by RP18. It was obtained as yellow UV absorbing needles. The ^{1}H NMR spectrum of compound **122** was different from **121** and showed instead of aliphatic multiplets two new aromatic doublets at δ 7.27 (H-4) and 7.09 (H-2), indicating another benzene ring with protons in *meta*-position. ESIMS delivered the molecular weight 318, while HRESIMS established the molecular formula $C_{20}H_{14}O_4$. A search in AntiBase[113] and comparison of the above spectroscopic data with literature values confirmed the structure **122**.[187]



122

Tetrangulol methyl ether (**122**) possessed antibacterial activity and is non-competitively inhibiting the prolyl-endopeptidases of *Flavobacterium meningosepticum* (IC_{50} = 8.9 μM) or enhanced the cytotoxicity of colchicine, respectively.[188] It has got special interest as an inhibitor of mycobacteria[189] and of *Helicobacter pylori*.[190,191] Tetrangulol methyl ether (**122**) is an aromatisation product of angucycline

precursors. The biosynthetic pathway of this type of angular quinones can be started with the condensation of a respective number of acetate residues via the acetate pathway by means of a minimal polyketide synthase (minPKS) complex, which consists of two ketosynthases and an acyl carrier protein (ACP) at which the growing polyketide chain is attached. Subsequently, the polyketide chain is folded to form different aromatic compounds by various ketoreductases, cyclises and aromatases.[192] The suggested biosynthetic pathway is drawn in Figure 116.

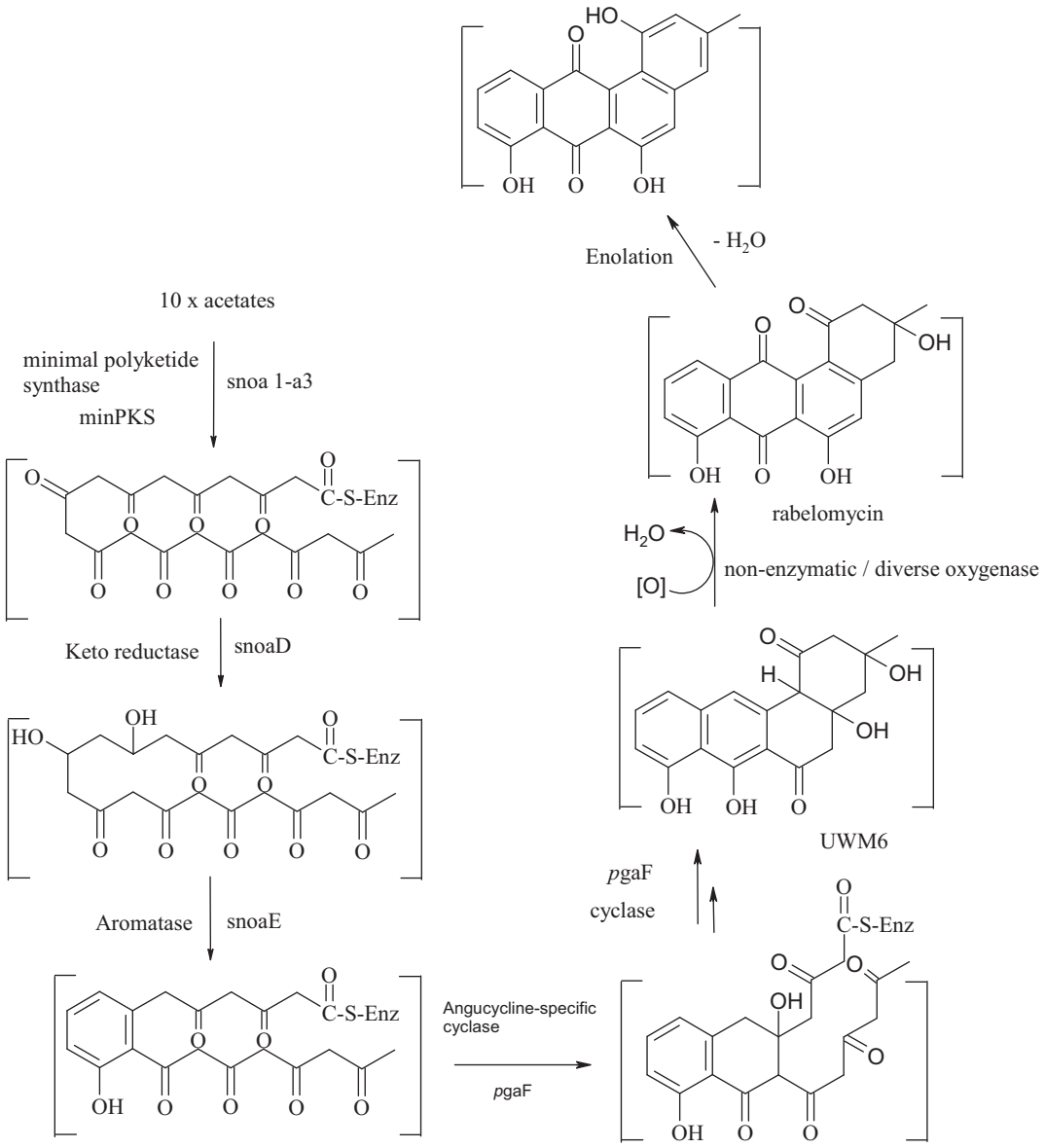


Figure 116: Biosynthetic pathway of angular quinones [192]

4.10 Marine derived *Streptomyces* sp. B5746

The crude extract of the marine derived *Streptomyces* sp. B5746 did not exhibit any antimicrobial activities in our tests. In the chemical screening it showed three UV absorbing bands, which turned to yellow with anisaldehyde/sulphuric acid.

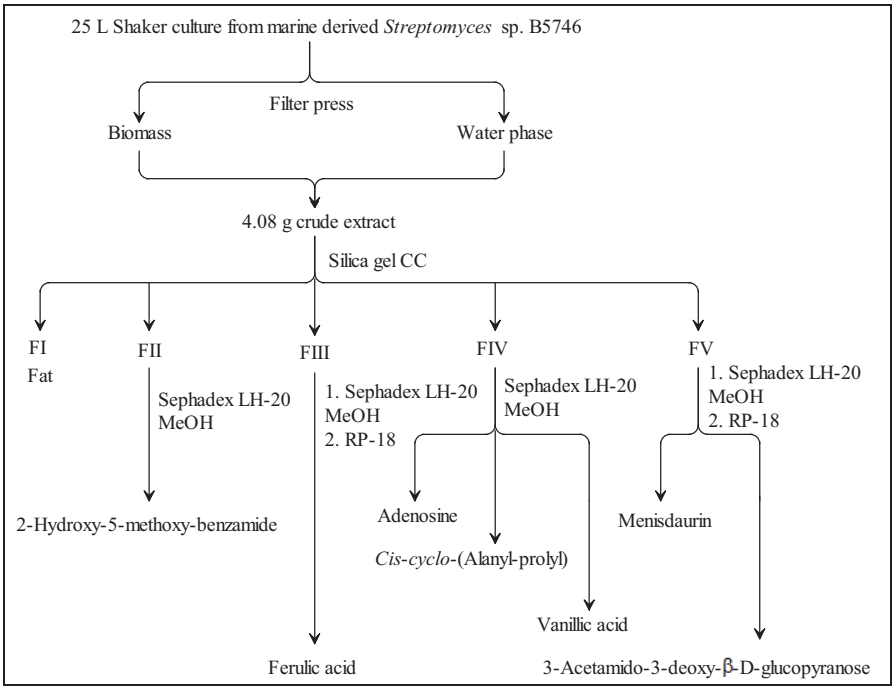


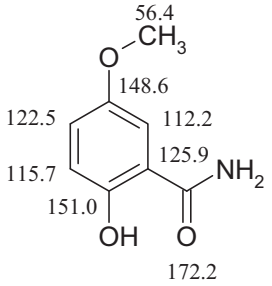
Figure 117: Work up procedure of B5746

The well grown agar culture of the marine derived *Streptomyces* sp. B5746 was used for the inoculation of 100 of 1 L Erlenmeyer flasks each contained 250 ml M_2^+ medium (with 50% sea water). The culture was kept at 28 °C on a linear shaker for 6 days. The dark brown culture broth was filtered to afford the filtrate and the biomass. Both fractions were extracted, evaporated and combined to deliver the crude extract (4.08g). The extract was fractionated over silica gel column chromatography using CH_2Cl_2/MeOH gradient to afford five fractions FI to FV. FII was separated on Sephadex LH-20 using MeOH to afford 2-hydroxy-5-methoxy-benzamide (**123**). Both FIII and FIV were purified on Sephadex LH-20 using MeOH to deliver ferulic acid (from FIII) adenosine and and *cis*-cyclo-(Alanyl-prolyl) and vanillic acid. Where FV was subjected to Sephadex LH-20 followed by reverse phase RP-18 to obtain menisdaurin (**124**) and 3-acetamido-3-deoxy-β-D-glucopyranose (**125**).

4.10.1 2-Hydroxy-5-methoxybenzamide

2-Hydroxy-5-methoxybenzamide (**123**) was isolated as colourless solid from a UV absorbing band, which turned to blue with anisaldehyde/sulphuric acid. The ^{1}H NMR

spectrum showed three aromatic protons; one of them appeared as doublet of doublet at δ 7.40 (H-4) and displayed an *ortho*-coupling with the neighbouring proton at δ 6.82 (H-3) and a *meta*-coupling with the proton at δ 7.47 (H-6). A methoxy singlet was observed at δ 3.89. The ^{13}C NMR spectrum revealed eight signals: one carbonyl appeared at δ 172.2 indicating an acid or amide derivative, and three quaternary carbons were seen at δ 151.0, 148.6 and 125.9. Additionally, three aromatic carbons appeared at δ 122.5, 115.7 and 112.2 and a methoxy carbon was seen at δ 56.4. ESIMS afforded the molecular weight 166 and HRESI MS established the molecular formula as $C_8H_9NO_3$.



123

Table 31: ^{13}C and ^{1}H NMR shifts of 2-hydroxy-5-methoxy-benzamide (**123**)

Position	δ_C	C type	δ_H multi.; *J* in [Hz]
1	125.9	C_q	
2	151.0	C_q	
3	115.7	CH	6.82 (d, 8.3)
4	122.5	CH	7.40 (dd, 8.3, 2.1)
5	148.6	C_q	
6	112.2	CH	7.47 (d, 2.0)
1-CO	172.2	C_q	
5-OCH_3	56.4	CH_3-O	3.89 (s)

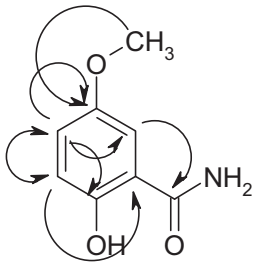
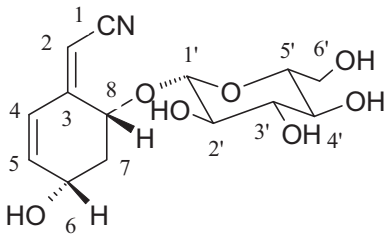


Figure 118: Selected H,H COSY and HMBC correlations of 2-hydroxy-5-methoxy-benzamide (**123**)

The connectivity of structure **123** was established on the basis of the H,H COSY and HMBC correlations as assigned in Figure 118. A search in AntiBase[113] and the Chemical Abstracts confirmed that compound **123** is known from synthesis,[193] but was now isolated from a biological source for the first time.

4.10.2 (Z)-1-Cyanomethylene-4(*2R*)-hydroxy-6(S)-(β-glucopyranosyloxy)-2-cyclohexene (Menisdaurin)

Menisdaurin (**124**) was isolated from fraction FV as white UV absorbing solid, which turned to yellow with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed two vinyl protons at δ (6.29, dd) and (6.20, dd) respectively; another vinyl proton appeared as singlet at δ 5.50. An oxymethine was observed as doublet of doublet at δ 4.93. It was obvious that the compound contained a sugar moiety due to the presence of six oxmethine signals. An anomeric proton signal appeared as doublet at δ 4.54. Two doublets of doublets of an oxymethylene appeared at δ 3.89 and 3.67, four oxymethine signals were obtained between δ 3.28 and 3.37 related to the sugar unit. Additionally an oxymethine appeared as triplet at δ 4.37. In the upfield region, a methylene moiety was observed at δ 2.26 and 2.02.



124

The ^{13}C NMR spectrum revealed fourteen signals: two quaternary carbons appeared at δ 157.0 and 117.9, three olefinic carbon signals were obtained at δ 140.5, 127.6 and 96.8. A typical anomeric carbon was observed at δ 101.5 along with six oxymethine signals between δ 65.4 and 78.2. An oxymethylene carbon of a sugar unit was found at δ 63.2 and a methylene carbon occurred at δ 36.2. ESIMS delivered the *pseud*omolecular ion peak at 359 $[M + Na]^{+}$, while HRESIMS established the molecular formula $C_{14}H_{19}NO_7$.

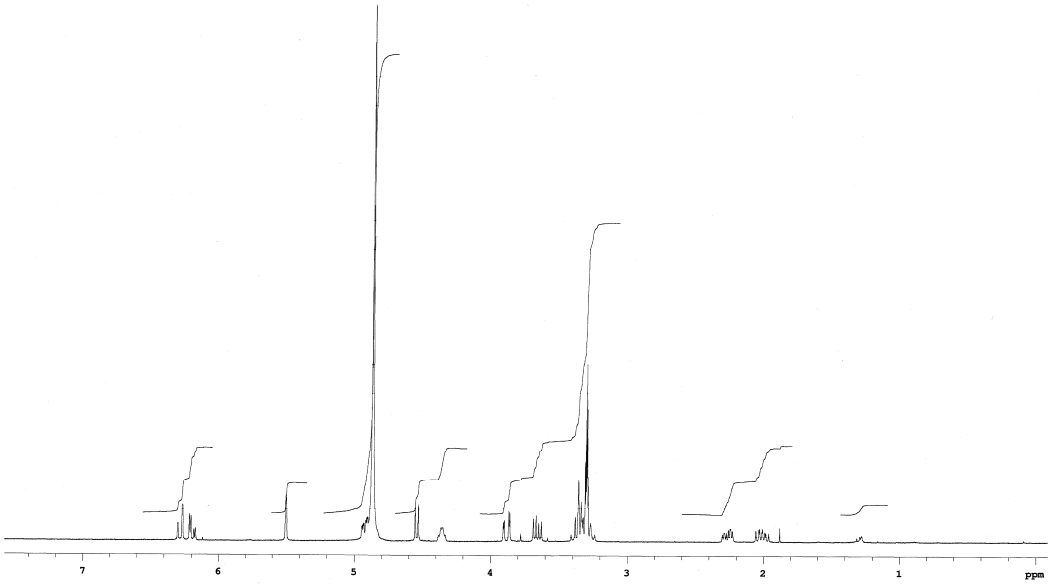


Figure 119: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of menisdaurin (**124**)

H,H COSY spectrum displayed several correlations from H-4 to H-8 and HMBC correlations assigned the sugar unit as a hexose. Further HMBC correlations confirmed the presence of hexene ring, which was connected with the sugar at C-8, where the proton H-1' showed ^{3}J correlations with the carbon C-8 at δ 72.5. A signal at δ 117.9 (C_q-1) gave solely a correlation with H-2. As an odd number of sp^2 carbon signals was found, this was tentatively assigned to a nitrile carbon: this was finally confirmed by the missing two double bond equivalents. The proton H-2 showed an additional ^{3}J coupling with the olefinic carbon at δ 127.6.

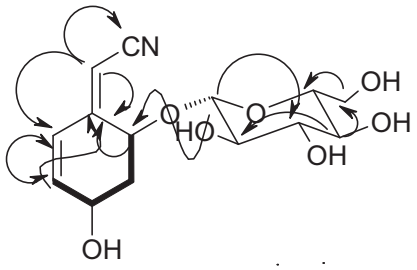


Figure 120: H,H COSY and HMBC correlations of menisdaurin (**124**)

Table 32: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) for menisdaurin (**124**) in CD_3OD

Position	δ_C	δ_H (mult.; *J* in Hz)
1	117.9	
2	96.8	5.50 (s)
3	157.0	
4	127.6	6.29 (dd, 10.5, 1.4)
5	140.5	6.20 (dd, 10.0, 3.6)
6	65.4	4.37 (dd, 10.0, 6.5)
7	36.2	2.26 (ABX, 13.3, 8.9) 2.02 (ABX, 13.3, 6.4)
8	72.5	4.93 (ddd, 5.1, 4.5, 1.5)
1'	101.5	4.54 (d, 7.3)
2'	71.7	3.28 (dd, 7.3, 8.6)
3'	78.0	3.32 (dd, 8.7, 1.9)
4'	74.5	3.33 (dd, 1.9)
5'	78.2	3.37 (dd, 6.0, 1.6)
6'	63.1	3.89 (ABX, 11.8, 2.0) 3.67 (ABX, 11.8, 6.0)

A search with the substructures in AntiBase[113] did not deliver a plausible hit, but the Dictionary of Natural Products and the Chemical Abstracts pointed to menisdaurin (**124**), a cyanide-releasing nitrile glucoside, which was reported as secondary metabolite from medicinal plants such as *Purshia tridentata* DC.,[194] *Cowania mexicana*,[195] *Bauhinia sirindhorniae*,[196] *Guazuma ulmifolia***,** *Ostrya virgininana***,** *Tiquilia plicata* and *Tiquilia canescens*[197] and *Ehretia longiflora* champ**.**[198]. The sugar moiety was reported to be β-glucopyranose. Compound **124** has now been isolated for the first time from bacteria.

These findings lead to the interesting relationship between endophytic bacteria and their host plants. Endophytic microorganisms can co-operate with their host plant by producing secondary metabolites that can protect the plant providing the ability to survive.[199] Such interactions can explain the presence of the same natural compounds in both microorganisms and host plants. *Streptomyces* is widely distributed, and the first endophytic *Streptomyces* sp. was isolated from *Lolium perenne*, a grass species: The plant afforded a diketopiperazine named methylalbonoursin.[200] Another endophytic *Streptomyces* sp. (NRRL 30566) was isolated from a fern-leaved *Grevillea pteridifolia* tree, and it delivered novel kakadumycin antibiotics, which are closely related with the echinomycins.[201] It is expectable in this respect that endophytes such as bacteria or fungi can produce these secondary metabolites also inside the host

plant; at least for compounds from *Erythrina crista-galli,* this has been confirmed.[202] Further studies and contributions of biologist, chemist and ecologist are still required in this exciting field.

The biosynthetic pathway of menisdaurin began from the precursor tyrosine (Tyr) via allylic rearrangement and ketonization as drawn in Figure 121.

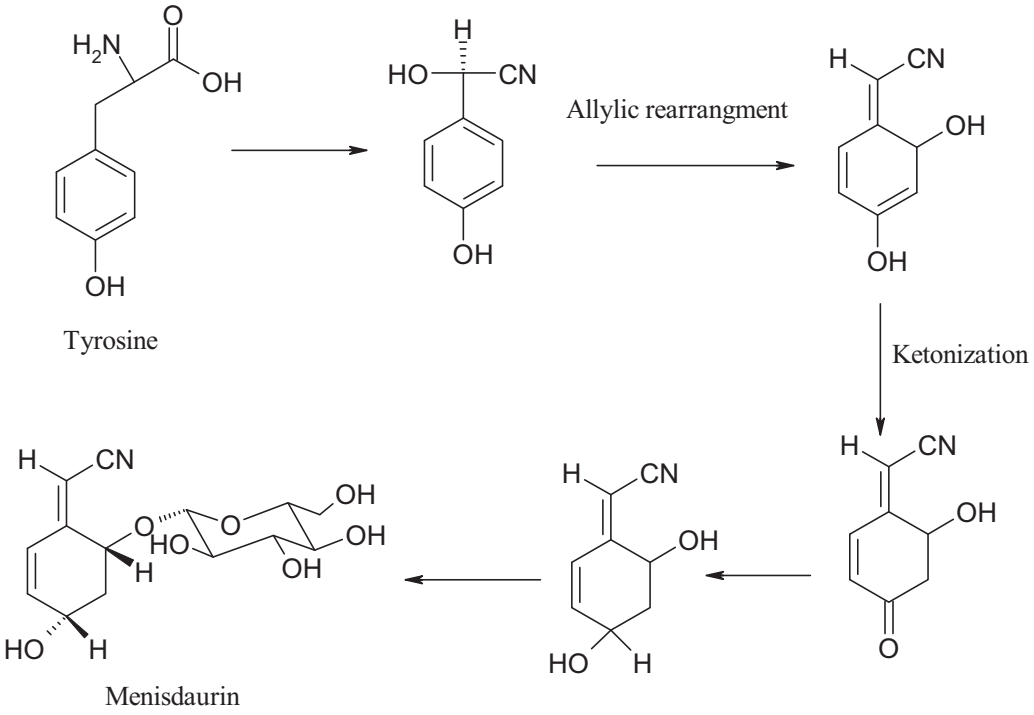
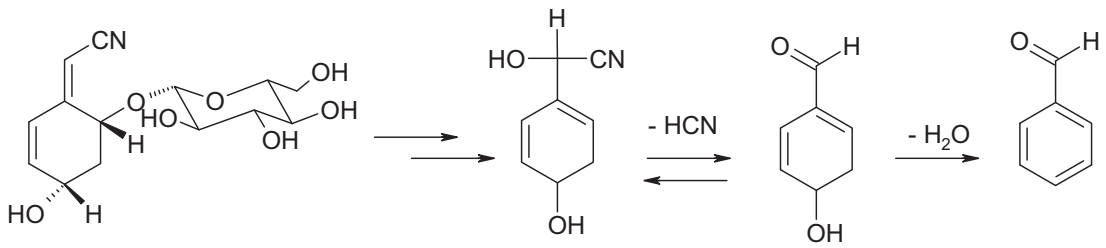


Figure 121: Proposed biosynthetic pathway of menisdaurin (**124**)

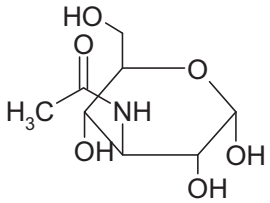
The reversion of this pathway would end up in a derivative of *p*-hydroxybenzaldehyde (or benzaldehyde) and the toxic HCN and explain the ecological purpose of this compound as cyanogenic glycoside:



4.10.3 3-Acetamido-3-deoxy-β-D-glucopyranose

3-Acetamido-3-deoxy-β-D-glucopyranose (**125**) was isolated as mixture with further sugar derivatives as white UV absorbing solid, which turned to blue with anisalde-

hyde/sulphuric acid. The ^{1}H NMR spectrum of **125** showed overlapping oxymethine protons between δ 3.30–4.00 indicating the presence of a sugar. A 1H doublet at δ 4.46 is a typical signal for an anomeric proton, and a methyl singlet at δ 1.98 could be connected to a carbonyl group, as no double bonds were visible. ESIMS delivered the *pseudo*molecular ion peak at 244 $[M + Na]^+$, and HRESIMS established the molecular formula $C_8H_{15}NO_6$. A search in AntiBase[113] using above spectroscopic information led to 3-acetamido-3-deoxy-β-D-glucopyranose, which was isolated from *Streptomyces* sp. for the first time in 1980. It was assigned as one of a very few antibiotics that are delivered by both a unicellular bacterium (family Bacillaceae) and a filamentous bacterium (family Streptomycetaceae).[203] If the isolated product contained indeed **125** remained open, as well as the nature of further components in the mixture.



125

5 Metabolites from Endophytic Fungi

5.1 Tropical Endophytic Fungus *Gaeumannomyces amomi* BCC4066

A zingiberaceous isolate BCC4066 of the fungus *Gaeumannomyces amomi* was obtained from healthy leaves and pseudostem of *Alpinia malaccensis* and was described as new endophyte.[204] *G. amomi* BCC4066 had weak activities against *Penicillium avellaneum* and *Bacillus subtilis* but was highly active against *Candida albicans*.

The sub-culture of the endophytic fungus *G. amomi* BCC4066 was used to inoculate 20 L of a corn meal medium and incubated for 21 days at 20 °C on the fermentor to give the mycelium and the filtrate. The mycelium was extracted three times with ethyl acetate and acetone, respectively. The organic phases were evaporated to dryness and combined to afford the crude extract (5 g), which was dissolved in methanol and defatted with cyclohexane. The filtrate was extracted using Amberlite XAD-16 resin followed by elution with MeOH. The methanolic extract was evaporated to dryness (55 g) and separated on silica gel column chromatography (CH_2Cl_2/ MeOH 8:2) to afford stemphol 1-*O*-*β*-D-galactoside (**127**). The ethyl acetate extract of the myce-

lium was separated on Sephadex LH-20 followed by silica gel to yield stemphol (**126**) indole-3-carboxylic acid (5.0 mg) and kojic acid (10.0 mg).

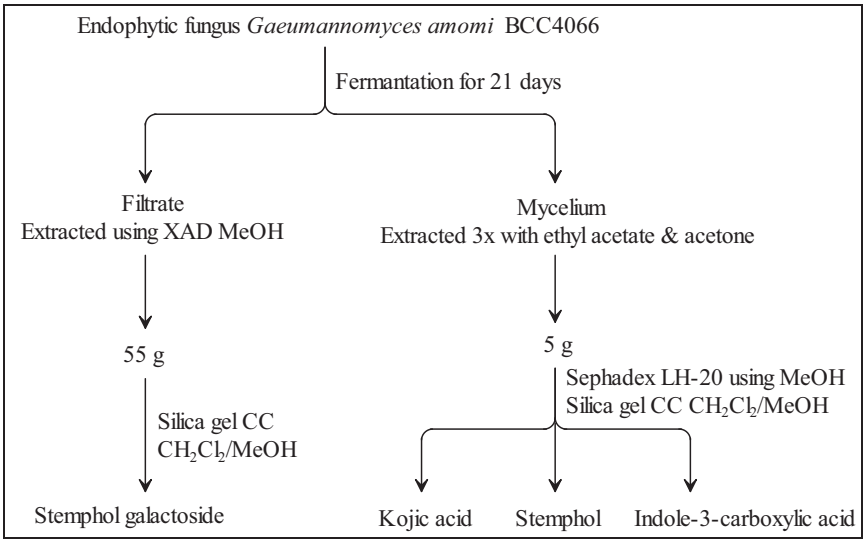
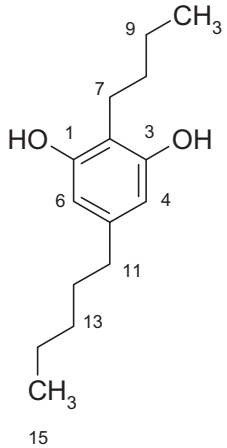


Figure 122: Work up scheme of endophytic fungus *G. amomi* BCC4066

5.1.1 Stemphol (2-butyl-1,3-dihydroxy-5-pentylbenzene)

Stemphol (**126**) was isolated as colourless crystals from a UV absorbing zone. The ^1H NMR spectrum revealed one aromatic signal at δ 6.20 with the intensity of two protons (H-6 and H-4). A signal of two exchangeable hydroxyl protons appeared at δ 4.77. In addition to two triplets at δ 2.56 (CH_2-7) and 2.44 (CH_2-11), and ten methylene protons were observed between δ 1.27-1.57 (CH_2-8, 9, 12, 13, 14). Two methyl groups appeared as triplets at δ 0.87 (CH_3-10) and 0.93 (CH_3-15).



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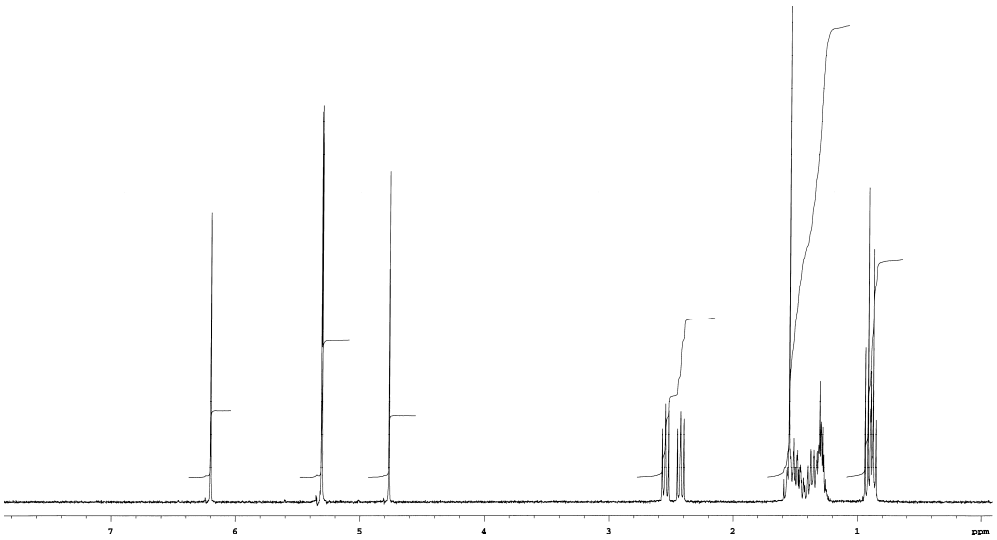
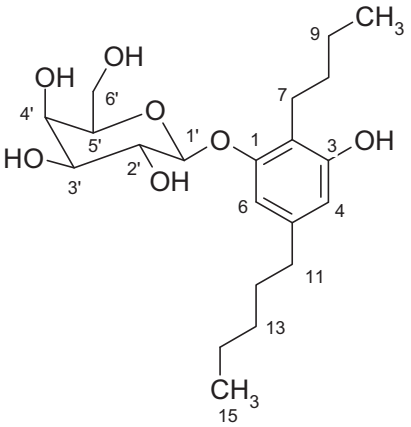


Figure 123: ^{1}H NMR spectrum (CD_2Cl_2, 300 MHz) of stemphol (**126**)

The ^{13}C NMR spectrum displayed three quaternary carbons at δ 154.9, 142.7 and 112.8, and an aromatic CH signal at δ 108.2. Additionally seven methylene carbon signals were observed, in addition to a methyl carbon of double intensity at δ 14.0. The ESI mass spectrum revealed *pseudo*molecular ions at *m/z* 237 $[M + H]^+$ and 235 $[M - H]^-$. The HRESI mass spectrum established the formula $C_{15}H_{24}O_2$. A search in AntiBase[113] led to stemphol (**126**), which was firstly isolated from *Stemphylium majusculum*[205] and later from *Pleospora herbarum*.[206] It is an alkylresorcinol, a member of a group of compounds with biopharmacological, biomedical, and biotechnological importance.[207] Stemphol (**126**) was tested in our lab and displayed significant growth inhibitory activity against brine shrimps (*Artemia salina*) in a concentration of ~10 μg/ml.

5.1.2 Stemphol galactoside (Stemphol 1-*O*-*β*-D-galactoside)

Stemphol galactoside (**127**) was isolated as colourless solid from a UV absorbing zone, which turned to red with anisaldehyde/sulphuric acid. In the ^{1}H NMR spectrum, two *meta*-coupled aromatic 1H signals at δ 6.50 and 6.31 were visible. Five oxymethine signals as typical for a sugar residue were observed between δ 3.2 and 4.0, and the signal of an anomeric proton was visible at δ 4.81. Six methylene signals were found in the aliphatic region, in addition to two methyl signals at δ 0.92 and 0.88.



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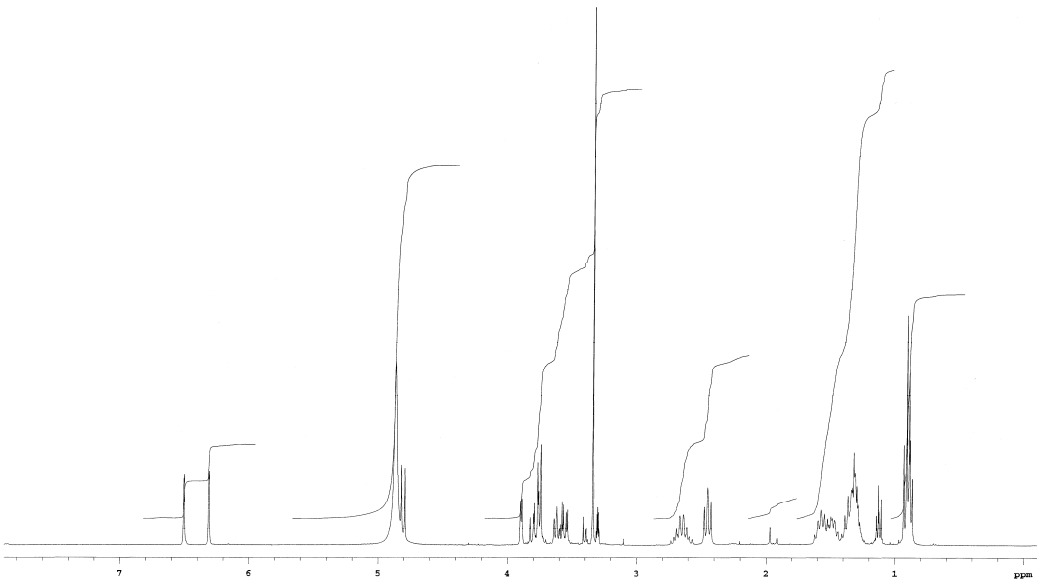


Figure 124: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of stemphol 1-*O*-*β*-D-galactoside (**127**)

The ^{13}C NMR spectrum revealed 21 signals, of which four were of quaternary carbons and two methine signals were due to olefinic or aromatic systems. Seven methylene signals and two methyl carbons indicated aliphatic chains; the signals between *δ* 62– 67 and at 103.2 were due to a sugar unit. HMBC correlations confirmed a tetra-substituted benzene ring with protons in *meta*-position.

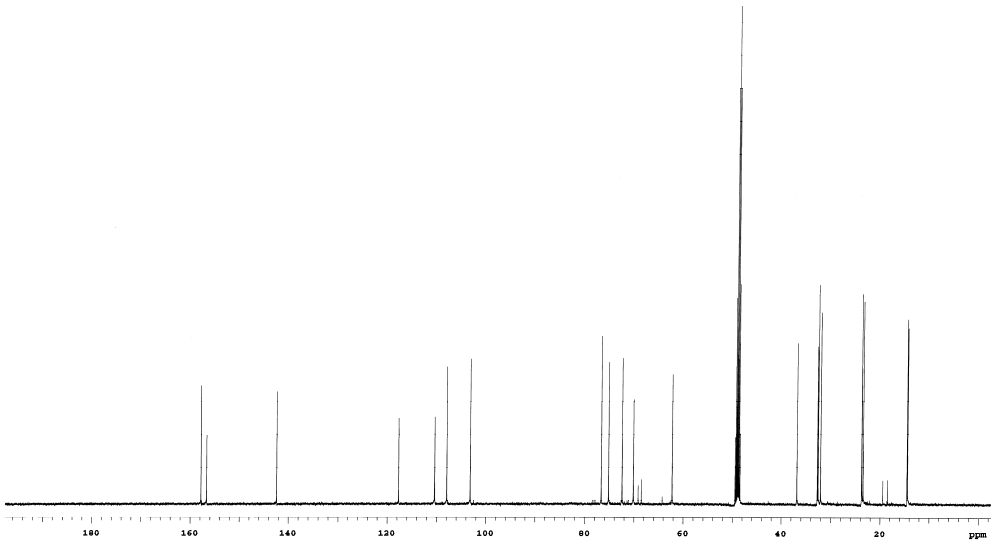


Figure 125: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of stemphol 1-*O*-*β*-D-galactoside (**127**)

$^{1}H,^{1}H$ COSY correlations showed the presence of an *n*-butyl and an *n*-pentyl moiety, which were according to the HMBC data located in *para*-position to each other. A search in AntiBase[113] with this information led again to the stemphol (**126**) skeleton, and the existence of a sugar moiety and the mass difference indicated a new stemphol hexoside. The planar structure of **127** was confirmed by interpretation of the $^{1}H,^{1}H$ COSY, HSQC and HMBC spectra. The coupling constant (^{3}J = 7.8 Hz) of the anomeric proton indicated a *β*-configuration, and the HMBC correlation of the anomeric proton H-1' with C-1 confirmed the 1,1'-linkage of the sugar residue with the aglycone. Further correlations are shown in Figure 126.

For the identification of the hexose, derivatization of the sugar has been done using MSTFA: For hydrolysis, a solution of about 0.01 mg of **127** in 50 μL of MeOH and 50 μL of 1 M HCl was kept at 80 °C. After 4 h, the sample was brought to dryness at 0.1 mbar at r.t., and the residue was derivatized with 50 μL of MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) at 40 °C for 60 min. For comparison, a number of hexoses were treated in the same way and analysed by GC/MS (Table 33).

Table 33: Retention times of silylated reference sugars

	R_t [min]		
Sugar	signal I	signal II	signal III
Sugar of **127**	18.29	18.74	19.20
Galactose	18.30	18.76	19.22
Glucose	18.58	19.16	19.93
Mannose	18.20	19.26	20.01

The retention times of the silylated sugar were compared with several aldohexoses (Table 33). After hydrolysis and silylation, the sugar of **127** revealed three peaks of isomeric α/β-pyranone/furanone derivatives, which were identical within the error limits with peaks delivered by TMS-galactose; all other investigated sugars were clearly different. Accordingly, **127** is stemphol-1-*O*-*β*-D-galactoside. According to the Klyne rule,[145] the β-configuration indicated a D-sugar, so that **127** was identified as stemphol-1-*O*-β-D-galactopyranoside.[146]

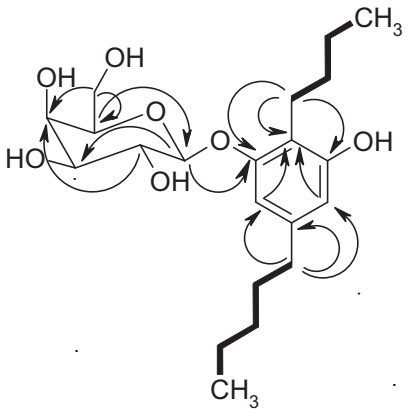


Figure 126: Selected ^{1}H-^{1}H COSY and HMBC and correlations observed for stemphol 1-*O*-*β*-D-galactoside (**127**)

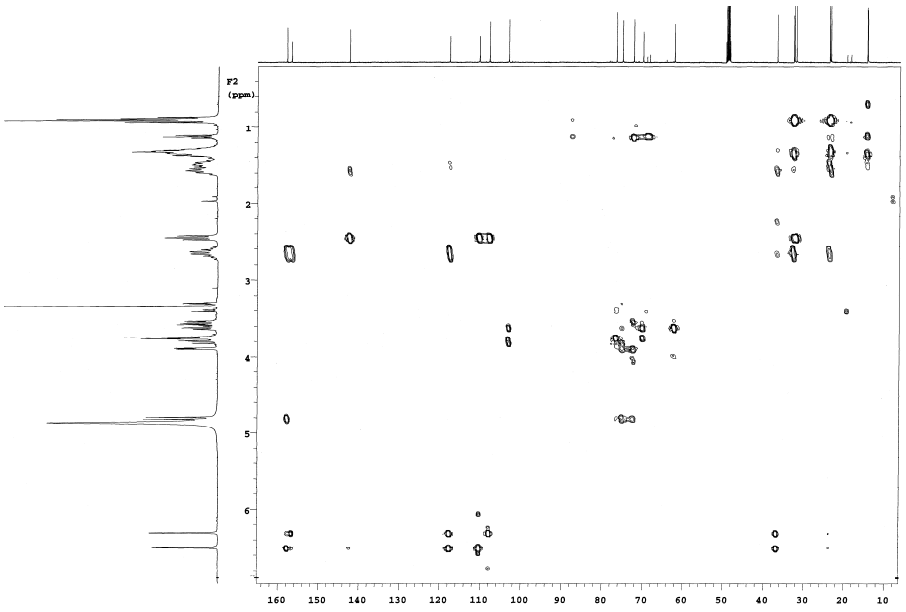


Figure 127: H,H COSY spectrum (CD_3OD, 300 MHz) of stemphol 1-*O*-*β*-D-galactoside (**127**)

5.2 Endophytic fungus LAF 12

The fungus LAF 12 was isolated from the Kenyan medicinal plant *Laggera alata* (Asteraceae) collected from the edges of the Mau forest in the Rift Valley Province. A culture of this fungus has been deposited at the Institute of Biomolecular and Organic Chemistry, University of Göttingen, Germany. In the pre-screening the crude extract showed antimicrobial activity against different microorganisms as well as larvicidal activity against *Anopheles gambiae* (66%).

The fungus LAF 12 was cultivated on 20 L of fermentation medium (M_2^+) in 20 P- Flasks as resting culture for one month at 28 °C. The resulting mycelium was extracted using ethyl acetate and MeOH, while the filtrate was extracted using Amberlite XAD-16 resin. The organic phases were evaporated to dryness. The crude extract of the ethyl acetate phase of the mycelium (1.5 g) was chromatographed on silica gel using a CH_2Cl_2/MeOH gradient to deliver three fractions; FI contained fat; FII was purified on Sephadex LH-20 to afford D-sorbitol (**130**). FIII was purified on silica gel followed by Sephadex LH-20 to deliver a compound, which is still under discussion.

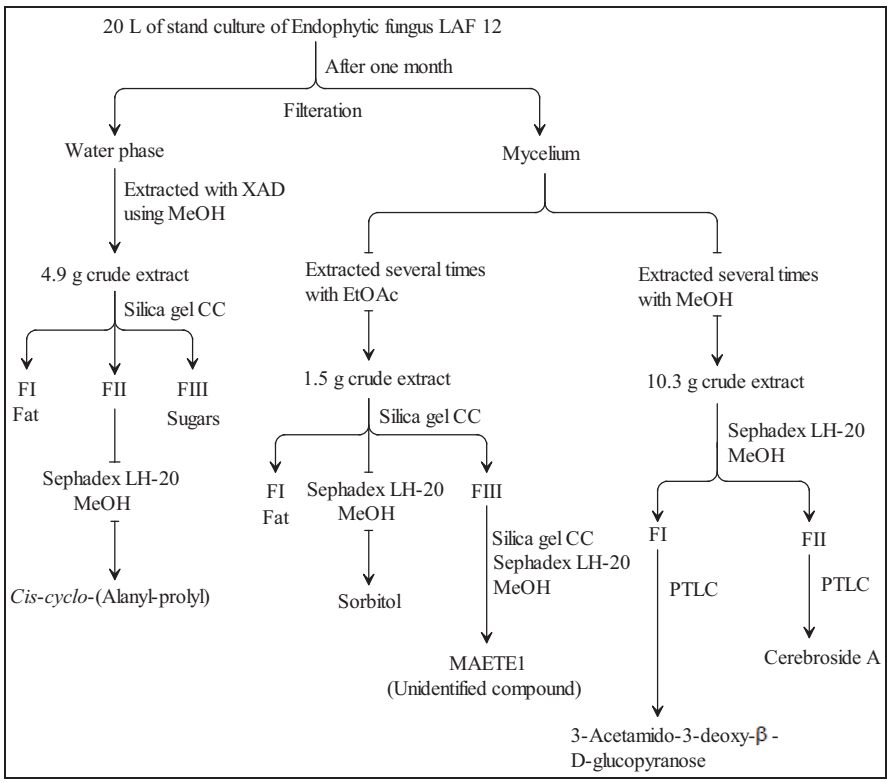


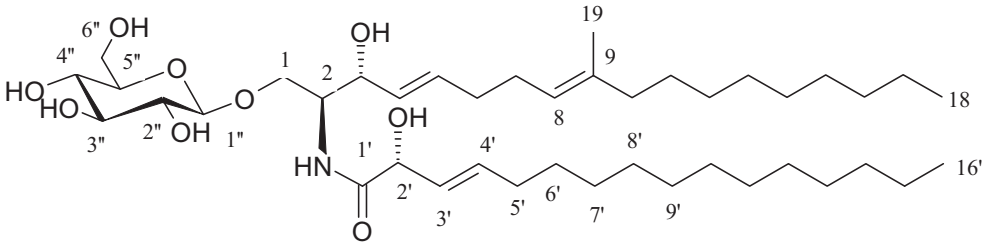
Figure 128: Work up procedure of endophytic fungus LAF 12

The MeOH extract (10.3 g) from the mycelium was chromatographed on silica gel using a CH_2Cl_2/MeOH gradient to deliver two fractions FI and FII, which were purified by PTLC to afford cerebroside A (**128**) and 3-acetamido-3-deoxy-β-D-glucopyranose (**125**). Moreover the crude extract (4.9) from the water phase was chromatographed on silica gel using CH_2Cl_2/MeOH gradient to deliver three fractions, where FI and FIII mainly contained fat and sugars, respectively; FII was purified on Sephadex LH-20 to obtain *cis*-*cyclo*-(alanyl-prolyl) (**129**).

5.2.1 Cerebroside A

Cerebroside A (**128**) was isolated as white solid from the methanol extract by using Sephadex LH-20 followed by PTLC. It was obtained as UV inactive substance, which turned to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed several proton signals in the aliphatic region as well as six olefinic protons downfield at δ 5.82-5.15. Oxymethine signals were observed at δ 4.43 (H-2') and 4.14 (H-3) in addition to a typical anomeric proton signal at δ 4.26 (H-1") with the coupling constant of 7.7 Hz indicating the presence of a sugar moiety. Five oxygenated proton signals between δ 3.86 - 3.20 (H-2" – H-6") could be attributed to the sugar unit. An oxymethylene proton at δ 4.11 (H-1) was observed in addition to a multiplet at δ 3.97 (H-2); the latter could be connected to nitrogen. Twentyone methylene sig-

nals were obtained, four methylene protons were observed between 1.98 –2.04 (CH_2-6, 7, 10, 5') and the rest was observed between δ 1.28 - 1.38 (CH_2-11, 12-15, 16, 17, 6'- 15'), in addition to methyl protons, which appeared as a 6H triplet at δ 0.90 (CH_3-18, 16') and a methyl singlet at δ 1.59 (CH_3-9).



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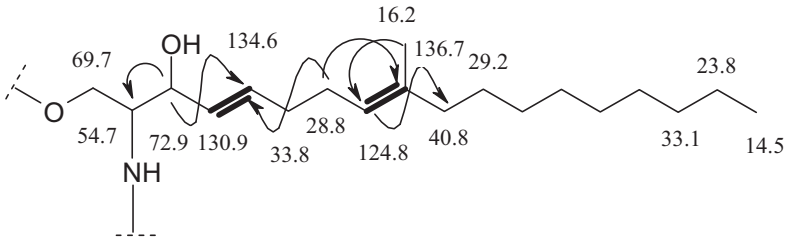
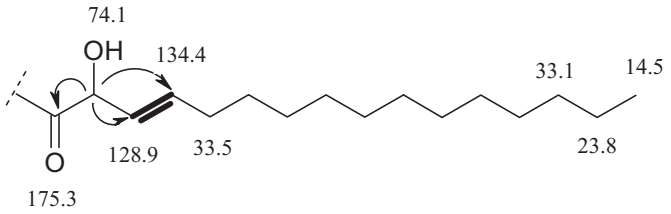
The ^{13}C NMR spectrum showed 41 carbon signals; five olefinc carbons were observed at δ 134.6, 134.4, 130.9, 128.9 and 124.8 in addition to two quaternary carbons at δ 136.7 and 175.3. The latter could be a carbonyl of an amide or acid derivative. Several oxygenated carbon signals appeared between δ 69.7 - 62.7 in addition to a signal at δ 104.6, which indicated the anomeric carbon of a sugar unit. Twenty-one methylene carbons were observed between δ 40.8-23.8, a methine carbon appeared at δ 54.7 and three methyl carbon signals were found at δ 16.2 and 14.5. The ESI mass spectrum afforded a *pseudo*molecular ion peak at 748 $[M + Na]^+$, which delivered the molecular weight of 725 Dalton. HRESIMS revealed the molecular formula $C_{41}H_{75}NO_9$.

Analysis of HSQC, COSY and HMBC spectra showed several strong correlations from which fragments a, b and c were established. Fragment a showed a sphingosine moiety, in which an oxymethine proton at δ 4.14 (H-3) showed a COSY correlation with the methine proton at δ 3.97 (H-2) and the olefinic proton at δ 5.82 (H-4) as well as HMBC correlations with an olefinic carbon at δ 134.6 (C-5). An olefinic proton at δ 5.15 (H-8) showed HMBC correlations with both the methyl carbon at δ 16.2 (CH_3-9) and a methylene carbon at δ 40.8 (C-10), both methylene protons at (H-10) and the methyl protons at CH_3-9 showed HMBC correlations with the quaternary carbon at δ 136.7 (C-9). The methylene protons (CH_2 11 - 16) displayed COSY correlations among each others (Fragment A).



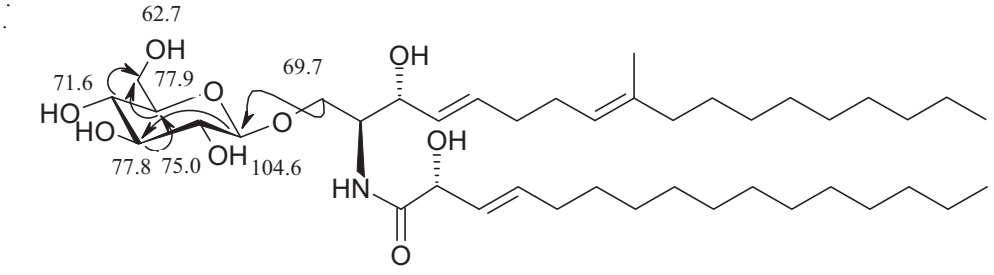
Fragment **A**

In the fatty acid moiety, an oxymethine proton at δ 4.43 (H-2') showed a strong HMBC correlation with a carbonyl at δ 175.3 (fragment B). Due to the presence of nine oxygen atoms, which were assigned by HRESIMS, the only possibility to connect the latter carbonyl with fragment A is an amide bond, as the remaining six oxygen atoms are located in a sugar part. The only nitrogen atom must be attached to the carbonyl at δ 175.3. The proton H-2' showed a COSY correlation with the olefinic proton at δ 5.44 (H-4) and a HMBC correlation with the olefinic carbon at δ 134.6. The methylene groups between δ 1.28 – 1.30 showed COSY correlation as well as HMBC correlations amongst each others. Both methyl triplets at δ 0.90 (H-18, 16') showed COSY correlations with the methylene protons H-17 and 15'.



Fragment **B**

The sugar moiety was established according to the HMBC correlations as shown in fragment C.



Fragment **C**

A search in AntiBase[113] with these substructures led to the cerebroside **128**, which was confirmed by comparison with the literature data.[208] Compound **128** is a bioactive cerebroside isolated previously from *Pachybasium* sp. It showed antifungal activity against *Candida albicans* and could also be responsible for the antifungal

activity against *C. albicans* of extracts of the endophytic fungus LAF 12. The sugar unit was identified previously as D-glucose in a β-pyranose linkage.[209] Cerebrosides are glycosphingolipids and were found in many phytopathogens as elicitors that induce the disease resistance in e.g. rice plants.[210]

Table 34: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of cerebroside A (**128**) in CD_3OD

Position	δ_C	δ_H (mult.; *J* in [Hz])
1	69.7	3.72 (dd, 10.5, 3.4) 4.11 (dd, 10.5, 2.9)
2	54.7	3.97 (m)
3	72.9	4.14 (dd, 7.8, 5.2)
4	130.9	5.44 (dt, 15.4, 7.3, 1.3)
5	134.6	5.72 (dt, 15.0, 6.1, 5.8)
6	33.8	2.04 (m)
7	28.8	2.04 (m)
8	124.8	5.15 (t, 6.7)
9	136.6	
10	40.8	1.98 (m)
11	29.2	1.38 (m)
12-15	30.9, 30.9, 30.8, 30.7	1.28 – 1.38 (m)
16	33.1	1.28 – 1.30 (m)
17	23.8	1.28 – 1.30 (m)
18	14.5	0.90 (t, 6.9)
CH_3-9	16.2	1.59 (s)
1'	175.3	
2'	74.1	4.43 (dd, 6.0, 1.6)
3'	128.9	5.51 (dt, 15.4, 6.1, 1.3)
4'	134.4	5.82 (dt, 15.4, 6.7, 1.3)
5'	33.5	2.04 (m)
6'-13'	30.88, 30.87, 30.82, 30.81, 30.7, 30.54, 30.48, 30.4, 30.3	1.28 – 1.38 (m)
14'	33.1	1.28 – 1.30 m
15'	23.8	1.28 – 1.30 m
16'	14.5	0.90 (t, 6.9)
1"	104.6	4.26 (d, 7.7)
2"	75.0	3.20 (dd, 9.3, 7.7)
3"	77.94	3.36 (dd, 9.3)
4"	71.6	3.30 (m)
5"	77.86	3.27 (m)
6"	62.7	3.86 (dd, 12.0, 1.2) 3.74 (dd, 12.0, 5.5)

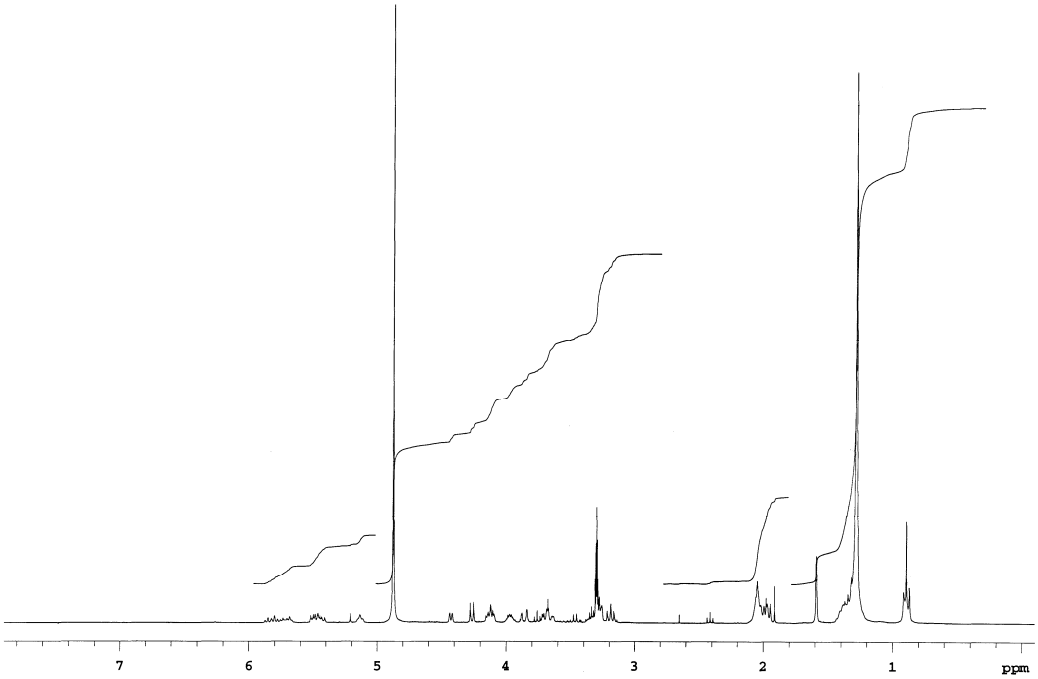


Figure 129: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of cerebroside A (**128**)

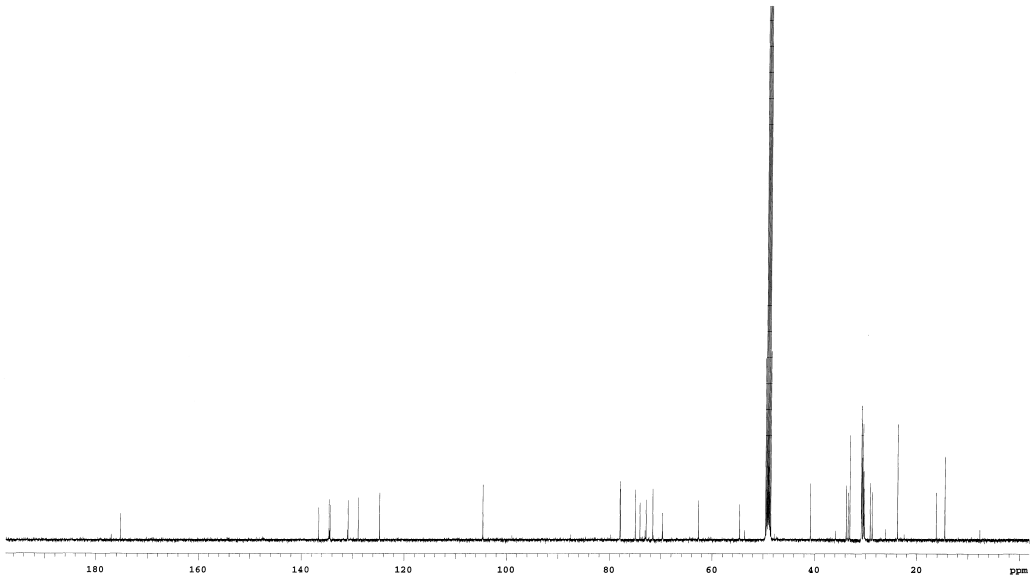
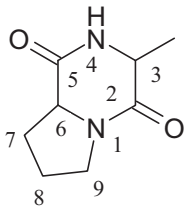


Figure 130: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of cerebroside A (**128**)

5.2.2 *Cis-cyclo* -(Alanyl-prolyl)

Cis-cyclo-(Alanyl-prolyl) (**129**) was isolated as white solid from fraction FII. The ^{1}H NMR spectrum showed a broad 1H signal at δ 6.5, which could be attributed to an NH proton, and two methine protons at δ 4.12 (CH-3 and CH-6), which could be connected with oxygen or nitrogen. Three methylene protons appeared as multiplets at δ 3.56 (CH_2-9) and between 1.86-1.36 (CH_2-7 and CH_2-8), and a methyl doublet was found at δ 1.45. The ^{13}C NMR spectrum revealed eight signals, two amide or lactone carbonyls at δ 170.5 (CO-5) and 166.2 (CO-2), two methine carbons at δ 59.3 (CH-6) and 51.2 (CH-3), three methylene carbons at δ 45.5 (CH_2-9), 28.2 (CH_2-7) and 22. 8 (CH_2-8) and a methyl carbon at δ 16.0 (CH_3-3). A search in AntiBase[113] confirmed the structure as **129** after comparing with the literature data.[211] Compound **129** was isolated previously from plants and fungi[212] and found to be one of many other diketopiperazines present in roasted coffee[213] as well as roasted cocoa nibs (*Theobroma cacao*), where they were found to be generated from amino acids during the roasting process. They are important factors responsible for the bitter taste of roasted cocoa.[214,215]



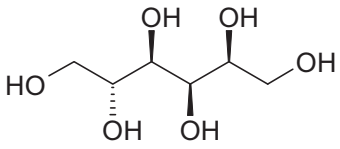
129

5.2.3 D-Sorbitol

A polar compound was isolated as white powder from a UV inactive zone, which turned to yellow with anisaldehyde/sulphuric acid. The ESI mass spectrum displayed the molecular weight 182, while HRESIMS established the molecular formula $C_6H_{14}NO_6$. The ^{13}C NMR spectrum showed, however, only three oxygenated carbons at δ 73.5, 72.0 and 65.8, so that a symmetrical hexitol was assumed. Among these sugar alcohols, D-sorbitol (D-glucitol, **130**) is the most common one, and the ^{1}H and ^{13}C NMR spectra resembled indeed closely our reference spectra.

Sorbitol is a well-known sugar alcohol, which is obtained by reduction of glucose.[216] Compound **130** is found in many stone fruits such as cherries as well as berries from trees of the genus *Sorbus*.[217] It was identified previously from the rust fungus *Puccinia graminis* f. sp. t*ritici* as one of the major components.[218]

It is important to mention that compound **130** is used as sweetening agent for the diets of diabetics since the 1920s. It causes only a slight increase in the blood sugar concentration,[219] provides the same number of calories per gram as sucrose, but is only 0.54 – 0.7 times as sweet.[220]



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6 Summary

Plants and microorganisms have a strong medical and ecological importance due to their production of bioactive metabolites. Because of the development of resistance problems and the appearance of new diseases, a steady flow of new drugs is needed. The search for new lead compounds is therefore of huge interest for modern pharmaceutical industry, where natural products are still an essential source of pharmaceuticals.[221] It is important in this context also to mention that novel molecules produced by nature provide challenges to organic chemists not only in the investigation of their structure and the development of total syntheses, but also in the identification of the pharmacophores, which could lead to more suitable chemotherapeutic agents.[222]

Although nature provides novel molecules with wide rang of important and interesting activities, the investigation of these bioactive drugs or the application of unique new compound requires a multidisplinary approach as well as international collaboration in the discovery process.[222]

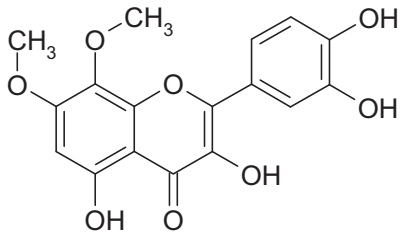
Ethnopharmacology is considered as a part of pharmacognosy that focus on the study of the biological effects of natural compounds isolated from ethnomedically used plants or endophytic fungi in order to establish a link between the traditional usage, chemical compounds and the biological system.[223]

In the present research work the crude extracts of four Sudanese medicinal plants were selected. *Xanthium brasilicum* Vell (local name Rantok) is used in rural regions of Sudan against endemic parasitic diseases, and recently an antimalarial activity of the crude extract *in vitro* and *in vivo* has been reported.[75] The powdered bark of *Albizia zygia* (local name Deghn El basha El ahmar) is used alone or as a decoction in the southern Sudan as an antimalarial and antiparasitic drug. The seeds of *Tephrosia apollinea* (local name Amayogha) are used as antimalarial drug in many parts of Sudan. The maceration of the roots of *Aristolochia bracteolata* (local name Um galagel) is used against malaria as well as anti-parasitic and anti-rheumatic drug.

In our tests, the crude extract of *X. brasilicum* exhibited zoosporicidal activity against *Plasmopara viticola* at a concentration of 100 μg/ml. This test indicates activity against an important plant-pathogenic fungus and was performed in cooperation with Dr. T. Islam.

On extensive chromatography, a methoxylated flavone **53** was isolated together with two sesquiterpenes, namely xanthatin (**56**) and 4-oxobedfordia acid (**57**), and two

steroids, stigmasterol (**51**) and *β*-sitosterol (**52**); compounds **53**, **56** and **57** were isolated for the first time from *Xanthium brasilicum*. Compound **53** displayed a high zoosporicidal activity against downy mildew (*P. viticola*) at a concentration of 5 *μ*g/ml, while compounds **56** and **57** were inactive. Also an antimalarial activity was not found. The ecological benefit of **53** as natural antifungal agent is obvious.



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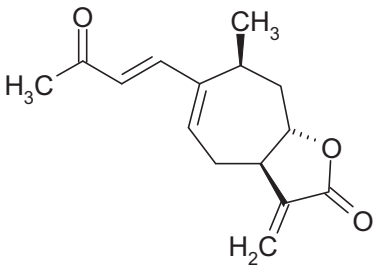
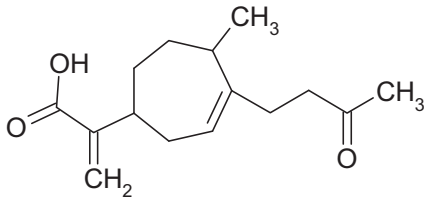
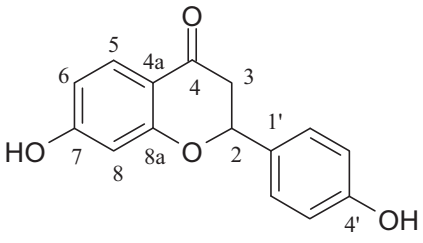


56



57

The crude extract of the antimalarial Sudanese medicinal plant *Albizia zygia* afforded three flavonoids, namely 4',7-dihydroxyflavanone (**58**), 7,3',4'-trihydroxyflavone (**59**) and 7,3',4'-trihydroxy-3-methoxyflavone (**60**) together with lupeol (**61**), chondrillasterol (**62**) and *p*-hydroxybenzoic acid. Compounds **59** and **60** were isolated for the first time from the genus *Albizia*. They were known compounds from synthesis and quite recently isolated as natural products from the heartwood of the traditionally used Taiwanese medicinal plant *Acacia confusa* Merr. (Leguminosae). Compound **58** is a known flavanone from many medicinal plants and was isolated now for the first time from *A. zygia*. In a recent report the methanolic extract of the stem bark of *A. zygia* exhibited antiprotozoal activity (IC_{50} 1.0 *μ*g/ml) against *Plasmodium falciparum* strain K1 and *Trypanosoma brucei rhodesiense* (IC_{50} 0.2 *μ*g/ml), which causes African trypanosomiasis. The flavonoids isolated here were tested against *P. falciparum* K1: only compound **59** showed high antimalarial activity (IC_{50} 0.078 *μ*g/ml), but unfortunately it displayed cytotoxicity (IC_{50} 0.405 *μ*g/ml) against L6 strain. Recently lupeol (**61**) was found to inhibit the growth of the malaria causing agent *Plasmodium falciparum* by 45% at 25 μg/ml. This agrees well with the fact that lupeol (**61**) was isolated from the antimalarial plant *A. zygia* also in the present investigation.



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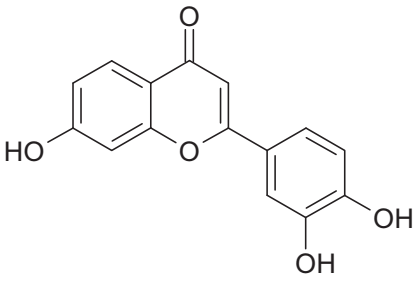
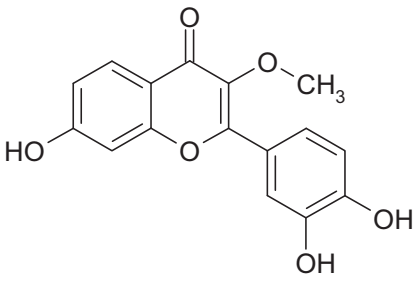


59



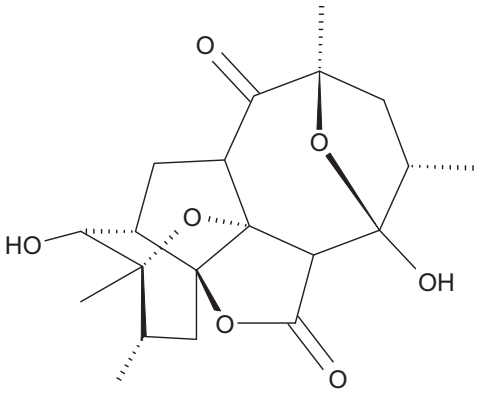
60

The other two plants were *Tephrosia apollinea* and *Aristolochia bracteolata*. *Tephrosia apollinea* delivered pseudosemiglabrin (**63**), semiglabrin (**64**), lanceolatin A (**65**), apollinine (**66**) and 3*β*-*O*-Glucosylsitosterol (**67**). *Aristolochia bracteolata* afforded aristolochic acid A (**68**) and B (**69**). With these results, the investigation of the plant extracts was therefore finished. In the second part of this thesis, metabolites from bacteria (mainly streptomycetes) and some endophytes were investigated.

The genus *Streptomyces* is an important group of actinomycetes because their high productivity of a wide range of secondary metabolites. The characterization and identification of new microbial metabolites is still of high importance, and also the re-investigation of already identified molecules for new activities is still of interest.[224]

In the present work two marine, seven terrestrial *Streptomyces* spp., a *Bacillus* species and two endophytic fungi have been selected due to their biological activities and results from the chemical screening. Accordingly fermentation, extraction, and isolation of metabolites were performed under standard conditions. Dereplication was done with the help of AntiBase[113] and for the structure elucidation NMR, MS, IR, HPLC/MS measurements were performed. The pure compounds were tested for different biological activities.

The crude extract of the terrestrial *Streptomyces* ANK 210 showed activity against cancer cell lines and was found to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57), and *Candida albicans* in the agar diffusion test. From a 25 L shaker culture, two new compounds were isolated, namely *ent*-homoabyssomicins A (**70**) and B (**72**) together with four known metabolites, namely 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**79**), indole-3-carboxylic acid, benadrostin (**80**) and polypropylenglycol (**81**). Compounds **70** and **72** were inactive against *Artemia salina*, *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57), *Candida albicans*. The related abyssomicins were found to be potent inhibitors of *p*-aminobenzoate (*p*ABA)/tetrahydrofolate biosynthesis. Compounds **70** and **72** were not yet tested.



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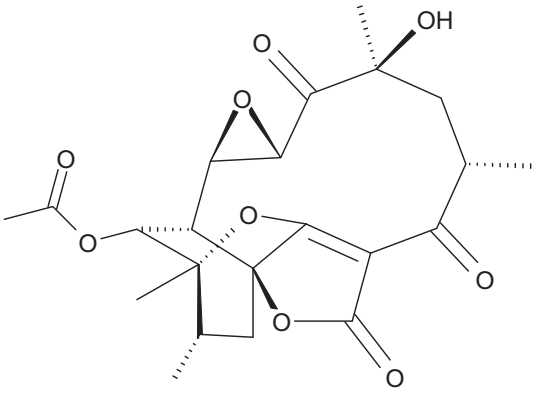
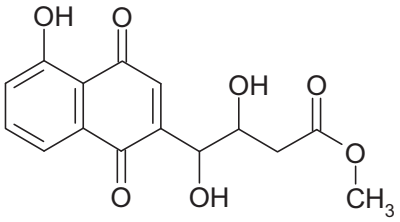


72

The crude extract of the terrestrial *Streptomyces* sp. ANK 313 displayed zoosporicidal activity against *P. viticola* at a concentration of 100 µg/ml as well as high antibacterial activity against *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57), and moderate activity against *Escherichia coli* and *Bacillus subtilis*. From a 30 L shaker culture, seven compounds were isolated, of which two were new compounds, namely khatmiamycin (**82**) and omdurmycin (**88**) together with GTRI-02 (**84**), 4-ethyl-5-methyl-heptanamide, aloesaponarin II (**85**), LL-C10037α (**86**), and LL-C10037β (**87**). Compounds **82**, **84** and **86** displayed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57) and *Escherichia coli*, which can accounted for the antibacterial activity of the crude extract. Compound **82** exhibited a strong motility inhibitory activity against the zoospores of *P. viticola* at 10 µg/ml, followed by GTRI-02 (**84**), aloesaponarin II (**85**), LL-C10037α (**86**), and *N*-(4,5-dihydroxy-cyclohex-1-ene-2-on)-acetamide (**87**).



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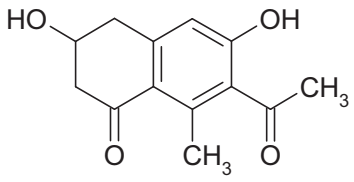

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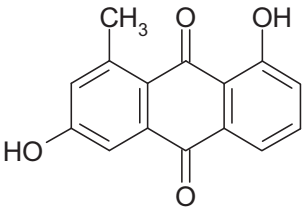

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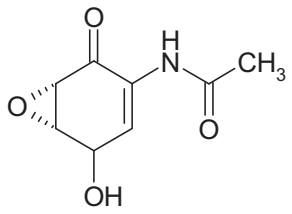

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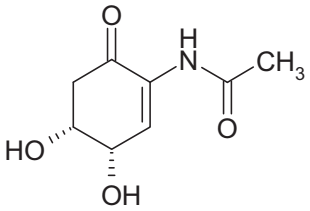

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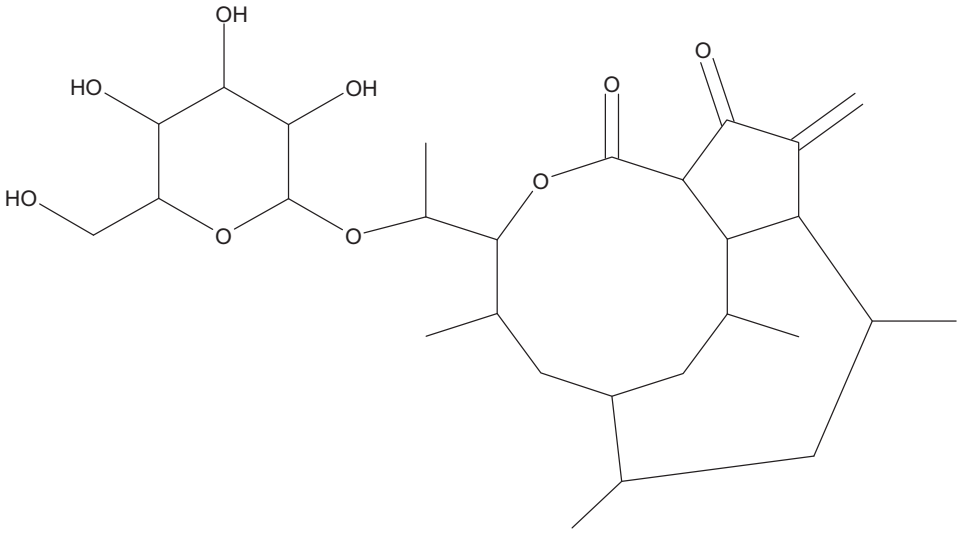
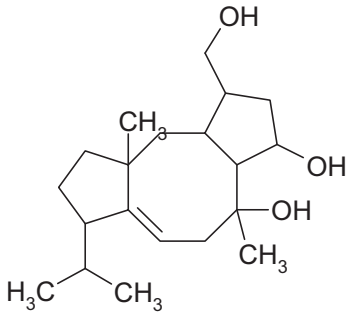


88

Cyclooctatin (**89**) was isolated from the terrestrial *Streptomyces* sp. GW08/253 together with the new 6-(2-but-1-enyl-4-methyl-pheny)-hex-5-enoic acid (**93**) and five known compounds, i.e. 6-[2-(1-hydroxy-butyl)-4-methyl-phenyl]-hex-5-enoic acid (**92**), phenazine-1-carboxamide (**90**), phenazine-1-carboxylic acid (**91**), 4-hydroxy-

benzoic acid methyl ester (**94**) and 3-(hydroxyacetyl)-indole (**95**). Cyclooctatin (**89**) displayed a very high activity against *Artemia salina* (100% at 10 µg/ml); this toxicity had not been reported before. It also showed low antibacterial activity against *Bacillus subtilis* and *Escherichia coli* by causing inhibition zones of 9.5 mm at 40 µg/disk.



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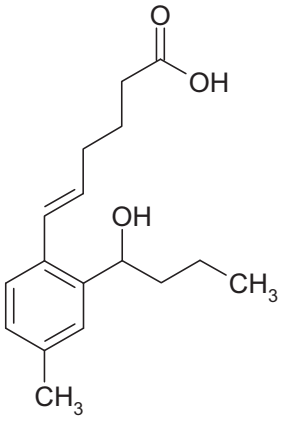

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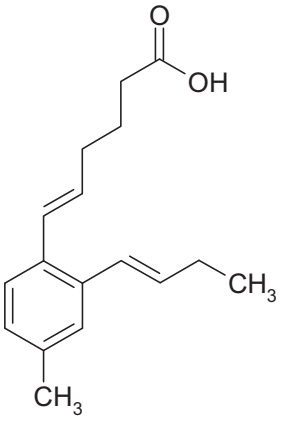
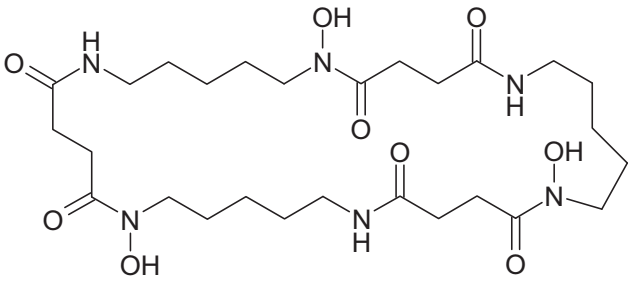


93

The crude extract of the terrestrial *Streptomyces* Red 202 showed activity against *Escherichia coli*. On TLC it showed three interesting UV absorbing spots, of which one turned to pink and was identified as the "the cyclic sideriphore" nocardamine (**96**). The other two red zones delivered diastovaricin I (**97**) and 6-hydroxy-5-methoxyisatine (**98**). The latter was known from synthesis and isolated recently as natural compound in our group.



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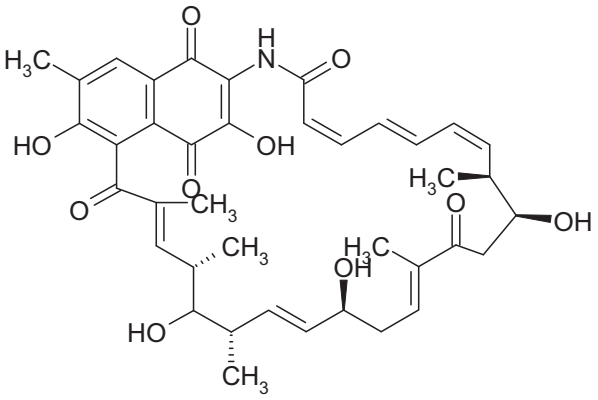

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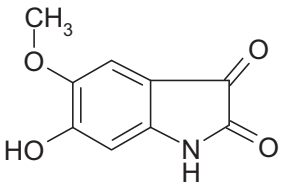
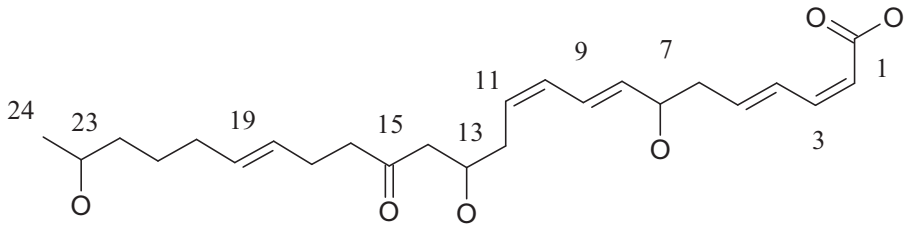


98

During the pre-screening, the crude extract of the terrestrial *Streptomyces* sp. WO1004 showed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. On TLC two UV absorbing bands appeared which turned to black with anisaldehyde/sulphuric acid and heating. After chromatography five compounds were isolated, namely isomacrolactinic acid (**100**), tyrosol, uridine, macrolactin A (**101**) and dihydromacrolactin F (**102**). The latter compound was synthesized previously by William Fenical by reduction of macrolactin F (**112**) using sodium borohydride; it is now isolated as new natural product. Compound **102** displayed very good activity against oomycetes in a concentration of 10 μg/ml: this is a new activity for this type of compounds. Macrolactin A (**101**) is a very active antibacterial, anticancer and antiviral compound. The antibacterial activity of the crude extract can be accounted for by the presence of macrolactin A (**101**).



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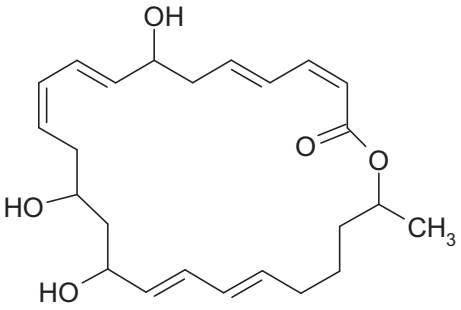

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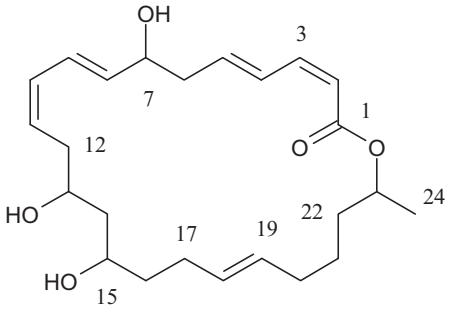
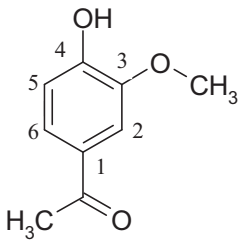


102

After the pre-screening, an optimisation of the culture conditions for the terrestrial *Streptomyces* sp. HO9 on different media was performed. Using M_2^+ medium afforded the best yield, and additionlly on TLC three interesting UV absorbing bands were observed, which turned to dark orange with anisaldehyde/sulphuric acid spray reagent. No biological activity was found. The crude extract afforded steroids, indole-3-carboxylic acid, 1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**103**), 2,6-dimethyl-oxepan-4-one (**104**), anthranilic acid, and indole-3-carboxylic acid methyl ester (**106**). Compound **104** was isolated as new compound; it exhibited low antibacterial activity against *Bacillus subtilis* and *Escherichia coli* by causing inhibition zones of 9.5 mm at 40 μg/disk.



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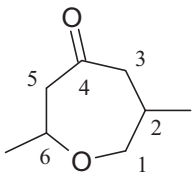
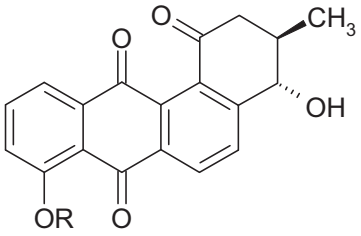


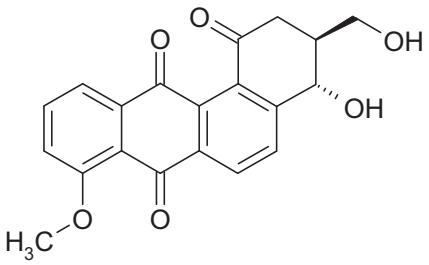
104

The crude extract of the marine derived *Streptomyces* sp. B6219 displayed high antitumor activity (cancer cell lines) as well as moderate antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü57) and showed antifungal activity against *Mucor miehei* (Tü 284). Six angular quinones were isolated: The new compound **119** was named fujianmycin C; further (known) compounds were fujianmycin A (**118**), fujianmycin B (**117**), ochromycinone (**120**), ochromycinone methyl ether (**121**), and tetrangulol methyl ether (**122**). Compound **119** displayed antibacterial activity against *Streptomyces viridochromogenes* (Tü57) and was found to be inactive against cancer cell lines, while

the other known metabolites were reported to have antibacterial and anticancer activities, which could account for the antibacterial and anticancer activity of the crude extract.



118 R = H
117 R = Me



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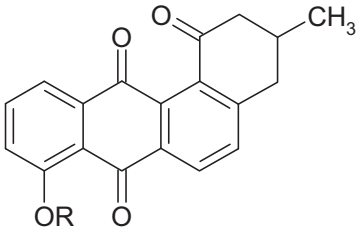
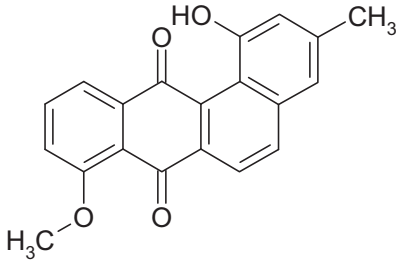
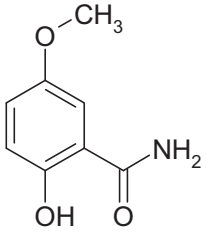


120 R = H
121 R = Me

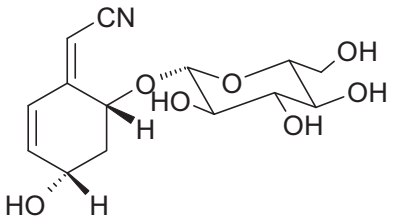


122

In the chemical screening, the crude extract of the marine derived *Streptomyces* B5746 showed two UV absorbing bands, which turned to yellow with anisaldehyde/sulphuric acid and heating. These two bands were found to contain 2-hydroxy-5-methoxy-benzamide (**123**), which is new natural compound, and menisdaurin (**124**), which was isolated previously from plants and is isolated now for the first time from bacteria.



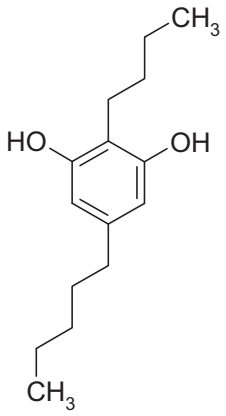
123



124

The endophytic fungus *Gaeumannomyces amomi* BCC4066 delivered a known compound stemphol (**126**) and it is derivative, the new stemphol galactoside (**127**). The sugar was identified by GC/MS. Compound **126** was found to be active against

Artemia salina. Compound **127** has been tested for antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, *B. cereus*, for cytotoxicity against a primate cell line (Vero), inhibition of cancer cell growth, herpes simplex virus type-1 (HSV-1), malaria, *Mycobacterium tuberculosis* H37Ra and plant pathogenic fungi (*Botrytis cinerea*, *Phytophthora infestans*, *Pyricularia oryzae* and *Septoria tritici*). Unfortunately **127** was inactive in all tests.



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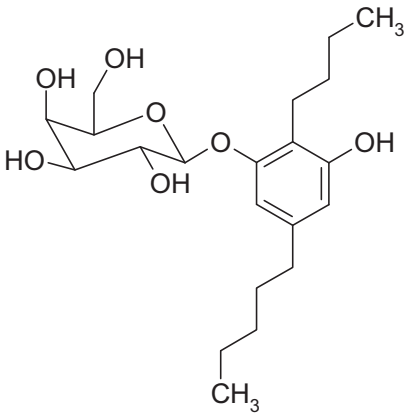


127

Table 35: Total number of isolated compounds from microorganisms and plants in the present research work

Strains/plants	No. of strains/plants	Total no. of compounds	No. of new compounds
Marine *Streptomyces*	2	10	3
Terrestrial *Streptomyces*	7	38	9
Bacillus sp. M10	1	4	-
Sudanese medicinal plants	4	18	3
Endophytic fungi	2	9	1

The present investigation confirmed again terrestrial and marine *Streptomyces* spp. as a rich sources of bioactive secondary metabolites as well as of interesting molecules such as the *ent*-homoabyssomicins A (**70**) and B (**72**). The productivity as well as the diversity of compounds is higher in the case of terrestrial and marine *Streptomyces* spp. in comparison with plants and endophytic fungi. Accordingly, both groups of streptomycetes represent still an important and amazing target for discovery of novel bioactive compounds. The investigation in the field of natural products should ur-

gently be continued. Nature produces potent compounds, and only a small part of the natural sources such as plants, animals, and microorganisms have been described and therefore dialogue between chemists and biologists is necessary for further research into the discovery of new bioactive compounds.

7 Materials and Methods

7.1 General

IR spectra: Perkin-Elmer 1600 Series FT–IR; Perkin-Elmer 297 infrared spectrophotometer; Beckman DU-640; Shimadzu FT-IR; (KBr tablet and film). - **UV/VIS spectra:** Perkin-Elmer Lambda 15 UV/VIS spectrometer. - **Optical rotations:** Polarimeter (Perkin-Elmer, model 243), the concentration were given in [mg/ml]. – **^{1}H NMR spectra:** Varian Unity 300 (300.145 MHz), Bruker AMX 300 (300.135 MHz), Varian Inova 500 (499.8 MHz), Varian Inova 600 (600 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet 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. Reserpine (MW = 608) and leucine-enkephalin (MW = 555) were used as standards in positive and negative mode. High-resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). ESI MS/MS was performed with normalized collision energy of 35%. EI MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for EI HRMS, samples were infused with a flow rate of 2 µL/min. **CD-Spectra:** The Circulardichroismus (CD)-spectra were measured on a Jasco J 500 Spectrometer with BMC IF 800 PC and Jasco IF 500 A/D-transformer. The molar ellipticity θ is given in [10^{-1} grad cm^2 mol^{-1}].– **Filter press:** Schenk Niro 212 B40.

7.2 Materials

Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). – **Glass plates for chemical screening:** Merck silica gel 60 F254, (10 × 20 cm). - **Preparative thin layer chromatography (PTLC):** 55 g Silica

gel P/UV_{254} (Macherey-Nagel & Co.) is added to 120 ml of demineralised water with continuous stirring for 15 minutes. 60 ml of the homogenous suspension is poured on a horizontal held (20 × 20 cm) glass plate and the unfilled spaces are covered by distributing the suspension. The plates are air dried for 24 hours and activated by heating for 3 hours at 130 °C. - **Column chromatography (CC):** MN silica gel 60: 0.05-0.2 mm, 70-270 mesh (Macherey-Nagel & Co); silica gel (230-400 mesh) for flash chromatography: 30-60 μm (J. T. Baker); 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.

7.3 Spray Reagents

Anisaldehyde/sulphuric acid: 1 ml anisaldehyde was added to 100 ml of a stock solution containing 85 ml methanol, 14 ml acetic acid and 1 ml sulphuric acid. **Ehrlich's reagent:** 1 g 4-dimethylaminobenzaldehyde was dissolved in a mixture of 25 ml hydrochloric acid (37%) and 75 ml methanol, give red colouration with indole and yellow for other N-heterocycles. **Ninhydrin:** 0.3 g ninhydrin (2,2-dihydroxyindan-1,3-dione) was dissolved in 95 ml *iso*-propanol. The mixture was added to 5 ml collidin (2,4,6-trimethylpyridin) and 5 ml acetic acid (96%). This reagent gave a blue to a violet colouration with amino acids, peptides and polypeptides with free amino groups. Ninhydrin in ethanol (0.1 %) was also directly used. **Chlorine/*o*-dianisidin reaction:** The reagent was prepared from 100 ml (0.032%) *o*-dianisidin in 1 N acetic acid, 1.5 g $Na_2WO_4 \cdot 2\ H_2O$ in 10 ml water, 115 ml acetone and 450 mg KI. The moistened TLC plate was kept ca. 30 min in a chlorine atmosphere (from 0.5 g $KClO_3$ + 2 ml conc. HCl) and then subjected to drying for ca. 1 h, till the excess of chlorine was evaporated and then dipped into the reagent. The reagent is specific for peptides as universal spraying reagent. **NaOH or KOH:** 2 N NaOH or KOH solutions are used to identify *peri*-hydroxyquinones by deepening of the colour from orange to violet or blue.

7.4 Microbiological Materials

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. (Zwijndrecht). - **Petri-dishes:** 94 mm diameter, 16 mm height, Fa. Greiner Labortechnik, Nürtingen. – **Celite:** Celite France S. A., Rueil-Malmaison Cedex. - **Sterile filters:** Midisart 2000, 0.2 µm, PTFE-Filter, Sartorius, Göttingen. - **Laminar-Flow-Box:** Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1. - **Brine shrimp eggs (*Artemia salina*):** SERA Artemia Salinenkrebseier, SERA Heinsberg (brine shrimp eggs can be obtained from aquarist shops).

7.5 Recipes

All cultures were autoclaved at 1.2 bar and 120 °C. Sterilisation time for 1 L shaker culture: 33 min, 2 L concentrated medium for fermentor: 50 min and fermentor containing 16 l water: 82 min.

Artificial sea water

Iron citrate	2 g (powder)
NaCl	389 g
$MgCl_2.6H_2O$	176 g
Na_2SO_4	68.8 g
$CaCl_2$	36.0 g
Na_2HPO_4	0.16 g
SiO_2	0.30 g
Trace element stock soln.	20 ml
Stock soln.	200 ml
Tap water	ad 20 L

Trace element stock solution

H_3BO_3	0.611 g
$MnCl_2$	0.389 g
$CuSO_4$	0.056 g
$ZnSO_4 \cdot 7\ H_2O$	0.056 g
$Al_2(SO_4)_3 \cdot 18\ H_2O$	0.056 g
$NiSO_4 \cdot 6\ H_2O$	0.056 g
$CO(NO_3)_3 \cdot 6\ H_2O$	0.056 g
TiO_2	0.056 g
$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	0.056 g
LiCl	0.028 g
$SnCl_2$	0.028 g
KI	0.028 g
Tap water	ad 1 L

Stock solution

KCl	110 g
$NaHCO_3$	32 g
KBr	16 g
$SrCl_2$. $6H_2O$	6.8 g (dissolved separately)
H_3BO_3	4.4 g
NaF	0.48 g
NH_4NO_3	0.32 g
Tap water	ad 2 L

7.6 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 bacto agar

M_2^+ medium (M_2 medium with sea water)

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

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

M_2 100% sea water + $CaCO_3$

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

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

$CaCl_2$-Medium

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

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

Luria-Bertani-Medium (LB)

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

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

Soja-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 bacto agar.

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

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

The pH was adjusted to 7.8 using 2N NaOH.

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

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

The pH was adjusted to 7.8 using 2N NaOH.

Nutritional solution A

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

Nutritional solution B

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

Stock Solutions and Media for Cultivation of algae

Fe-EDTA

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

Trace element Solution II:

Solution A:

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

Salts are dissolved in 10 ml of demineralised water.

Solution B:

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

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

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

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

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

7.7 Microbiological and Analytical Methods

7.7.1 Storage of Strains

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

7.7.2 Pre-screening

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

7.7.3 Biological Screening

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

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

7.7.4 Chemical and Pharmacological Screening

Samples of the extracts were separated on silica gel glass plates (10 × 20 cm) with two solvent systems $CHCl_3$/5% MeOH and $CHCl_3$/10% MeOH. After drying, the plates were photographed under UV light at 254 nm and marked at 366 nm, and subsequently stained by anisaldehyde and Ehrlich's reagent. Finally, the plates were scanned for documentation. For the pharmacological investigations, approximately 25 mg of the crude extract was sent to industrial partners.

7.7.5 Brine Shrimp Microwell Cytotoxicity Assay

To a 500 ml separating funnel, filled with 400 ml of artificial sea water, 1 g of dried eggs of *Artemia salina* L. and 1 g food were added. The suspension was aerated by bubbling air into the funnel and kept for 24 to 48 hours at room temperature. After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining unhatched eggs dropped. In order to get a higher density of larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. 30 to 40 shrimp larvae were transferred to a deep-well microtiter plate (wells diameter 1.8 cm, depth 2 cm) filled with 0.2 ml of salt water and the dead larvae counted (number N). A solution of 20 µg of the crude extract in 5 to 10 µL DMSO was added and the plate kept at r.t. in the dark. After 24 h, the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of *ca*. 0.5 ml methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in%. Each test row was accompanied by a blind sample with pure DMSO (number B) and a control sample with 1 µg/test actinomycin D. The mortality rate M was calculated using the following formula:

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

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

The mortality rate with actinomycin must be 100%.

7.7.6 Production of Zoospores and Bioassy

Sporangia of *Plasmopara viticola* was isolated from the infected leaves of grapevine *Vitis vinifera* cv. Müller-Thurgau. The strain was maintained by regular culturing on the lower surface of young grapevine leaves on petri dish containing 1.5% agar at 25°C and 95% relative humidity. At day 6 of cultivation, the sporangiophores bearing lemon-shaped sporangia were harvested into an eppendorf by a micro-vacuum cleaner. The freshly harvested sporangia were separated from sporangiophores by filtration through 50 μm nylon mesh, washed twice and then incubated in sterilized water (10^4 sporangia/mL) in the dark for 6 h at room temperature (23°C) to release zoospores. These zoospores remained motile for 10-12 h in sterilized water and used for bioassay. Stock solution of crude extracts and pure compounds were first dissolved in dimethyl sulfoxide (DMSO) and then diluted with distilled water. The concentration of DMSO in the zoospore suspension never exceeded 1% (v/v), a condition that does not affect zoospore motility. The bioassay was carried out as described earlier.[170] Briefly, 40 μL of sample suspension was directly added to the 360 μL zoospore suspension (*ca.* 10^5/mL) taken in a dish of plant tissue culture multi dish plate to make a final volume of 400 μL and then quickly mixed with a glass rod. 1% aqueous DMSO was used as a control. The motility of zoospores was observed under a light microscope at 10× magnification. Quantification of time-course changes of motility and lysis of zoospores were carried out as described earlier.[142] Each treatment was replicated thrice. The mean value (%) ± SE (standard error) of the affected spores in each treatment was calculated.

7.7.7 Antitumor Test

A modified propidium iodide assay was used to examine the antiproliferative activity of the compounds against human tumor cell lines. The test procedure was described else where[225]. Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice, or obtained from American Type Culture Collection, Rockville, MD, USA, National Cancer Institute, Bethesda, MD, USA, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

7.7.8 Fermentation in 20 L Fermentor

The 20 L glass fermentor was filled with 16 L of water and closed with the metal lid. The aeration, acid/base and antifoam systems were connected to the fermentor and the inlet and outlet openings and tubes were closed with aluminium-foil and clamps.

The pH electrode port was closed with a glass stopper. The fermentor was autoclaved for 90 minutes at 120 °C, after that it was taken out of the autoclave and the air supply, stirring motor and water circulation pumps were switched on. The acid (2N HCl), base (2N NaOH) and antifoam flasks (1% Niax/70% EtOH) were connected and filled. The pH electrode was sterilised for 30 minutes with 70% EtOH and then connected with the lid. Parallel to the preparation of the fermentor itself, two litres of medium containing suitable nutrients were prepared and autoclaved for 50 minutes at 120 °C. After cooling, the medium was added to the fermentor and the pre-culture was used to inoculate the fermentor.

7.8 Primary Screening of Results

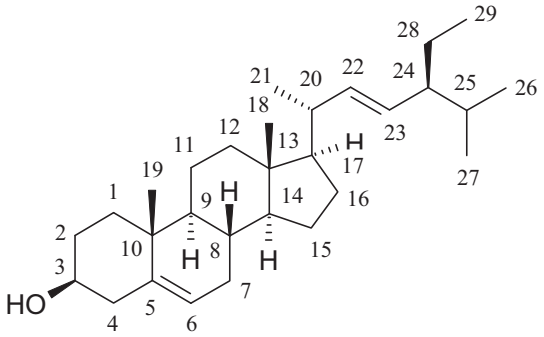
7.8.1 Bases of Evaluation

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 (+++).

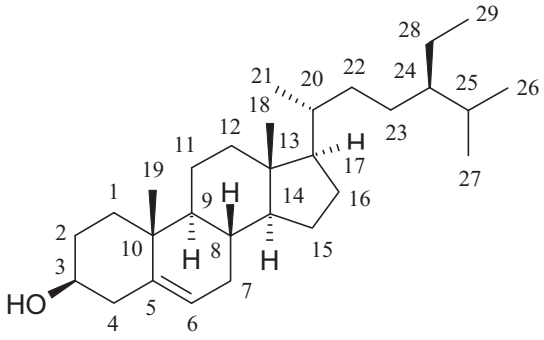
8 Plant Metabolites

8.1 *Xanthium brasilicum* Vell (Asteraceae)

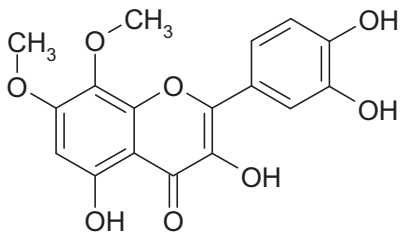
Aerial parts of the plant were dried, crushed and extracted with CH_2Cl_2 and EtOAc. Both organic phases were combined and brought to dryness under reduced pressure to afford the crude extract (99.8 g). The crude extract was subjected to silica gel column chromatography (7.5 × 60 cm) using cyclohexane/EtOAc gradient (0 to 100 EtOAc) to yield four fractions namely (FI to FIV). FI was mainly fat (40.0 g), FII was separated on slica gel column (3 × 60 cm) using cyclohexane: EtOAc gradient (0 to 100 EtOAc) to deliver fat, Stigmasterol (**51**) and *β*-sitosterol (**52**). FIII was purified on Sephadex LH-20 using MeOH followed by reverse phase RP-18 using MeOH/H_2O gradient 10 to 70 MeOH to obtain zanthoxyl flavone **53**. FIV was purified on Sephadex LH-20 using MeOH followed by reverse phase RP-18 using MeOH/H_2O gradient 10 to 80 MeOH to afford two sesquiterpenes **56** and **57**.



Stigmasterol (51): White solid. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 5.35 (d, ^3J = 5.2 Hz, 1H, H-6), 5.16 (dd, ^3J = 15.0, ^3J = 8.4 Hz, 1H, H-22), 5.02 (dd, ^3J = 15.0, 8.4 Hz, 1H, H-23), 3.53 (m, 1H, H-3), 1.01 (s, 3H, CH_3-19), 0.93 (d, ^3J = 6.4 Hz, 3H, CH_3-21), 0.85 (d, J = 6.0 Hz, 3H, CH_3-29), 0.83 (d, ^3J = 7.6 Hz, 3H, CH_3-27), 0.81 (d, ^3J = 6.8 Hz, 3H, CH_3-26), 0.68 (s, 3H, CH_3-18). – **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 140.7 (C-5), 138.3 (22-C), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 55.9 (C-17), 50.2 (C-9), 45.8 (C-24), 42.3 (C-13), 42.2 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.4 (C-20), 31.9 (C-8), 31.9 (C-7), 31.9 (C-2), 28.9 (C-25), 28.2 (C-16), 24.4 (C-15), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.0 (C-29), 12.2 (C-18). – **EIMS** (70 eV) *m/z* 412 ([M]$^+$, 18), 255 (10).

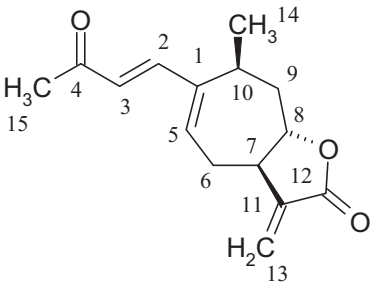


***β*-Sitosterol (52):** White solid. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 5.35 (d, ^3J = 5.2 Hz, 1H, H-6), 3.53 (1H, m, H-3), 1.01 (s, 3H, CH_3-19), 0.93 (d, ^3J = 6.4 Hz, 3H, CH_3-21), 0.85 (d, ^3J = 6.0 Hz, 3H, CH_3-29), 0.83 (d, ^3J = 7.6 Hz, 3H, CH_3-27), 0.81 (d, ^3J = 6.8 Hz, 3H, CH_3-26), 0.68 (s, 3H, CH_3-18). – **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 55.9 (C-17), 50.2 (C-9), 45.8 (C-24), 42.3 (C-13), 42.2 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.4 (C-20), 33.9 (C-22), 31.9 (C-8), 31.9 (C-7), 31.9 (C-2), 28.9 (C-25), 28.2 (C-16), 26.1 (C-23), 24.4 (C-15), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.2 (C-29), 11.9 (C-18). – **EIMS** (70 eV) *m/z* 414 ([M]$^+$, 100), 396 (32), 255 (62).

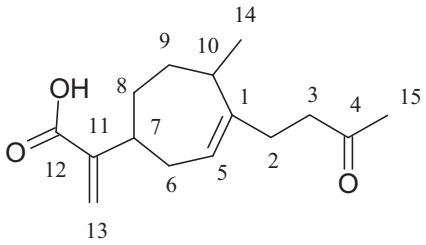


Zanthoxyl flavone (53): Yellow needles, 4.62 mg, UV absorbing, yellow colour with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.29 (CH_2Cl_2/5 % CH_3OH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 1. – **H,H COSY** and **HMBC** see Figure 3. – **(-)-ESI MS** *m/z*

345 ([M - H]$^-$, 100), 691 ([2M - H]$^-$, 70). – **(+)-HRESI/MS** *m/z* 345.0619 [M - H]$^-$ (calcd for $C_{17}H_{13}O_8$, 345.0616).



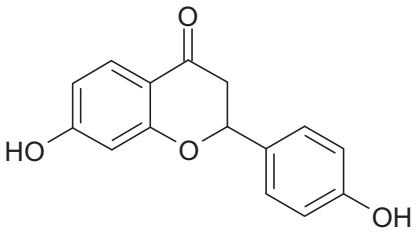
Xanthatin (56): White solid, 2.3 g, UV absorbing at 254 nm, pink colour with anisaldehyde/sulphuric acid spray reagent. – $\boldsymbol{R_f}$ = 0.65 (CH_2Cl_2/ 5%MeOH). – **^{1}H** ($CDCl_3$, 300 MHz) and **^{13}C NMR** ($CDCl_3$, 125 MHz) see Table 3. – **H,H COSY** and **HMBC** see Figure 7. **-(+)- ESIMS** *m/z* 269 ([M + Na]$^+$ 100), 515 ([2M + Na]$^+$, 30). – **(+)-HRESI MS** *m/z* 269.11502 [M + Na]$^+$, (calcd 269.11482 for $C_{15}H_{18}O_3Na$).



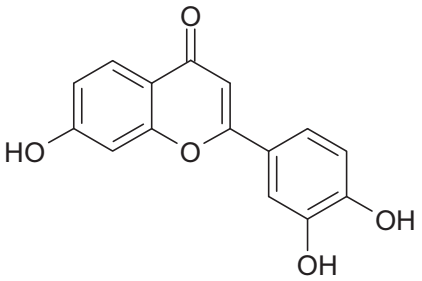
4-Oxobedfordia acid (57): white solid, 1.1 g**, UV** absorbing, pink colour with anisaldehyde/sulphuric acid spray reagent. – $\boldsymbol{R_f}$ = 0.68 (CH_2Cl_2/5% MeOH). – **^{1}H** ($CDCl_3$, 300 MHz) and **^{13}C NMR** ($CDCl_3$, 125 MHz) see Table 4. – **H,H COSY** and **HMBC** see Figure 9. – **(+)-ESIMS** *m/z* 273 ([M + Na]$^+$ 100), 523 ([2M + Na]$^+$ 50); **(-)-ESIMS** *m/z* 249 ([M - H]$^-$, 100), 521 ([2M – 2H + Na]$^+$ 70). – **(+)-HRESI MS** *m/z* 273.14630 [M + Na]$^+$, (calcd 273.14612 for $C_{15}H_{22}O_3Na$).

8.2 *Albizia zygia* (Leguminosae subfamily Mimosoideae)

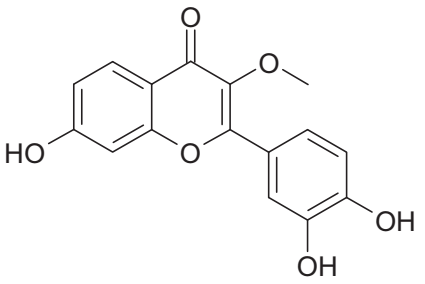
An air-dried sample (1.0 kg) of the bark of *A. zygia* was powdered and extracted (3×) with 50% CH_2Cl_2/MeOH. The extracts were pooled and evaporated under reduced pressure to give a dark brown residue (148.6 g). The reside was partitioned between ethyl acetate and water which delivered 7.3 g of crude extract after evaporation of the organic phase and 139.5 g from the water phase. Fractionation of the organic extract was carried out using silica gel chromatography eluted with a gradient of cyclohexane: EtOAc. Fractions were combined according to their TLC profiles yielding three major fractions. Fraction 1 was further purified on Sephadex LH-20 using CH_2Cl_2: MeOH (6:4) followed by gradient chromatography on RP-18 eluted with MeOH:H_2O (10:90 to 50:50), which afforded compound **58**. Fraction II was separated using LiChroprep RP-18 eluted with MeOH/H_2O (10:90 to 50:50) to give compound **59** and **60**. The gum from the water phase was washed with MeOH and the evaporated washing then subjected to gradient chromatography on silica gel eluted with cyclohexane/EtOAc gradient (10 to 100 EtOAc). This afforded compounds **61** and **62**.



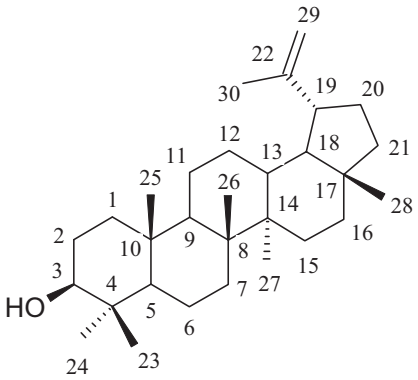
4'7-Dihydroxyflavanone (58): Yellow needles, 1.0 mg, turned to yellow with anisaldehyde/sulphuric acid. – R_f = 0.10 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.72 (d, ^3J = 8.6 Hz, 1H, H-5), 7.32 (d, ^3J = 8.6 Hz, 2H, H-2', H-6'), 6.81 (d, ^3J = 8.6, 2H, H-3',5'), 6.48 (dd, ^3J = 8.6, ^4J = 2.2 Hz, 1H, H-6), 6.34 (d, ^4J = 2.3 Hz, 1H, H-8), 5.38 (dd, ^3J = 13.1, ^3J = 3.1 Hz, 1H, H-2), 3.06 (dd, ^2J = 17.5, ^3J = 13.1 Hz, 1H, H_a-3), 2.68 (dd, ^2J = 17.5, ^3J = 3.0 Hz, 1H, H_b-3). – **(-)-ESI MS** *m/z* 255 ([M - H]$^-$, 40), 511 ([2M - H]$^-$, 100). – **(-)-HRESIMS** *m/z* 255.06633 [M - H]$^-$ (calcd 255.06628 for $C_{15}H_{11}O_4$).



7,3',4'-Trihydroxyflavone (59): Light yellow amorphous solid, 2.7 mg, yellow colour with anisaldehyde/sulphuric acid. – R_f = 0.14 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 7.95 (d, ^3J = 8.5 Hz, 1H, H-5), 7.38 (m, 2H, H-2',6'), 6.92 (m, 1H, H-8), 6.89 (m, 1H, H-6), 6.88 (m, 1H, H-5'), 6.61 (s, 1H, H-3); OH signals at very broad signals at $\delta \sim 10$. – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 176.1 (C_q, C-4), 162.6 (C_q, C-2), 162.5 (C_q, C-7), 157.3 (C_q, C-8a), 149.1 (C_q, C-4'), 145.6 (C_q, C-3'), 126.4 (CH, C-5), 122.0 (C_q, C-1'), 118.4 (CH, C-6'), 116.0 (C_q, C-4a), 115.9 (CH, C-5'), 114.7 (CH, C-6), 113.1 (CH, C-2'), 104.4 (CH, C-3), 102.3 (CH, C-8). – **(-)-ESI MS** *m/z* 269 ([M - H]$^-$, 100), 539 ([2M - H]$^-$, 85), 809 ([3M - H]$^-$, 62). – **(+)-HRESIMS** *m/z* 271.06019 [M + H]$^+$ (calcd 271.06010 for $C_{15}H_{11}O_5$).



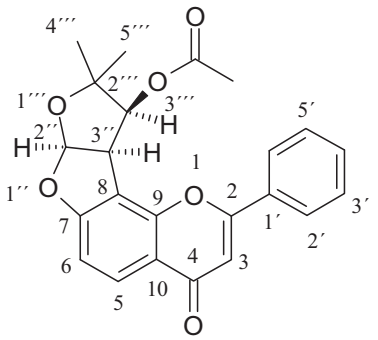
3-*O*-Methylfisetin (60): Light yellow amorphous solid, 1.7 mg, yellow colour with anisaldehyde/sulphuric acid. – R_f = 0.11 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz; data from 2D spectra in mixture with **59**): δ 7.89 (d, ^3J = 8.8 Hz, 1H, 5-H), 7.53 (d, ^4J = 2.0 Hz, 1H, H-2'), 7.43 (dd, ^3J = 8.4, ^4J = 2.1 Hz, 1H, H-6'), 6.90 (m, 2H, H-5',6), 6.88 (m, 1H, H-8), 3.77 (s, 3H, 3-OCH_3). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 172.9 (C_q, C-4), 162.6 (C_q, C-7), 156.3 (C_q, C-8a), 154.5 (C_q, C-2), 148.1 (C_q, C-4'), 145.1 (C_q, C-3'), 139.3 (C_q, C-3), 126.5 (CH, C-5), 121.3 (C_q, C-1'), 120.2 (C_q, C-6'), 116.2 (CH, C-5'), 115.6 (C_q, C-4a), 116.2 (CH, C-6), 115.3 (CH, C-2'), 101.9 (CH, C-8), 59.3 (3-OCH_3). – **(-)-ESI MS** *m/z* 299 ([M - H]$^-$). – **(+)-HRESIMS** *m/z* 301.07070 [M + H]$^+$ (calcd 301.07066 for $C_{16}H_{13}O_6$).



Lupeol (61): White powder, 2.85 mg, turned to violet with anisaldehyde/sulphuric acid. – R_f = 0.66 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 4.69 (d, J = 1.7 Hz, 1H, H_a-29) 4.57 (m, 1H, H_b-29), 3.18 (dd, J = 10.8, J = 5.4 Hz, 1H, H-3), 2.37 (m, 1H, H-19), 2.25-139 (m, 2H, CH_2-20), 1.93 (m, 1H, H_a-21), 1.91, 1.88 (m, 2H, CH_2-2), 1.66 (s, CH_3-30), 1.64 (m, 1H, H_a-1), 1.64 (m, 1H, H_a-12), 1.59 (m, 1H, H-13), 1.57-1.30 (m, 2H, CH_2-15), 1.54 - 1.44 (m, 3H, CH_2-6, H_a-16), 151-1.33 (m, 1H, H_b-21), 1.42-1.26 (m, 2H, CH_2-11), 1.40 (m, 2H, CH_2-7), 1.40 (m, 1H, H-18), 1.35 (m, 2H, H-18, H_b-16), 1.18 (1H, m, H-9), 1.07 (m, H, H_b-12), 1.01 (s, 3H, CH_3-27), 0.95 (s, 3H, CH_3- 26), 0.92, 0.81, 0.77 (s, 9H, CH_3-25, CH_3-24, CH_3-23), 0.90 (d, J = 5.5, 1H, H_b-1), 0.74 (s, CH_3-28), 0.66 (d, J = 8.9 Hz, H-5). – **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 150.9 (C-22), 109.3 (C-29), 79.0 (C-3), 55.3 (C-5), 50.5 (C-9), 48.4 (C-18), 48.0 (C-19), 43.1 (C-17), 42.9 (C-14), 40.9 (C-8), 40.1 (C-21), 38.9 (C-4), 38.8 (C-1), 38.1 (C-13), 37.2 (C-10), 35.6 (C-16), 34.3 (C-7), 29.9 (C-20), 28.1 (C-23), 27.5 (C-15), 27.5 (C-2), 25.2 (C-12), 21.0 (C-11), 19.4 (C-30), 18.4 (C-6), 18.1 (C-28), 16.2 (C-24), 16.1 (C-25), 15.5 (C-26), 14.6 (C-27). – **EI MS** (70 eV) *m/z* 426 ([M]$^+$, 85). – **(+)-HRESI MS** *m/z* 427.39361 [M + H]$^+$, (calcd 427.39344 for $C_{30}H_{50}O$).

8.3 *Tephrosia apollinea* (Del.) Link (Leguminosae)

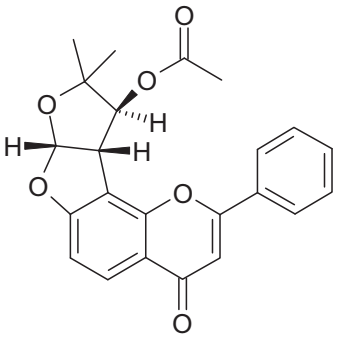
An aerial part of the plant (500 g) was dried, crushed, and extracted with CH_2Cl_2, EtOAc and MeOH. The three organic phases were combined and brought to dryness under reduced pressure. The crude extract (80 g) was defatted using cyclohexane and subjected to silica gel vacuum chromatography (7.5 × 60 cm) using cyclohexane/EtOAc gradient (0/100 %) to yield four fractions. Fraction (FII) was purified on silica gel column chromatography using cyclohexane/EtOAc gradient (0 to 90 EtOAc) to afford compound **63** and **64**. While fraction (FIII) was purified on silica gel column chromatography using cyclohexane/EtOAc gradient 0 to 100 EtOAc followed by Sephadex LH-20 using MeOH to afford compound **65**. Fraction (FIV) was separated on Sephadex LH-20 using MeOH to afford two sub-fractions named FIVa and FIVb, FIVa was purified on silica gel CC using cyclohexane/EtOAc gradient 0 to 100 EtOAc to afford compound **66**, while FIVb was purified on Sephadex LH-20 using MeOH to deliver compound **67**.

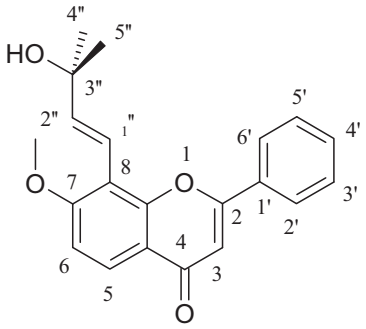


Pseudosemiglabrin (63): White amorphous, 6.88 mg, blue-greenish with anisaldehyde/sulphuric acid. –

R_f = 0.76 (CH_2Cl_2/5% MeOH). – **^{1}H** ($CDCl_3$, 300 MHz) and **^{13}C NMR (**$CDCl_3$, 125 MHz) see Table 5. – **(+)-ESI MS** *m/z* 393 ([M + H]$^+$, 5), 807 ([2M + H]$^+$, 100). – **(+)-HRESI MS** *m/z* 393.13324 [M + H]$^+$, (calcd 393.13326 for $C_{23}H_{21}O_6$).

Semiglabrin (64): White amorphous, 30.0 mg, blue-greenish with anisaldehyde/sulphuric acid. – R_f = 0.76 (CH_2Cl_2/5% MeOH). – **^{1}H** ($CDCl_3$, 300 MHz) and **^{13}C NMR** ($CDCl_3$, 125 MHz), see Table 6. – **(+)- ESI MS** *m/z* 393.6 ([M + H]$^+$, 35), 807 ([2M + H]$^+$, 100). – **(+)-HRESI MS** *m/z* 393.13345 [M + H]$^+$, (calcd 393.13345 for $C_{23}H_{21}O_6$).

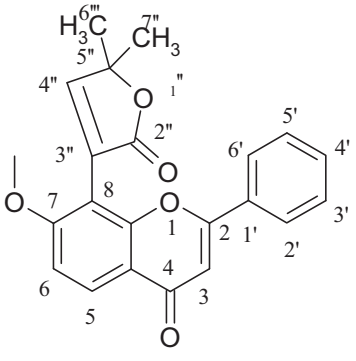




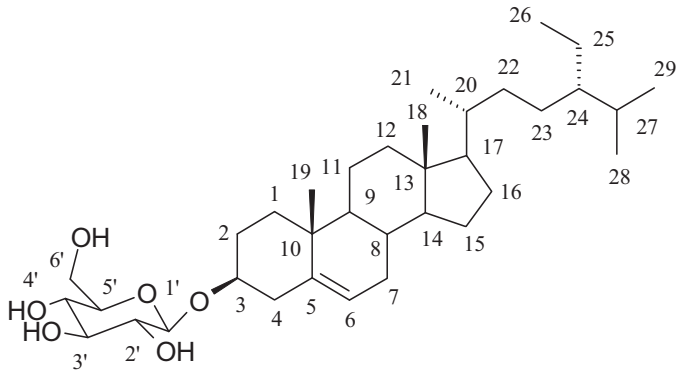
Lanceolatin A (65): White amorphous, 10.05 mg, turned to violet with anisaldehyde/sulphuric acid. – R_f = 0.7 (CH_2Cl_2/5% MeOH). – **^{1}H** ($CDCl_3$, 300 MHz) and **^{13}C NMR (**$CDCl_3$, 125 MHz), see Table 7. – **(+)-ESIMS** *m/z* 359 ([M + Na]$^+$, 6), 695 ([2M + Na]$^+$, 100), 1031 ([3M + Na]$^+$, 30).

Apollinine-Precursor: – **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.55 (dd, ^3J = 5.9, 1.7 Hz, 2H), 8.18 (d, ^3J = 9.0 Hz, 1H), 7.71 (d, ^3J = 7.8, 1.9 Hz, 2H), 7.63 (ddd, ^3J = 9.4, 7.6, 1.8 Hz, 1H), 7.44 (m, 3H), 7.23 (dd, ^3J = 4.4, 1.5 Hz, 1H), 7.21(dd, ^3J = 4.2, 1.5 Hz, 1H), 7.02 (d, ^3J = 9.0, 1H), 6.67 (s, 1H), 3.89 (s, 3H), 1.62 (s, 6H).

Apollinine (66): White amorphous, 30.0 mg, turned to green with anisaldehyde/sulphuric acid. – R_f = 0.66 (CH_2Cl_2/5% MeOH). – **^{1}H** (300 MHz, $CDCl_3$) and **^{13}C NMR** ($CDCl_3$, 125 MHz), see Table 8. – **H,H COSY** and **HMBC** see Figure 20. – **(+)-ESI MS** *m/z* 363 ([M + H]$^+$, 15), 747 ([2M + Na]$^+$, 60), 1109 ([3M + H]$^+$, 100). – **(+)-HRESI MS** *m/z* 363.12270 [M + H]$^+$, (calcd 363.12270 for $C_{22}H_{19}O_5$.).



3β-*O*-Glucosylsitosterol (67): White amorphous, 5.63 mg, turned to dark violet with anisaldehyde/sulphuric acid. – R_f = 0.33 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 5.33 (d, ^3J = 5.1, 1H, H-6), 4.43 (s br, 2 H, 2 OH?), 4.27 (d, ^3J = 7.7, 1H, H-1'), 3.98 (t, ^3J = 5.9, 1H, H-4'), 3.69 (dd, ^2J = 11.7, ^3J = 4.3, 1H, H_a-6'), 3.49 (m,



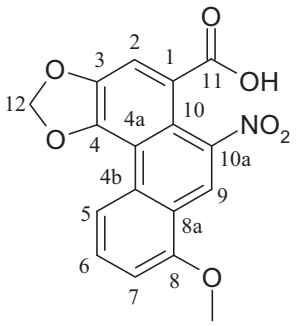
2H, H-3, H_b-6'), 3.21 (m, 1H, H-3'), 3.18 (dd, ^3J = 8.4, ^3J = 7.7, 1H, H-2'), 3.11 (dd, ^3J = 8.8, ^3J = 4.5 Hz, 1H, H-5'), 2.13, 2.17 (m, 2H, CH_2-4), 1.93 (m, 2H, CH_2-12), 1.93, 1.49, 1.45 (m, 2H, CH_2-7) 1.82-1.79 (m, 2H, CH_2-2), 1.82, 176 (m, 2H, CH_2-16), 1.58 (m, 1H, H-27), 1.55, 120 (m, 2H, CH_2-15), 1.45 (m, 2H, CH_2-11), 1.42, 1.33 (m, 1H, H-8), 1.38, 129 (m, 1H, H-20), 1.28 (m, 2H, CH_2-25), 1.17, 0.75 (m, 2H, CH_2-23), 1.05 (m, 1H, H-17), 0.98 (m, 2H, CH_2-1), 0.97 (m, 1H, H-24), 0.95 (m, 2H, CH_2-22), 0.94 (m, 1H, H-14), 0.93 (d, ^3J = 6.5, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.85 - 0.80 (m, 1H, H-9), 0.85 (d, ^3J = 6.5 Hz, 3H, CH_3-29), 0.84 (t, ^3J = 6.7, 3H, CH_3-26), 0.82 (d, ^3J = 6.6 Hz, 3H, CH_3-28), 0.69 (s, 3H, CH_3-18). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 140.3 (C_q, C-5), 120.4 (CH, C-6), 100.6 (CH, C-1'), 76.9 (CH, C-3), 76.6 (CH, C-5'), 76.6 (CH, C-3'), 73.2 (CH, C-2'), 70.2 (CH, C-4'), 61.1 (CH_2, C-6'), 55.8 (CH_2, C-7), 55.3 (CH, C-8), 50.0 (CH, C-9), 49.4 (CH, C-14), 45.1(CH, C-17), 38.9 (CH_2, C-2), 38.7 (CH_2, C-4), 38.1 (CH, C-20), 36.4 (C_q, C-13), 35.9 (C_q, C-10), 34.9 (CH_2, C-1), 33.2 (CH_2, C-15), 31.9 (CH, C-24), 30.1 (CH_2, C-16), 28.8 (CH_2, C-11), 28.7 (CH_2, C-25), 27.1 (CH_2, C-12), 24.1 (CH_2, C-22), 23.3 (CH_2, C-23), 20.4 (CH_3, C-28), 20.0 (CH_3, C-19), 19.0 (CH_3, C-18), 18.6 (CH_3, C-29), 18.5 (CH_3, C-21), 11.3 (CH_3, C-26), 10.8 (CH, C-27). – **(+)-ESIMS** *m/z* 599 ($[M + Na]^+$, 15), 1175 ($[2M + Na]^+$, 100). – **(+)-HRESI MS** *m/z* 599.42781 [M + Na]$^+$, (calcd 599.42780 for $C_{35}H_{60}O_6Na$).

8.4 *Aristolochia bracteolata* Lam (Aristolochiaceae)

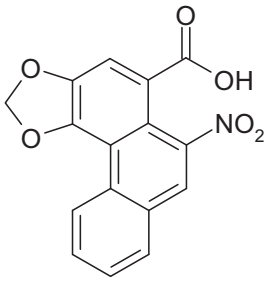
The roots were dried and crushed (500 g) and extracted exhaustively using methanol by Soxhlet apparatus. The methanolic extract was concentrated and evaporated till dryness. The crude extract (70 g) was defatted with cyclohexane; the fat was about 30 g. The crude extract was fractionated on silica gel column chromatography (7.5 × 60 cm) using CH_2Cl_2/MeOH 0:20 % to deliver two fractions. FII was purified on silica gel column using CH_2Cl_2/MeOH gradient 0 to 10 MeOH to give two sub-fractions (FIIa and FIIb), FIIa was purified on silica gel column using CH_2Cl_2/MeOH gradient 0 to 10 MeOH to afford a yellow powder of aristolochic acids mixture. FIIb was sepa-

rated on Sephadex LH-20 using MeOH to offer aristolochic A acid (**68**), while FIII was mainly sugars (20 g).

Aristolochic acid A (68): Yellow amorphous, 5.4 g, yellow colour with anisaldehyde/sulphuric acid. – R_f = 0.22 (CH_2Cl_2/10% MeOH). – **^{1}H** (CD_3OD, 300 MHz) and **^{13}C NMR (**CD_3OD, 125 MHz) see Table 8. – **H,H COSY** and **HMBC** see Figure 25. – **(+)-ESIMS** *m/z* 342 ([M + H]$^+$ 70), 683 ([2M + H]$^+$, 10). – **(+)- HRESIMS** *m/z* 364.04283 [M + Na]$^+$ (calcd 364.04277 for $C_{17}H_{11}NO_7Na$).



Aristolochic acids B (69) and **A (68) mixture:** Yellow amorphous, 160 g, yellow colour with anisaldehyde/sulphuric acid. – R_f = 0.22 (CH_2Cl_2/10% MeOH). – **(+)-ESIMS** *m/z* 334 ([M + H]$^+$ 50), 645 ([2M + H]$^+$, 100). – **(+)-HRESIMS** *m/z* 334.03225 [M + Na]$^+$ (calcd 334.03221 for $C_{16}H_9O_6Na$).



9 Metabolities from Selected Bacterial Strains

9.1 Origin of the Investigated Strains

All Streptomycetes with names starting with the signature "ANK" were obtained from Prof. Dr. H. Anke, Institute for Biotechnology and Drug Research, Kaiserslautern. *Streptomyces* spp. starting with the signature "GW" are of terrestrial origin and were obtained from the collection of the laboratory of Dr. Iris Grün-Wollny, Giessen. The marine *Streptomyces* spp. "B" are obtained from the collection of Dr. E. Helmke, Alfred-Wegener Institute for Polar and Marine Research, Bremerhaven. The origin of a few further strains is mentioned at the corresponding place.

9.2 Terrestrial *Streptomyces* sp. ANK 210

The well-grown agar plate of the terrestrial *Streptomyces* sp. ANK 210 isolate was used to inoculate 1 L of M_2^+ medium and the culture was incubated for 96 hours at 28 °C. The culture broth was extracted with ethyl acetate to afford the crude extract. The crude extract was used for the pre-screening.

9.2.1 Pre-screening

In the biological activity the crude extract showed weak antimicrobial activity against different microorganisms and *Artemia salina* as mentioned in Figure 131.

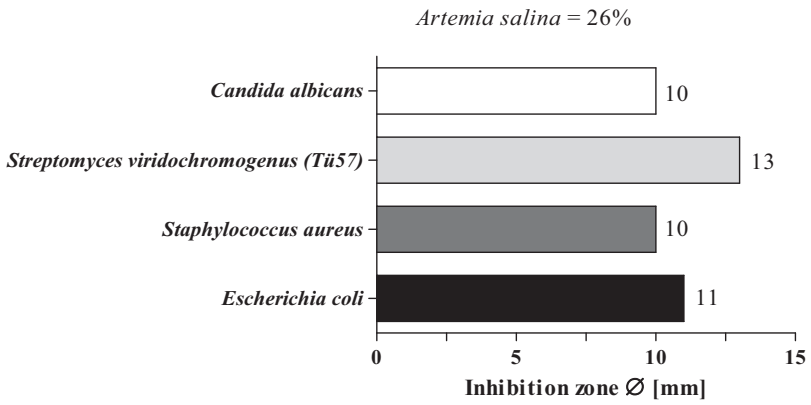


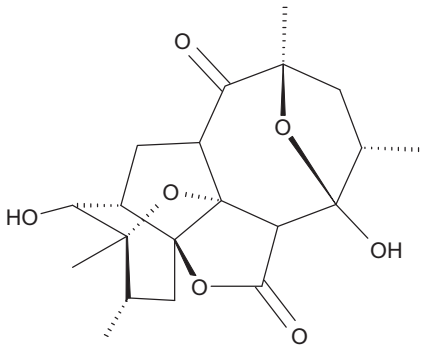
Figure 131: Biological activity of the crude extract from the terrestrial *Streptomyces* sp. ANK 210

In the chemical screening on Thin Layer Chromatography (TLC) the crude extract exhibited two interesting UV active bands, which gave pale yellow colour with anisaldehyde/sulphuric acid reaction.

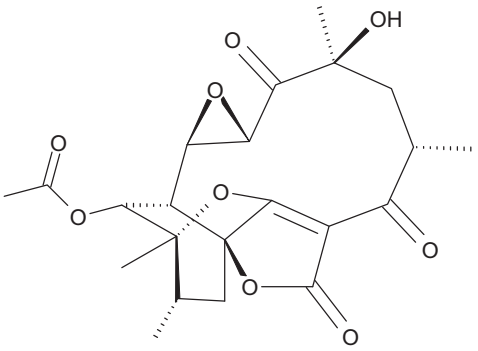
9.2.2 Fermentation and Isolation

The well-developed colonies of the agar plates were used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2^+ medium.The culture was incubated on the shaker culture (95 rpm) at 28 °C for 7 days. The resulting brown culture broth was mixed with *ca.* 1 kg diatomaceous earth (Celite) and pressed through a filter press to afford the aqueous filtrate and a mycelial fraction. The aqueous fraction was extracted by the Amberlite XAD-16 resin using MeOH. The mycelium was extracted (3×) with EtOAc followed by acetone (1×). The EtOAc and acetone phases were evaporated till dryness. The MeOH fraction from the aqueous residue was evaporated and extracted using EtOAc and evaporated to dryness. On TLC the three crude extracts showed no differences, accordingly they were combined to obtain 5.8 g of a brown yellowish crude extract. The crude extract was dissolved in methanol, defatted with cyclohexane and subjected to silica gel column chromatography using CH_2Cl_2/MeOH gradient (column 3 × 60 cm, 0 to 20 % MeOH). Fraction FII was purified on Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O gradient (10 to 50 % MeOH) to deliver *ent*-homoabyssomicins A (**70**) and B (**72**), while FIII was purified on Sephadex LH-20 using MeOH followed by silica gel using cyclohex-

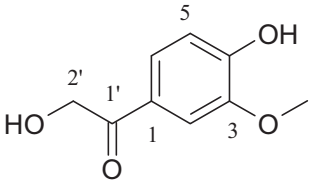
ane/EtOAc (gradient 0 to 100 % EtOAc) to afford indole-3-carboxylic acid, 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**79**) and Niax (**81**). FIV was purified on RP-18 using MeOH/H_2O (4:6) to afford benadrostin (**80**).



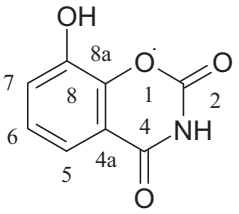
***ent*-Homoabyssomicin A (70):** White crystals, 12.05 mg, UV absorbing at 254 nm, pale yellow colour with anisaldehyde/sulphuric acid reagent. – R_f = 0.45 (CH_2Cl_2/5% MeOH). – $[\alpha]_D^{20}$ = -19 (c = 0.1, CH_3OH). – **CD**: (c 1.89 × 10^{-4} M, MeOH) λ max 202.4 nm ($\Delta\varepsilon$ 3.35). – **IR (KBr):** ν_{max} = 3428, 2928, 1762, 1726, 1636, 1460, 1383, 1250, 1111, 1075, 1030 cm^{-1}. – **^{1}H** and **^{13}C NMR** (CD_3OD, 600, 125 MHz) see Table 10. – **H,H COSY** and **HMBC** see Figure 36. – **(+)-ESIMS** *m/z* 379 ($[M + H]^+$, 90), 779 ([2M + Na] $^+$, 50). – **(+)-HRESIMS** *m/z* 379.17512 $[M + H]^+$, (calcd. 379.17513 for $C_{20}H_{27}O_7$.



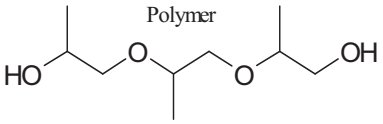
***ent*-Homoabyssomicin B (72):** White solid, 1.31 mg, UV absorbing at 254 nm, yellow colour with anisaldehyde/sulphuric acid. – R_f = 0.48 (CH_2Cl_2/5% MeOH). – $[\alpha]_D^{20}$ = +39 (c = 0.1, CH_3OH). – **CD**: (c 1.085 × 10^{-4} M, MeOH) λ max 235.8 nm ($\Delta\varepsilon$ 7.48), λ max 206.6 nm ($\Delta\varepsilon$ 14.57). – **IR (KBr)**: ν_{max} = 3428, 1742, 1656, 1454, 1396, 1020, 666 cm^{-1}. – **^{1}H** and **^{13}C NMR** (CD_3OD, 600, 125 MHz) see Table 11. – **H,H COSY** and **HMBC** see Figure **41**. – **(+)-ESIMS** *m/z* 457 ($[M + Na]^+$, 100), 891 ([2M + Na] $^+$, 50). – **(+)-HRESIMS** *m/z* 457.14686 $[M + Na]^+$, (calcd. 457.14690 for $C_{22}H_{26}O_9Na$).



2-Hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (79): Colourless solid, 4.7 mg, UV absorbing, greenish-blue with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.52 (CH_2Cl_2/3% MeOH). – **^{1}H** (300 MHz, CD_3OD): δ 7.52 (d, ^4J = 2.0 Hz, 1H, H-2), 7.50 (dd, ^3J = 8.2 Hz, ^4J = 2.0 Hz, 1H, H-6), 6.85 (d, ^3J = 8.2 Hz, 1H, H-5), 4.83 (s, 2H, CH_2-2'), 3.90 (s, 3H, OCH_3-3). – **(-)-ESIMS** *m/z* 385.2 ([2M - 2H + Na]$^-$, 100), 181.2 ([M - H]$^-$, 100). – **(+)-HRESIMS** *m/z* 183.06517 $[M + H]^+$ (calcd. 183.06519 for $C_9H_{11}O_4$).



Benadrostin (80): Colourless solid, 6.3 mg, UV absorbing band at 254 nm, yellow colour with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.24 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.46 (dd, ^3J = 6.7 Hz, ^4J = 2.7 Hz, 1H, H-5), 7.21 (dd, ^3J = 8.0 Hz, ^4J = 2.7 Hz, 1H, H-7), 7.17 (t, ^3J = 6.7 Hz, 1H, H-6). – **(+)-ESIMS** *m/z* 234 ($[H + Na]^+$); **(-)-ESIMS** *m/z* 178 ($[M - H]^-$). – **(+)-HRESIMS** *m/z* 180.02913 $[M + H]^+$ (calcd. 180.02911 for $C_8H_6NO_4$).



Niax (Polypropylenglycol) (81): Colourless oil, 17.3 mg, UV absrobing zone, turned to pink with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.16 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 3.51 (m, CH_2), 1.10 (d, ^3J = 2.3 Hz, CH_3).

9.3 Terrestrial *Streptomyces* sp. ANK 313

The agar plate of the terrestrial *Streptomyces* sp. ANK 313 was used to inoculate 1 L culture of M_2^+ medium and kept at 28 °C on the shaker culture for 7 days. The resulting culture broth was extracted with ethyl acetate and used for pre-screening.

9.3.1 Pre-screening

The terrestrial *Streptomyces* sp. ANK 313 was selected due to the chemical and biological pre-screening, in which the crude extract exhibited three interesting UV active zones, which gave yellow colour with anisaldehyde/sulphuric acid spray reagent. Additionally, the crude extract showed promising antibacterial activity as mentioned in Figure 132, additionally the crude extract of the terrestrial *Streptomyces* sp. ANK 313 showed zoosporicidal activity against *P.viticola* at the concentration of 100 μg/ml.

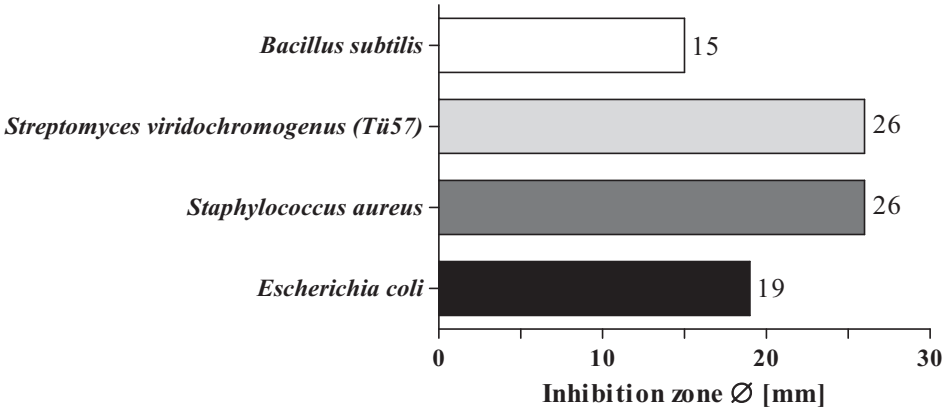
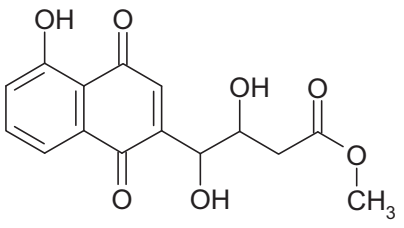


Figure 132: Antibacterial activity of the crude extract from terrestrial *Streptomyces sp.* ANK 313

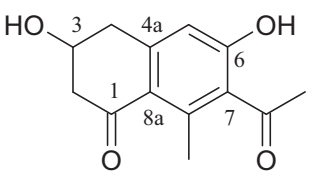
9.3.2 Bacterial Culturing and Isolation

The well-grown agar plate of the terrestrial *Streptomyces* sp. ANK313 was used to inoculate 100 of 1 L Erlenmeyer flasks each containing 300 ml of M_2^+ medium. The culture was incubated on the linear shaker culture at 28 °C for 7 days. The resulting culture broth was pressed through a filter press to deliver the filtrate and a biomass phases. The biomass was extracted with ethyl acetate followed by acetone. The filtrate was passed through XAD-16 resin and extracted with MeOH. Both fractions were evaporated till dryness. The combined extract (7.33 g) was defatted using cyclohexane and chromatographed on silica gel (column 3 × 60 cm) using CH_2Cl_2/MeOH gradient (0 to 20 % MeOH) to obtain three fractions. On TLC fraction F1 showed two yellow components, which was further purified on Sephadex LH-20 using MeOH followed by PTLC CH_2Cl_2/5% MeOH and again Sephadex LH-20 using MeOH to get three compounds; khatmiamycin (**82**), GTRI-02 (**84**) and 4-Ethyl-5-methyl-heptanamide. Fraction II was subjected to Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O (4:6) to deliver compounds aloesaponarin II (**85**) and LL-C10037α (**86**). Moreover, purification of FIII on RP-18 using MeOH/H_2O (3:7) followed by PTLC revealed compound LL-C10037β (**87**) and omdurmycin (**88**).

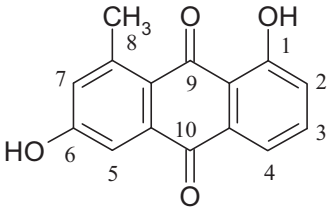


Khatmiamycin (82): Yellow solid, 1.28 mg, UV absorbing band at 254 nm, black with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.49 (CH_2Cl_2/5% MeOH). – **UV/VIS**: λ_{max} (log ε) = (MeOH): 243 (4.11), 265 (4.02), 322 (3.51), 412 (3.38). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 13. – **H,H COSY** and

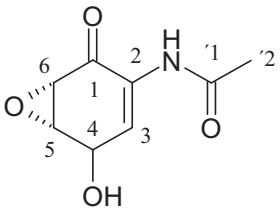
HMBC see Figure 46. – **(+)-ESIMS** *m/z* 329 ([H + Na]$^+$, 100), 635 ([2M + Na]$^+$, 10). – **(+)- HRESIMS** *m/z* 329.06311 [M + Na]$^+$ (calcd. 329.06319 for $C_{15}H_{14}O_7Na$).



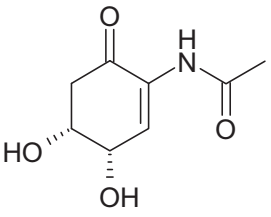
GTRI-02 (84): Yellow solid, 4.83 mg, UV absorbing band at 254 nm, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.33 (CH_2Cl_2/7% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 14. – **H,H COSY** and **HMBC** see Figure 51. – **(+)-ESIMS** *m/z* 257 ([H + Na]$^+$, 40), 491([2M + Na]$^+$, 100).



Aloesaponarin II (85): Yellow solid, 2.10 mg, UV absorbing band at 254 nm, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.43 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (300 MHz, CD_3OD) see Table 15. – (-)-**ESIMS** *m/z* 253 ([H - H]$^-$, 100), 529 ([2M - 2H + Na]$^-$, 10).

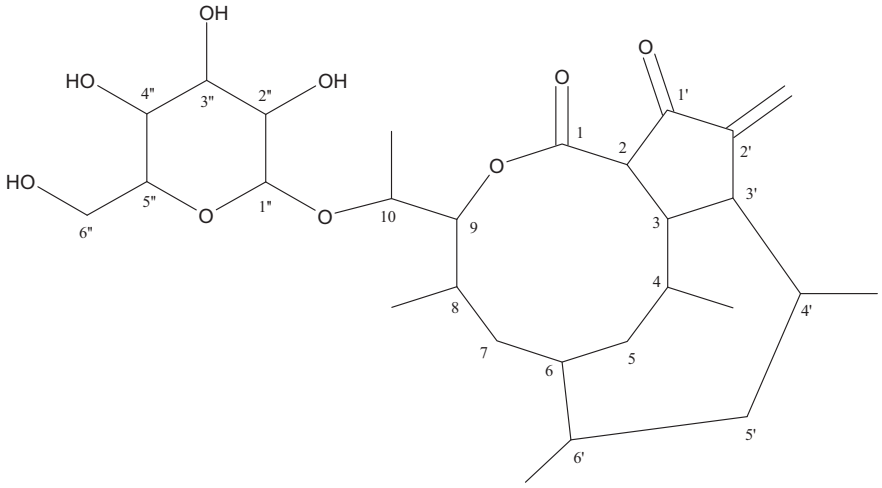


LL-C10037α (86): Yellow solid**,** 18.49 mg, UV absorbing band at 254 nm, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.20 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 16. – **H,H COSY** and **HMBC** see Figure 54. – **EIMS** (70 eV) *m/z* 183 ([M]$^+$, 40). – **(+)-HRESIMS** *m/z* 206.04240 [M + Na]$^+$ (calcd. 206.04238 for $C_8H_9NO_4Na$).



LL-C10037β (87): Yellow solid**,** 8.92 mg, UV absorbing band at 254 nm, yellow with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.30 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.51 (dd, ^3J = 3.8 Hz, ^4J = 1.8 Hz, 1H, H-3), 4.58 (t, ^3J = 7.3 Hz, ^4J = 3.7 Hz, 1H, H-5), 4.16 (ddd, ^3J = 4.8 Hz, ^3J = 3.7 Hz, ^4J = 1.8 Hz, 1H, H-4), 2.80 (dd, ^2J = 16.6 Hz, ^3J = 6.5 Hz, 1H, H_a-6), 2.69 (dd, ^2J = 16.6 Hz, ^3J = 3.7 Hz, 1H, H_b-6), 2.09 (s, CH_3-2'). – **(+)-ESIMS** *m/z* 208 ([M + Na]$^+$, 20), 393 ([2M + Na]$^+$, 100).

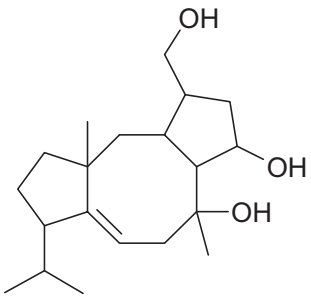
Omdurmycin (88): White solid, 3.4 mg, UV absorbing band at 254 nm, violet colour with anisaldehyde /sulphuric acid and heating. – R_f = 0.18 (CH_2Cl_2/10% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 19. – **(+)-ESIMS** *m/z* 547 ([M + Na]$^+$, 100), 1071 ([2M + Na]$^+$, 50). – **(+)- HRESIMS** *m/z* 547.28762 [M + Na]$^+$ (calcd. 547.28775 for $C_{28}H_{44}O_9Na$).



9.4 Terrestrial *Streptomyces* sp. GW08/253

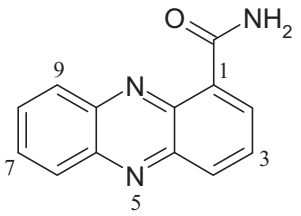
9.4.1 Bacterial Culturing, Work up and Isolation

An agar plate of the terrestrial *Streptomyces* sp. isolate GW 08/253 was used to inoculate 100 of 1 L Erlenmeyer flasks each containing 300 ml of M_2^+ medium (30 L). The culture was incubated for 8 days at 28 °C on a linear shaker. The culture broth was mixed with ca. 1 Kg diatomaceous earth and filtered using a filter press to deliver the mycelium and the water phase. The solid phase and the filtrate were extracted separately by using ethyl acetate. Both organic phases were combined and concentrated under reduced pressure to yield 7.10 g of a dark brown oily crude extract. The resulting crude extract was defatted by using cyclohexane and subjected to flash column chromatography using a CH_2Cl_2/MeOH gradient and separated into three fractions. Further purification has been done of the three fractions on Sephadex LH-20 MeOH, PTLC CH_2Cl_2/MeOH (95/5 %), silica gel (CC/EtOAc) and RP18 to afford six known compounds and one new compound. These compounds are: cyclooctatin (**89**), phenazine-1-carboxamide (**90**), phenazine-1-carboxylic acid (**91**), 6-[2-(1-hydroxy-butyl)-4-methyl-phenyl]-hex-5-enoic acid (**92**), a new compound 6-(2-but-1-enyl-4-methyl-pheny)-hex-5-enoic acid (**93**), 4-hydroxy-benzoic acid methyl ester (**94**) and 3-(hydroxyacetyl)-indole (**95**).

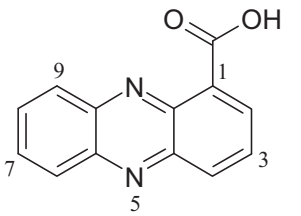


Cyclooctatin (**89**)**:** White solid, 5.23 mg, UV absorbing, turned to dark violet with anisaldehyde/sulphuric acid. – R_f = 0.10 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR**

(CD_3OD, 300, 125 MHz) see Table 20. – **H,H COSY** and **HMBC** see Figure 67. – **(+)-EIMS** *m/z* 345 ($[M + Na]^+$, 5), 667 ($[2M + Na]^+$, 100). – **(+)-HRESIMS** *m/z* 345.24024 $[M + Na]^+$ (calcd. 345.24002 for $C_{20}H_{34}O_3Na$).

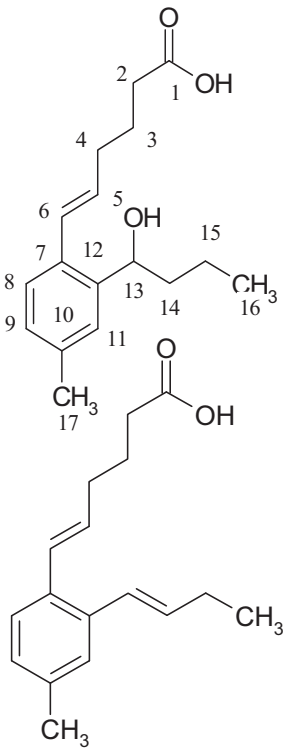


Phenazine-1-carboxamide (90): Yellow needles, 1.66 mg, UV absorbing zone, turned to yellow with anisaldehyde/sulphuric acid. – R_f = 0.29 (CH_2Cl_2/5% MeOH. – **^{1}H NMR** (300 MHz, $CDCl_3$): δ 10.74 (brs, 1H, 1-NH), 9.02 (dd, ^3J = 7.1, J = 1.5 Hz, 1H, H-2), 8.42 (dd, ^3J = 8.7, J = 1.5 Hz, 1H, H-4), 8.28 (m, 1H, H-6), 8.23 (m, 1H, H-9), 7.97 (dd, ^3J = 7.1, J = 1.5 Hz, 1H, H-3), 7.93 (dd, ^3J = 7.0 Hz, J = 1.6 Hz, 1H, H-7), 7.89 (dd, ^3J = 7.7 Hz, J = 1.0 Hz, 1H, H-8). – **(+)-ESIMS** *m/z* 246 ($[M + Na]^+$, 13), 469 ($[2M + Na]^+$, 100). – **(+)-HRESIMS** *m/z* 246.06383 $[M + Na]^+$ (calcd. 246.06378 for $C_{13}H_9N_3ONa$).



Phenazine-1-carboxylic acid (91): Yellow needles, 4.05 mg, UV absorbing zone, turned to yellow with anisaldehyde/sulphuric acid. – R_f = 0.35 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ 15.58 (brs, 1H, 1-OH), 8.98 (dd, ^3J = 7.1 Hz, J = 1.5 Hz, 1H, H-2), 8.54 (dd, ^3J = 8.8 Hz, J = 1.5 Hz, 1H, H-4), 8.33 (m, 1H, H-9), 8.29 (m, 1H, H-6), 8.00 (m, 3H, H-3,7,8). – **(+)-ESIMS** *m/z* 245 ($[M + Na]^+$, 10), 469 ($[2M - 2H + Na]^+$, 100).

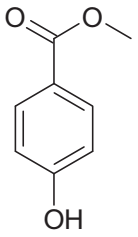
6-[2-(1-Hydroxy-butyl)-4-methyl-phenyl]-hex-5-enoic acid (92): Colourless solid, 1.38 mg, strong UV absorbing substance, turned to blue with anisaldehyde/sulphuric acid. – R_f = 0.15 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 600, 125 MHz), see Table 21. – **H,H COSY** and **HMBC** see Figure 68. – **EIMS** (70 eV) *m/z* 276 ($[M]^+$, 10), 169 (60), 215 (100), 171 (39), 233 (61), 260 (22) . – **(+)-HRESIMS** *m/z* 299.16198 $[M + Na]^+$ (calcd. 299.16177 for $C_{17}H_{24}O_3Na$).



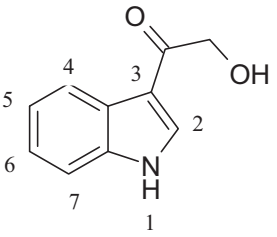
6-(2-But-1-enyl-4-methyl-pheny)-hex-5-enoic acid (93): Colourless solid, 1.1 mg, strong UV absorbing substance, turned to blue with anisaldehyde/sulphuric acid.

– R_f = 0.32 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz), see Table 22. – **H,H COSY** and **HMBC** see Figure 73. – **(+)-ESIMS** *m/z* 257 ([M - H]$^-$, 100), 515 ([2M - H]$^-$, 26). – **(+)-HRESIMS** *m/z* 259.16949 [M + H]$^+$ (calcd. 259.16926 for $C_{17}H_{23}O_2$).

***p*-Hydroxy-benzoic acid methyl ester (94):** Colourless solid, 13.7 mg, strong UV absorbing substance, no colour with anisaldehyde/sulphuric acid. – R_f = 0.30 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.94 (d, ^3J = 8.9 Hz, 2H, H-2, H-6), 6.85 (d, ^3J = 8.9 Hz, 2H, H-3, H-5), 5.70 (s, 1H, OH), 3.87 (s, 3H, 1-OCH_3). – **(+)-HRESIMS** *m/z* 153.05475 [M + H]$^+$ (calcd. 153.05462 for $C_8H_9O_3$).



3-(Hydroxyacetyl)-indole (95): Pale yellow solid, 2.6 mg, UV absorbing, turned to green yellowish with anisaldehyde/sulphuric acid. – R_f = 0.43 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.26 (m, 1H, H-4), 7.90 (d, J = 3.1 Hz, 1H, H-2), 7.44 (m, 1H, H-7), 7.32 (m, 2H, H-5,6), 4.77 (s, 1H, CH_2-3). – **(+)-ESIMS** *m/z* 198 ([M + Na]$^+$, 100), 373 ([2M + Na]$^+$, 75).



9.5 Terrestrial *Streptomyces* sp. Red 202

The strain Red 202 gave a reddish mycelium after incubation on M_2^+ medium at 28 °C for three days. One agar plate was used to inoculate 4 of 1 litre-Erlenmeyer flasks each containing 250 ml of M_2^+ medium at 28 °C on a linear shaker with 95 rpm for seven days. The yellow reddish crude extract obtained from the 1 L shaker culture was used for the biological and chemical screening.

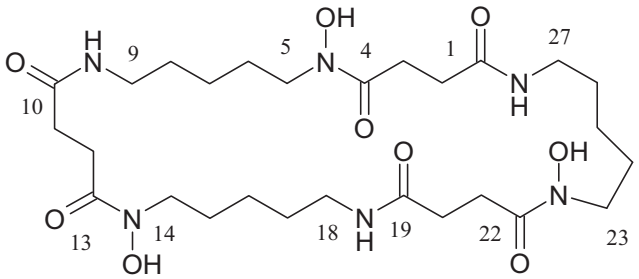
9.5.1 Pre-screening

The crude extract of the terrestrial *Streptomyces* Red 202 showed three interesting UV absorbing bands, which turned to black and red colour with anisaldehyde/sulphuric acid. Additionally, the crude extract showed moderate biological activity against *Escherichia coli*.

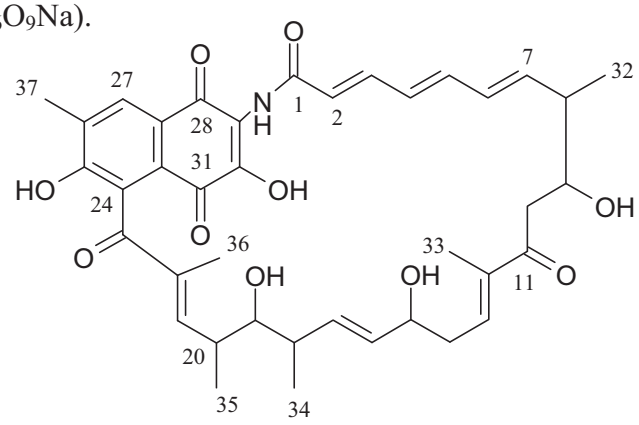
9.5.2 Bacteria Culturing and Isolation

Twenty-five litres of M_2^+ medium were inoculated and incubated at 28 °C on a linear shaker for seven days. The resulting yellow reddish culture broth was mixed with Celite and filtered using filter press. The water phase was passed over an XAD-16 column (65 × 8 cm), the resin washed with distilled water and eluted with metha-

nol. The mycelium was extracted with ethyl acetate followed by acetone. Both organic phases were evaporated till dryness to obtain two crude extracts. On TLC both of them showed different UV absorbing bands, which gave different colours with anisaldehyde/sulphuric acid and heating. In this respect they were chromatographed separately. The crude extract obtained from the filtrate (dark reddish, 4.50 g) was chromatographed on silica gel column chromatography (column 3 x 60 cm) CH_2Cl_2/MeOH gradient 0 to 20 % MeOH) to obtain two fractions namely FI-FII, FI was fat, while FII was purified on Sephadex LH-20 using MeOH to deliver nocardamine (**96**) and diastovaricin I (**97**). Where the crude extract obtained from the biomass (dark brown, 3.73 g) was chromatographed on silica gel column (3 x 60 cm) cyclohexane/EtOAc gradient 0 to 100 to obtain two fractions namely MI-MII, MI contained fat; MII was subjected to Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O (gradient 10 to 50 MeOH) to afford 6-hydroxy-5-methoxyisatine (**98**) and the metabolites thymidine and xanthosine (**99**).

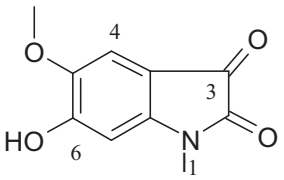


Nocardamine (96): White amorphous solid, 158.4 mg, UV inactive, turned to pale violet with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.29 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 9.55 (s, 3 H, OH), 7.68 (t, ^3J = 4.9 Hz, 3 H, 9, 18, 27-NH), 3.47 (t, ^3J = 6.6 Hz, 6 H, 5, 14, 23-CH_2), 2.99 (q, ^3J = 6.2 Hz, 6H, 9,18,27-CH_2), 2.59 (m, 6H, 2,11,20-CH_2), 2.29 (t, ^3J = 7.2 Hz, 6H, 3, 12,21-CH_2), 1.50 (t, ^3J = 7.0 Hz, 6H, 6,15,24-CH_2), 1.38 (qn, ^3J = 7.2 Hz, 6H, 8,17,26-CH_2), 1.22 (m, 6 H, 7,16,25-CH_2). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 171.8 (CO, C-4,13,22), 171.2 (CO, C-1,10,19), 46.8 (CH_2, C-5,14,23), 38.3 (CH_2, C-9,18,27), 30.0 (CH_2, C-3,12,21), 28.6 (CH_2, C-8,17,26), 27.5 (CH_2, C-2,11,20), 25.8 (CH_2, C-6,15,24), 23.2 (CH_2, C-7,16,25). – **(+)-ESIMS** *m/z* 623 ([M + Na]$^+$, 100); **(-)-ESIMS** *m/z* 599 ([M - H]$^-$, 100). – **(+)-HRESIMS** *m/z* 623.33731 [M + Na]$^+$ (calcd. 623.33741 for $C_{27}H_{48}N_6O_9Na$).

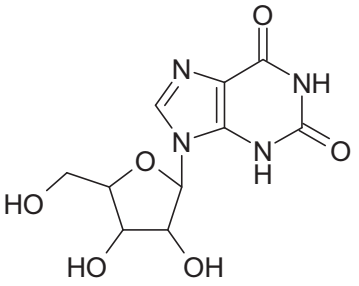


Diastovaricin I (**97**)**:** Red solid, 12.74 mg, UV absorbing, black with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.18 (CH_2Cl_2/5%

MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 600, 125 MHz) see Table 23. – **H,H COSY** and **HMBC** see Figure 82. – **(+)-ESIMS** *m/z* 710 ([M + Na]$^+$, 100), 1397 ([2M + Na]$^+$, 10). – **(-)-ESIMS** *m/z* 686 ([M - H]$^-$, 100). – **(+)-HRESIMS** *m/z* 710.29330 [M + Na]$^+$ (calcd. 710.29357 for $C_{39}H_{45}NO_{10}Na$).



6-Hydroxy-5-methoxyisatine (98): Reddish solid, 5.23 mg, UV absorbing at 254 nm, reddish colour by anisaldehyde/sulphuric acid and heating. – R_f = 0.36 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 Hz): δ 6.70 (s, 1H, H-4), 5.83 (s, 1H, H-7), 3.69 (s, 3H, 5-CH_3). – **^{13}C NMR** (CD_3OD, 125 Hz): δ 178.9 (C_q, CO-3), 175.0 (C_q, C-6), 168.0 (C_q, CO-2), 154.0 (C_q, C-7a), 150.0 (C_q, C-5), 105.5 (CH, C-4), 103.5 (C_q, C-3a), 102.7 (CH, C-7), 55.8 (CH_3, 5-OMe). – **H,H COSY** and **HMBC** see Figure 84. – **(+)-ESIMS** *m/z* 216 ([M + Na]$^+$, 100), 409 ([2 M + Na]$^+$, 100); **(-)-ESIMS** *m/z* 192 ([M - H]$^-$, 100). – **(+)-HRESI MS** *m/z* 216.02684 [M + H]$^+$ (calcd. 216.02673 for $C_9H_7NO_4Na$).



Xanthosine (99): Colourless oil, 1.4 mg, UV absorbing at 254 nm, blue by anisaldehyde/sulphuric acid and heating. – R_f = 0.10 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 Hz): δ 7.83 (s, 1H, H-2), 6.28 (t, ^3J = 7.0 Hz, 1H, H-1'), 4.24 (dt, ^3J = 8.4, 10.7 Hz, 1H, H-4'), 4.12 (dd, ^3J = 5.4, 3.6 Hz, 1H, H-3'), 3.52 (m, 1H, H_a-5'), 3.50 (m, 1H, H-2'), 22.2 (m, 1H, H_b-5'). – **(+)-ESIMS** *m/z* 307 ([M + Na]$^+$), 591 ([2 M + Na]$^+$).

9.6 Terrestrial *Streptomyces* sp. WO 1004

The agar plate of the terrestrial *Streptomyces* sp. WO 1004 was used to inoculate 4 of 1 litre-Erlenmeyer flasks each containing 250 ml of M_2^+ medium. The flasks were kept at 28 °C on a rotary shaker (95 rpm) for five days. The resulting culture broth was filtered under vacuum and extracted with ethyl acetate to yield brown oily crude extract, which used for chemical and biological pre-screening.

9.6.1 Pre-screening

On TLC the crude extract of the terrestrial *Streptomyces* sp. WO 1004 isolate showed two interesting bands, which gave black colour with anisaldehyde/sulphuric acid. Additionally, the crude extract showed antibacterial activity as mentioned in Figure 133.

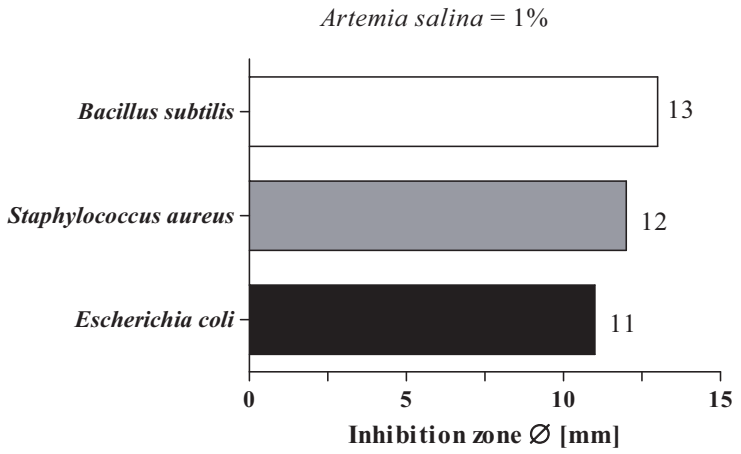
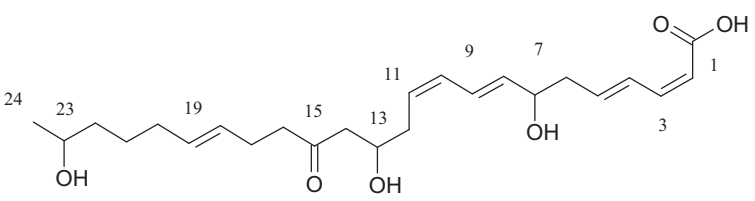


Figure 133: Biological activities of the crude extract of the terrestrial *Streptomyces* WO 1004

9.6.2 Bacterial Culturing and Isolation

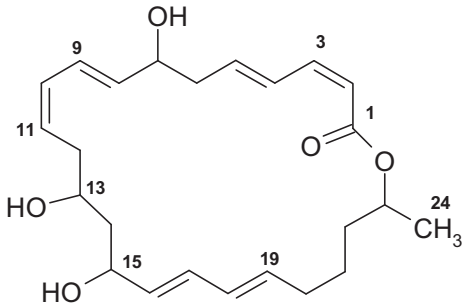
The agar cultures of the terrestrial *Streptomyces* sp. WO 1004 isolate was used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2^+ medium (25 L): After incubated at 28 °C on a linear shaker for seven days. The dark brown culture broth was mixed with 1.2 kg diatomaceous earth and filtered under vacuum. The biomass was extracted three times with ethyl acetate (3 L) followed by acetone. The filtrate was subjected to XAD-16 column; the resin has been washed with distilled water. The combined extracts were concentrated under vacuum to obtain brown oily crude extract (7.3 g). The crude extract was subjected to silica gel column chromatography (CC) eluting with CH_2Cl_2 followed by stepwise addition of CH_3OH to yield five fractions namely FI to FV. FII was subjected to Sephadex LH-20 eluted with CH_3OH followed by reverse phase RP-18 MeOH/H_2O (gradient 10 to 40% MeOH) to afford isomacrolactinic acid (**100**), FIII was purified on silica gel column chromatography using cyclohexane/EtOAc gradient 0 to100 EtOAc to yield tyrosol and uridine. Moreover FIV was purified on Sephadex LH-20 using MeOH followed by RP-18 MeOH/H_2O (3/7) to deliver macrolactin A (**101**), while FV was purified on RP-18 MeOH/H_2O gradient (10 to 50 MeOH) to deliver dihydromacrolactin F (**102**).

Isomacrolactinic acid (100): Yellow oil, 7.18 mg, UV absorbing, black with anisaldehyde/sul-

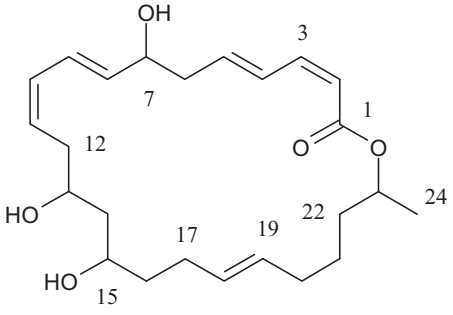


phuric acid spray reagent. – R_f = 0.29 (CH_2Cl_2/10% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) spectroscopic data see Table 24. – **H,H COSY** and **HMBC** see Figure 89. – **(-)-ESIMS** *m/z* 419 ([M - H]$^-$, 100), 839 ([2M - H]$^-$, 15). – **(+)-HRESIMS** *m/z* 443.24052 [M + Na]$^+$ (calcd. 443.24041 for $C_{24}H_{36}O_6Na$).

Macrolactin A (**101**)**:** Oily substance, 4.42 mg, UV absorbing, black colour with anisaldehyde/sulphuric acid. – R_f = 0.26 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 600 MHz): δ 7.23 (dd, ^3J = 14.4, ^3J = 11.3 Hz 1H, H-4), 6.64 (dd, ^3J = 11.6, ^3J = 11.3 Hz 1H, H-3), 6.58 (dd, ^3J = 15.0, ^3J = 11.3 Hz, 1H, H-9), 6.18 (dd, ^3J = 14.7, ^3J = 10.6, Hz, 1H, H-17), 6.17 (ddd, ^3J = 14.3, ^3J = 7.9, ^3J = 7.5 Hz, 1H, H-5), 6.09 (dd, ^3J = 10.6, ^3J = 14.7 Hz, 1H, H-18), 6.05 (dd, ^3J = 11.5, ^3J = 11.0 Hz, 1H, H-10), 5.76 (dd, ^3J = 15.2, ^3J = 5.7 Hz, 1H, H-8), 5.65, 5.54 (2 m, 2H, H-16, 19), 5.56 (m, 1H, H-11), 5.53 (d, ^3J = 11.6 Hz, 1H, H-2), 5.01 (m, 1H, H-23), 4.29 (m, 1H, H-15), 4.21 (m, 1H, H-7), 3.84 (m, 1H, H-13), 2.48 (m, 1H, H_a-12), 2.33 (m, 1H, H_b-12), 2.41 (m, 1H, H-6), 2.18 (m, 1H, H_a-20), 2.10 (m, 1H, H_b-20), 1.63 (m, 1H, H_a-22), 1.59 (m, 2H, CH_2-14), 1.52 (m, 1H, H_b-22), 1.49 (m, 2H, CH_2-21), 1.24 (d, ^3J = 6.3 Hz, 3H, CH_3-24). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 168.0 (CO, C-1), 145.0 (CH, C-3), 142.2 (CH, C-5), 137.5 (CH, C-8), 135.2 (CH, C-16), 135.1 (CH, C-19), 131.7 (CH, C-18), 131.4 (CH, C-17), 131.2 (CH, C-10), 130.2 (CH, C-4), 128.4 (CH, C-11), 125.9 (CH, C-9), 118.0 (CH, C-2), 72.3 (CH, C-23), 72.2 (CH, C-7), 69.8 (CH-15), 69.2 (CH, C-13), 43.9 (CH_2, C-14), 42.9 (CH_2, C-6), 36.5 (CH_2, C-12), 36.0 (CH_2-22), 33.0 (CH_2-20), 25.7 (CH_2, C-21), 20.1 (CH_3, C-24). – **H,H COSY** and **HMBC** see Figure 92. – **(+)-ESIMS** *m/z* 827 ([2 M + Na]$^+$, 100), 425 ([M + Na]$^+$, 40). – **(+)-HRESIMS** *m/z* 425.22988 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_5Na$, 425.22985).



Dihydromacrolactin F (**102**)**:** Yellow oil, 11.73 mg, UV absorbing, brown with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.14 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 25. – **H,H**



COSY and **HMBC** see Figure 96. – **(+)- ESIMS** *m/z* 427 ([M + Na]$^+$, 70), 831 ([2M + Na]$^+$, 100). – **(+)-HRESIMS** *m/z* 427.24575 [M + Na]$^+$ (calcd. 427.24549 for $C_{24}H_{36}O_5Na$).

9.7 Terrestrial *Streptomyces* sp. HO9

The terrestrial *Streptomyces* sp. HO9 isolate showed white colonies on the agar plate.

9.7.1 Pre-screening

In the pre-screening the terrestrial *Streptomyces* sp. HO9 isolate was optimised using different media as listed in Table 36 to get the optimal yields. Six of 1L Erlenmeyer flasks were inoculated each containing 300 ml of different media at 28 °C for 6 days using vigorous shaking at 95 rpm. The resulting culture broth was extracted with ethyl acetate and used for pre-screening.

The strain delivered high yield on M_2^+ medium, additionally on TLC three UV absorbing zones were observed, one turned to violet and two of them gave dark orange colour with anisaldehyde/sulphuric acid and heating. Unfortunately the crude extract of M_2^+ medium didn't show any biological activity, while the crude extracts of B and D media showed moderate activity against *S. aureus*, *Candida albicans*, *Chlorella vulgaris* and *Chlorella sorokiniana*. M_2^+ medium was selected for the large-scale shaker culture.

Table 36: Optimisation of the terrestrial *Streptomyces* sp. HO9

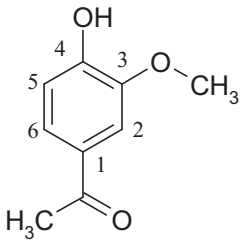
Medium	PH	Ingredients	Temp.	Cult. Period
M_2^+(A)	7.8	10 g/1L malt extract, 4g/1L glucose, 4g/1L yeast	28 ºC	6 days
Meat extract (B)	7.8	10g/1L glucose, 2g/1L pepton, 1g/1L yeast, 1g/1L meat extract	28 ºC	6 days
LB (C)	7.8	10g/1L Trypton, 5g/1L yeast, 10g/1L NaCl, 5g/1L glucose	28 ºC	6 days
B (D)	7.8	20g/1L Soya (low fat), 20g/1L Mannitol	28 ºC	6 days
$CaCl_2$ (E)	7.8	40g/1L yeast, 5g/1L glucose, 45g/1L $CaCl_2$	28 ºC	6 days
Fish flour (F)	7.8	21g/1L glucose, 5g/1L fish flour, 10g/1L flour (Type 405), 1g/1L yeast, 0.5g/1L $MgSO_4$, 1g/1L NaCl, 0.5g/1L $CaCl_2$	28 ºC	6 days

Figure 134: Optimisation of the terrestrial *Streptomyces* sp. HO9 isolate using different media

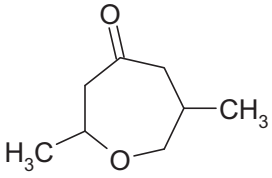
9.7.2 Fermentation, Work-up and Isolation

The well-grown agar plates were used to inoculate 100 of 1L Erlenmeyer flasks each containing 250 ml M_2^+ medium (25 L), the culture was incubated at 28 °C for 8 days using a linear shaker. The dark brown culture broth was filtered under vacuum using filter press. The biomass was extracted three times with ethyl acetate followed by acetone. The filtrate was subjected to XAD-16 column, the resin washed with dis-

tilled water and extracted with MeOH. The combined extracts were concentrated under vacuum till dryness to obtain a crude extract of 3.5 g. The crude extract was subjected to silica gel column chromatography eluting with CH_2Cl_2 followed by stepwise addition of CH_3OH (gradient 0 to 20 %) to yield four fractions. Fraction II was subjected to silica gel column chromatography eluted with cyclohexane/ EtOAc gradient 0 to 100 % to afford two *β*-sitosterol and stigmasterol. On the other hand FIII was purified on Sephadex LH-20 column chromatography eluted with CH_3OH followed by reverse phase RP-18 eluted with MeOH/water gradient 10 to 50 to afford indole-3-carboxylic acid and 1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**103**). FIV was chromatographed on Sephadex LH-20 using MeOH followed by PTLC (CH_2Cl_2/5% MeOH) to obtain anthranilic acid and 2,6-dimethyl-oxepan-4-one (**104**). Moreover, FV was purified on Sephadex LH-20 using MeOH to afford 3H-Indole-carboxylic acid methyl ester (**106**).

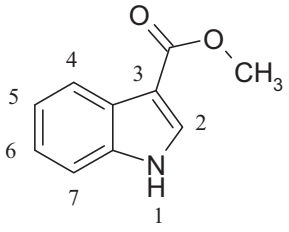


1-(4-Hydroxy-3-methoxy-phenyl)-ethanone (103): Colourless solid, 1.0 mg, UV absorbing, dark orange with anisaldehyde/sulphuric spray reagent. – R_f = 0.61 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 26. − **H,H COSY** and **HMBC** see Figure 99. –**EIMS** (70 eV) *m/z* 166 ($[M]^{+\cdot}$, 20), 151 ($[M\text{-}CH_4]^{+\cdot}$, 40) 43 (CH_3CO, 100).



2,6-Dimethyl-oxepan-4-one (104): Colourless solid, 1.64 mg, UV absorbing, dark orange with anisaldehyde/sulphuric spray reagent. – R_f = 0.24 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (300 MHz, CD_3OD): δ 4.20 (dd, ^3J = 12.8, ^3J = 6.5 Hz, 1H, H-6), 3.41, (ABX, J_{AB} = 10.7, ^3J = 5.6 Hz, 1H, H_a-1), 3.35 (ABX, J_{AB} = 10.7, ^3J = 6.5 Hz, 1H, H_b-1), 2.63-2.52 (m, 2H, CH_2-5), 2.29 (dd, ^2J = 16.6, ^3J = 7.8 Hz, 1H, H_a-3), 2.21 (dd, ^2J = 16.6, ^3J = 6.5 Hz, 1H, H_b-3), 2.15 (dd, ^3J = 12.8, ^3J = 6.5 Hz, 1H, CH-2), 1.18 (dd, ^3J = 6.7 Hz, 3H, 6-CH_3), 0.90 (d, ^3J = 6.7 Hz, 3H, 2-CH_3). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 211.7 (C_q, C-4), 67.7 (CH_2, C-1), 65.0 (CH, C-6), 52.9 (CH_2, C-5), 48.2 (CH_2, C-3), 33.0 (CH, C-2), 23.5 (6-CH_3), 17.1 (2-CH_3). − **H,H COSY** and **HMBC** see Figure 101. − **(+)-CIMS** *m/z* 143 $[M + H]^+$. – **(+)-HRESIMS** *m/z* 143.10666 $[M + H]^+$ (calcd. 143.10679 for $C_8H_{15}O_2$).

Indole-3-carboxylic acid methyl ester (106): Orange solid, 1.14 mg, UV absorbing, orange colour with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.15 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.54 (d, ^3J = 8.4 Hz, 1H, H-4), 7.34 (d, ^3J = 8.1 Hz, 1H, H-7), 7.18 (s, 1H, H-2), 7.08 (ddd, J = 8.0, J = 7.0, J = 1.2 Hz, 1H, H-5), 7.02 (ddd, J = 8.1, J = 7.0, J = 1.1 Hz, 1H, H-6), 3.65 (s, 3H, 3-OCH_3). – **EIMS** *m/z* 175 ($[M]^{+\cdot}$).

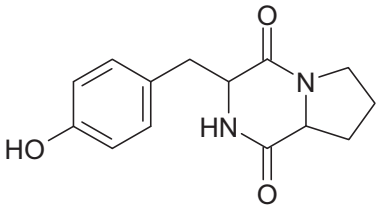


9.8 *Bacillus* sp. M10 strain

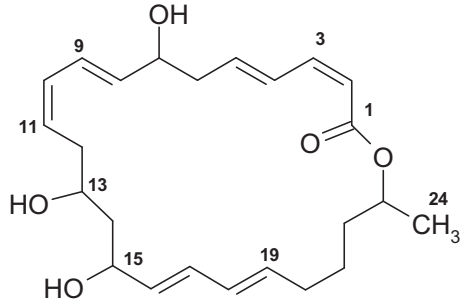
9.8.1 Fermentation, Extraction and Isolation

The well-grown agar plates of *Bacillus* sp. were used to inoculate 60 of 1 L Erlenmeyer flasks, each containing 250 ml of LB medium at PH 7.0. The cultures were cultivated on the linear shaker culture for 72 hours at 34 °C. The resulting culture broth was filtered using filter press. The mycelium was extracted with ethyl acetate and acetone. The filtrate was subjected to XAD-16 column and extracted with MeOH. The two combined phases was brought to dryness under reduced pressure. The crude extract (8.64 g) was defatted with cyclohexane and chromatographed on silica gel column to afford two fractions. FII was purified on silica gel column chromatography using cyclohexane/EtOAc gradient 0 to 100 EtOAc followed by Sephadex LH-20 using MeOH to deliver compounds **107** and **109** in addition to Adenosine and indole 3-carboxylic acid. Moreover, fraction III was purified on Sephadex LH-20 using MeOH to afford macrolactin F (**112**) and macrolactin B (**113**).

***Cis-cyclo*(Tyr, Pro) (107):** Colourless oil, 35.24 mg, UV absorbing, violet with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.35 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.03 (d, ^3J = 8.5 Hz, 2H, H-3', 5'), 6.70 (d, ^3J = 8.5 Hz, 2H, H-2', 6'), 4.32 (m, 1H, H-3), 4.03 (t, ^3J = 4.5 Hz, 1H, H-6), 3.60-3.49 (m, 2H, CH_2-9), 3.37 (m, 1H, H_a-10), 3.05 (AB, J = 9.2 Hz, 5.1 Hz, 1H, H_b-10), 2.10 - 1.78 (2 m, 1 + 3 H, CH_2-7, 8). – **(-)-ESIMS** *m/z* 259 ($[M - H]^-$, 80), 519 ($[2M - H]^-$, 100).

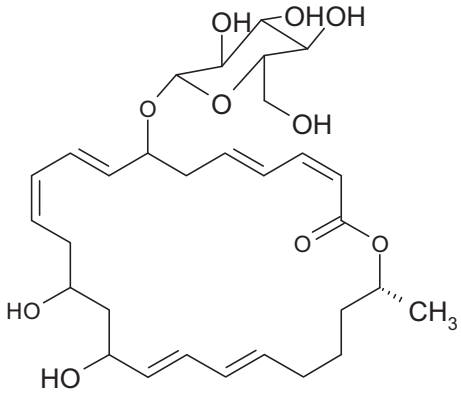


Macrolactin F (112): Yellow oil, 21.78 mg, UV absorbing, dark brown with



anisaldehyde/ sulphuric acid. − R_f = 0.14 (CH_2Cl_2/5% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz) see Table 27. − **(+)-ESIMS** *m/z* 425 ([M + Na]$^+$, 100), 827 ([2M + Na]$^+$, 20).

Macrolactin B (113): Colourless solid, 3.27 mg, UV absorbing, black colour with anisaldehyde/sulphuric acid, decomposed on storage. − R_f = 0.10 (CH_2Cl_2/5% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz) see Table 28. − **(+)-ESIMS** *m/z* 587 ([M + Na]$^+$, 100), 1151 ([2M + Na]$^+$, 20).



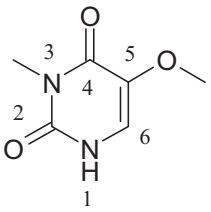
9.9 Terrestrial *Streptomyces* sp. WO 521

9.9.1 Pre-screening

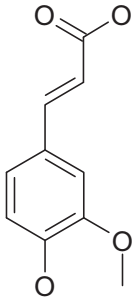
In the biological pre-screening the crude extract of the *Streptomyce*s sp. WO 521 isolate exhibited activity against *Mucor miehei* (14 mm) and *A. Cochlioides and* high activity against *Artemia salina* (100%). On TLC two interesting spots were observed, they turned to violet and brown yellowish with anisaldehyde/sulphuric acid .

9.9.2 Fermentation, Work up and Isolation

The terrestrial *Streptomyces* sp. WO 521 subculture was used to inoculate a 25 L shaker culture using M_2^+ medium at 28 °C (PH 7.8). The culture broth was harvested after seven days. Biomass and filtrate were separated with the aid of the filter press. The biomass was extracted several times with ethyl acetate followed by aceton (2 x). The water phase was subjected to XAD-16 column and extracted with MeOH. Both crude extracts showed identity on TLC, so they were combined (7.57 g) and chromatographed on silica gel column using CH_2Cl_2/MeOH gradient (0 to 15 % MeOH). The resulting fractions (FII to FIV) were purified separately. FII was purified on Sephadex LH-20 using MeOH followed by reverse phase RP-18 using MeOH/H_2O gradient (10 to 40 % MeOH) to afford 5-methoxy-3-methyl-1H-pyrimidine-2,4-dione (**115**) and tyrosol. FIII was purified on Sephadex LH-20 using MeOH followed by reverse phase RP-18 using MeOH/H_2O gradient (20 to 50% MeOH) to give indole-3-carboxylic acid and indole-3-acetic acid. FIV was separated on Sephadex LH-20 using MeOH to deliver tryptophol and ferulic acid (**116**).



5-Methoxy-3-methyl-1H-pyrimidine-2,4-dione (115): Brown solid, 1.0 mg, UV absorbing, turned to pale brown with anisaldehyde/sulphuric spray reagent. – R_f = 0.23 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (300 MHz, CD_3OD): δ 7.92 (s, 1H, H-6), 3.79 (s, 3H 5-OCH_3), 2.39 (s, 3H, 3-CH_3). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 170.0 (C_q-4), 152.2 (C_q-2), 148.0 (C_q-5), 122.0 (CH-6), 57.5 (5-OCH_3), 40.9 (3-CH3). – **EIMS** (70 eV) *m/z* 156 ([M]$^{+\cdot}$, 100). – **(+)-HRESIMS** *m/z* 157.04953 [M + H]$^+$ (calcd. 157.06077 for $C_8H_9O_2N_3$).



Ferulic acid (116): Colourless oil, UV absorbing substance, violet with anisaldehyde/sulphuric acid spray reagent. – R_f = 0.30 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.55 (d, ^3J = 15.8 Hz, 1H, H-2'), 7.09 (d, ^4J = 1.8 Hz, 1H, H-2), 7.04 (dd, ^3J = 8.2, ^4J = 1.8 Hz, 1H, H-6), 6.80 (d, ^3J = 8.2 Hz, 1H, H-5), 6.32 (d, ^3J = 15.8 Hz, 1H, H-1'), 3.92 (s, 3H, 3-OCH_3). – **EIMS** (70 eV) *m/z* 194 ([M]$^{+\cdot}$, 100).

9.10 Marine derived *Streptomyces* sp. B6219

The agar plate of the marine derived *Streptomyces* sp. B6219 showed white colonies, it used to inoculate one L of M_2^+ medium (with 50% sea water) and kept at 28 °C for three days. The culture broth was extracted with ethyl acetate and the resulting crude extract was used for pre-screening.

9.10.1 Pre-screening

In the biological screening the crude extract of the marine derived *Streptomyces* sp. B6219 showed antimicrobial activity as mentioned in Figure 135.

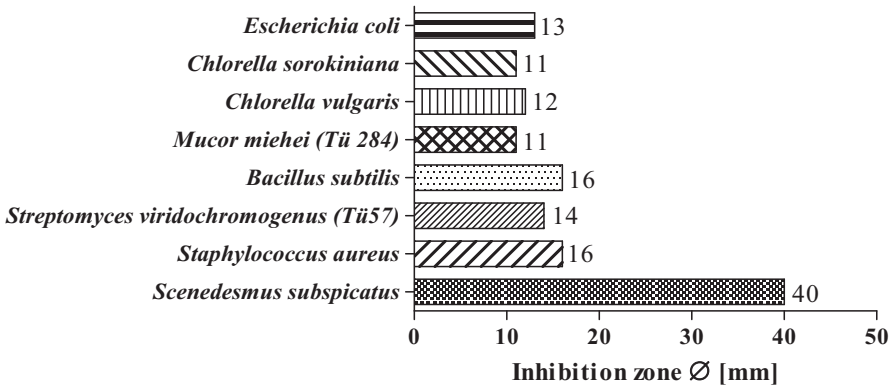
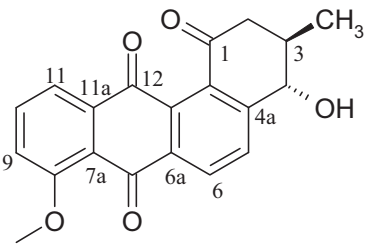


Figure 135: Antimicrobial activity of the crude extract from marine derived *Streptomyces* sp. B6219

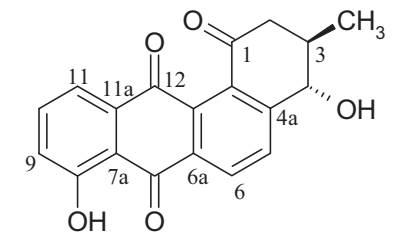
Regarding the chemical screening of the marine derived *Streptomyces* sp. B6219 on TLC the crude extract showed one interesting orange fluorescent zone, which turned to violet with 2N NaOH.

9.10.2 Fermentation and Isolation

The well-grown agar plates were used to inoculate 100 of 1 L Erlenmeyer flasks each containing 300 ml of M_2^+ medium (with 50% sea water). The culture was incubated on the linear shaker culture at 28 °C for 8 days. The culture broth was pressed through a pressure filter to afford biomass and filtrate. The filtrate was passed through XAD-16 resin and extracted with MeOH, while the biomass was extracted with EtOAc three times followed by acetone. The organic phases were brought to dryness. The combined crude extract (6.43 g) was defatted by cyclohexane and chromatographed on a silica gel column chromatography (column 3 x 60 cm) using CH_2Cl_2/MeOH gradient (0 to 20 % MeOH) to deliver four fractions FI-FIV. FII was purified on Sephadex LH-20 using MeOH to afford two sub-fractions, FIIa was later purified using PTLC (CH_2Cl_2/5% MeOH) and again Sephadex LH-20 using MeOH to afford a new bioactive angucyclinone, fujianmycin C (**119**), while FIIb after purification on Sephadex LH-20 using MeOH afforded fujianmycin A (**118**) and ochromycinone (**120**). Separation of FIII on silica gel (CH_2Cl_2/MeOH gradient 0 to 10 % MeOH) and Sephadex LH-20 using MeOH delivered two sub-fractions, FIIIa on PTLC (CH_2Cl_2/5% MeOH) afforded fujianmycin B (**117**), while FIIIb was purified on Sephadex LH-20 using MeOH to deliver ochromycinone methyl ether (**121**). FIV was purified on Sephadex LH-20 using MeOH followed by RP-18 (MeOH/30% H_2O) to deliver tetrangulol methyl ether (**122**).



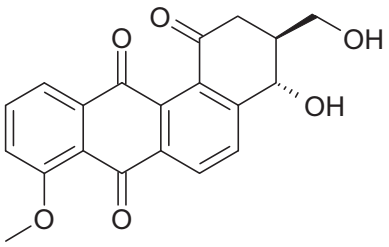
Fujianmycin B (117): Yellow needles, 7.97 mg, UV absorbing band at 254 nm, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.33 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR** (DMSO-d_6, 300, 125 MHz) see Table 23. – **(+)-ESIMS** *m/z* 359 ($[M + Na]^+$, 10), 695 ($[2 M + Na]^+$, 100). – **(+)-HRESI MS** *m/z* 337.10702 $[M + H]^+$ (calcd. 337.10705 for $C_{20}H_{17}O_5$).



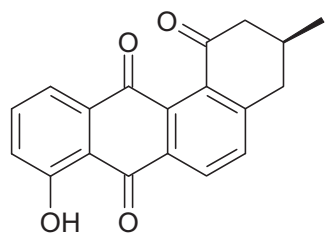
Fujianmycin A (118): Yellow needles, 6.4 mg, UV absorbing band at 254 nm, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.43 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (DMSO-d_6, 300

MHz, in mixture with ochromycinone (**120**)?): δ 12.16 (br s, 1H, 8-OH), 8.23 (d, ^3J = 8.0 Hz, 1H, H-6), 7.80 (m, 2H, H-5,10), 7.62 (d, ^3J = 7.7 Hz, 1H, H-11), 7.53 (d, ^3J = 7.5 Hz, 1H, H-9), 4.91 (d, ^3J = 5.3 Hz, 1H, H-4), 3.07, 2.74 (ABX, J_{AB} = 16.3, J_{AX} = 4.2 Hz , J_{BX} = 10.1 Hz 2H, CH_2-2), 2.18 (1H, m, H-3), 1.15 (d, ^3J = 6.5 Hz, 3H, 14-CH_3). – **(-)-ESIMS** *m/z* 321 ([M - H]$^-$, 100), 643 ([2M - H]$^-$, 10). – **(+)- HRESIMS** *m/z* 323.09146 $[M + H]^+$ (calcd. 323.091400 for $C_{19}H_{15}O_5$).

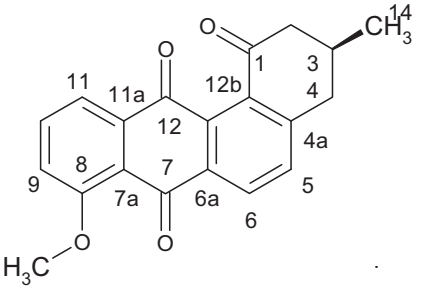
Fujianmycin C (119): Yellow needles, 1.0 mg, UV absorbing band at 254 nm, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.38 (CH_2Cl_2/5% MeOH). **UV/VIS**: λmax (log ε) = (MeOH): 225 (4.3), 256 (4.4), 375 (3.8). – $[\alpha]_D^{20}$ = +18 (c = 0.1, CH_3OH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 30. – **H,H COSY** and **HMBC** see Figure 113. – **(+)-CIMS** *m/z* 353 ($[M + H]^+$, 100). – **(+)-HRESIMS** *m/z* 353.10205 $[M + H]^+$ (calcd. 353.10196 for $C_{20}H_{17}O_6$).



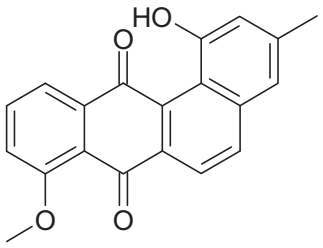
Ochromycinone (120): Yellow needles, 1.41 mg, orange fluorescent under UV light, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.36 (CH_2Cl_2/3% MeOH). – **^{1}H NMR** (CD_2Cl_2, 600 MHz): δ 12.42 (br s, 1H, 8-OH), 8.22 (d, ^3J = 7.9 Hz, 1H, H-6), 7.78 (dd, J = 7.6, 1.1 Hz, 1H, H-11), 7.68 (t, ^3J = 8.0 Hz, 1H, H-10), 7.57 (d, J = 8.0 Hz, 1 H, H-5), 7.38 (dd, J = 8.2, 1.1 Hz, 1H, H-9), 2.98, 2.86, 2.67, 2.38 (4m, 2 CH_2), 1.90 (m, 1 H, H-3), 1.13 (d, ^3J = 6.5 Hz, 3H, CH_3-14); signals tentatively assigned. – **(+)-ESIMS** *m/z* 329 ($[M + Na]^+$ 10), 635 ($[2M + Na]^+$ 100). – **(+)-HRESIMS** *m/z* 307.09653 $[M + H]^+$ (calcd. 307.09649 for $C_{19}H_{15}O_4$).



Ochromycinone methyl ether (121): Yellow needles, 1.38 mg, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.40 (CH_2Cl_2/3% MeOH). – **^{1}H NMR** (CD_2Cl_2, 300 MHz): δ 8.20 (d, ^3J = 8.1 Hz, 1H, H-6), 7.70 (m, 2H, H-5,10), 7.53 (d, ^3J = 8.4 Hz, 1H, H-11), 7.32 (dd, ^3J = 8.4 Hz, ^4J = 1.5 Hz, 1H, H-9), 3.98 (s, 3H, 8-OCH_3), 2.98, 2.89, 2.67, 2.45 (4m, 2 CH_2),



1.55 (m, 1H, H-3), 1.16 (d, ^3J = 6.3 Hz, 3H, CH_3-14). – **(+)-ESIMS** *m/z* 343 ([M + Na]$^+$ 10), 663 ([2M + Na]$^+$ 100). – **(+)-HRESIMS** *m/z* 321.11207 [M + H]$^+$ (calcd. 321.11214 for $C_{20}H_{17}O_4$).



Tetrangulol methyl ether (122): Yellow needles, 1.0 mg, yellow with anisaldehyde/sulphuric acid spraying reagent. – R_f = 0.30 (CH_2Cl_2/3% MeOH). – **^{1}H NMR** (CD_2Cl_2, 600 MHz): δ 11.08 (br s, 1H, 1-OH), 8.20 (d, ^3J = 8.6 Hz, 1H, H-6), 8.08 (d, ^3J = 8.8 Hz, 1H, H-5), 7.88 (dd, ^3J = 7.7 Hz, ^4J = 1.0 Hz, 1H, H-11), 7.73 (dd, ^3J = 8.4, ^3J = 7.7 Hz, 1H, H-10), 7.37 (dd, ^3J = 7.7 Hz, ^4J = 1.0 Hz, 1H, H-9), 7.23 (d, 1H, J = 1.5 Hz, H-4), 7.04 (d, J = 1.7 Hz, H-2), 4.03 (s, 3H, 8-OCH_3), 2.45 (s, 3H, CH_3-14). – **(+)-ESIMS** *m/z* 319 ([M + H]$^+$, 10), 659 ([2M + Na]$^+$, 100), 977 ([3M + Na]$^+$, 10). – **(+)-HRESIMS** *m/z* 319.09641 [M + H]$^+$ (calcd. 319.09649 for $C_{20}H_{15}O_4$).

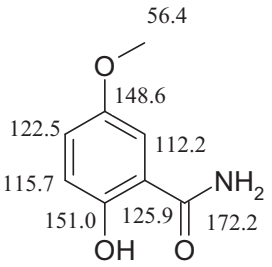
9.11 Marine derived *Streptomyces* sp. B5746

The well-grown agar culture of the marine derived *Streptomyces* sp. B5746 was used to inoculate 1 L fermentation using M_2^+ medium (with 50% sea water) for five days at 28 °C. The culture broth was extracted with ethyl acetate and used for pre screening. In antimicrobial screening using the agar disk diffusion method, the crude extract doesn't exhibit any biological activity. In the chemical pre-screening the crude extract showed two interesting UV absorbing band, which turned to yellow and blue colour after spraying with anisaldehyde/sulphuric acid and heating.

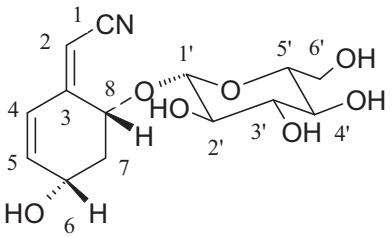
9.11.1 Fermentation and Isolation

The well grown agar culture of the marine derived *Streptomyces* sp. B5746 was used for the inoculation of 100 of 1 L Erlenmeyer flasks each contained 250 ml M_2^+ medium (with 50% sea water). The culture was kept at 28 °C on a linear shaker for 6 days. The dark brown culture broth was filtered over Celite with the aid of the filter press. The filtrate was passed over an XAD-16 column (65 × 8 cm), 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 (4.08g) was fractionated over silica gel column chromatography using CH_2Cl_2/MeOH gradient (0 to 20% MeOH) to afford five fractions namely (FI to FV). FII was separated on Sephadex LH-20 using MeOH to afford 2-hydroxy-5-methoxy-benzamide (**123**). Both FIII and FIV were purified on Sephadex LH-20 using MeOH to deliver ferulic acid (from FIII) adenosine and and *cis-cyclo*-(Alanyl-prolyl) and

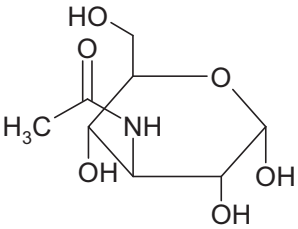
vanillic acid. Where FV was subjected to Sephadex LH-20 using MeOH followed by reverse phase RP-18 using MeOH/H_2O gradient (10 to 30% MeOH) to obtain menisdaurin (**124**) and 3-acetamido-3-deoxy-β-D-glucopyranose (**125**).



2-Hydroxy-5-methoxy-benzamide (123): Colourless solid, 1.0 mg, UV absorbing, blue with anisaldehyde/sulphuric spray reagent. – R_f = 0.22 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 31. – **H,H COSY** and **HMBC** see Figure 118. – **(+)-ESIMS** *m/z* 168 ($[M + H]^+$, 100), 335 ($[2M + Na]^+$, 10). – **(-)-ESIMS** *m/z* 166 ($[M - H]^-$, 100). – **(+)-HRESI MS** *m/z* 166.0508 $[M - H]^-$, (calcd. 166.0510 for $C_8H_8NO_3$).



(Z)-1-Cyanomethylene-4(*2R*)-hydroxy-6(S)-(β-glucopyranosyloxy)-2-cyclohexene (Menisdaurin) (124): Colourless solid, 1.2 mg, UV absorbing, turned to yellow with anisaldehyde/sulphuric spray reagent. – R_f = 0.14 (CH_2Cl_2/10% MeOH). – **^{1}H** and **^{13}C NMR** (CD_3OD, 300, 125 MHz), see Table 32. – **H,H COSY** and **HMBC** see Figure 120. – **(+)-ESIMS** *m/z* 336 ($[M + Na]^+$, 100), 649 ($[2M + Na]^+$, 40). – **(+)-HRESIMS** *m/z* 336.10556 $[M + Na]^+$ (calcd. 336.10537 for $C_{14}H_{19}NO_7Na$).



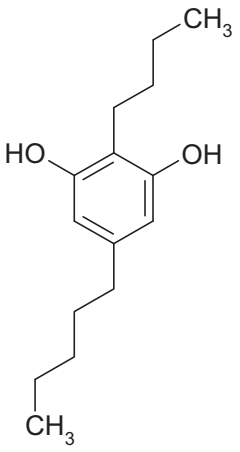
3-Acetamido-3-deoxy-β-D-glucopyranose (125): Colourless solid. 1.6 mg**,** UV inactive, yellow colour by anisaldehyde/sulphuric acid and heating. – R_f = 0.10 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300): δ 4.46 (d, ^3J = 7.8 Hz, 1H, H-1), 3.82-3.46 (m, 6H, H-2, H-3, H-4, H-5, CH_2-6), 1.98 (s, 3H, 3-CH_3). – **(+)-ESIMS** *m/z* 244 ($[M + Na]^+$, 40), 465 ($[2M + Na]^+$, 100). – **(+)-HRESIMS** *m/z* 244.07927 $[M + Na]^+$ (calcd. 244.07916 for $C_8H_{15}NO_6Na$).

10 Metabolites from Endophytic Fungi

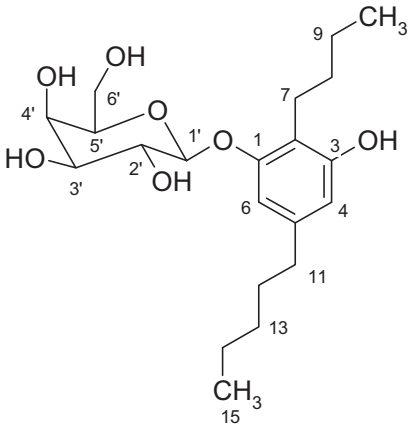
10.1 Tropical Endophytic Fungus *Gaeumannomyces amomi* BCC4066

10.1.1 Fermentation, Extraction and Isolation

The fungus *G. amomi* BCC4066 was cultivated on 20 L of fermentation medium (25 g corn meal, 20 g sucrose, 1 g yeast extract per litre) for 21 days at 20 °C to give the mycelium and filtrate. The mycelium was extracted three times with ethyl acetate and acetone, respectively. The organic phases were evaporated to dryness and combined to afford the crude extract (5 g), which dissolved in methanol and defatted with cyclohexane. The filtrate was extracted using Amberlite XAD-16 resin followed by elution with MeOH. The methanolic extract was evaporated to dryness (55 g) and separated on silica gel column chromatography (CH_2Cl_2/ MeOH 8:2) to afford stemphol 1-*O*-*β*-D-galactoside (**127**). The ethyl acetate extract of the mycelium was separated on Sephadex LH-20 (MeOH) followed by silica gel (column, CH_2Cl_2) to yield stemphol (**126**) indole-3-carboxylic acid (5.0 mg) and kojic acid (10.0 mg).



Stemphol (126): Colourless crystals, 30.0 mg, UV absorbing. – R_f = 0.73 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.20 (s, 2 H, H-6, H-4), 4.77 (s, 2 OH), 2.56 (t, ^3J = 7.3 Hz, CH_2-7), 2.44 (t, ^3J = 7.5 Hz, CH_2-11), 1.57 (m, CH_2-12) , 1.52 (m, CH_2-8), 1.48-127 (m, 6 H, CH_2-9, 13, 14) 0.87 (t, ^3J = 7.0 Hz, CH_3-10), 0.93 (t, ^3J = 7.2 Hz, CH_3-15). – **^{13}C NMR** ($CDCl_2$, 125 MHz): δ 154.9 (CH-1, CH-3), 142.7 (Cq-5), 112.8 (Cq-2), 108.2 (CH-4, 6), 35.7 (CH_2-11), 31.8 (CH_2-8), 31.7 (CH_2-13), 31.1 (CH_2-12), 23.1 (CH_2-7), 22.9 (CH_2-9), 22.8 (CH_2-14), 14.0 (CH_3-10, 15). – **(+)-ESIMS** *m/z* 237 ($[M + H]^+$ 100), 495 ($[2M + Na]^+$, 30).



Stemphol 1-*O*-*β*-D-galactopyranoside (127): Colourless solid, 20.0 mg, UV absorbing, red colour with anisaldehyde/sulphuric acid and heating. – R_f = 0.23 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.50 (d, ^4J = 1.3 Hz, 1H, H-4), 6.31 (d, ^4J = 1.3 Hz, 1H, H-6), 4.81 (d, ^3J = 7.8 Hz, 1H, H-1'), 3.90 (d, ^3J = 2.9 Hz, 1H, H-4'), 3.76 (dd, ^3J = 7.8, ^3J = 7.3 1H, H-2'), 3.74 (d, ^3J = 5.1 Hz, 2H, CH_2-6'), 3.63 (d, ^3J = 5.3 Hz, 1H, H-5'), 3.57 (dd, ^3J = 7.3, ^3J = 2.1 1H, H-3'), 2.64 (m, 2H, CH_2-7), 2.45

(t, ^3J = 7.4 Hz, 2H, CH_2-11), 1.58 (m, 2H, CH_2-12), 1.50 (m, 2H, CH_2-8), 1.40-1.25 (m, 6H, CH_2-9,13,14), 0.92 (t, ^3J = 7.2 Hz, 3H, CH_3-15), 0.88 (t, ^3J = 7.0 Hz, 3H, CH_3-10). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 157.8 (C_q, C-1), 156.7 (C_q, C-3), 142.4 (C_q, C-5), 117.7 (C_q, C-2), 110.4 (CH, C-6), 107.9 (CH, C-4), 103.2 (CH, C-1'), 76.7 (CH, C-5'), 75.2 (CH, C-3´), 72.5 (CH, C-2'), 70.2 (CH, C-4'), 62.3 (CH_2, C-6'), 36.9 (CH_2, C-11), 32.8 (CH_2, C-8), 32.6 (CH_2, C-13), 32.1 (CH_2, C-12) 23.9 (CH_2, C-7) 23.8 (CH_2, C-9) 23.7 (CH_2, C-14) 14.5 (CH_3, C-15) 14.4 (CH_3, C-10). – **H,H COSY** and **HMBC** see Figure 126. – **(+)-ESIMS** *m/z* 421 ($[M + Na]^+$ 40), 819 ($[2M + Na]^+$, 100). – **(+)-HRESIMS** *m/z* 421.21958 $[M + Na]^+$ (calcd 421.21967 for $C_{21}H_{34}O_7Na$).

10.2 Tropical Endophytic Fungus LAF

Small pieces of the agar plates of the tropical entophytic fungus LAF 12 were used to inoculate 1 L of M_2^+ medium and incubated on a small rotary shaker (95 rpm) at 28 °C for two weeks. The culture broth was extracted with ethyl acetate and the resulting crude extract was used for pre-screening.

10.2.1 Pre-screening

On the agar diffusion test, the crude extract of the tropical entophytic fungus LAF 12 exhibited promising biological activity against *Chlorella vulgaris* and *Chlorella sorokiniana* as well as plant pathogen fungus *Aphanomyces cochlioides* and antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü57) as well as antifungal activity against *Candida albicans* as mentioned in Figure xx. On TLC two interesting high polar spots were observed, one of them was UV inactive, turned to violet with anisaldehyde/sulphuric acid spray reagent and the second one was UV active spot and turned to yellow colour with anisaldehyde/sulphuric acid and heating.

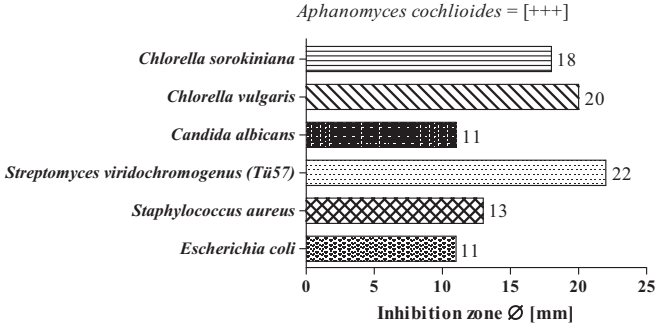
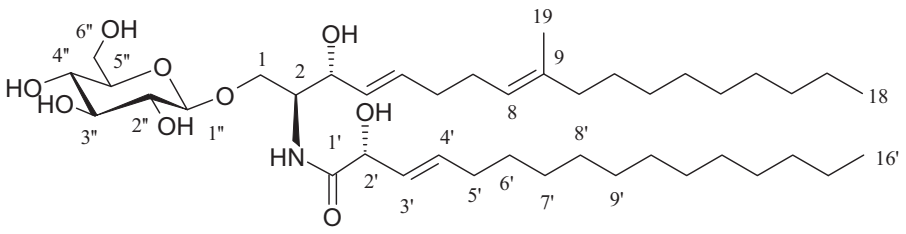


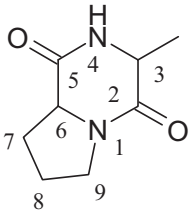
Figure 136: Biological activity of the crude extract of the tropical endophytic fungus LAF 12

10.2.2 Large Scale Fermentation, Extraction and Isolation

The endophytic fungus LAF 12 was cultivated on 20 L of fermentation medium (M_2^+) on 20 P- Flasks and incubated for one month at 28 °C. The resulting mycelium was extracted using ethyl acetate (8 x) and MeOH (4 x), while the filtrate was extracted using Amberlite XAD-16 resin followed by elution with MeOH and extracted again using ethyl acetate. The organic phases were evaporated to dryness. The crude extract of the ethyl acetate phase of the mycelium (1.5 g) was chromatographed on silica gel (column 3 x 60 cm) using CH_2Cl_2/MeOH gradient 0 to 20 % MeOH) to deliver three fractions; FI was fat, while FII was purified on Sephadex LH-20 to afford D-sorbitol (**130**) and FIII was purified on silica gel using CH_2Cl_2/10% MeOH followed by Sephadex LH-20 using MeOH to deliver a compound under discussion. On the other hand the MeOH extract (10.3 g) from the mycelium was chromatographed on silica gel (column 3 x 60 cm) using CH_2Cl_2/MeOH gradient 0 to 10% MeOH) to deliver two fractions namely (FI and FII) both were purified on PTLC using CH_2Cl_2/10% MeOH to afford cerebroside A (**128**) and 3-acetamido-3-deoxy-β-D-glucopyranose (**125**). Moreover the crude extract (4.9) from the water phase was chromatographed on silica gel (column 3 x 60 cm) using CH_2Cl_2/MeOH gradient 0 to 10% MeOH) to deliver three fractions, FI and FIII were mainly contain fat and sugars respectively, while FII was purified on Sephadex LH-20 using MeOH to obtain *cis*-*cyclo*-(Alanyl-prolyl) (**129**).

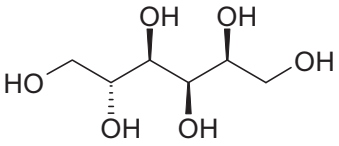


Cerebroside A (128): White solid, 2.86 mg, UV inactive, violet colour by anisaldehyde/sulphuric acid and heating. – R_f = 0.14 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz) and **^{13}C NMR** (CD_3OD, 125 MHz) see Table 34. – **(+)-ESIMS** *m/z* 748 ($[M + Na]^+$, 100), 1473 ($[2 M + Na]^+$, 5). – **(+)-HRESI MS** *m/z* 726.55132 $[M + H]^+$ (calcd. 726.55146 for $C_{41}H_{76}NO_9$).



***Cis*-cyclo-(Alanyl-proline) (129):** Colourless oil**,** 5.0 mg, UV absorbing at 254 nm, brown colour by anisaldehyde/sulphuric acid and heating. – R_f = 0.28 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 6.5 (br s, 1H, NH), 4.12 (m, 2H, H-3, H-6),), 3.56 (m, 2H, CH_2-9), 1.86-1.36 (m, 4H, CH_2-7, 8). 1.45 (d, ^3J = 6.9 Hz, 3-CH_3). – **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 170.5 (C_q, CO-5), 166.2 (C_q, CO-2), 59.3

(CH, C-6), 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).



D-Sorbitol (130): White powder, 81.74 mg, UV in active, yellow colour by anisaldehyde/sulphuric acid and heating. – R_f = 0.10 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (D_2O, 300 Hz): δ 3.93 (dd, ^3J = 11.3 Hz, 2.5 Hz, 1H, Ha-1), 3.84 (dd, ^3J = 8.6 Hz, 5.6 Hz, 1H, H-3), 3.80 (dd, ^3J = 8.6 Hz, 2.5 Hz, 1H, H-2), 3.73 (dd, ^3J = 11.3 Hz, 5.6 Hz, 1H, Hb-1). – **^{13}C NMR** (D_2O, 125 Hz): δ 73.5 (CH, C-2), 72.0 (CH, C-3), 65.8 (CH_2, C-1). – **(+)-ESIMS** *m/z* 205 ($[M + Na]^+$, 20), 387 ($[2 M + Na]^+$, 100), 181 ($[M - H]^-$, 80), 363 ($[2 M - H]^-$, 100). – **(+)-HRESI MS** *m/z* 181.07173 $[M - H]^-$ (calcd. 181.07176 for $C_6H_{13}O_6$).

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12 Spectra

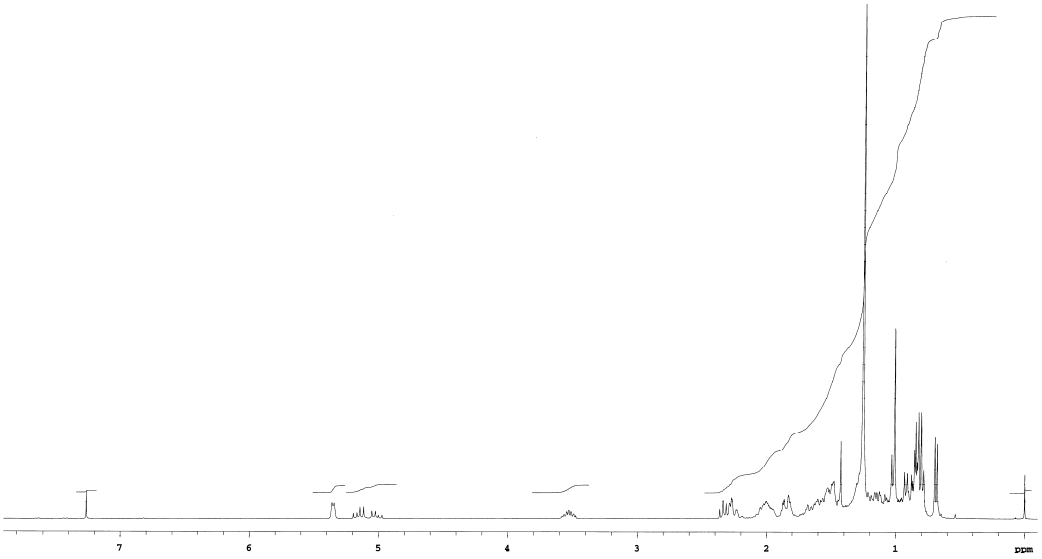

Figure 137: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of a mixture of compounds **51** and **52**

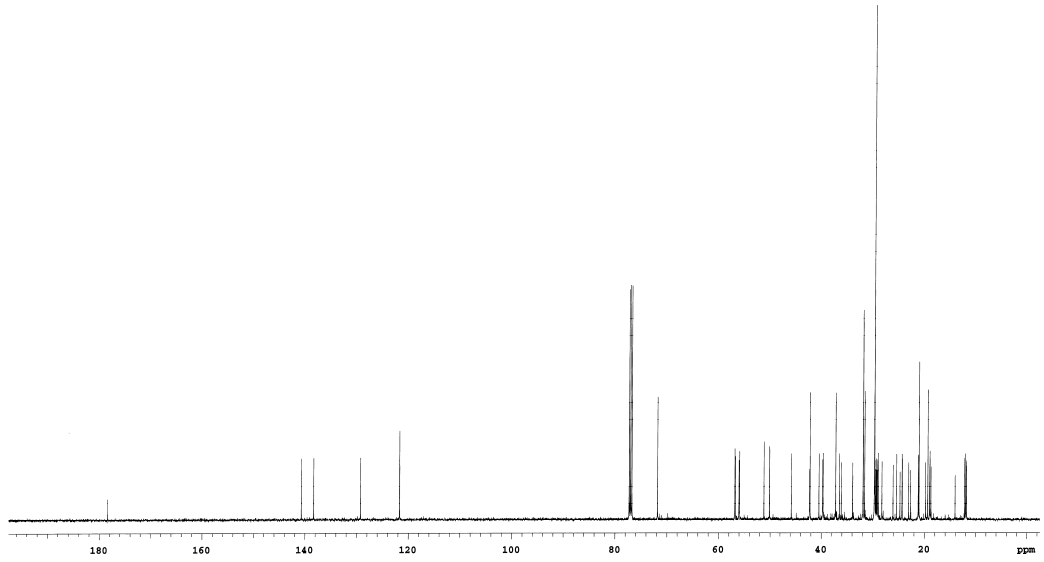

Figure 138: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of mixture of compounds **51** and **52**

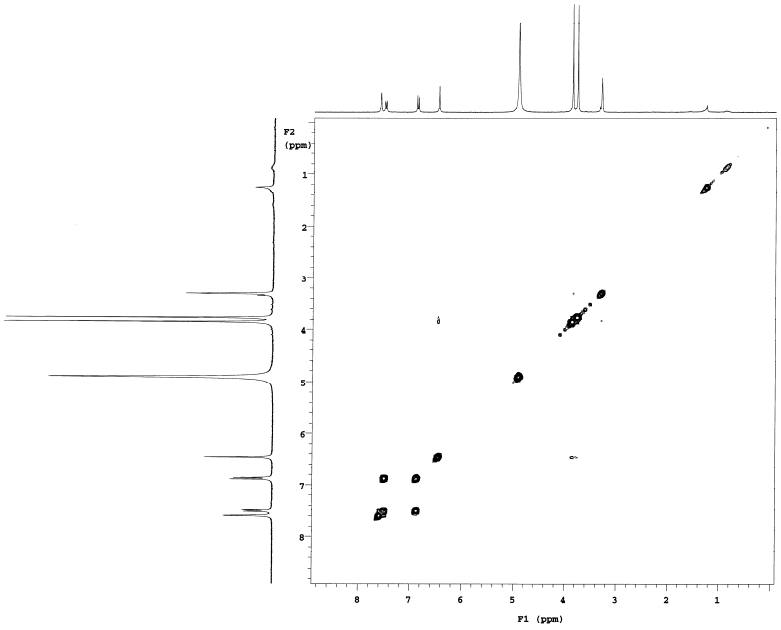

Figure 139: H,H COSY spectrum (CD_3OD, 300 MHz) of compound **53**

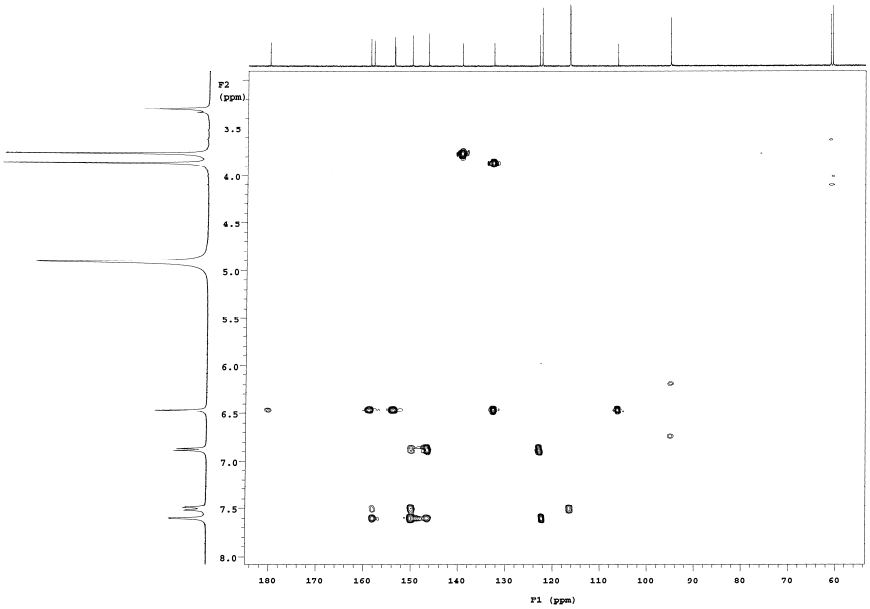

Figure 140: HMBC spectrum (CD_3OD, 300 MHz) of compound **53**

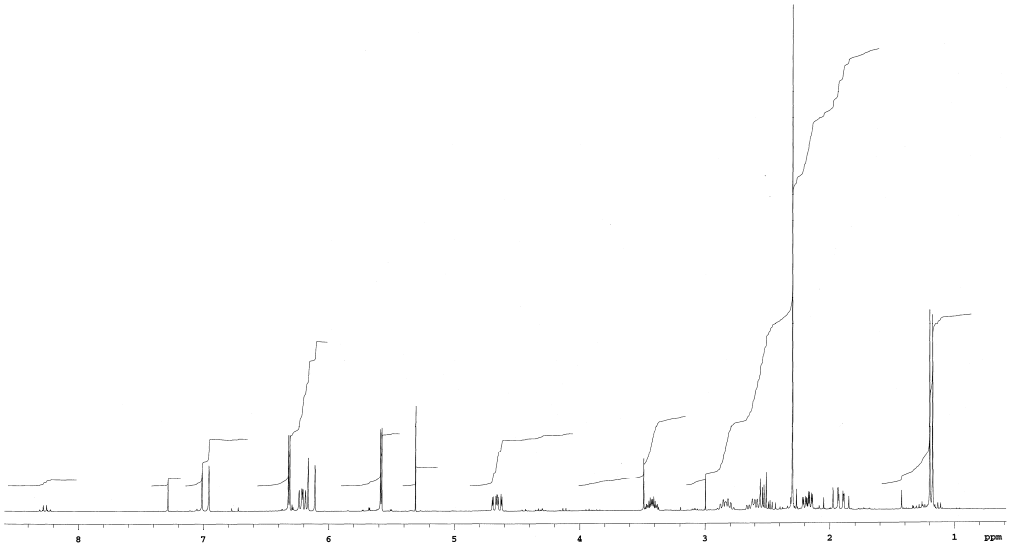

Figure 141: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of xanthatin (**56**)

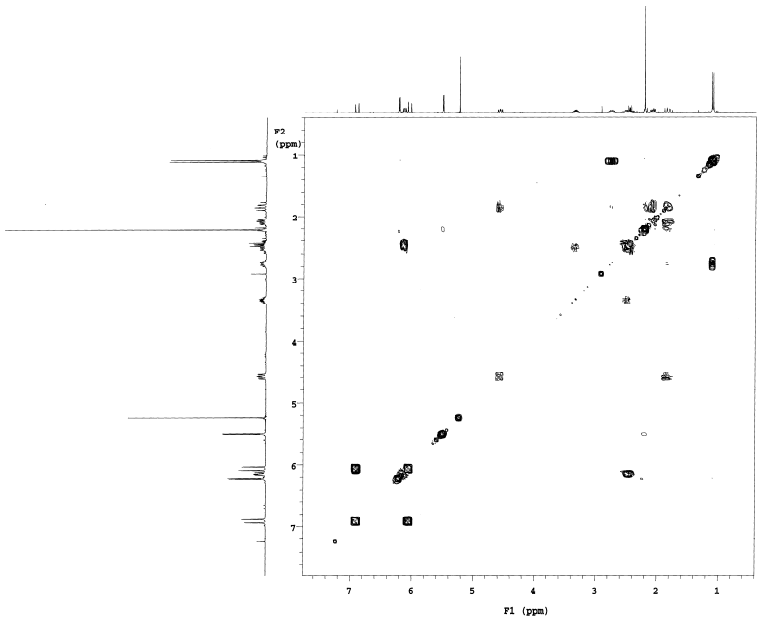

Figure 142: $^{1}H,^{1}H$ COSY spectrum ($CDCl_3$, 300 MHz) of xanthatin (**56**)

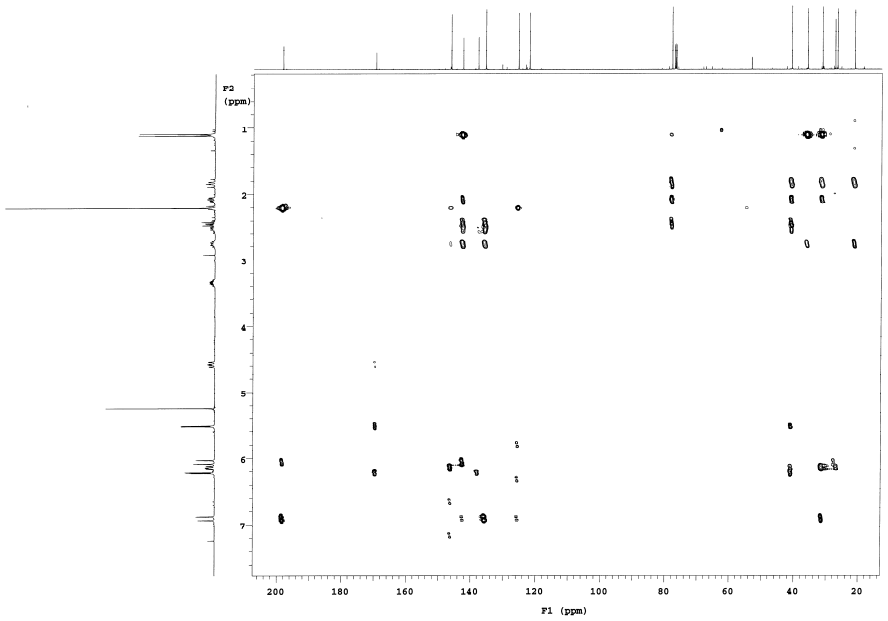

Figure 143: HMBC spectrum ($CDCl_3$, 300 MHz) of xanthatin (**56**)

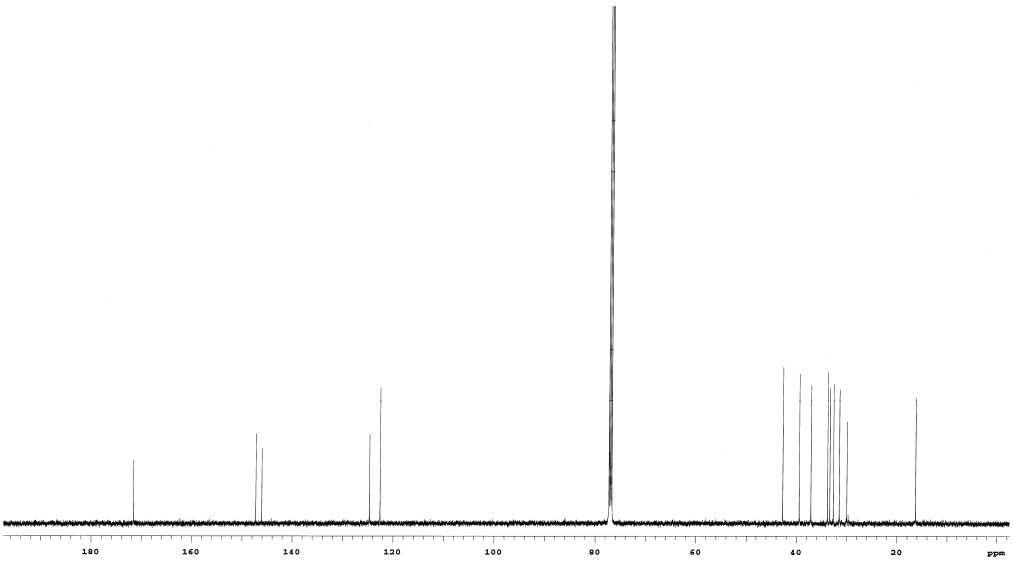

Figure 144: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of 4-oxobedfordia acid (**57**)

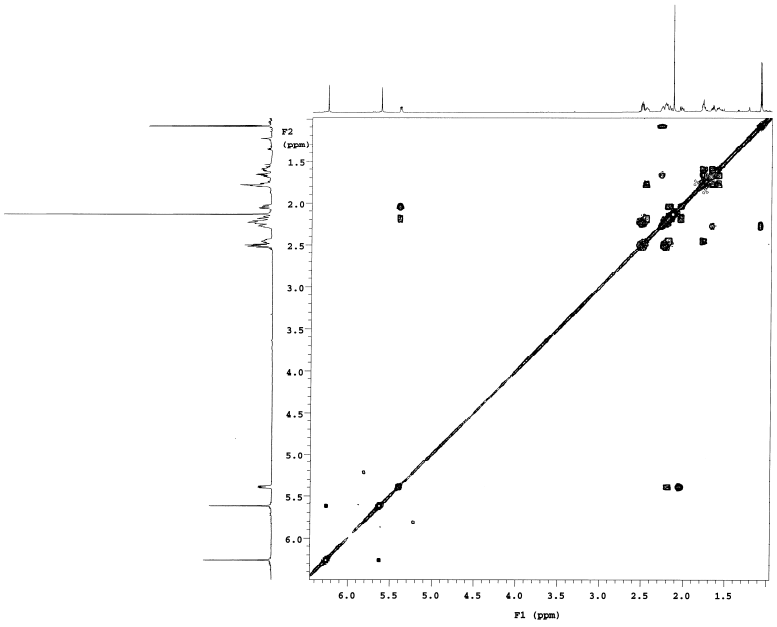

Figure 145: H,H COSY spectrum ($CDCl_3$, 300 MHz) of 4-oxobedfordia acid (**57**)

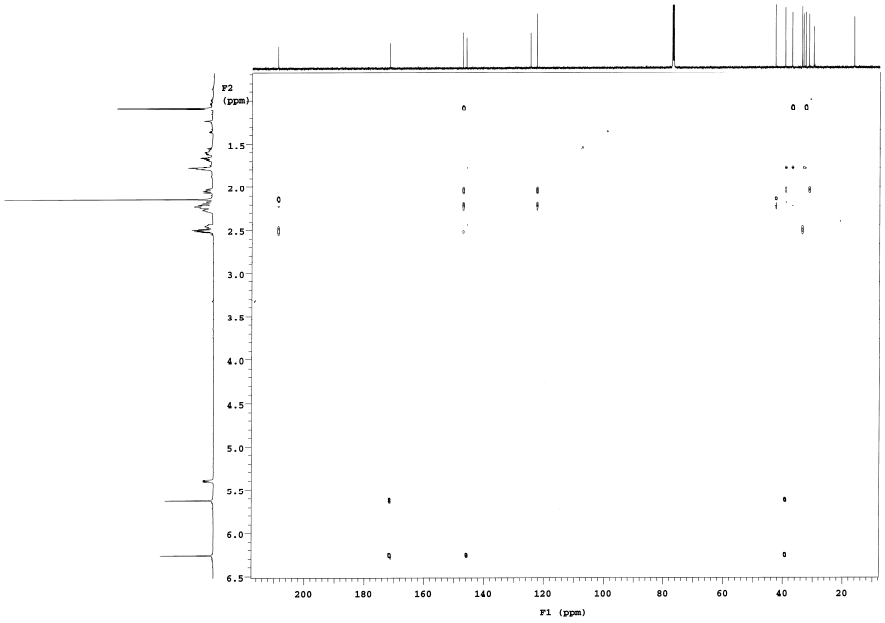

Figure 146: HMBC spectrum ($CDCl_3$, 300 MHz) of 4-oxobedfordia acid (**57**)

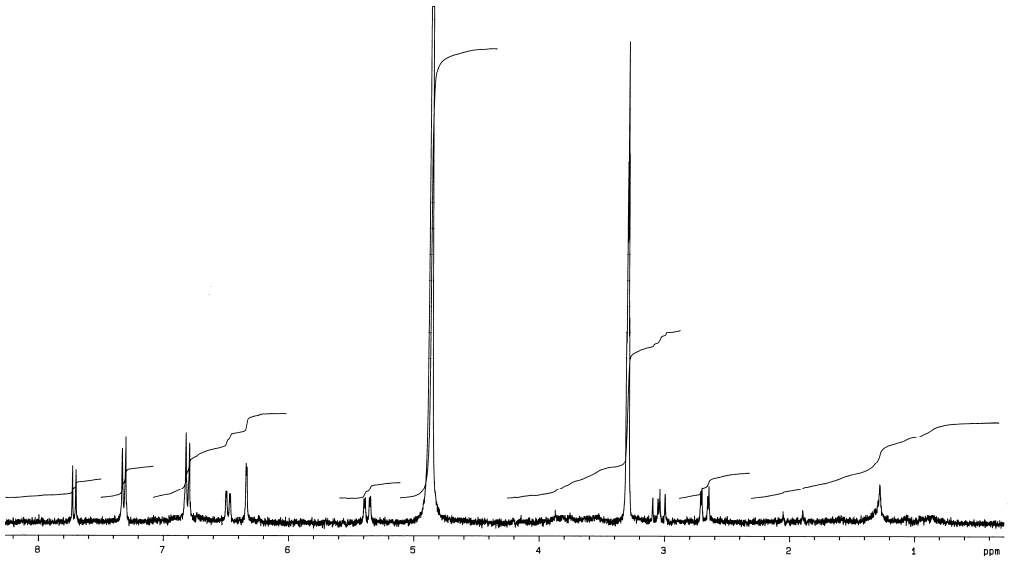

Figure 147: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 4',7–dihydroxyflavanone (**58**)

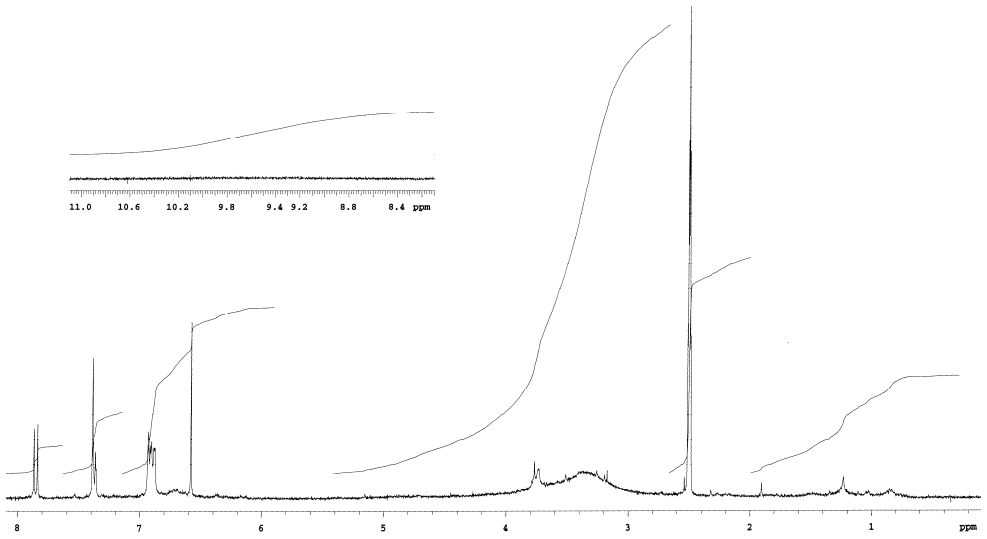

Figure 148: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of 7,3',4'-trihydroxyflavone (**59**)

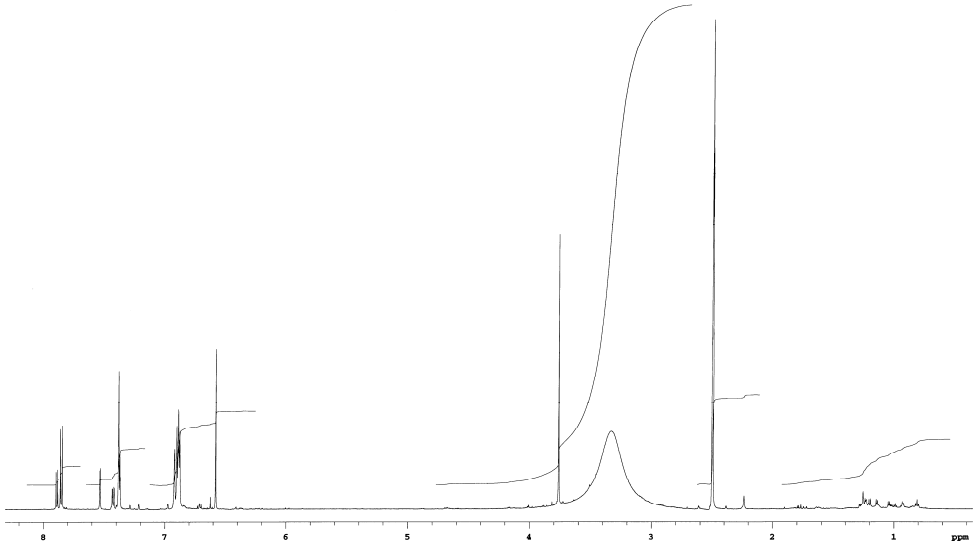

Figure 149: ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of 3-*O*-methylfisetin (**60**)

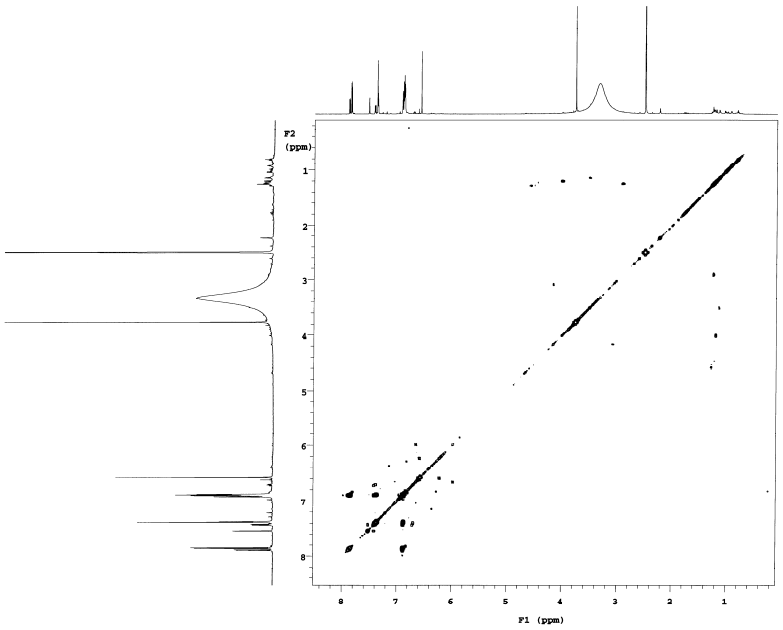

Figure 150: H,H COSY spectrum (DMSO-d_6, 600 MHz) of 3-*O*-methylfisetin (**60**)

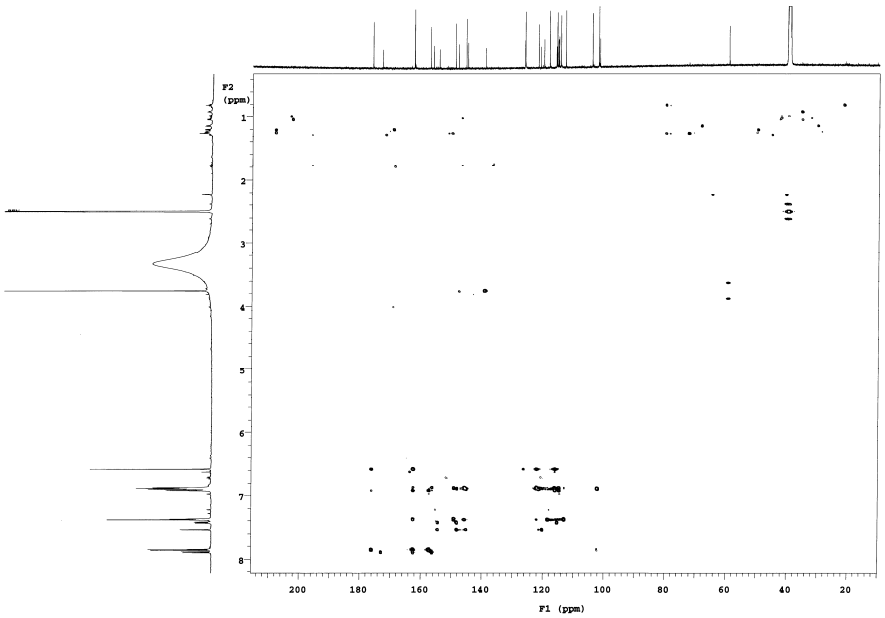

Figure 151: HMBC spectrum (DMSO-d_6, 600 MHz) of 3-*O*-methylfisetin (**60**)

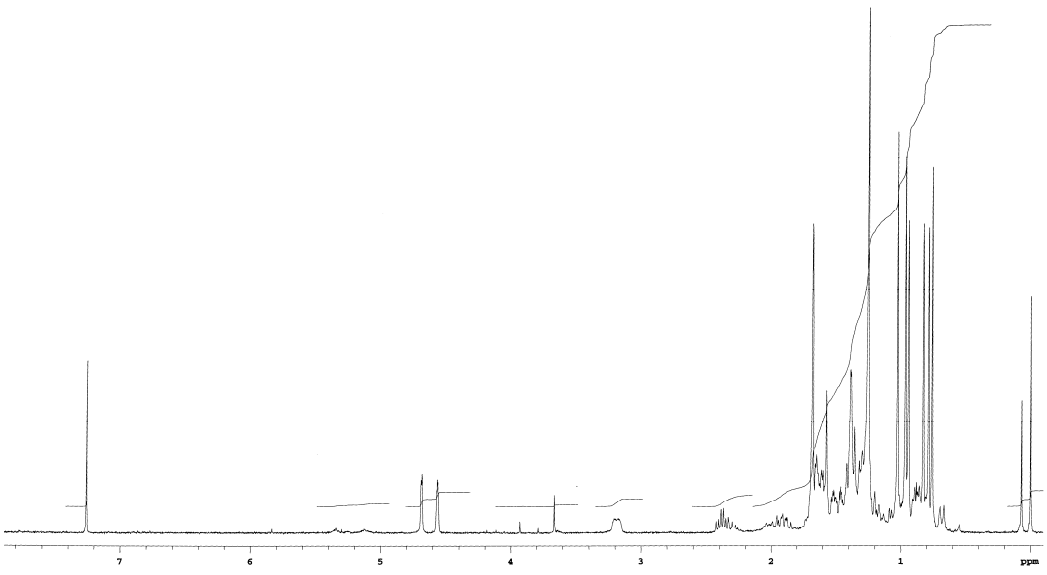

Figure 152: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of lupeol (**61**)

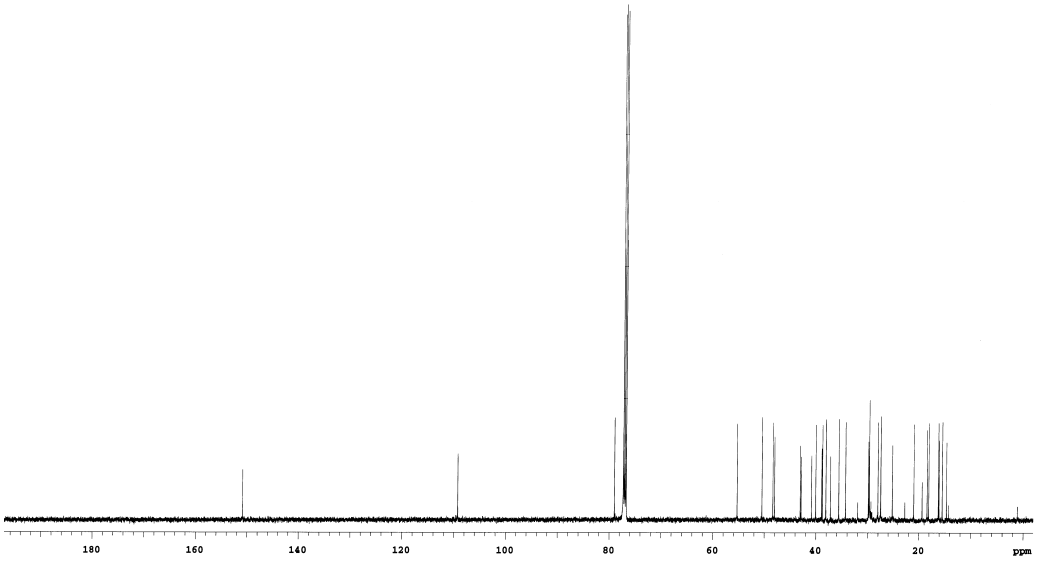

Figure 153: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of lupeol (**61**)

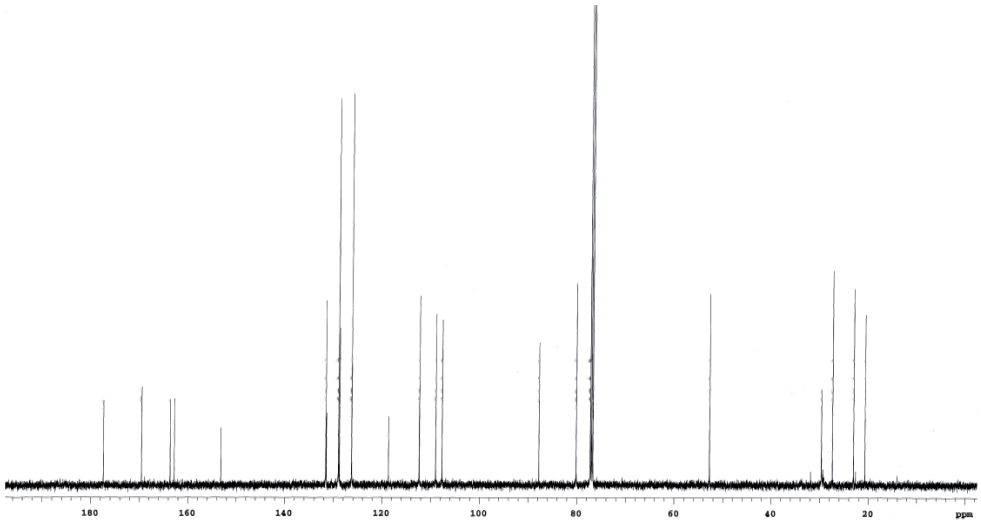

Figure 154: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of semiglabrin (**64**)

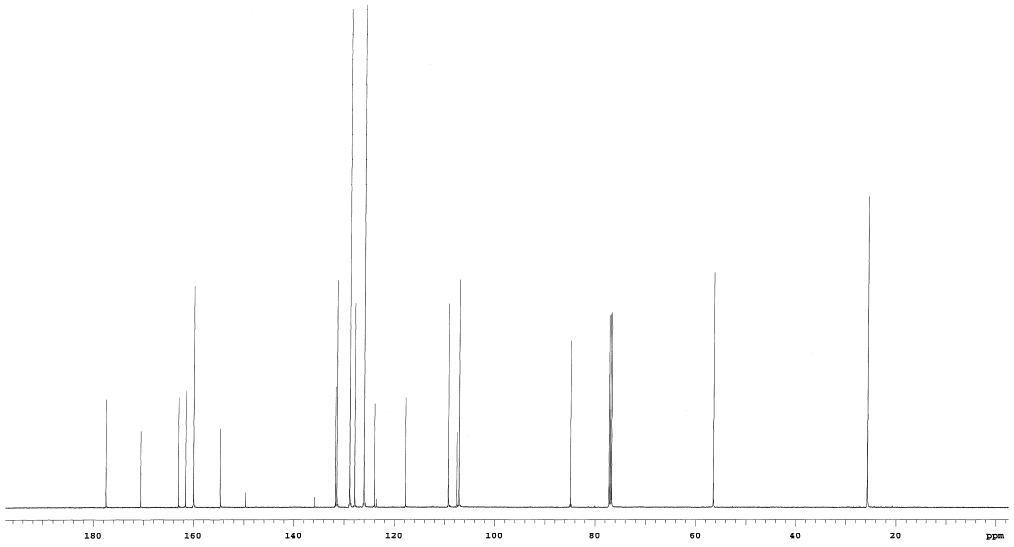

Figure 155: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of apollinine (**66**)

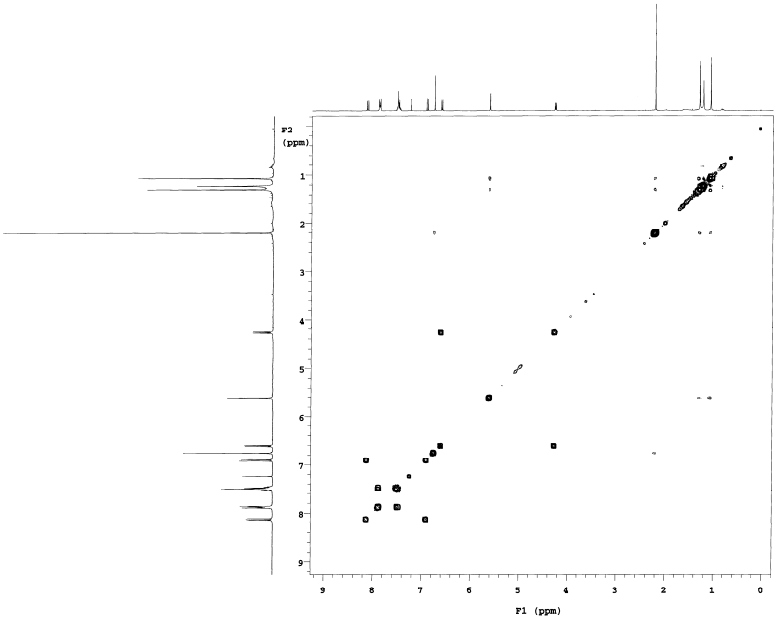

Figure 156: H,H COSY spectrum ($CDCl_3$, 300 MHz) of apollinine (**66**)

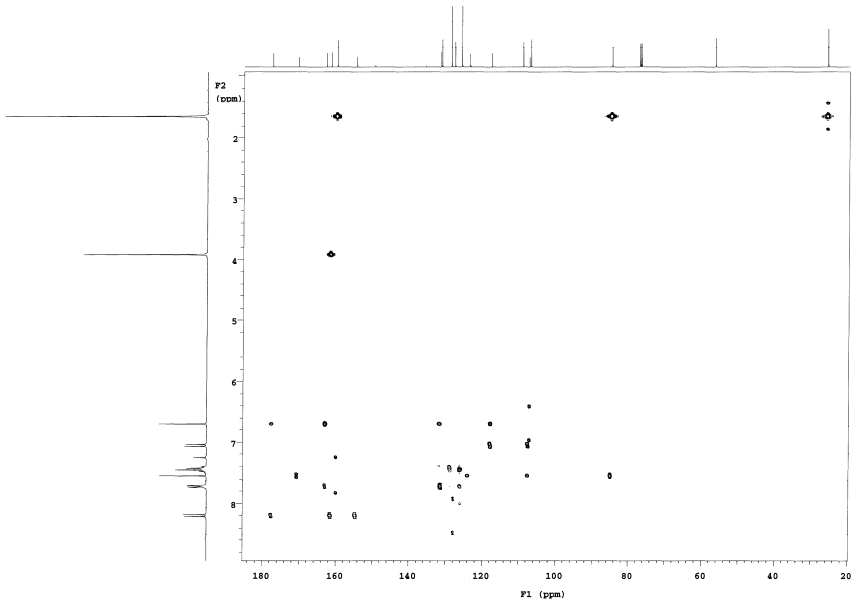

Figure 157: HMBC spectrum ($CDCl_3$, 300 MHz) of apollinine (**66**)

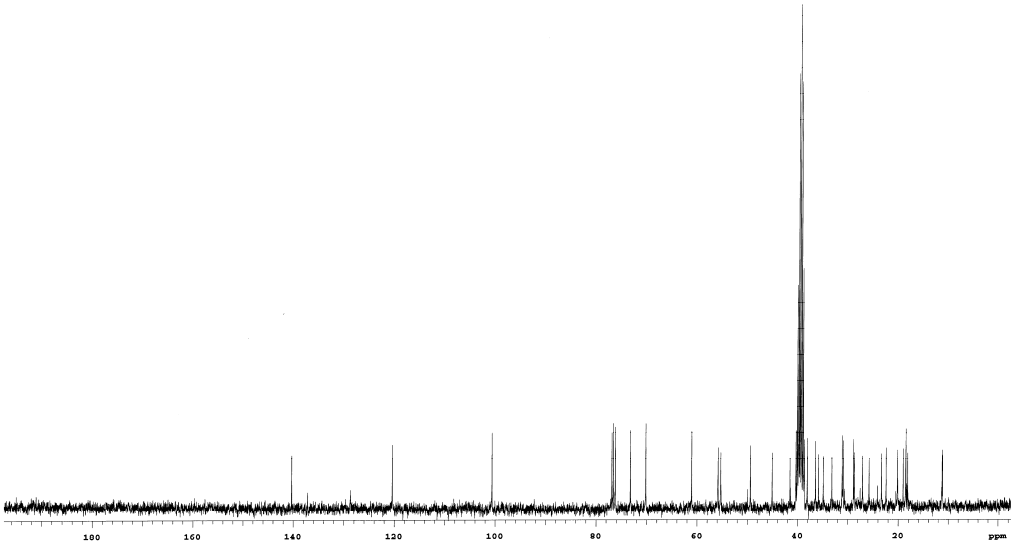

Figure 158: ^{13}C NMR spectrum of 3*β*-*O*-glucosylsitosterol (**67**)

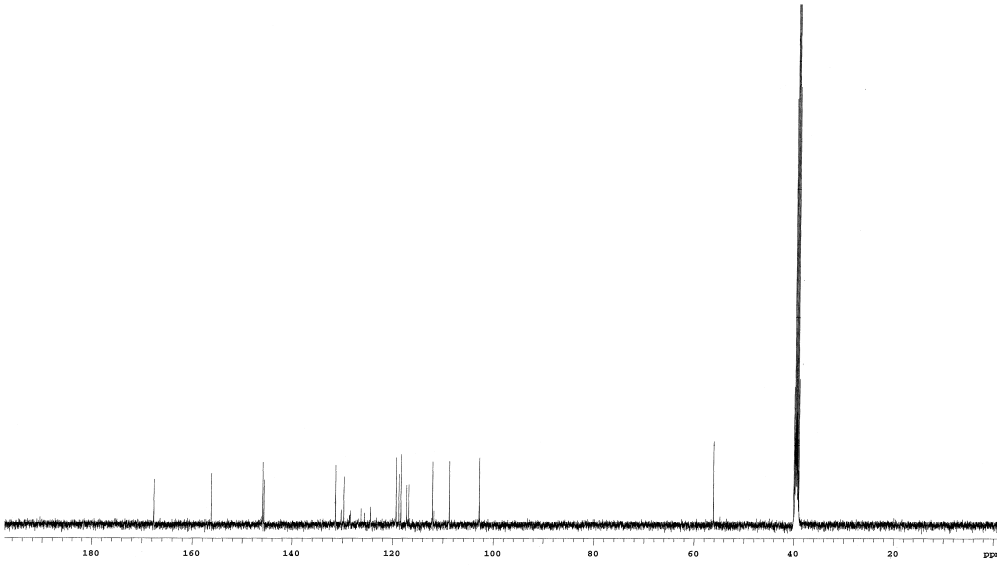

Figure 159: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of aristolochic acid A (**68**)

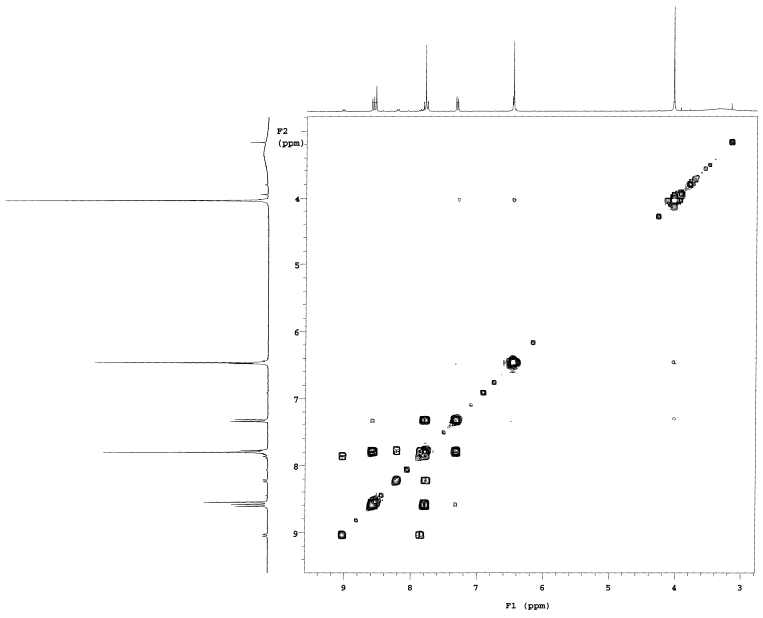

Figure 160: H,H COSY spectrum (DMSO-d_6, 300 MHz) of aristolochic acid A (**68**)

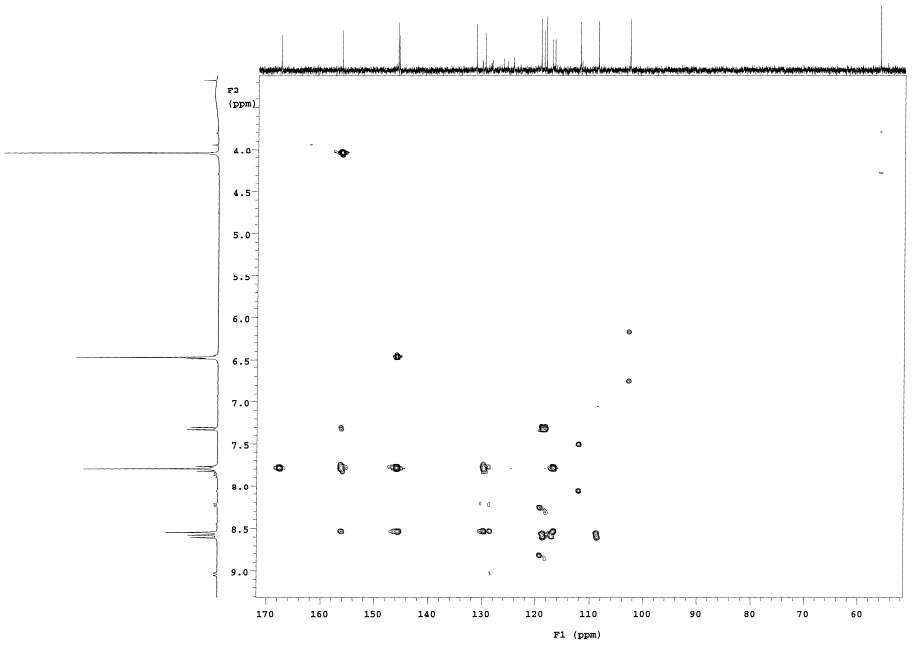

Figure 161: HMBC spectrum (DMSO-d_6, 300 Hz) of aristolochic acid A (**68**)

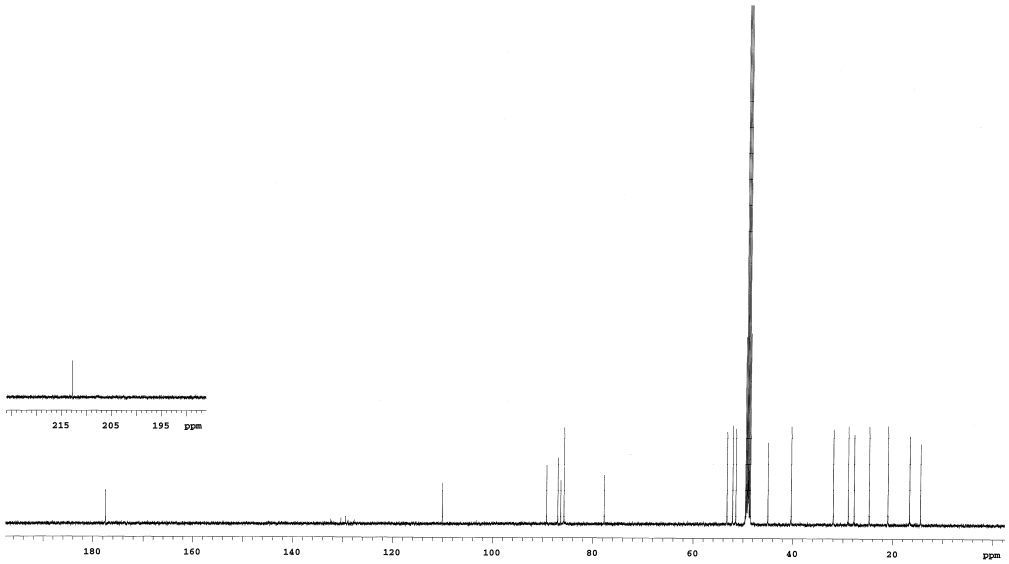

Figure 162: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of *ent*-homoabyssomicin A (**70**)

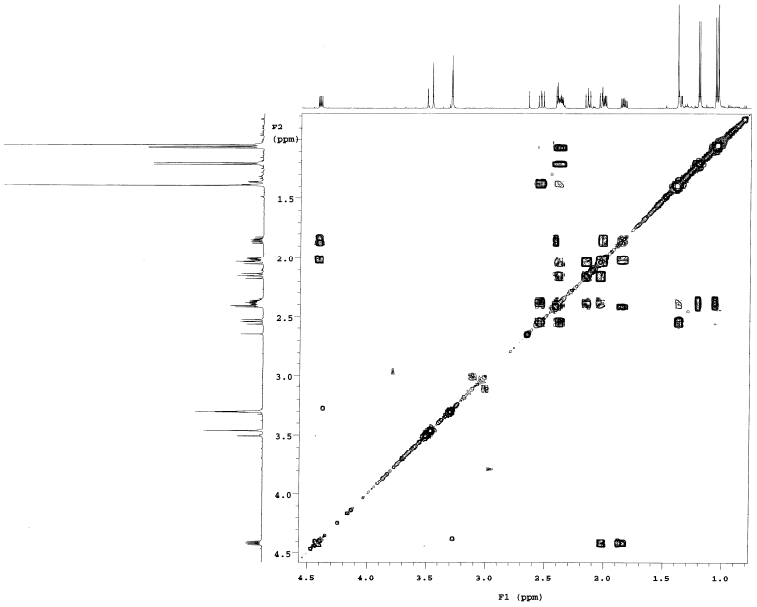

Figure 163: H,H COSY spectrum (CD_3OD, 600 MHz) of *ent*-homoabyssomicin A (**70**)

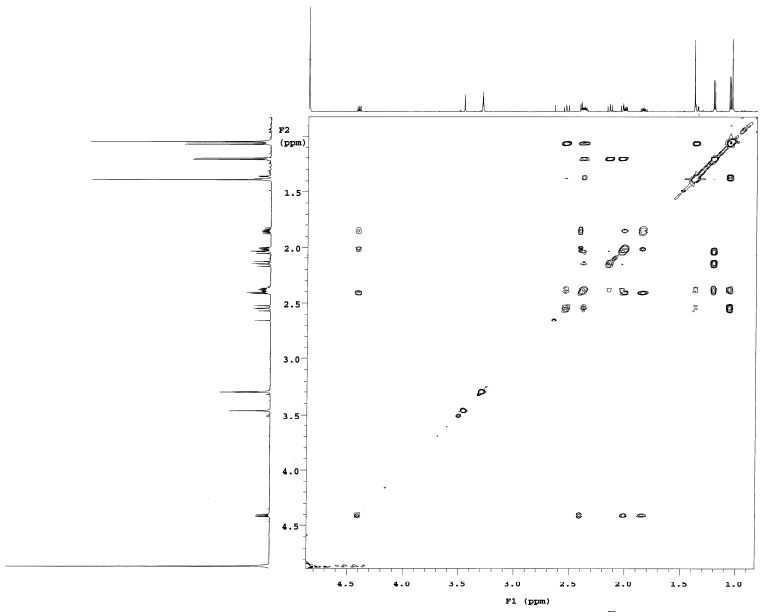

Figure 164: TOCSY spectrum (CD_3OD, 600 MHz) of *ent*-homoabyssomicin A (**70**)

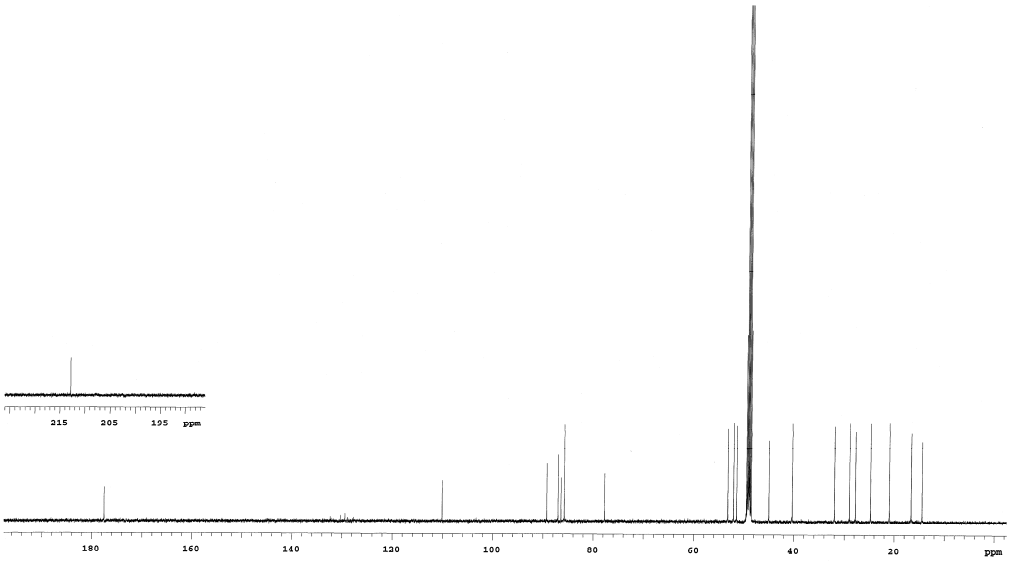

Figure 165: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of *ent*-homoabyssomicin B (**72**)

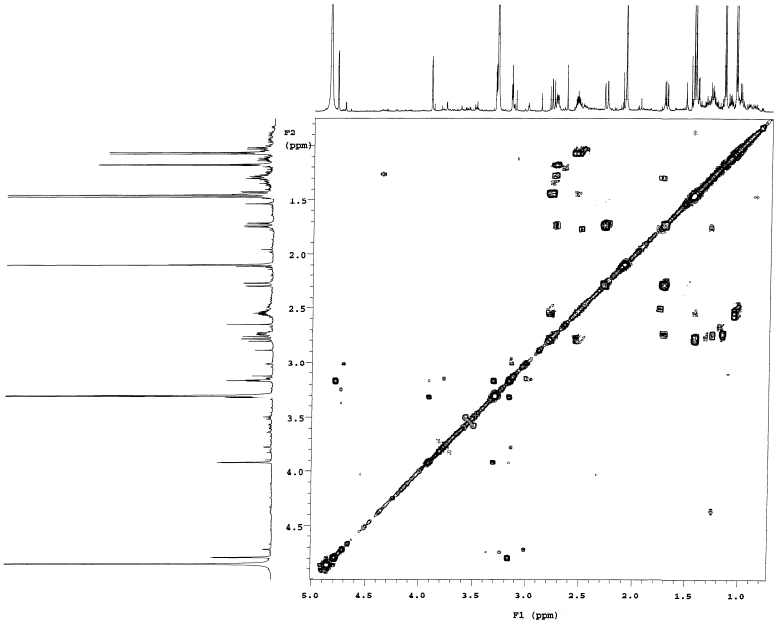

Figure 166: NOSEY spectrum (CD_3OD, 600 MHz) of *ent*-homoabyssomicin B (**72**)

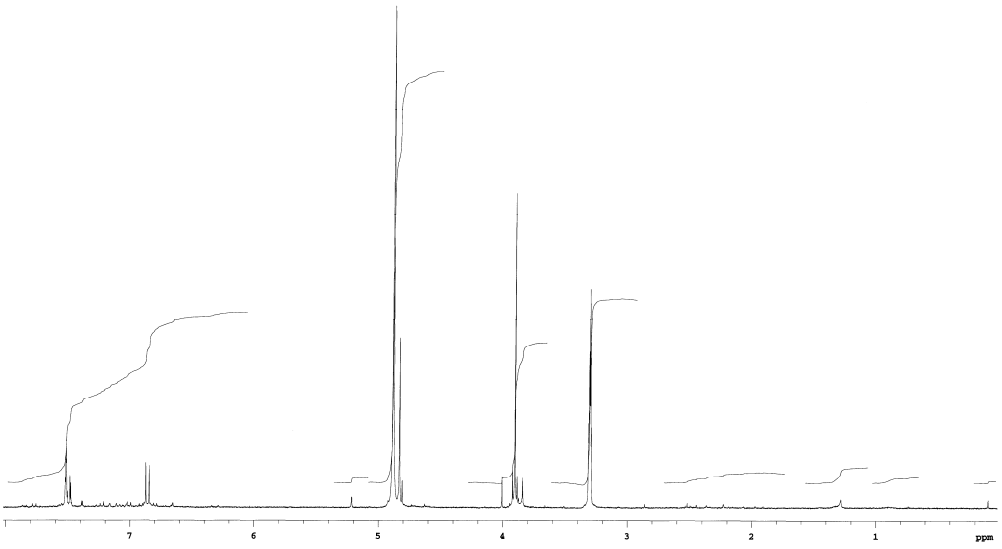

Figure 167: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**79**)

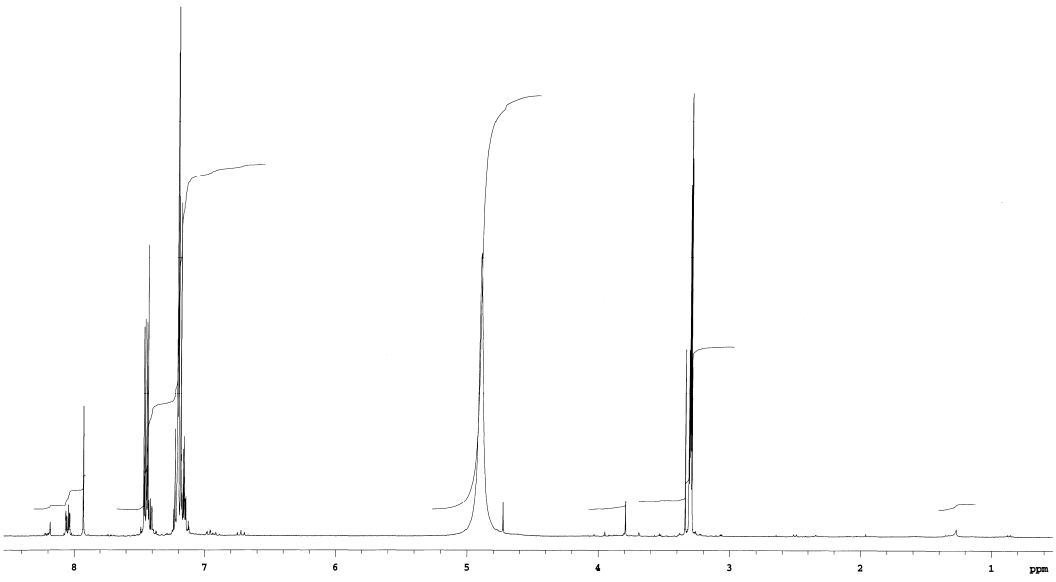

Figure 168: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of benadrostin (**80**)

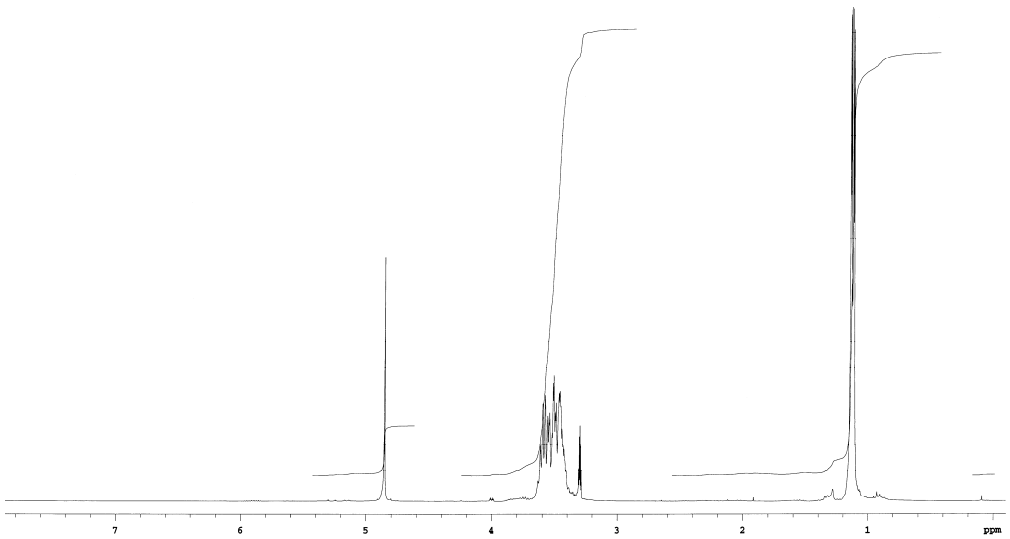

Figure 169: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of polypropylenglycol (**81**)

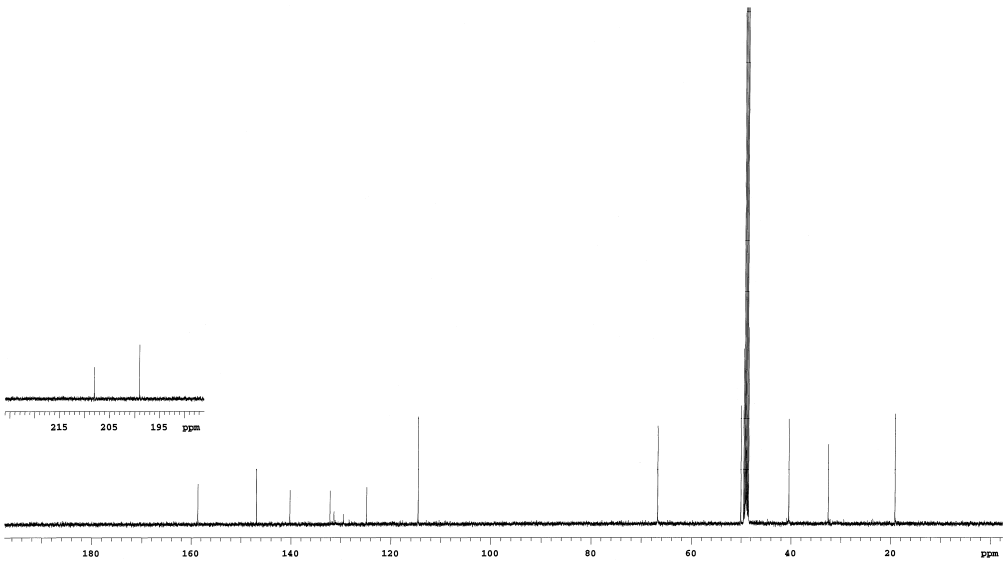

Figure 170: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of GTRI-02 (**84**)

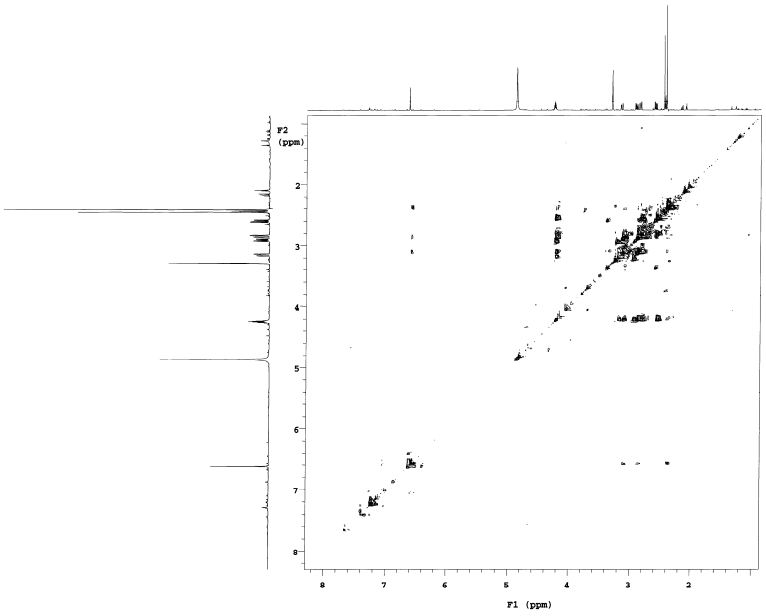

Figure 171: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) of GTRI-02 (**84**)

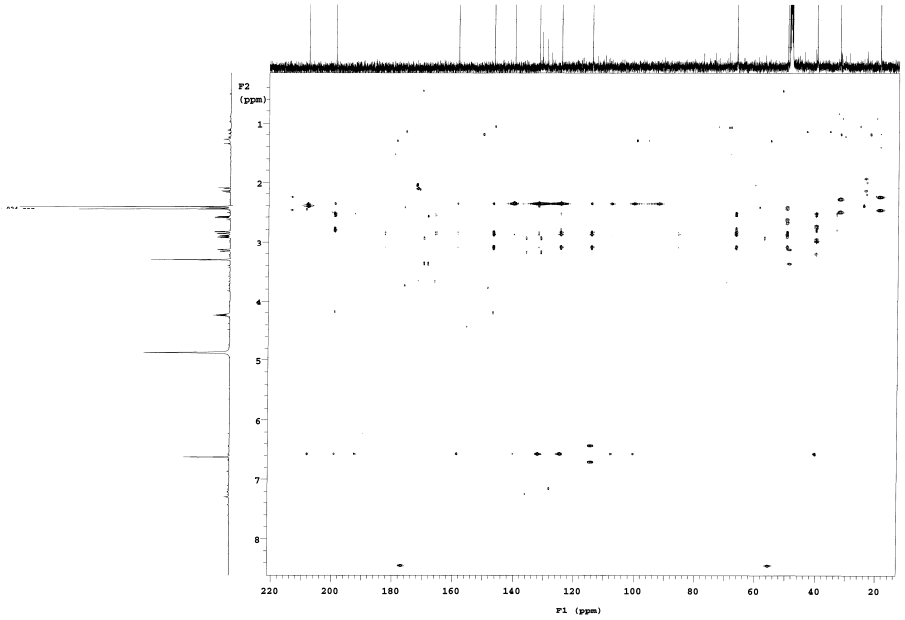

Figure 172: HMBC spectrum (CD_3OD, 300 MHz) of GTRI-02 (**84**)

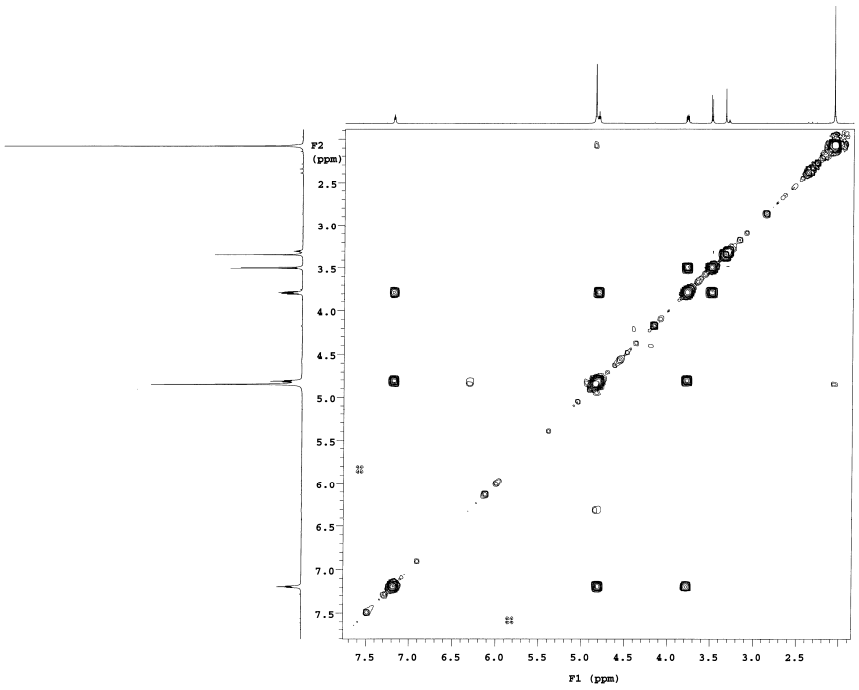

Figure 173: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) of LL-C10037α (**86**)

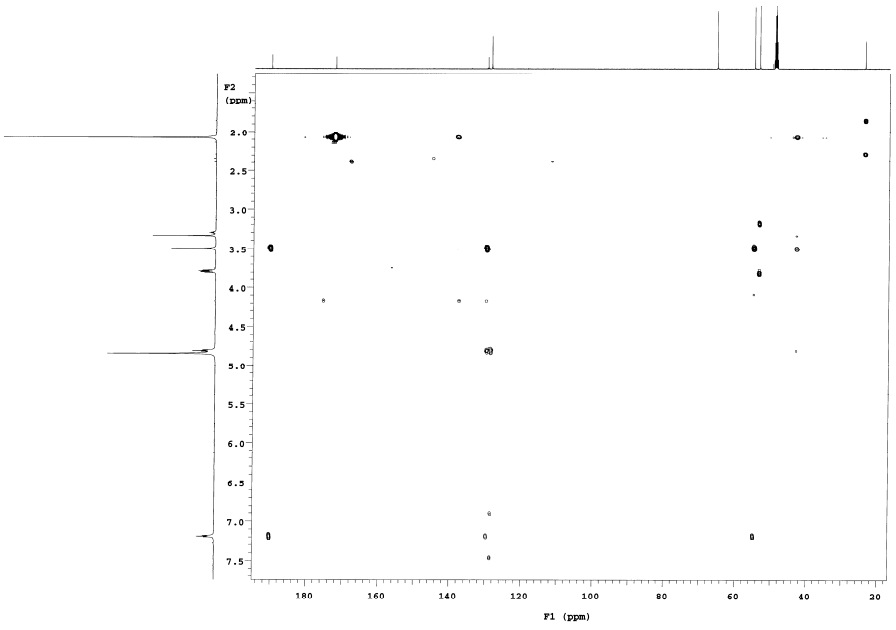

Figure 174: HMBC spectrum (CD_3OD, 300 MHz) of LL-C10037α (**86**)

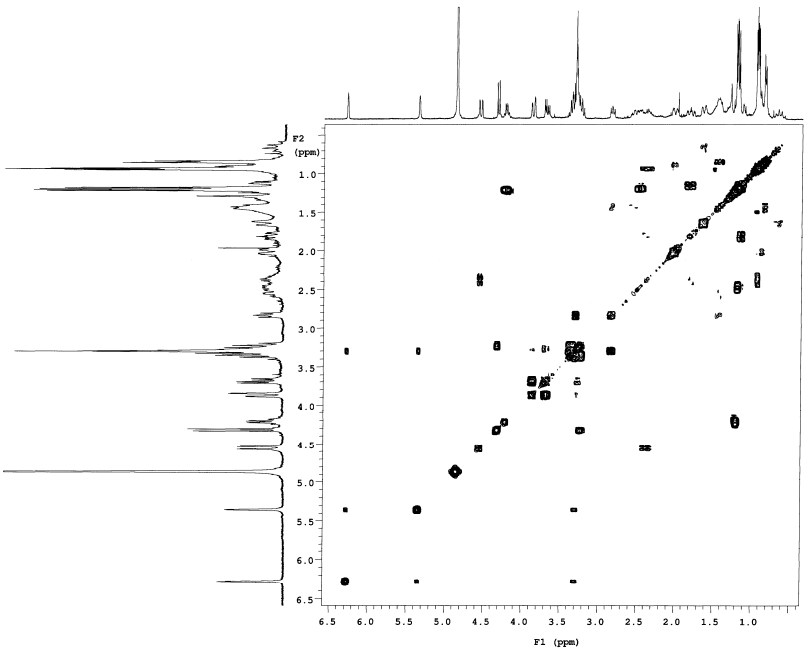

Figure 175: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) of omdurmycin (**88**)

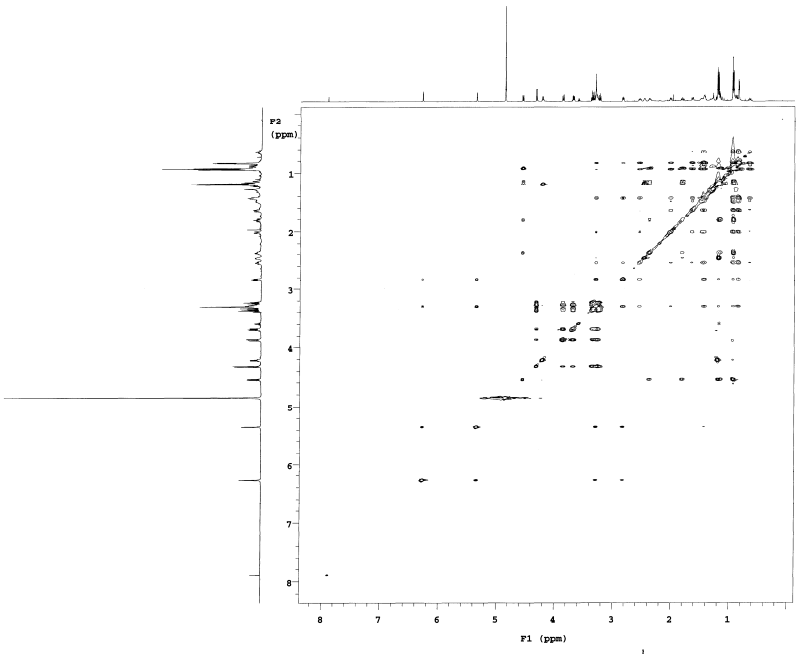

Figure 176: TOCSY spectrum (CD_3OD, 300 MHz) of omdurmycin (**88**)

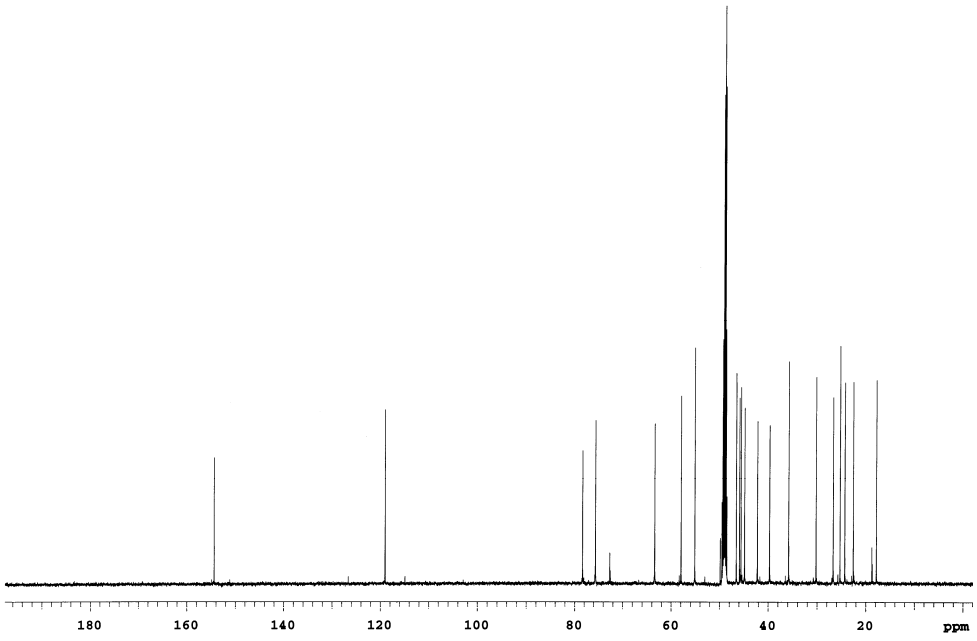

Figure 177: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of cyclooctatin (**89**)

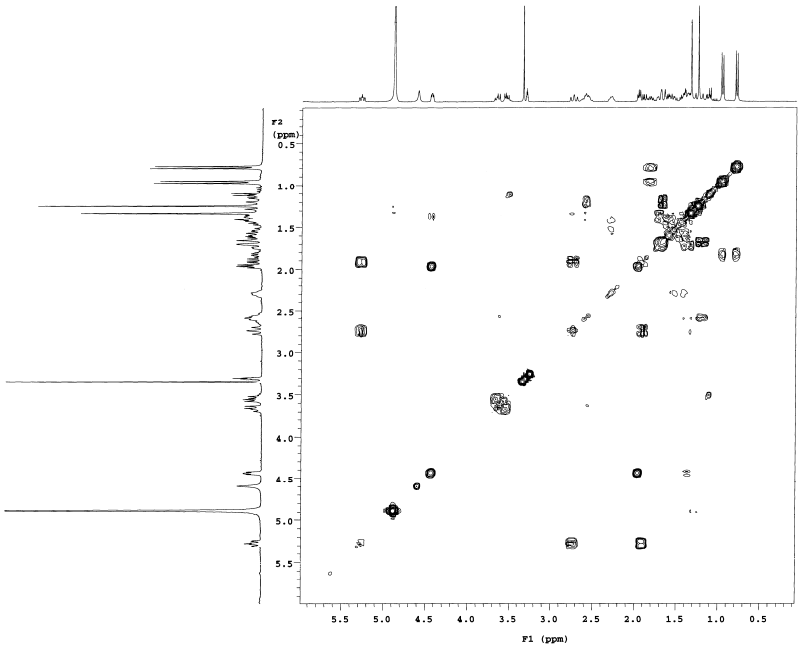

Figure 178: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) spectrum of cyclooctatin (**89**)

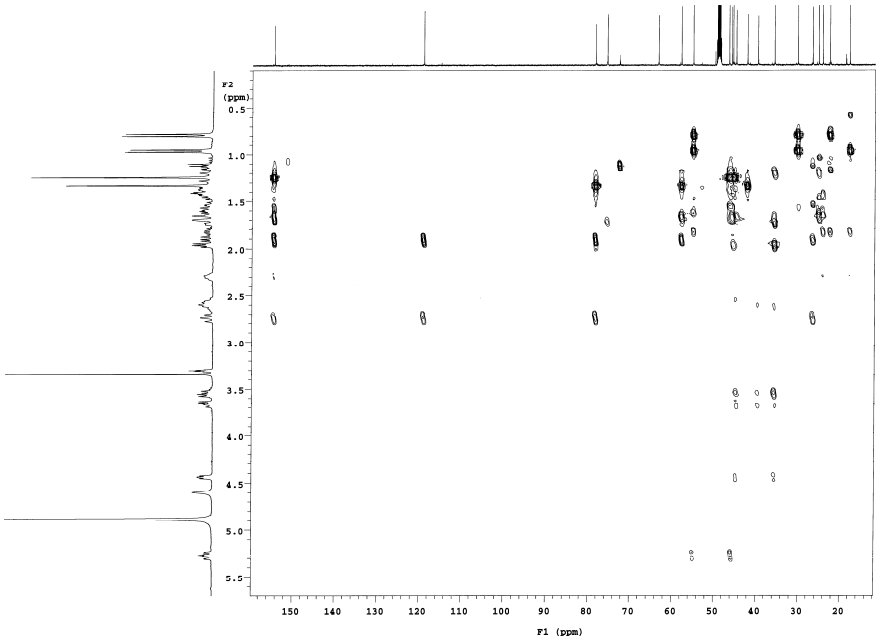

Figure 179: HMBC spectrum (CD_3OD, 300 MHz) spectrum of cyclooctatin (**89**)

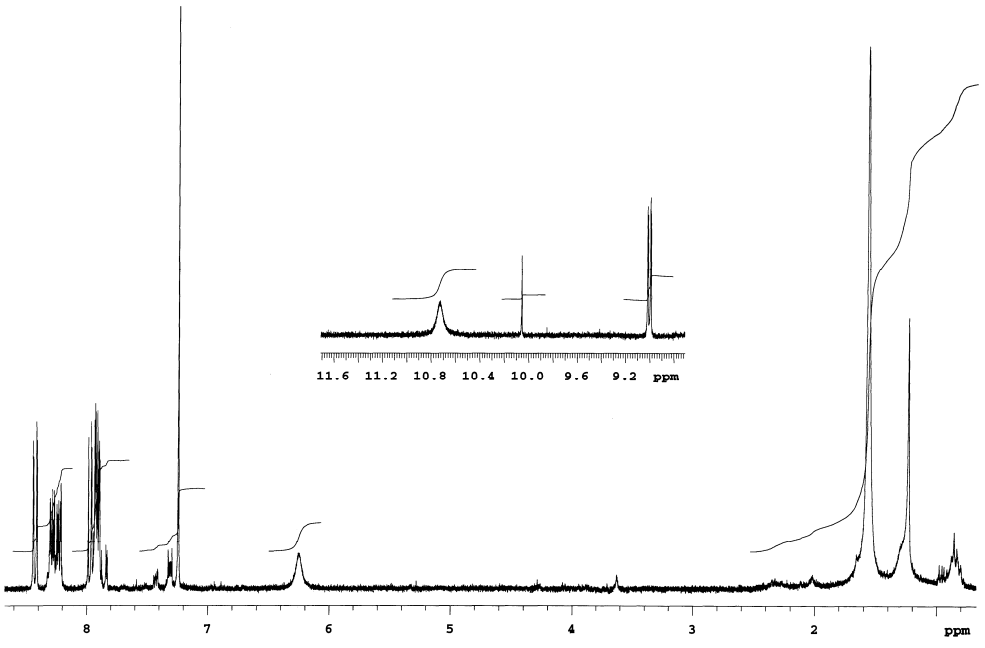

Figure 180: ^1H NMR spectrum ($CDCl_3$, 300 MHz) spectrum of phenazine-1-carboxamide (**90**)

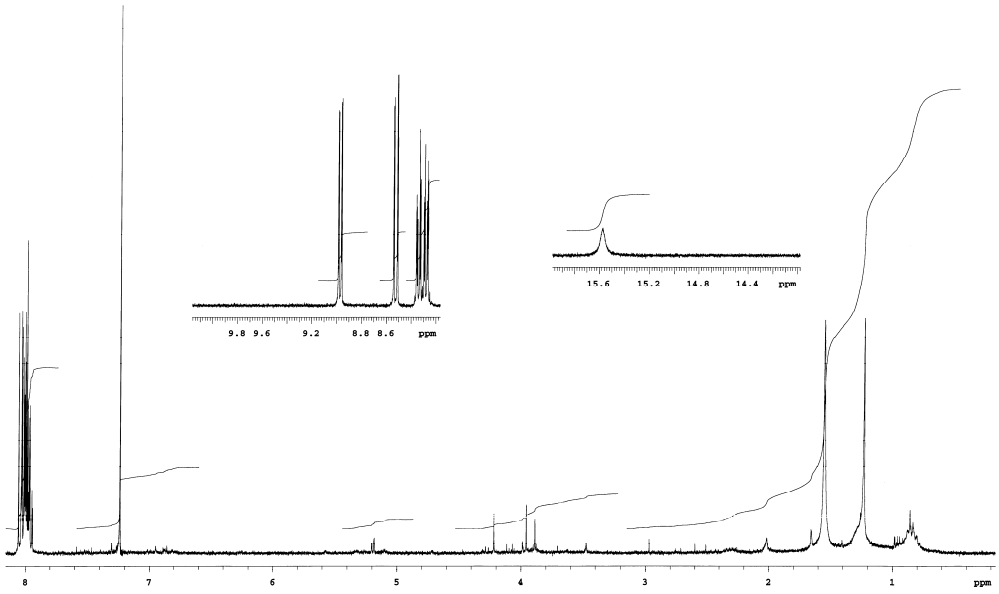

Figure 181: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of phenazine-1-carboxylic acid (**91**)

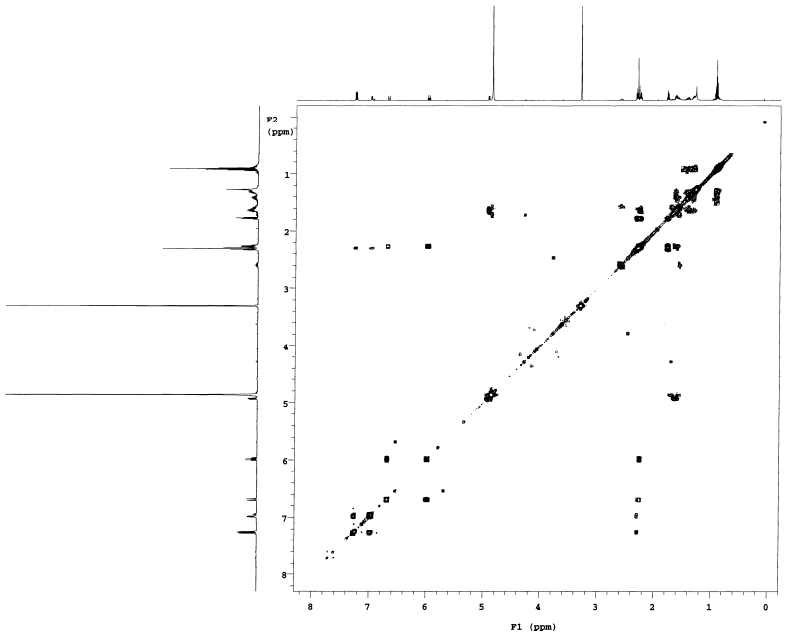

Figure 182: ^{1}H,^{1}H COSY spectrum (CD_3OD, 600 MHz) spectrum of **92**

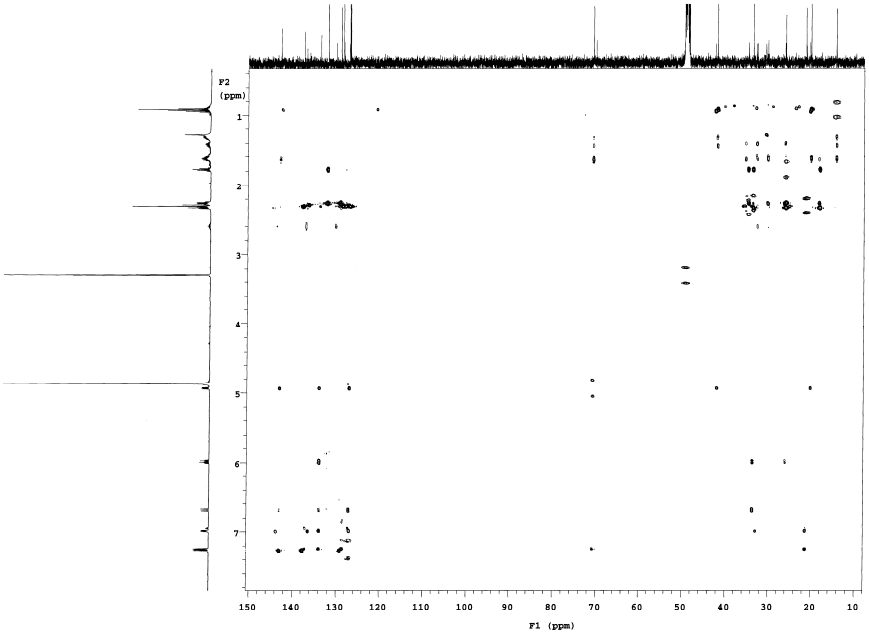

Figure 183: HMBC spectrum (CD_3OD, 600 MHz) spectrum of **92**

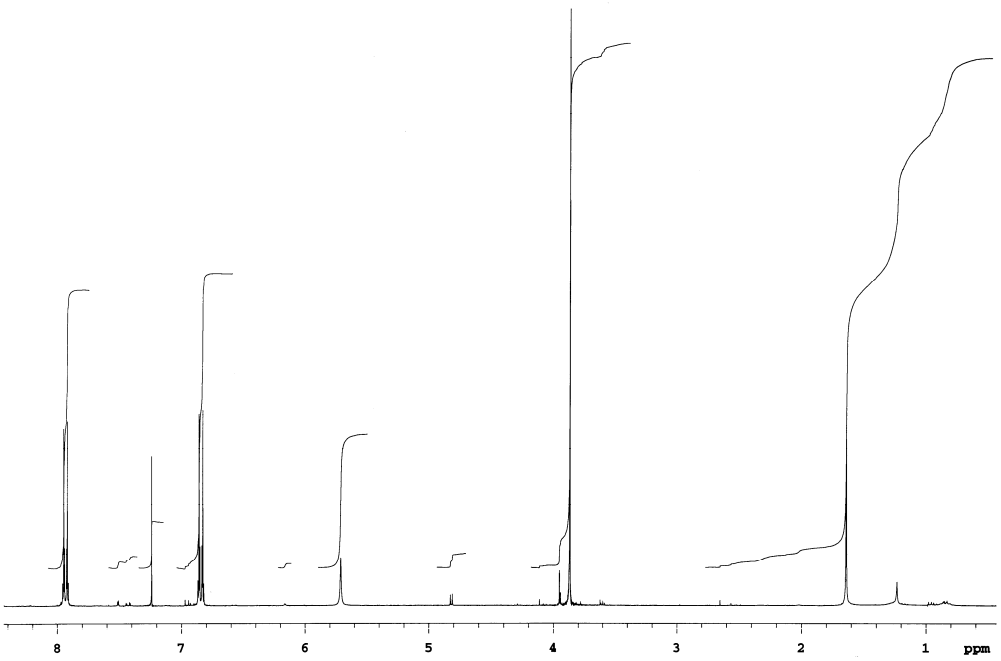

Figure 184: ^1H NMR spectrum (CD_3OD, 300 MHz) of compound **94**

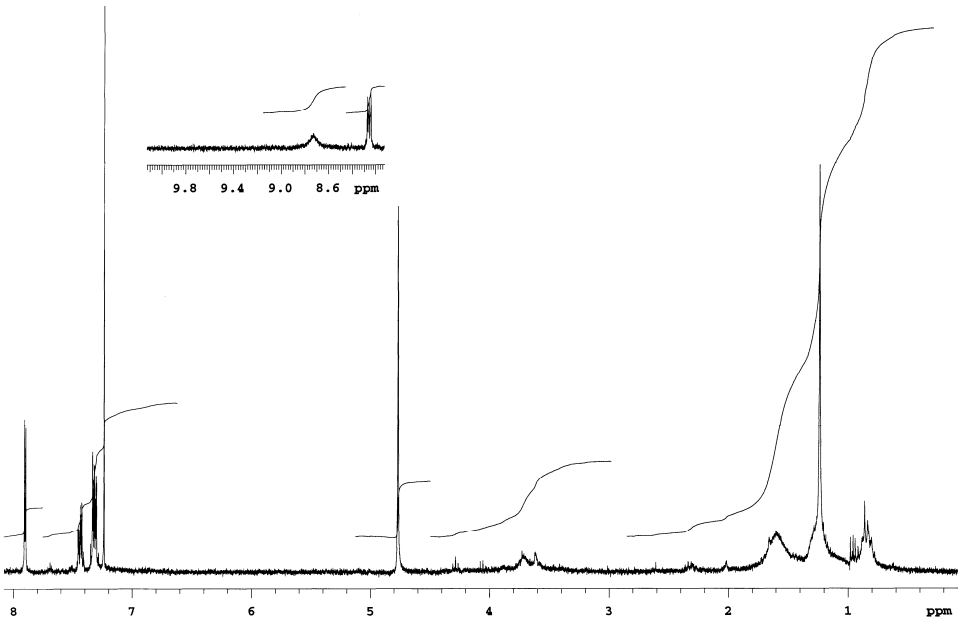

Figure 185: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 3-(hydroxyacetyl)-indole (**95**)

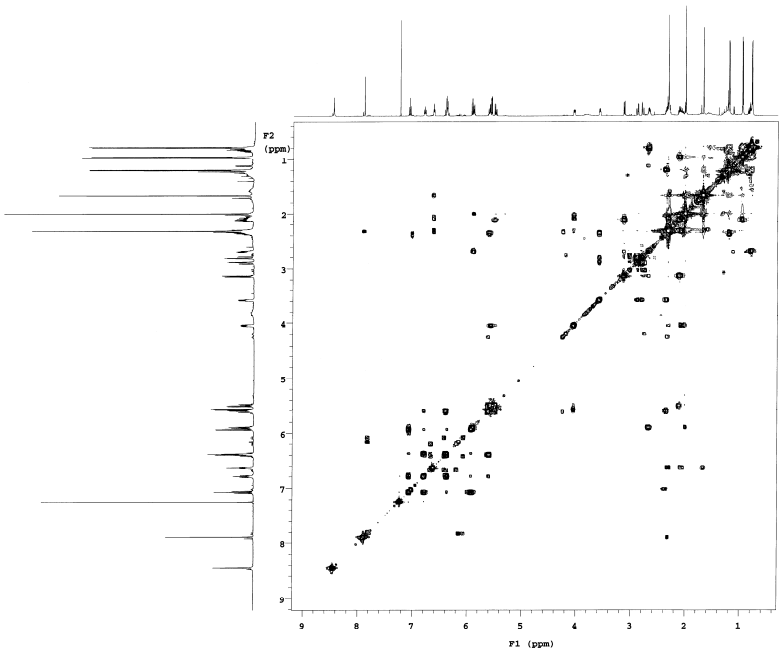

Figure 186: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 600 MHz) of diastovaricin I (**97**)

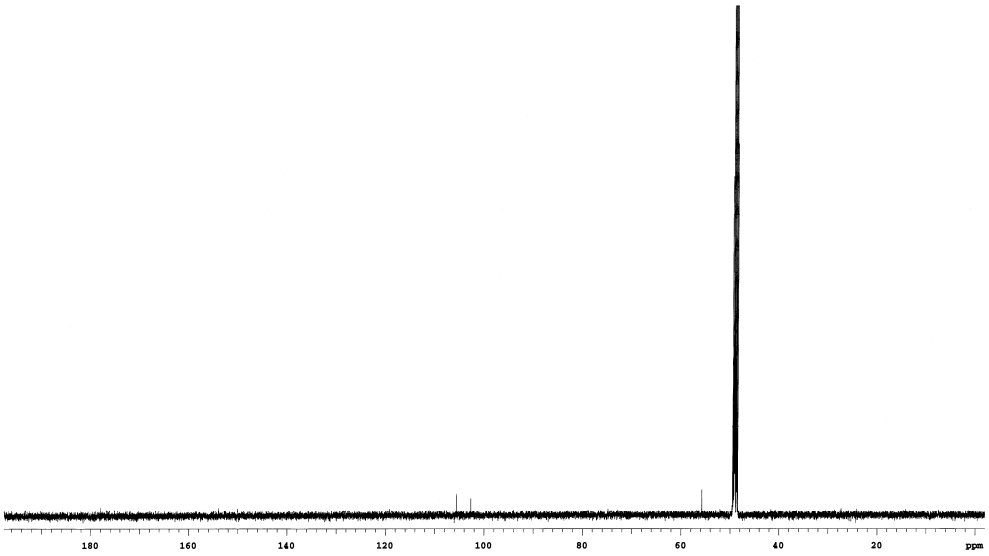

Figure 187: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 6-hydroxy-5-methoxyisatine (**98**)

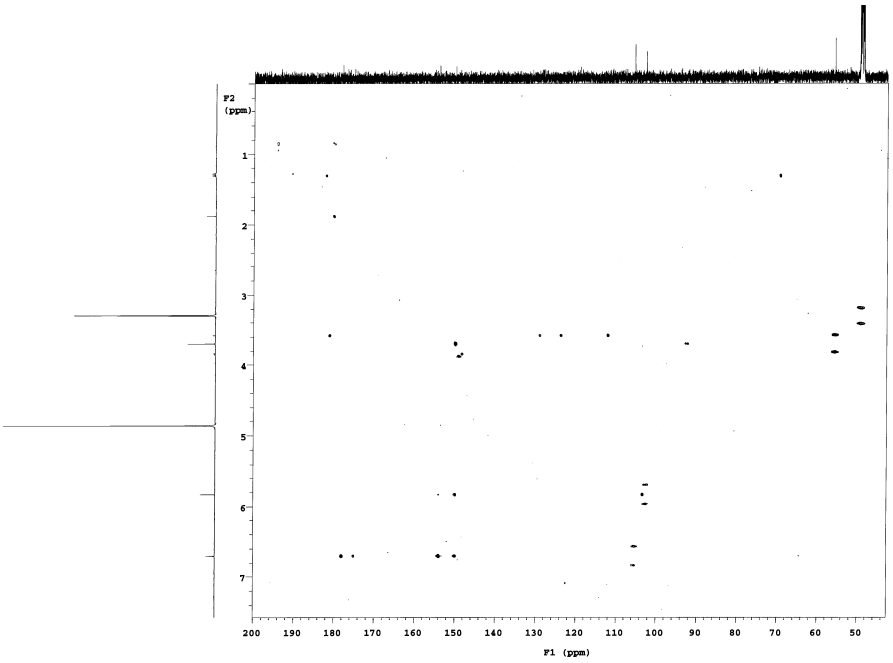

Figure 188: HMBC spectrum (CD_3OD, 300 MHz) of 6-hydroxy-5-methoxyisatine (**98**)

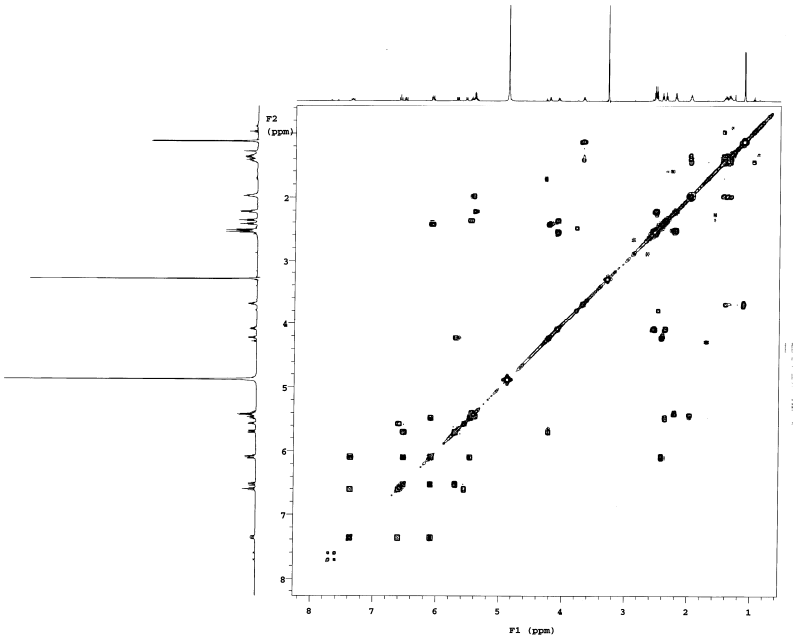

Figure 189: H,H COSY spectrum (CD_3OD, 300 MHz) of isomacrolactinic acid (**100**)

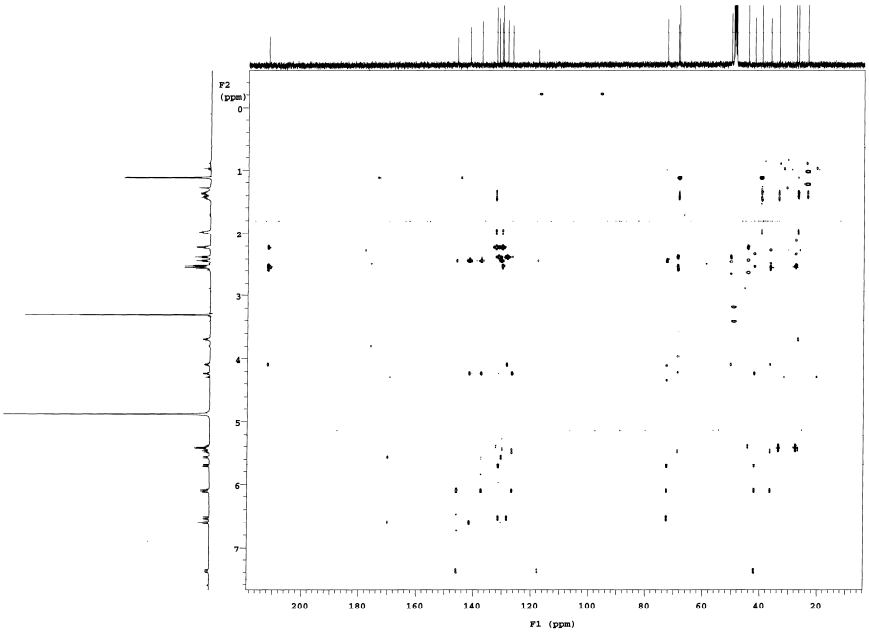

Figure 190: HMBC spectrum (CD_3OD, 300 MHz) of isomacrolactinic acid (**100**)

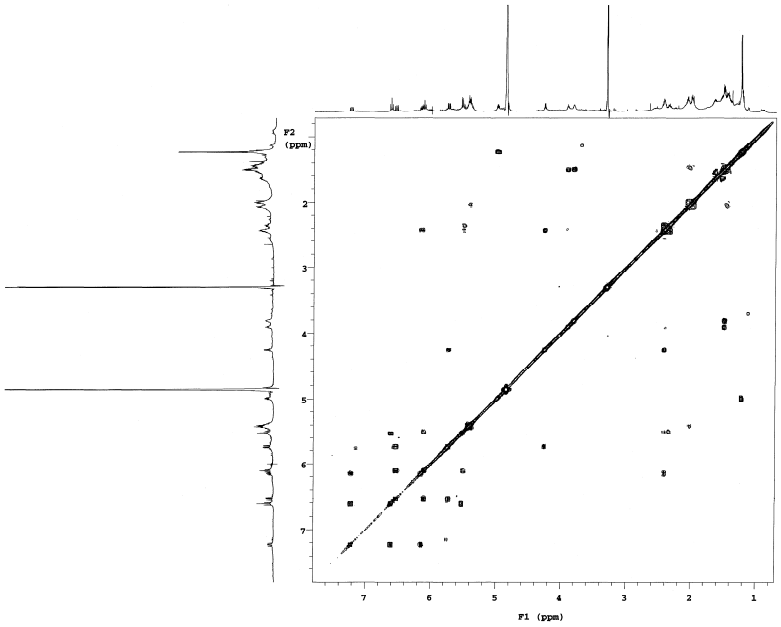

Figure 191: H,H COSY spectrum (CD_3OD, 300 MHz) of dihydromacrolactin F (**102**)

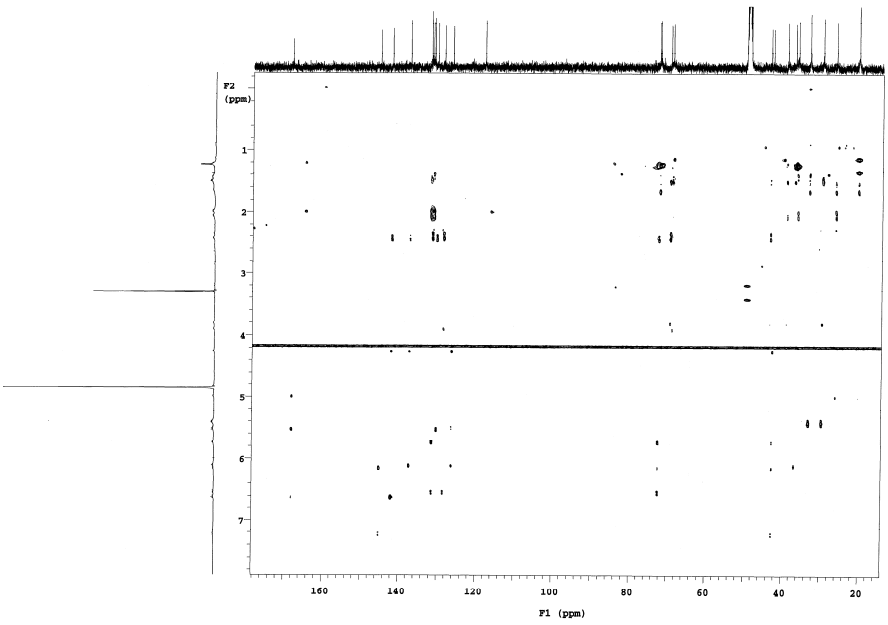

Figure 192: HMBC spectrum (CD_3OD, 300 MHz) of dihydromacrolactin F (**102**)

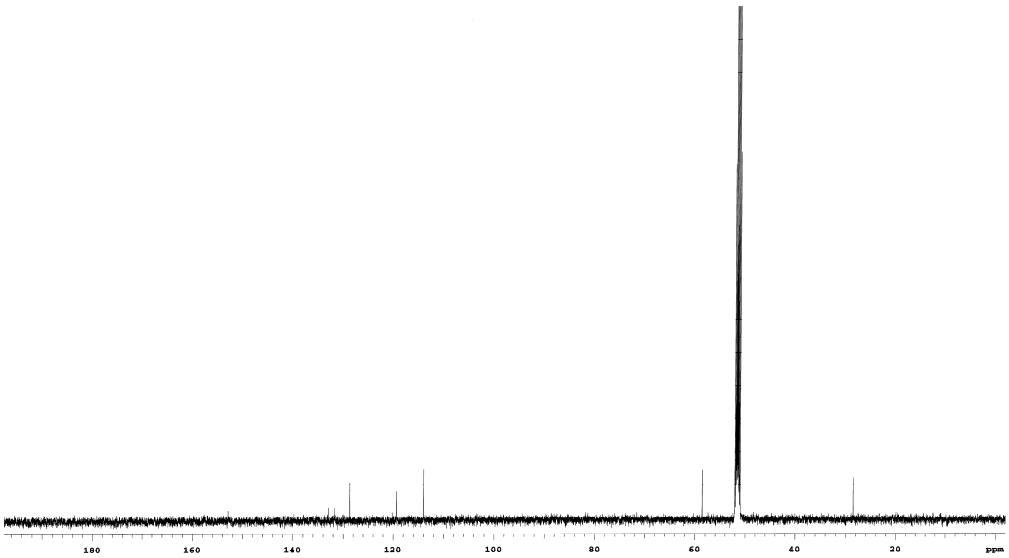

Figure 193: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **103**

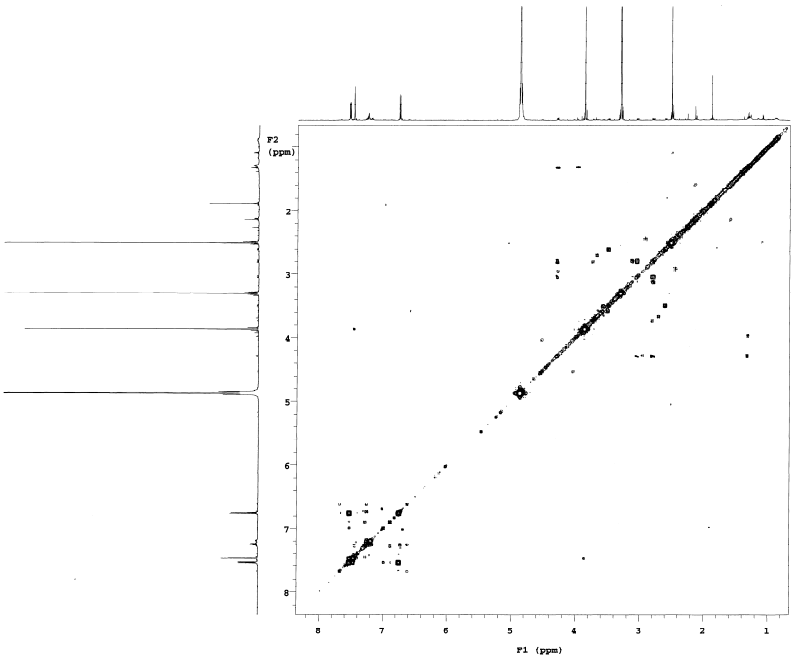

Figure 194: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) of compound **103**

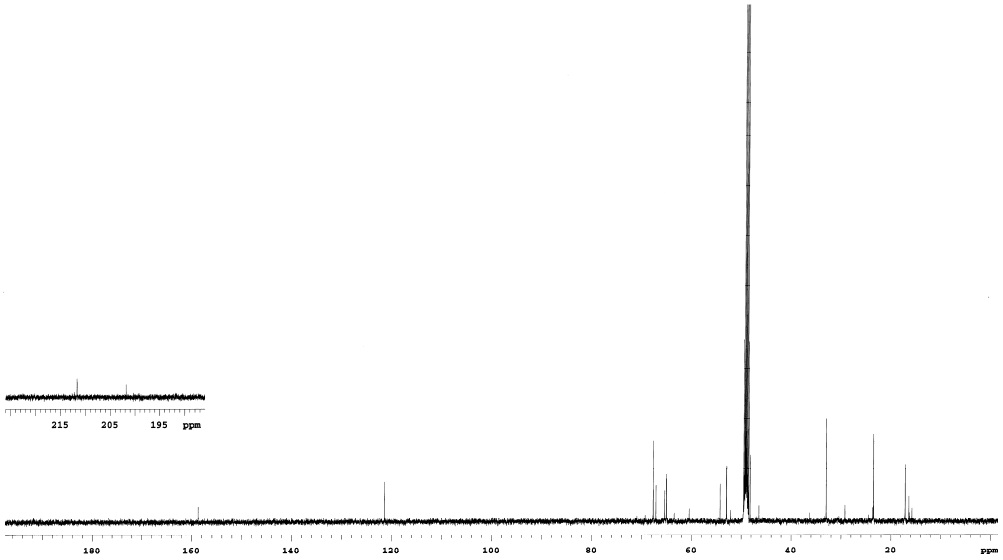

Figure 195: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 2,6-dimethyl-oxepan-4-one (**104**)

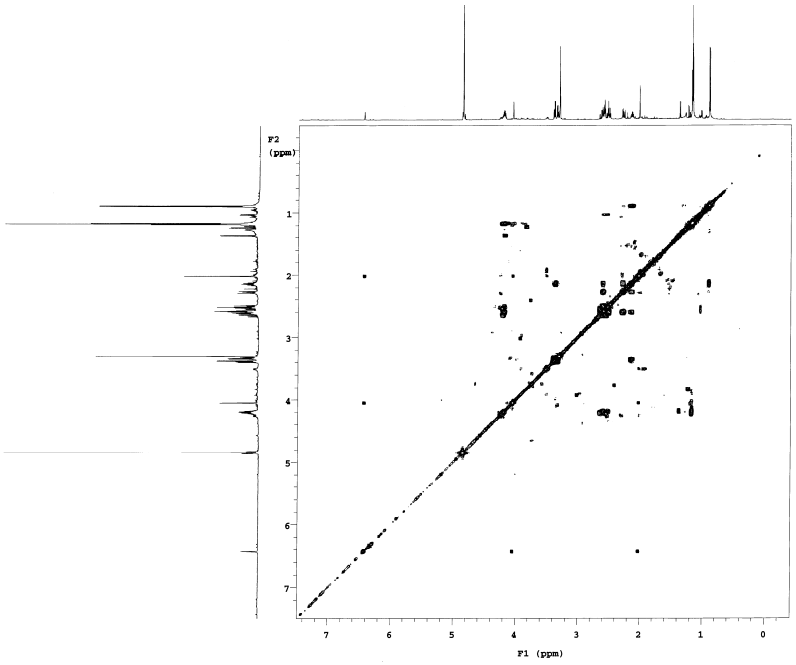

Figure 196: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) of 2,6-dimethyl-oxepan-4-one (**104**)

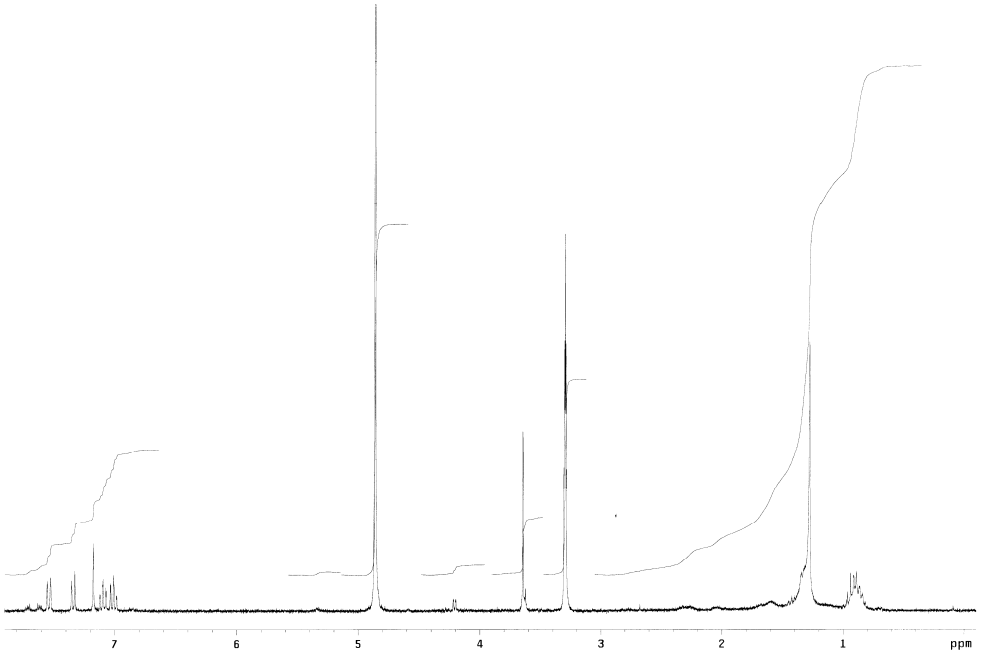

Figure 197: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of compound **106**

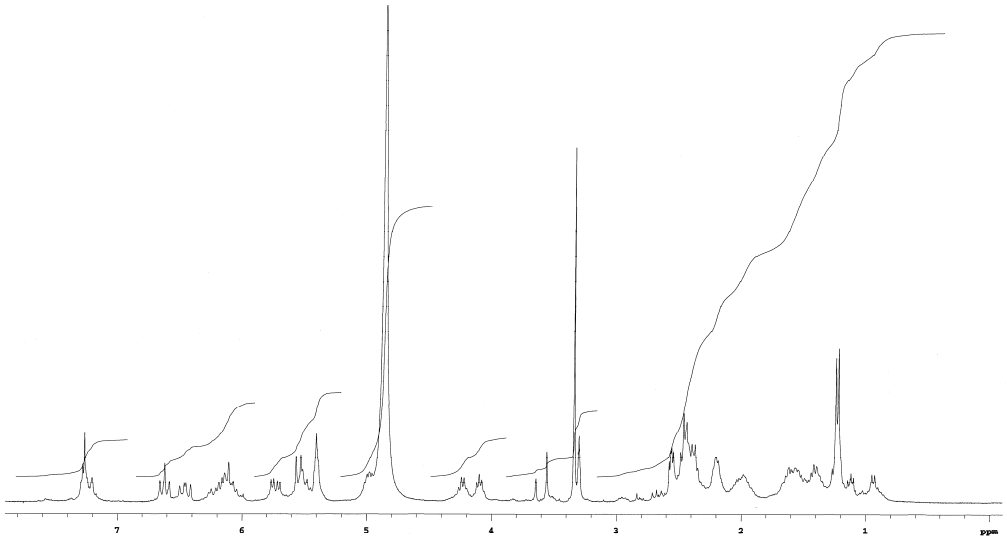

Figure 198: ^{1}H NMR spectraum (CD_3OD, 300 MHz) of macrolactin F (**112**)

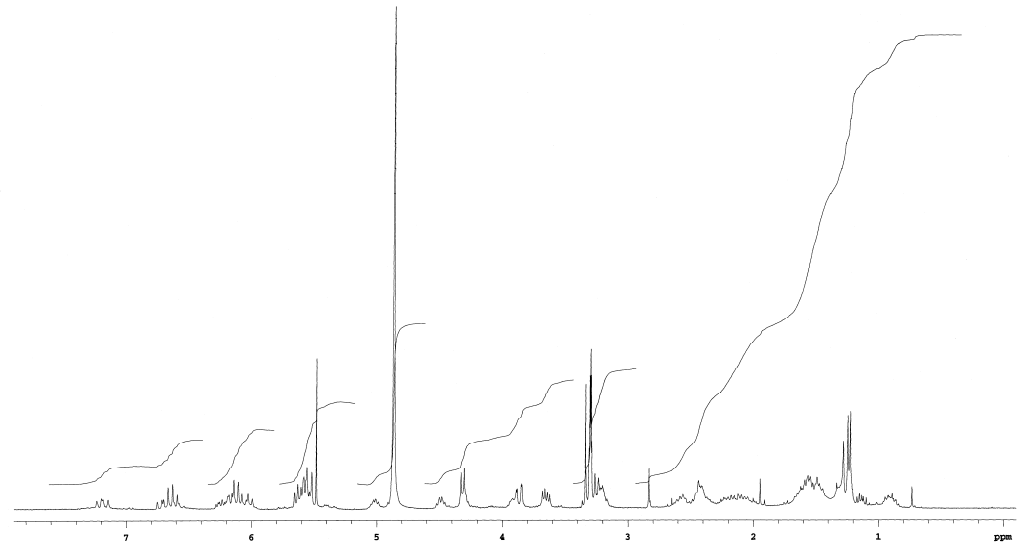

Figure 199: ^{1}H NMR spectrum of macrolactin B (**113**)

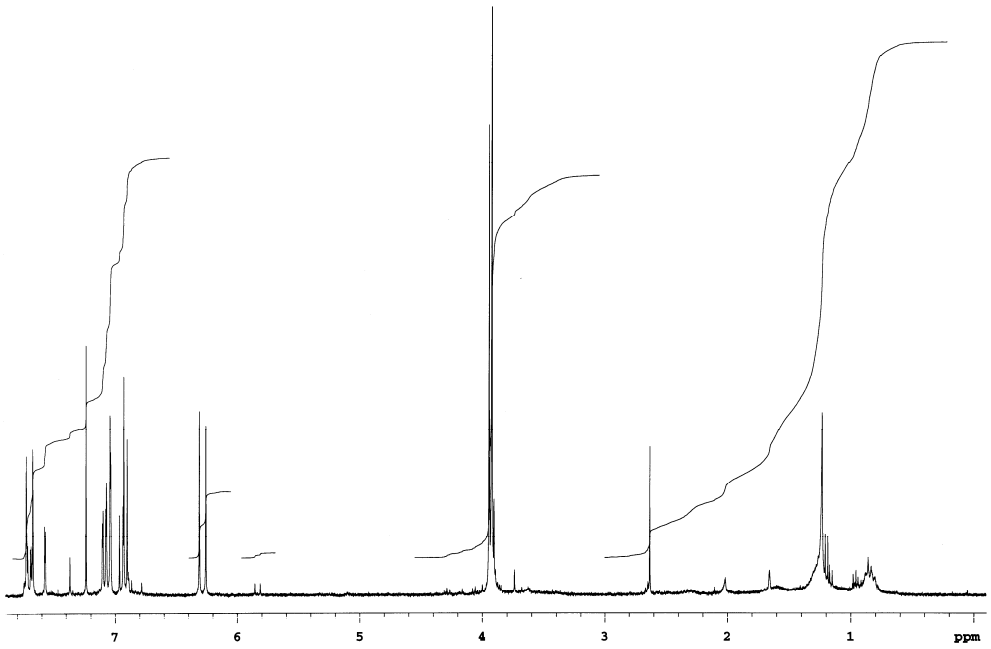

Figure 200: ^{1}H NMR spectrum of compound ferulic acid (**116**)

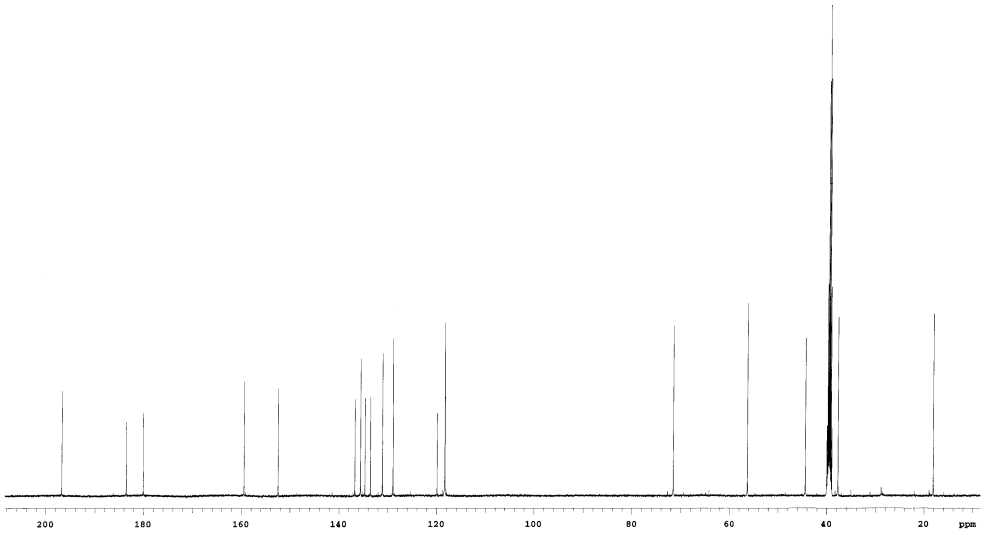

Figure 201: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of fujianmycin B (**117**)

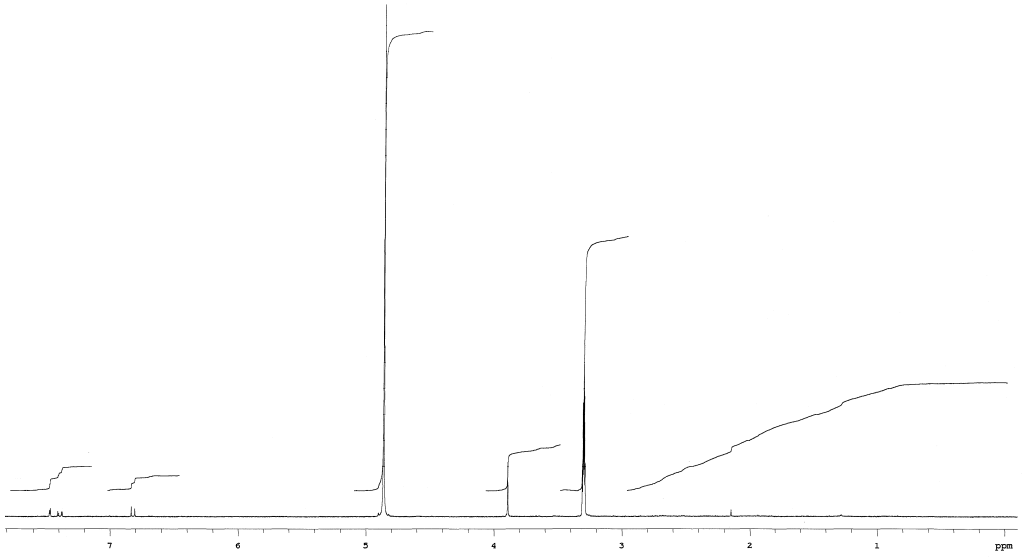

Figure 202: ^{1}HNMR spectrum (CD_3OD, 300 MHz) of 2-hydroxy-5-methoxybenzamide (**123**)

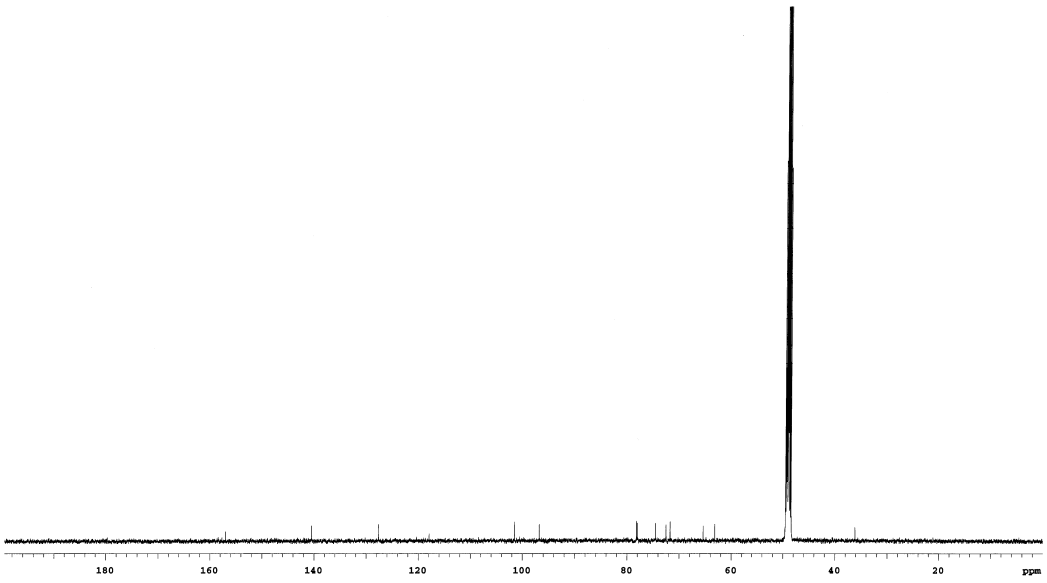

Figure 203: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of menisdaurin (**124**)

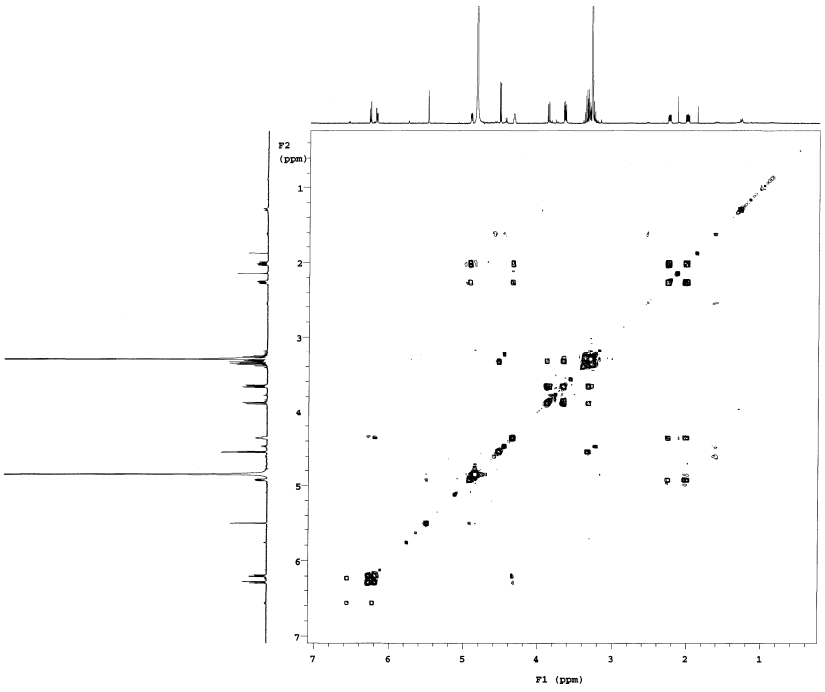

Figure 204: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 300 MHz) spectrum of menisdaurin (**124**)

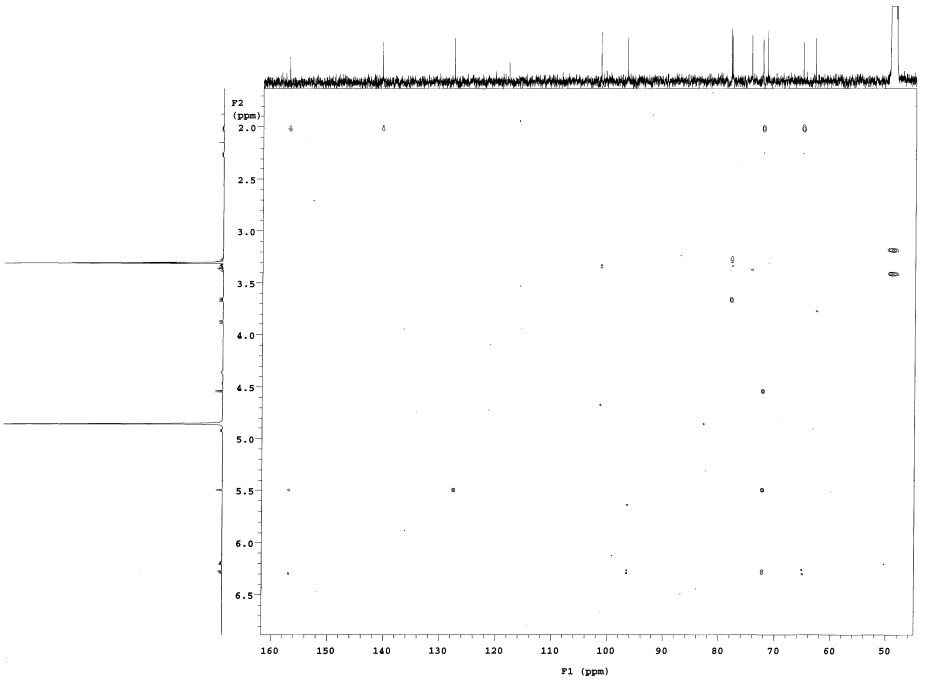

Figure 205: HMBC spectrum (CD_3OD, 300 MHz) spectrum of menisdaurin (**124**)

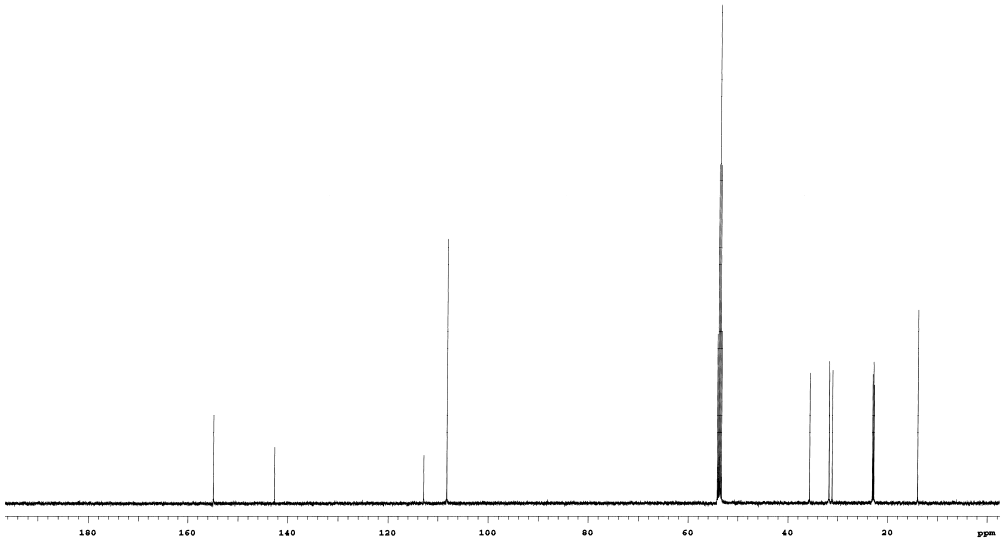

Figure 206: ^{13}C NMR spectrum (CD_2Cl_2, 125 MHz) of stemphol (**126**)

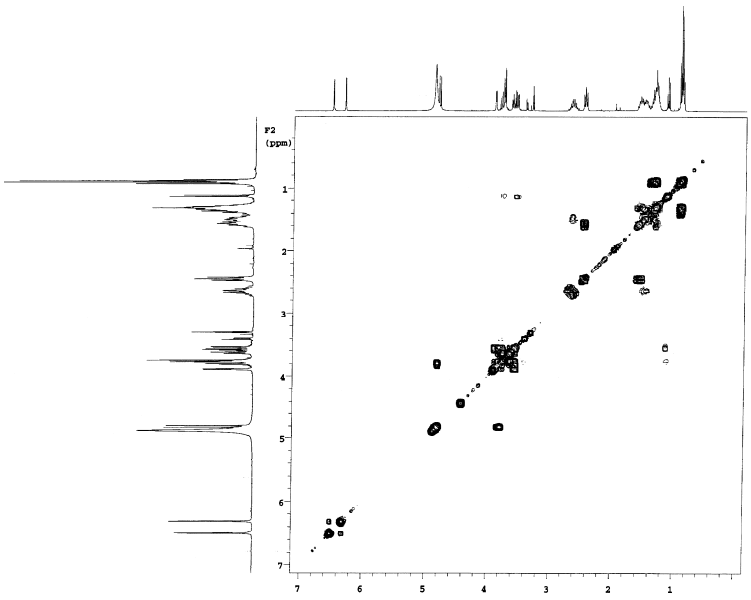

Figure 207: H,H COSY spectrum (CD_3OD, 300 MHz) of stemphol 1-*O*-*β*-D-galactoside (**127**)

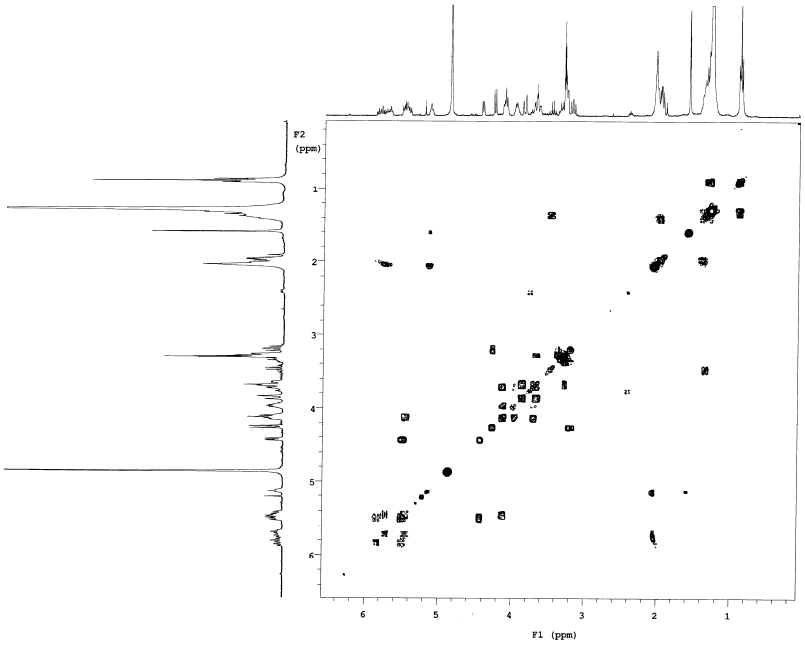

Figure 208: H,H COSY spectrum of cerebroside A (**128**)

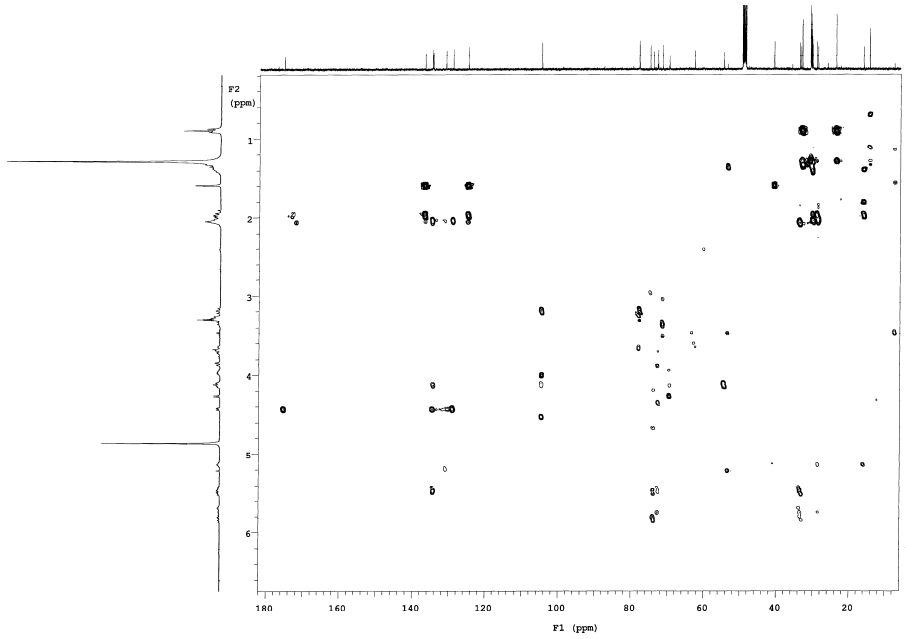

Figure 209: HMBC spectrum of cerebroside A (**128**)

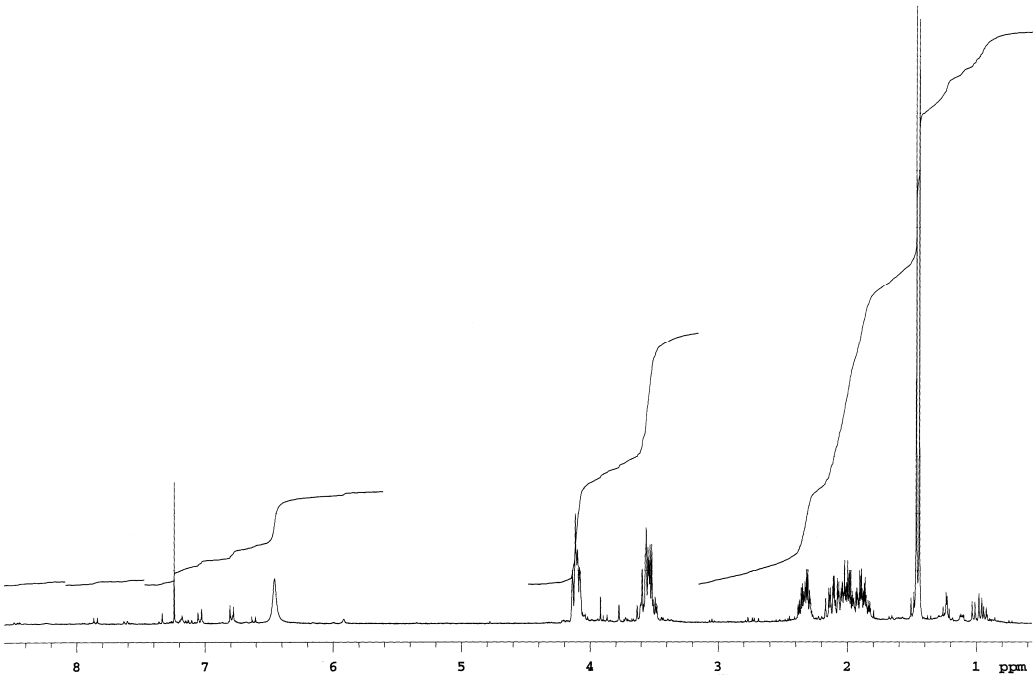

Figure 210: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of *cis-cyclo*-(Alanyl-prolyl) (**129**)

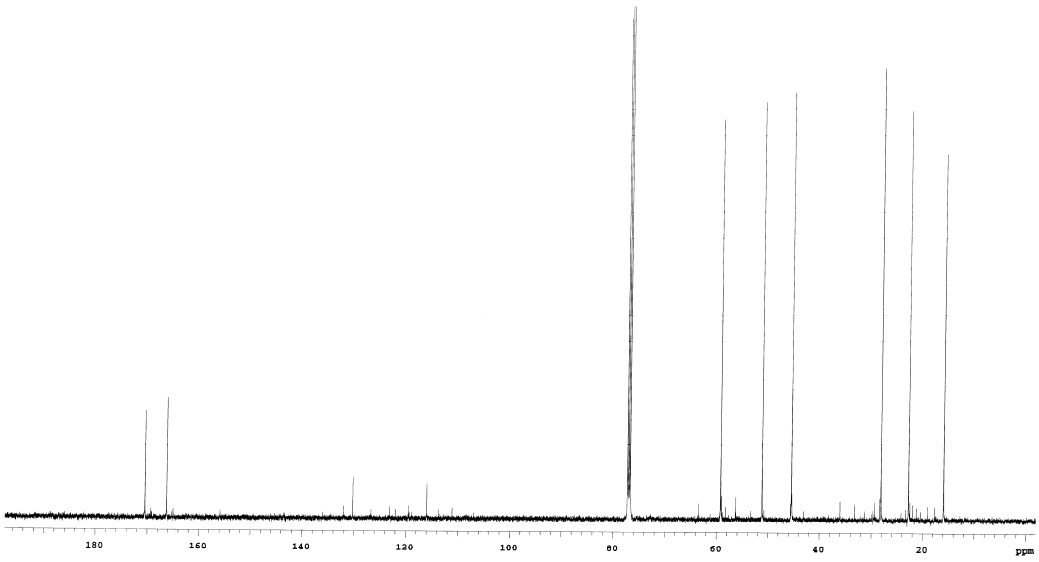

Figure 211: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of *cis*-*cyclo*-(Alanyl-prolyl) (**129**)

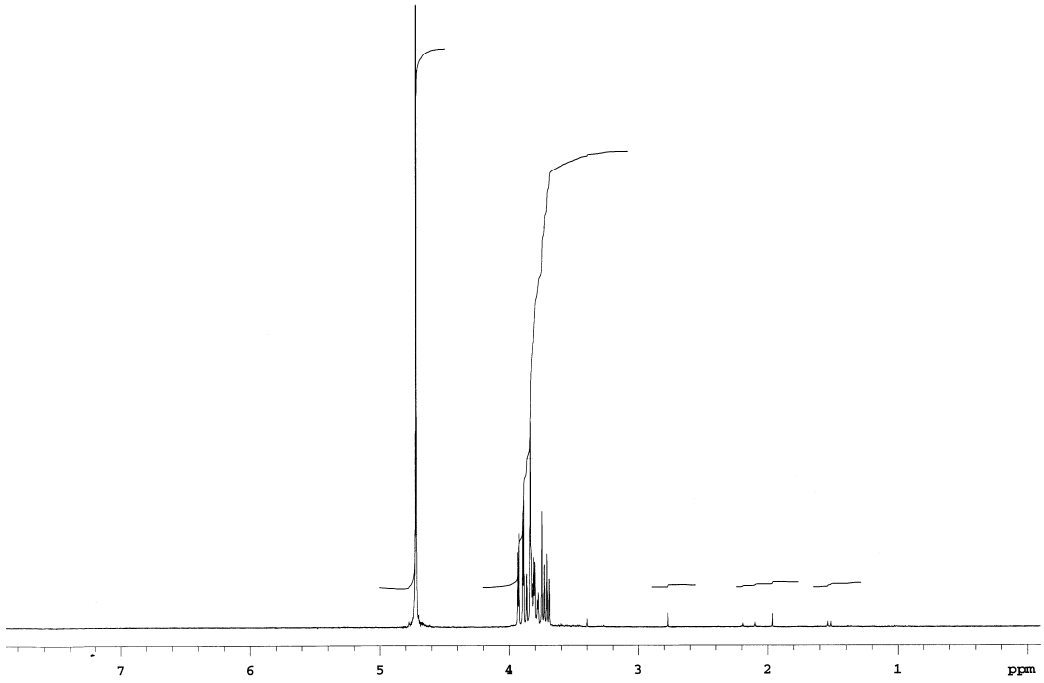

Figure 212: ^{1}H NMR spectrum (D_2O, 300 MHz) of D-sorbitol (**130**)

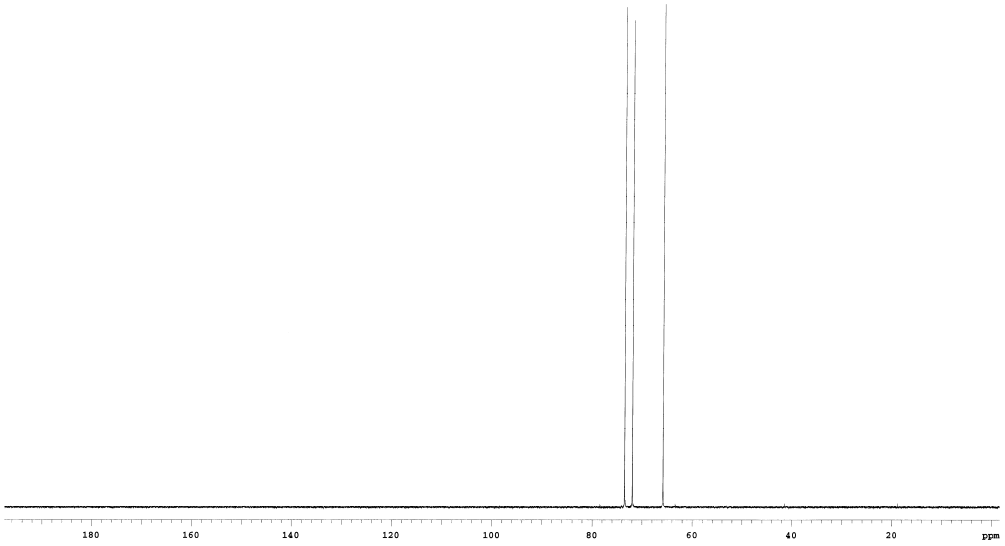

Figure 213: ^{13}C NMR spectrum (D_2O, 125 MHz) of D-sorbitol (**130**)

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CURRICULUM VITAE

Name	Muna Ali *ABDALLA*
Nationality	Sudanese
Marital status	Married
Language	Arabic, English, German Written and Spoken
Position held	Lecturer, University of Khartoum, Faculty of Agriculture, Department of Food Science and Technology
Mailing address	Institute of Organic and Biomolecular Chemistry, Georg-August-University of Göttingen, Tammannstrasse 2, 37075 Göttingen
Mailig address in Sudan	University of Khartoum, Faculty of Agriculture, Department of Biochemistry and Food Science P.O. Box 13314, Shambat, Khartoum North, Sudan
E-mail address	munalsamahoni@yahoo.com munalsamahoni@daad-alumni.de

ACADEMIC QUALIFICATION

Second University Degree	Master of Science in Agriculture (August 2004)
Specialization	Biochemistry and Nutrition.
Title of Thesis	Evaluation of the Nutritional Status of Children under Age of Five in El Fau Rural Area in Relation to their Feeding Patterns.

First University Degree Bachelor of Science (Agric.) Honours, Class I
Date of Degree Awarded August 2000
Specialization Biochemistry and Food Science.

PRIZES AND AWARDS

Organization	Type of ward	Awarded for	Date
Conferring award (German Academic Exchange Service) (DAAD)	Award Scholarship	PhD Study	2006-2010
Conferring award (German Academic Exchange Service) (DAAD)	Award Scholarship	Master Study	2002/2004
Arab Organization for Agricultural Development	Prize	Best Academic Performance in Biochemi-stry and Food Science in Fifth Year, Faculty of Agriculture	1999/2000
University of Khartoum	Prize	Best Academic Performance in Fifth Year, Faculty of Agriculture	1999/2000
University of Khartoum	Prize	Best Academic Performance in Biochemi-stry and Food Science in Fourth Year, Faculty of Agriculture	1998/99
Arab Organization for Agricultural Development	Prize	Best Academic Performance in Third Year Faculty of Agriculture	1997/98
University of Khartoum	Prize	Best Academic Performance in Second Year, Faculty of Agriculture	1996/97
University of Khartoum	Prize	Best Academic Performance in First Year, Faculty of Agriculture	1995/1996

EMPLOYMENT

- August 2006 – Up to Date: Lecturer, University of Khartoum, Faculty of

Agriculture, Department of Biochemistry and Food Science.

- April 2001 – 2006: Teaching Assistant, University of Khartoum, Faculty of Agriculture, Department of Biochemistry and Food Science.

- August 2006 – Up to Date: PhD student (Natural Products Isolation and Structure Elucidation) at the Institute of Organic and Biomolecular Chemistry, University of Göttingen

SCIENTIFIC PUBLICATIONS

Posters

[1] Muna A. ABDALLA, A. H. KHATTAB, A. H. El TINAY (**2007**). Evaluation of the Nutritional Status of Children under Age of Five in Relation to their Feeding Patterns.**Tropentag**, Witzenhausen, Germany.

[2] Muna A. ABDALLA, Muhammad BAHI, Mahmoud Al REFAI, Petrea FACEY and Hartmut LAATSCH (**2008**). Flavonoids from Sudanese *Albizia zygia* (Mimosoideae): A plant used as antimalarial remedy. **Chemie Forum**, Institute of Organic and Biomolecular Chemistry, Georg-August University Göttingen, Tammannstrasse 2, 37077 Göttingen, Germany.

[3] Muhammad BAHI, Muna A. ABDALLA, Mahmoud Al REFAI, Heidrun ANKE , and Hartmut LAATSCH (**2008**). A Bioactive Oxadiaziridine or N-Oxydiazenyl Derivative together with other New Phenolic Compounds Isolated from a Terrestrial *Streptomyces* sp. **Chemie Forum**, Institute of Organic and Biomolecular Chemistry, Georg-August University Göttingen, Tammannstrasse 2, 37077 Göttingen, Germany.

[4] Mahmoud Al REFAI, Muna A. ABDALLA, Muhammad BAHI, Hafizur RAHMAN, E.HELMKE and Hartmut LAATSCH (**2008**). The marine derived *Streptomyces* sp. B8041, a source of new butanolides. **Chemie Forum**, Institute of Organic and Biomolecular Chemistry, Georg-August University Göttingen, Tammannstrasse 2, 37077 Göttingen, Germany.

[5] Muna Ali ABDALLA , Saad Abdel Rahman SULIEMAN, Abdelgader H. KHATTAB (**2008**). Socio-economic and Cultural Aspects Affecting the Nutrition of Children under Age of Five in the Rural Area of Sudan. **Tropentag**, Hohenheim, Germany.

[6] Dhafer Saber ZINAD, Khaled A. SHAABAN, Muna A. ABDALLA, Hamdi A. NASR، Muhammad BAHI, Heidrun ANKE and Hartmut LAATSCH (**2009**). New Isocoumarins from a Terrestrial *Streptomyces* sp. **Chemie Forum**, Institute of Organic and Biomolecular Chemistry, Georg-August University of Göttingen, Tammannstrasse 2, 37077 Göttingen, Germany.

[7] Muna A. ABDALLA, E. HELMKE and Hartmut LAATSCH (**2009**). Fujianmycin C, A New Bioactive Drug from a Marine Derived *Streptomyces* sp. B6219. **Chemie Forum**, Institute of Organic and Biomolecular Chem-

istry, Georg-August University of Göttingen, Tammannstrasse 237077 Göttingen, Germany.

[8] Muna A. ABDALLA, MD. Tofazzal ISLAM and Hartmut LAATSCH (**2009**). Strong Zoosporicidal Activity of a New Flavone Isolated from Sudanese *Xanthium brasilicum* Vell (Asteraceae). **13th International Congress PHYTOPHARM**, July 29-31, Bonn, Germany.

[9] Muna A. ABDALLA, Prem P. YADAV, Birger DITTRICH, Heidrun ANKE and Hartmut LAATSCH (**2010**). Homoabyssomicins A and B two new Spirotetronate Metabolites from an *Actinomyces* sp. ANK 210. **22 Irseer Naturstofftage**, February 24-26, Irseer, Germany.

[10] K. FANDI, M. BAHI, M. A. ABDALLA, M. MASSADEH, H. LAATSCH. (**2010**). Screening Metabolites of Thermophiles from Thermal Springs Jordanian Bacteria by LC-MS/MS Profile. **The American Society for Microbiology (ASM) 110th General Meeting**, May 23-27, in San Diego, California, USA.

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[1] Muna A. Abdalla, Saad A. Sulieman, Abdullahi H. El Tinay, Abdul Gader H. Khattab **(2009**). Socio-Economic Aspects Influencing Food Consumption Patterns Among Children under Age of Five in Rural Area of Sudan. *Pakistan Journal of Nutrition*, 8 (5): 653-659.

[2] Juangjun Jumpathong, Pascal Retailleau, Muna Ali Abdalla, Jamal Ouazzani and Saisamorn Lumyong (**2009**). 2-Butyl-5-pentylbenzene-1,3-diol. *Acta Crystallographica Section* E65, o1366.

[3] Saad A. Sulieman, Muna A. Abdalla, El Tahir A. Omer and Tageldin E.M. Hago **(2009**). Phosphorus Supply and *Phaseolus vulgaris* Performance Grown in Shambat Clayalkaline Soil and Influenced by Farmyard Manure. *Australian Journal of Basic and Applied Sciences*, 3(3): 2598-2606.

[4] Juangjun Jumpathong, Muna A. Abdalla, Saisamorn Lumyong and Hartmut Laatsch (**2010**) Stemphol Galactoside, a New Stemphol Derivative Isolated from the Tropical Endophytic Fungus *Gaeumannomyces amomi*. *Natural Products Communications, 5*, 567–57.

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[6] Dhafer Saber Zinad, Khaled A. Shaaban, Muna Ali Abdalla, Md. Tofazzal Islam, Anja Schüffler, and Hartmut Laatsch. Bioactive Isocoumarins from a Terrestrial *Streptomyces* sp. ANK 302. Submitted (August **2010**) to *Natural Products Communications*.