Unusual Sesquiterpenes: Gorgonenes and Further Bioactive Secondary Metabolites Derived from Marine and Terrestrial Bacteria

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Dedicated to my beloved parents
Md Fazlur Rahman and Mrs. Fazilatunnessa
Contents

1 Introduction....................................................................................................... 1
  1.1 History of the use of natural products for medical applications .......... 2
  1.2 Approaches for the search of new natural products ......................... 4
  1.3 Antibiotic screening ............................................................................. 6
  1.4 Natural Products from Marine Bacteria ................................................ 6
  1.5 Therapeutic areas influenced by natural products .............................. 9
  1.6 Natural products against infectious diseases; bacterial pathogens .......... 9
  1.7 Antifungal compounds from natural sources ...................................... 10
  1.8 Antibacterial compounds from natural sources .................................. 11
  1.9 Potential antiparasitic drugs originated from natural products .......... 13
  1.10 Marine derived anticancer drugs ....................................................... 14
  1.11 Antiviral compounds from natural sources ...................................... 15
  1.12 Sequiterpenes from marine microorganism ........................................ 17

2 Objectives of the present investigation .................................................... 18

3 General techniques..................................................................................... 20
  3.1 Collection of strains ............................................................................. 20
  3.2 Pre-screening ........................................................................................ 20
  3.3 Chemical screening .............................................................................. 20
  3.4 Pharmacological and biological assays ................................................ 22
  3.5 Cultivation and scale-up ....................................................................... 22
  3.6 Isolation methods ............................................................................... 23
  3.7 Partial identification and dereplication ............................................... 23
  3.8 Description of Strain Codes ................................................................. 25

4 Investigation of selected marine and terrestrial Streptomyces ............... 25
  4.1 Terrestrial Streptomyces sp. ADM 14 .................................................... 25
  4.1.1 1-Hydroxy-4-methoxynaphthalene-2-caboylic acid ...................... 27
  4.1.2 5′-Methyl-thioadenosine ................................................................. 28
  4.1.3 5,7,9,11-Tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid .... 30
  4.2 Streptomyces sp. B7880 ........................................................................ 33
  4.2.1 ζ-Pyromycinone; Galirubinone C ..................................................... 33
  4.2.2 Cinerubin B; Ryemycin B ................................................................. 35
4.2.3 Cinerubin A/ Spartanamicin B .................................................................37
4.2.4 Cinerubin X .................................................................................................38
4.3 Terrestrial Streptomyces sp. ACT 7619 .........................................................39
4.3.1 Dinactin ........................................................................................................40
4.3.2 Bonactin ........................................................................................................41
4.3.3 4,10-Dihydroxy-10-methyl-dodec-2-en-1,4-olide ........................................42
4.4 Terrestrial Streptomycete GW 6311 ...............................................................43
4.4.1 β-Indomycinone ..........................................................................................43
4.4.2 Saptomycin A ...............................................................................................44
4.5 Terrestrial Streptomyces sp. GT 2005/079 .......................................................45
4.5.1 N-(7-Hydroxy-6-methyl-octyl)-acetamide ..................................................48
4.5.2 N-(7-Hydroxy-octyl)-acetamide ..................................................................50
4.5.3 N-(6-Hydroxy-6-methyl-heptyl)-acetamide .................................................53
4.5.4 Homononactin acid amide ..........................................................................56
4.5.5 2-Phenylbenzoxazole-4-carboxylic acid ....................................................59
4.5.6 2-(3-Chloro-2-hydroxy phenyl) benzoxazole-4-carboxylic acid ....................61
4.6 Terrestrial Actinomycete N 435 .................................................................63
4.6.1 Cyclo (dehydroalanyl-isoleucyl) .................................................................64
4.6.2 Cis-Cyclo(leucyl-tyrosyl) ..........................................................................65
4.6.3 Ramthacin B .................................................................................................69
4.7 Streptomyces sp. B7857 ..............................................................................71
4.7.1 Bacteriogorgonene A ..................................................................................73
4.7.2 Bacteriogorgonene B ..................................................................................78
4.7.3 Bacteriogorgonene C ..................................................................................81
4.8 Terrestrial Streptomycete GW5127 .............................................................84
4.8.1 13-Deoxy-1,3-dihydrodaunomycinone ......................................................85
4.8.2 4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one ......................................87
4.8.3 N-[2-(3-Hydroxy-4-methoxy-phenyl)-ethyl]-acetamide ................................90
4.8.4 4-(2-Hydroxyethyl)-2-methoxy-phenol .....................................................92
4.8.5 β-Rubromycin .............................................................................................93
4.8.6 γ-Rubromycin .................................................................95
4.9 Terrestrial *Streptomyces* sp. GW 18/1811 .................................................96
4.9.1 Komodoquinone B ...........................................................97
4.9.2 Feudomicinone C ..............................................................98
4.9.3 α-Hydroxyacetovanillone .....................................................99
4.9.4 N-Acetylhomocysteine thiolactone ..........................................103
4.9.5 Lysolipin I .................................................................105
5 Fungal metabolites ...........................................................................107
5.1 Bikaverin ..............................................................................107
6 Plant Metabolites from *Tephrosia* sp. .................................................110
6.1 Spinoflavanone B ..................................................................110
6.2 Caliphione A ........................................................................113
6.3 Tephcalostan ........................................................................117
6.4 Tephcalostan A ....................................................................118
6.5 Tephcalostan B and C ...........................................................121
6.6 Miletone .............................................................................124
6.7 Glabranine ...........................................................................127
6.8 Betulinic acid and Betulinic acid methyl ester ..............................129
6.9 Stigmasterol and γ-Sitosterol ...................................................131
6.10 2-Methoxymaackiain ............................................................133
6.11 Tephrosol (2-methoxy medicagol) ..............................................136
6.12 Di dehydrovillozin (mixture of two stereoisomers at C-6) .........138
6.13 Dehydrorotenone ................................................................141
6.14 Substituted Dichromen-7-one ................................................144
6.15 Obovatin methyl ether .........................................................146
6.16 Obovatin ...........................................................................149
6.17 7-Methylglabranin ................................................................150
6.18 Candidone .........................................................................152
6.19 Ovalichalcone ....................................................................153
6.20 Praecansone B ....................................................................155
7 Summary ................................................................................158
7.1 Terrestrial *Streptomyces* sp. ADM 14 ....................................158
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>Marine Streptomyces B7880</td>
<td>159</td>
</tr>
<tr>
<td>7.3</td>
<td>Terrestrial <em>Streptomyces</em> sp. ACT 7619</td>
<td>160</td>
</tr>
<tr>
<td>7.4</td>
<td>Terrestrial Streptomyces GW6311</td>
<td>160</td>
</tr>
<tr>
<td>7.5</td>
<td>Terrestrial <em>Streptomyces</em> sp. GT 2005/079</td>
<td>160</td>
</tr>
<tr>
<td>7.6</td>
<td>Terrestrial Actinomyces sp. N 435</td>
<td>162</td>
</tr>
<tr>
<td>7.7</td>
<td>Marine-derived <em>Streptomyces</em> sp. B 7857</td>
<td>162</td>
</tr>
<tr>
<td>7.8</td>
<td>Terrestrial streptomyces GW5127</td>
<td>163</td>
</tr>
<tr>
<td>7.9</td>
<td>Terrestrial <em>Streptomyces</em> sp. GW 18/1811</td>
<td>163</td>
</tr>
<tr>
<td>7.10</td>
<td>Fungal metabolite</td>
<td>164</td>
</tr>
<tr>
<td>7.11</td>
<td>Plant metabolites from <em>Tephrosia calophylla</em></td>
<td>165</td>
</tr>
<tr>
<td>8</td>
<td>Experimental Part</td>
<td>166</td>
</tr>
<tr>
<td>8.1</td>
<td>General</td>
<td>166</td>
</tr>
<tr>
<td>8.2</td>
<td>Materials</td>
<td>166</td>
</tr>
<tr>
<td>8.3</td>
<td>Spray reagents</td>
<td>166</td>
</tr>
<tr>
<td>8.4</td>
<td>Microbiological materials</td>
<td>167</td>
</tr>
<tr>
<td>8.5</td>
<td>Recipes</td>
<td>168</td>
</tr>
<tr>
<td>8.5.1</td>
<td>Nutrients</td>
<td>169</td>
</tr>
<tr>
<td>8.6</td>
<td>Microbiological and analytical methods</td>
<td>173</td>
</tr>
<tr>
<td>8.6.1</td>
<td>Storage of Strains</td>
<td>173</td>
</tr>
<tr>
<td>8.6.2</td>
<td>Pre-Screening</td>
<td>173</td>
</tr>
<tr>
<td>8.6.3</td>
<td>Biological screening</td>
<td>173</td>
</tr>
<tr>
<td>8.6.4</td>
<td>Chemical and pharmacological screening</td>
<td>173</td>
</tr>
<tr>
<td>8.6.5</td>
<td>Brine shrimp microwell cytotoxicity assay</td>
<td>174</td>
</tr>
<tr>
<td>8.6.6</td>
<td>Primary screening</td>
<td>174</td>
</tr>
<tr>
<td>8.7</td>
<td>Terrestrial <em>Streptomyces</em> sp. ADM 14</td>
<td>175</td>
</tr>
<tr>
<td>8.7.1</td>
<td>Primary Screening</td>
<td>175</td>
</tr>
<tr>
<td>8.7.2</td>
<td>Cultivation, work-up and isolation</td>
<td>175</td>
</tr>
<tr>
<td>8.7.3</td>
<td>Synthesis of 1-hydroxy-4-methoxynaphthoic acid (43)</td>
<td>176</td>
</tr>
<tr>
<td>8.8</td>
<td>Marine <em>Streptomyces</em> sp. B 7880</td>
<td>177</td>
</tr>
<tr>
<td>8.8.1</td>
<td>Primary screening</td>
<td>177</td>
</tr>
<tr>
<td>8.8.2</td>
<td>Isolation and cultivation</td>
<td>177</td>
</tr>
</tbody>
</table>
8.9 Terrestrial *Streptomyces* sp. ACT 7619 ................................................................. 179
  8.9.1 Primary Screening .......................................................................................... 179
  8.9.2 Fermentation and isolation ............................................................................ 179
8.10 Terrestrial *Streptomyces* sp. GW 6311 ............................................................ 180
  8.10.1 Primary Screening ....................................................................................... 180
  8.10.2 Cultivation and isolation ............................................................................. 181
8.11 *Streptomyces* sp. GT 2005/079 ........................................................................ 182
  8.11.1 Primary screening ........................................................................................ 182
  8.11.2 Cultivation and isolation ............................................................................. 182
8.12 Terrestrial Actinomycete N 435 ...................................................................... 185
  8.12.1 Primary Screening ....................................................................................... 185
  8.12.2 Fermentation and Isolation .......................................................................... 185
8.13 Marine *Streptomyces* sp. B 7857 ..................................................................... 186
  8.13.1 Primary screening ........................................................................................ 186
  8.13.2 Fermentation and Isolation .......................................................................... 187
8.14 Terrestrial *Streptomyces* sp. GW 5127 ............................................................ 188
  8.14.1 Primary screening ........................................................................................ 188
  8.14.2 Fermentation and isolation .......................................................................... 188
8.15 Terrestrial *Streptomyces* sp. GW 18/1811 ....................................................... 191
  8.15.1 Primary screening ........................................................................................ 191
  8.15.2 Cultivation and isolation ............................................................................. 191
8.16 Fungal metabolite bikaverin ............................................................................ 193
8.17 Plant metabolites .............................................................................................. 193
9 References ......................................................................................................... 200
1 Introduction

The seas and oceans occupy almost three quarters of the earth’s surface. They have numerous resources, which partly comprise of vegetation, algae, bacteria, fish shellfish and fungi. Recently these natural resources have drawn attention as a potential reservoir of many biologically active compounds. As the habitats for the marine organism are substantially different from that of terrestrial organisms, it can be supposed that their secondary metabolites will also differ considerably from terrestrial organisms.1-3

Research on the chemistry of natural products derived from marine microorganism has increased tremendously in recent years due to the demand of compounds having potential pharmaceutical applications or economical value such as for cosmetics, drugs, fine chemicals, and functional personal-care products.4 Until now, quiet a number of species have been assayed for their activity and a huge number of bioactive molecules with unique structural features has been isolated. Those bioactive compounds often showed toxic properties.5 To this end, more than 15 compounds from marine source are in human trials.1

Natural products were the basis of most early traditional medicines and have played an important role in drug discovery. Natural products have contributed significant value to the pharmaceutical industries over the last decades. The term “natural product” implies the compounds derived from organism, plants or animals. Human societies have been using natural products for millennia. Historically therapeutic formulations from relatively crude plant extracts were common in pharmaceutical companies but with the advancement of technology, drug formulations of fairly purified compounds have become more popular, cost effective and convenient.

Numerous drug classes derived from natural product sources have benefited the therapeutic areas of oncology, immunosuppression, metabolic and infectious diseases.14 Modern techniques like X-ray crystallography, NMR spectroscopy and alternative drug discovery methods e.g. rational drug design, combinatorial chemistry have provided significant advances in natural product drug discovery research over the last 15 to 20 years. The development of resistance in infectious microorganisms to the available drugs and the increase of new diseases such as AIDS, Ebola and SARS require the discovery and development of new drugs.6 Microorganisms continue to be a productive and successful focus for marine natural products research and in particular the number of marine derived drug candidates remains high.7
1.1 History of the use of natural products for medical applications

Research of natural product has enormous unexploited potential, and the significant advantages and disadvantages of natural product derived molecules as drug candidates for development has been reported in many articles. The blossoming of natural product discovery efforts came into focus after the large-scale production of penicillin (1) during World War II. The pharmaceutical companies engaged their efforts during the wartime to build stocks of penicillin but after the war ended, they refocused on the search for new antibiotics. Natural product discovery programs were developed by all of the major pharmaceutical companies. Their screening programs included anti-bacterial and anti-fungal targets, as well as targets for other diseases. In the 1970s for example, cholesterol biosynthesis inhibitors, compactin (2) and mevinolin (3) were discovered. The discovery of compactin and mevinolin made it possible to develop the hugely successful statin (4) therapeutics, which even today are considered as successful drugs in both medical treatment and in pharmaceutical business fortunes. The discovery of streptomycin, gentamicin, omegamycin (5) and other antibiotics pushed the industry to implement large research and developing programs around natural product discovery, particularly microbial fermentation based technologies.

1

2

3
Shu’s review\(^{12}\) revealed that at least twenty-one natural products and natural product-derived drugs have been introduced onto the market in the USA, Europe or Japan in the last 8 years. In addition, a semi-synthetic camptothecin derivative, belotecan\(^{13}\) (6) was launched in Korea in 2004. Table 1 represents twenty-one drugs which can be classified as 3 natural products, 10 semi-synthetic natural products and 8 natural product-derived drugs and include the “first in kind” drug caspofungin which functions as echinocandin antifungal, galantamine used as novel anti-Alzheimer’s drug, nitisinone (new treatment for the hereditary disease tyrosinemia type I), miglustat (new treatment of Type 1 Gaucher Disease).

**Table 1:** NP-derived drugs launched in the United States, Europe or Japan since 1998 by year with reference to their lead compound, classification and therapeutic area Year Generic name (trade name) Lead compound Classification Disease area.

<table>
<thead>
<tr>
<th>Year</th>
<th>Generic name</th>
<th>Lead compound</th>
<th>Classification</th>
<th>Disease area</th>
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<tbody>
<tr>
<td>1998</td>
<td>Orlistat (Xenical(^{R}))</td>
<td>Lipstatin</td>
<td>semi-synthetic-NP</td>
<td>antiobesity</td>
</tr>
<tr>
<td>1998</td>
<td>Cefoselis (Wincel(^{R}))</td>
<td>cephalosporin</td>
<td>semi-synthetic-NP</td>
<td>antibacterial</td>
</tr>
<tr>
<td>1999</td>
<td>Dalfopristin and quinupristin</td>
<td>Streptogramin and B Streptogramin A</td>
<td>semi-synthetic-NP</td>
<td>antibacterial</td>
</tr>
<tr>
<td>1999</td>
<td>Valrubcin (Valstar(^{R}))</td>
<td>doxorubicin</td>
<td>NP-derived</td>
<td>oncology</td>
</tr>
<tr>
<td>1999</td>
<td>colforsin daropate (Adele, Adehi(^{R}))</td>
<td>forskolin</td>
<td>semi-synthetic-NP</td>
<td>cardiotonic</td>
</tr>
<tr>
<td>2000</td>
<td>arteether (Artemotil(^{R}))</td>
<td>artemisinin</td>
<td>semi-synthetic NP</td>
<td>antimalarial</td>
</tr>
<tr>
<td>2001</td>
<td>ertapenem (Invanz(^{TM}))</td>
<td>thienamycin</td>
<td>NP-derived</td>
<td>antibacterial</td>
</tr>
<tr>
<td>2001</td>
<td>caspofungin (Cancidas (^{R}))</td>
<td>pneumocandin B</td>
<td>semi-synthetic NP</td>
<td>antifungal</td>
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</table>
### Approaches for the search of new natural products

To obtain new bioactive compounds in nature is not a single-step procedure. However, the first step is to select suitable sources. Physical, chemical or biological investigation of the best sources is important. After selecting the best source, the next step is to isolate the bioactive compounds, which may be obtained by common techniques such as chromatography, crystallization, bioassay guided isolation, fermentation, and so on. Once the isolated compounds are obtained, the next step is to evaluate their bioactivity. Modern techniques such as high-throughput screening, bioinformatics, and functional genomics are used to screen large numbers of compounds for their bioactivity. Once a bioactive compound is identified, its structure is elucidated by various techniques such as NMR, MS, and X-ray crystallography. The next step is to optimize the structure of the compound to improve its bioactivity, which is usually done by chemical modification. Once the optimized structure is obtained, the final step is to synthesize the compound, which is usually done by semi-synthetic methods. The synthesized compound is then evaluated for its bioactivity, and if it is found to be bioactive, it is patented and marketed as a new drug.
Interactions of metabolites with test systems can then be quantitatively or qualitatively evaluated.

In recent times, the genes for example of polyketide synthesis are being located and isolated. In addition to that, genetic engineering technology has made it possible to produce genetically manipulated microorganisms, which could be used to generate new metabolites by realigning the synthetic capacities of different species. Depending on the biological activities, novel natural products could be optimised to yield effective chemotherapeutic as well as other bioactive agents. To investigate new natural products biotransformation techniques are also being used. Biotransformation enables derivatisation of known compounds by esterification, reduction, oxidation, demethylation, or glycosylation utilizing the enzymes of living microorganisms. For example, antibiotics or antineoplastic agents, e.g. bleomycin A1 and glycopeptide antitumor metabolites were isolated from *Streptomyces verticillus*. Currently bleomycins are being used for clinical treatment of Hodkin’s lymphoma, carcinomas of the head, skin, neck, and tumors of testis. To achieve the same goal by chemical transformations would be time consuming as well as involving many steps and produces by-products.

In order to optimise their pharmacological properties, some antibiotics derived from natural resources need structural transformation. The antibacterial macrolide erythromycin (7) was isolated in 1952 while its semi-synthetic derivative telithromycin is 10-40 times more active than the parent antibiotic, which contains a 3-keto substituent instead of cladinose. The ketolides are a new class of antibiotics having excellent inhibitory abilities against Gram-negative or Gram-positive cocci like *Staphylococcus*, and *Enterococcus*, which are resistant against macrolides and other antibiotics.
1.3 Antibiotic screening

A new research field “antibiotic screening” became important after the discovery of penicillin and the actinomycins. In the continuous search to discover new bioactive compounds, screening approaches are applied to crude extracts to evaluate chemical, biological and pharmaceutical characteristics. The pharmaceutical industries focused on bioactive substances and often contributed the advantages of greater sensitivity and sample throughput that is High-throughput-Screening (HTS). However, it is plausible that novel compounds could be neglected which may be active against other targets. To solve this problem, in the 1980s, Zähner and Zeeck introduced a systematic chemical screening of the crude extracts. The chromatographic features of metabolites on thin layer chromatography (TLC) plates followed by their detection with staining reagents under defined reaction conditions approved the visualization of an almost complete fingerprint of the secondary metabolite content of that particular crude extract. The application of this method made it possible to isolate nearly all metabolites of a given strain, and the variety of unidentified compounds could then be biologically tested in uncontaminated state. Due to the rapid developments in the sensitivity of mass spectroscopy (MS), nuclear magnetic resonance (NMR) instruments, and the recent growing chemical databases (AntiBase, DNP, CA), the dereplication of known chemical compounds has greatly improved. In current years, screening is accomplished in combination with ultraviolet (UV), high performance liquid chromatography (HPLC), HPLC-MS, HPLC-CD, HPLC-NMR-MS or GC-MS methods.

At present, substantial concentration has been placed on marine microorganisms as the most permissible source for new bioactive natural products. However, under normal incubation conditions unlike the terrestrial bacteria the marine bacteria show a low production rate. To amplify the efficacy of screening for secondary metabolites of marine bacteria, polymerase chain reaction (PCR)-based screening is being used by microbiologists for assaying genes for example, non-ribosomal polypeptide syntheses (NRPSs), dNDP-glucose dehydratases, polyketide synthases and halogenases. The dilemma of this method however, is, that all these genes occur very frequently, but may not be expressed in every case (silent genes).

1.4 Natural Products from Marine Bacteria

It is not so easy to define exactly what a marine bacterium is. But marine bacteria must even not have common criteria such as sodium chloride requirements, which should be unequivocal physiological needs for autochthonous marine bacteria of the
free water column. Certain marine bacteria like *Salinospora* spp. use respiratory chain-linked sodium dependent NADH: quinone reductase instead of the common proton-coupled enzyme. Korormycin\textsuperscript{24} (8), which was isolated from a marine *Vibrio alginolyticus*, is a specific inhibitor of this enzyme and may be a tool to identify such sodium-dependent bacteria. \textsuperscript{25}

![Chemical structure of Korormycin](image)

Highly biologically active quinones are commonly found in streptomycetes; these compounds are easily detected by naked eyes because for their pink, yellow or red colours. Many quinones also have been isolated from marine sources. The C-glycosides himalomycin A (9) and B\textsuperscript{26} (10) are two recent examples of anthraquinones with the rare fridamycin E chromophor which act as a precursor of the anthracycline antibiotics. These two compounds were reported from *Streptomyces* sp. B 6921 which was isolated from a littoral sample from Mauritius having strong antibacterial activity.

![Chemical structure of himalomycin A](image)

![Chemical structure of himalomycin B](image)
Among the first findings from the deep-sea microbiota were the macrolactins\textsuperscript{27} (11 \textbf{a,b,c}), macrolactin E (12), macrolactin D (13), macrolactin F (14) and isomacrolactinic acid (15), which corresponded to a class of remarkable antiviral and cytotoxic macrocyclic lactones produced by an un-identified Gram-positive marine bacterium.

\begin{align*}
11a & \quad R_1 = R_2 = H \\
11b & \quad R_1 = \beta\text{-glucosyl}, R_2 = H \\
11c & \quad R_1 = H, R_2 = \beta\text{-glucosyl}
\end{align*}

Utilization of marine derived natural products for medicinal purposes is not a new initiative as seahorses were being utilized in China, Japan and Taiwan for centuries.
in long-established treatments for sexual disorders, circulatory and respiratory problems as well as kidney and liver dysfunctions, amongst other ailments. Compounds derived from marine sources usually have small to medium molecular weight. Many of the compounds produced by marine plants, invertebrates or microbes have inspired interdisciplinary studies by chemists and biologists. Some bacteria are collected from tropical and cold temperate ocean habitats and continue to be the subject of extensive chemical investigation. The extracts found from this type of bacteria are being examined as a source of new cytotoxic secondary metabolites that are prospective to guide for the development of new drugs.

In 1966, from a culture of *Pseudomonas bromoutilis* Burkholder and coworkers reported the highly brominated pyrrole antibiotic pentabromopseudiline \(^{28}\) (16), which was the first marine bacterial natural product. It exhibited remarkable antibiotic activity against Gram-positive bacteria.

\[
\begin{align*}
\text{OH} & \\
\text{Br} & \\
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\text{Br} & \\
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\end{align*}
\]

1.5 Therapeutic areas influenced by natural products

It can be said that it is the evolutionary selection, which makes many of the secondary metabolites highly potent and selective. For example, some are inferred to be signal molecules or demonstrate protecting mechanisms against competitors, herbivores or pathogens. Reproductivity of the producing organism is being assisted by these compounds and they help in maintaining survival. These characteristics make them ideal as lead compounds for development as therapeutic agents for oncology and infectious diseases wherein death or injury to populations of cells is desired.

1.6 Natural products against infectious diseases; bacterial pathogens

During the last century natural products were considered as the “silver bullet” for treating bacterial diseases because natural product directed discovery provided a vast majority of the antibiotics being used today. Nevertheless, many famous pharmaceutical companies ended their HTS (high-throughput screening) efforts for discovery of antibiotics. As a result, they did not update their natural product libraries. A re-
cent survey revealed that from pharmaceutical companies and academic laboratories conducting HTS, only a handful of anti-viral candidates were identified from activities originally identified but no anti-bacterial drug candidates were identified through HTS. A significant number of pathogens gradually arose and become resistant to antibiotics. In a recent report the US Center for Disease Control and Prevention indicated the emergence of *Mycobacterium tuberculosis* strains within the United States and the rest of the world, which are resistant to all available therapeutics. Due to the growing resistance to existing antibiotics and the indispensable medical needs the development of new classes of antibiotics which are in urgent need and new targets are therefore vital for drug discovery. In this context, the recently reported discovery of platensimycin\(^{29}\) (17), a new class of inhibitor, by a group at Merck can be considered as very encouraging. Platensimycin (17) demonstrated strong, broad-spectrum anti-bacterial activity by inhibiting cellular lipid biosynthesis. Platensimycin (17) was first time isolated and elucidated in our group,\(^{30}\) however, had not been published. It has been shown that cerulenin and thiolactomycin inhibit condensing enzymes in the biosynthesis pathways of fatty acids (Fab F/B and Fab H), however till now no drugs targeting these condensing enzymes are used clinically. Selective and specific activity against the Fab/F enzyme was shown by platensimycin in the synthetic pathway of fatty acids.

![Chemical Structure of Platensimycin](attachment:chemical_structure.png)

17

### 1.7 Antifungal compounds from natural sources

Existing antifungal drugs, which are in use to date have some connection to natural products. For example, some polyenes as well as griseofulvin are natural products, whereas the echinocandin derivatives are *semi*-synthetically originated from natural products. Another example is 5-fluorocytosine, which is a nucleoside and interferes with DNA and RNA synthesis, is chiefly used together with the polyene amphotericin B. Even though in general the azoles are considered to be synthetic in origin, their drug prototype pathway belongs to the *Streptomyces* metabolite azomycin. The antifungal activity of azoles is due to the inhibition P450 3A-dependant C14-a-methylase, which is an enzyme that changes lanosterol to ergosterol that leads to ergosterol depletion and disruption of the fungal cell membrane integrity.\(^{31-33}\)
Various fungi produce a group of naturally occurring lipopeptides called echino-
candins which display potent antifungal activity by inhibition of 1,3-β-D-glucan
synthesis in the fungal cell wall.\textsuperscript{34,35} Until now, two echinocandin-based drugs have
been approved for clinical use. A \textit{semi}-synthetic drug “caspofungin” which is a de-
rivative of pneumocandin B, was first introduced by Merck in the US in 2001,\textsuperscript{36,37}
whereas micafungin is a \textit{semisynthetic} derivative of FR901379 that was first
launched by Fujisawa in Japan in 2002. There are two echinocandins, anidulafungi
and aminocandin (structure not available), currently undergoing clinical evaluation.

\textit{2H}-Purin-2-one (18) and \textit{7H}-pyrrolo[2,3-d]pyrimidine-5-carboximidamide (19) are
two further examples of antifungal compounds from natural sources.

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\begin{tabular}{c}
\textbf{18} & \textbf{19}
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\end{center}

1.8 Antibacterial compounds from natural sources

In the development of antibacterial drugs, natural products have played a central
role, as most have been derived from natural product leads. For example, β-lactams
were first introduced in 1941, aminoglycosides in 1944, cephalosporins in 1945,
chloramphenicol in 1949, tetracyclines in 1950, macrolides in 1952, lincosamides in
1952, streptogramins in 1952, glycopeptides in 1956, rifamycins in 1957 and
lipopeptides in 2003 which is quite recent. Only a few antibacterials are syntheti-
cally derived such as sulphonamides (20) (1935), nitroimidazole (21) (1959), quin-
olone (1962), trimethoprim (1968) and oxazolidinones (2000).

\begin{center}
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\textbf{20}
\end{tabular}
\end{center}
The immense pressure for developing new and improved antibacterial drugs has increased tremendously due to widespread antibacterial drug resistance. However, only a few major pharmaceutical companies are actively engaged in the field despite the introduction of only a few recent novel antibacterial classes since 1968. Due to the huge investments required to bring an antibacterial drug from clinical trials to the market, an acceptable financial return may not be forthcoming and this may be a concern for major pharmaceutical companies. Anxieties also have been elevated that nowadays the regulatory procedures make approval complicated for new antibacterials as they need to compete head to head with the established drugs in sensitive strains, while activity against resistant strains is not satisfactorily weighted. After all, most mechanism based antibacterial screening programmes accepted to date have not been successful in identifying new in vivo active antibacterial drugs.\textsuperscript{17,38}

$\beta$-Lactam antibacterials derived from total synthesis, \textit{semi}-synthesis or from a natural product drugs can be further classified into seven subclasses: penicillins, cephalosporins, cephemycins, cephems, carbapenems, penems and monobactams. At present, four cephalosporins are existing (ceftizoxime alapivoxil (23), ceftobiprole\textsuperscript{39} (24), RWJ-442831 and PPI-0903), one penem (faropenem daloxate) and three carbapenems (CS-023, tebipenem and doripenem) in clinical development.
1.9 Potential antiparasitic drugs originated from natural products

Since ancient times, medicinal plants are being used for the treatment of parasitic diseases, which is well known and documented. For example, Cinchona succiruba (Rubiaceae) has been used as antimalarial. A very promising compound licochalcone A\(^{40}\) (26), isolated from Glycyrrhiza glabra (Fabaceae) is now in intensive pre-clinical studies for the treatment of intestinal microsporidiosis, Phase III clinical trials of fumagillin had been done by Sanofi-Aventis which was granted EU orphan drug status for this use in March 2002. The spore-forming unicellular parasite Enterocytozoon bieneusi causes microsporidiosis, which is of major concern to immuno-compromised patients as it causes chronic diarrhoea. In year 1949, fumagillin was first isolated from Aspergillus fumigatus, which was used shortly after its discovery as an effective drug for intestinal amoebiasis. Amphotericin B (27) is used as a drug for the therapy of visceral and mucocutaneous leishmaniasis. Different analogues of amphotericin B (27) such as hamycins A\(^{41}\) (28) also inhibit parasite growth. Hamycin is now extensive used in the treatment of candidiasis and otomycosis, which is found to be satisfactorily effective in killing Leishmania donovani promastigotes.
1.10 Marine derived anticancer drugs

The marine environment is infinitely diversified according to their chemical and biological characteristics. Consequently it is an astonishing resource for the discovery of new anticancer drugs. Modern technology and methodology in structure elucida-
tion, organic synthesis, and biological assay have made it possible for the isolation and clinical evaluation of various novel anticancer agents.

**Table 2.** Some examples of marine natural products as anticancer agents in preclinical or clinical trials

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Class</th>
<th>Molecular Target</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecteinascidin 743</td>
<td>Tetrahydroisoquinolone Alkaloid</td>
<td>Tubulin</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Dolastatin</td>
<td>Linear peptide</td>
<td>Tubulin</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Bryostatin</td>
<td>Macrocyclic lactone</td>
<td>PKC</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Synthadotin</td>
<td>Linear peptide</td>
<td>Tubulin</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Kahalalide F</td>
<td>Cyclic dipeptide</td>
<td>Lysosomes</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Squalamine</td>
<td>Aminosteroid</td>
<td>Phospholipid bilayer</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Dehydrodidemnin B</td>
<td>Cyclic dipeptide</td>
<td>Ornithine decarboxylase</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Didemnin B</td>
<td>Cyclic dipeptide</td>
<td>FK-506 bp</td>
<td>Phase 2</td>
</tr>
</tbody>
</table>

1.11 Antiviral compounds from natural sources

Viral diseases such as HIV, hepatitis B and C (HCV), Ebola, influenza, dengue fever and yellow fever cause some of the greatest health risks known to humans. The 2003 outbreak of the new Severe Acute Respiratory Syndrome (SARS) illustrates the potential danger and disruption, which can be caused by viral epidemics. Consequently, there has been considerable effort over the last 20 years invested into antivi-
ral drug discovery, especially in the field of HIV. In addition to development of small molecule antiviral drugs, vaccines also are commonly used to try to prevent diseases like influenza, measles, mumps, polio and smallpox. One of the most promising compounds being evaluated to treat HIV is PA-457, a semi-synthetic derivative of the plant triterpenoid betulinic acid. Betulinic acid was found to be a weak inhibitor of HIV replication and a concerted medicinal chemistry programme by Lee and co-workers at the University of North Carolina identified a semi-synthetic derivative as the promising candidate for further evaluation. Panacos Pharmaceuticals licensed and started antiretroviral Phase I clinical trials in March 2004 and plans for Phase II clinical development were announced in December 2004. Workers at Panacos have reported that the antiretroviral activity of PA-457 was due to a novel mechanism of action targeting a late step in the Gag processing. This finding has prompted great interest as it represents a new class of HIV inhibitor.42

In 1992, workers at the National Cancer Institute (NCI), a branch of the US National Institutes for Health, reported the isolation of coumarins, which they named calanolides, with potent activity against HIV-1. The calanolides were obtained from the tree, *Calophyllum lanigerum*, collected in Sarawak, Malaysia. The right to develop these compounds was licensed to Sarawak Medichem Pharmaceuticals who have progressed with the most promising candidate, (+)-calanolide A (31), through to Phase II clinical trials in combination with other anti-HIV agents. Calanolide A for use in preclinical and clinical studies was produced by total synthesis as the original plant source was not readily accessible and produced only small quantities of calanolide A. The later was also found to have activity against all *Mycobacterium tuberculosis* and anti HIV strains tested, including some which are resistant to standard antitubercular drugs. This property is unique amongst and may allow more efficient treatment of patients infected with both HIV and tuberculosis. The related coumarins calanolide B (30) (costatolide), dihydrocalanolide B (31) and oxocalanolide45 (32) are also under preclinical development by Sarawak Medichem and the NCI.
Introduction

1.12 Sequiterpenes from marine microorganism

During the last two decades sesquiterpenoids and their biological activities from *Asteraceae* species have been the focus of numerous phytochemical, pharmacological and synthetic studies. Because the sesquiterpenoids exhibit a wide range of biological activities, and include compounds that are plant growth regulators, insect antifeedants, antifungals, anti-tumour compounds and antibacterials, there has been much interest in relating structure and oxygenation patterns to function. Eudistomins C (33) and eudistomins E46 (34) are two examples of antiviral compounds isolated from nature.

To date, only a few sesquiterpenes have been reported from marine microorganism although they are common metabolites from fungi or plants. From the few reported sesquiterpenes, africantriol (35), 4S,7R-germacra-1E,5E-dien-11-ol (36), 1-epibicyclesquiphellandrene (37), 2-methylisoborneol (39) and the antitumor pentalenolactones pentalenolactone H (40) and arenamycin-E (41) are shown as examples. Marine-derived *Streptomyces* sp. M491 yielded new sesquiterpenes; leukotriene biosynthesis can be inhibited by some sesquiterpenes and some exhibit activity against human colon tumor cell line.
2 Objectives of the present investigation

The goal of this research is the isolation, purification and structure elucidation of new secondary metabolites with pharmacological activity from marine microorganisms. To attain this goal, a series of sequential chemical (TLC/LC-MS) and biological screening processes should be undertaken.

For the isolation and purification of the different constituents of crude extracts obtained from the fermentation, various chromatographic methods (silica gel, Sephadex LH-20, PTLC, HPLC, etc.) will be used. Various spectroscopic methods
Objectives of the present investigation

(NMR, MS, IR, UV, X-ray crystal analysis if possible) are utilized for the structure elucidation in addition to databases (AntiBase, Dictionary of Natural Products and Chemicals Abstracts), for rapid dereplication.

Fractions and pure compounds are submitted for different bioassays (i.e. antimicrobial test, antifungal, brine shrimp test) to explore new bioactivities against targets.
3 General techniques

3.1 Collection of strains

Through the cooperation with different microbiological groups the strains to be investigated were supplied. All the streptomycetes reported in this dissertation were obtained from the strain collection of the former BioLeads Company in Heidelberg and the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. Some plant extracts were obtained from Prof. S. Ganapaty, Andhra University, India. In most cases, the taxonomy on the species level was unknown in the beginning.

3.2 Pre-screening

From the strains received, only few were expected to produce metabolites with significant bioactivity or other interesting properties. Therefore, in order to select such strains, a pre-screening was performed. The pre-screening method includes a number of suitable qualitative or quantitative criteria, such as physical, biological, or chemical interactions of metabolites with different test systems.

To perform these tests, sub-culturing of the strains was done on agar plates for 3-7 days, which then were checked for contaminations under the microscope. In 1 L Erlenmeyer flasks with inflections containing 250 ml of medium, small pieces of the agar culture were then used for inoculation, followed by incubation on a rotary shaker at 28 °C. The culture broth was lyophilised and the dried residue was extracted with ethyl acetate. The crude extract obtained was used for chemical, biological, and pharmacological screenings. TLC with different spray reagents as well as HPLC played a vital role in the chemical screening.

3.3 Chemical screening

It is an expensive task and a multiple step procedure, to search for and isolate pure bioactive compounds from bacteria. Therefore, it is wise to reduce the unnecessary work, such as the re-isolation of known metabolites either from the crude extract or from a partially purified fraction. The most important step for this purpose is the chemical screening, which allows reaching this goal at the earliest stages of separation. One of the cheapest and simplest methods is TLC (thin layer chromatography), which is used for the detection of bacterial constituents in the crude extract. TLC is quick and easy to perform and requires simple equipment with results that are suffi-
ciently reproducible, when compared with other methods like HPLC or LC MS. Based on the $R_f$ value, a suitable organic solvent system is chosen for elution. After developing, the TLC plate is visualized under UV light. Further interesting zones are localized by exposure to different spray reagents. Numerous spray reagents are available for the detection of specific compounds while others are universal. Only the following spray reagents listed below were used in this study.

Many structural elements show different colour reactions with anisaldehyde/sulphuric acid.

Indoles turn pink, blue or violet, when Ehrlich’s reagent is sprayed on TLC. It is a rather specific reagent for these compounds. Some other nitrogen containing compounds and furans become brown, anthranilic acid derivatives change to yellow.

Addition of concentrated sulphuric acid on TLC is especially used for polyenes. Short conjugated chains show a brown or black colour while carotenoids develop a blue or green colour.

Sodium hydroxide can be used for the detection of peri-hydroxyquinones, which turn red, blue or violet, while the deep red prodigiosins show a yellow colour.

For the detection of peptides, the chlorine/o-dianisidin reaction is used as a universal reagent for the amide bond.

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Figure 1: General screening of the selected strains
3.4 Pharmacological and biological assays

An appropriate test is needed to screen a crude extract for bioactive substances. The screening programs developed in natural product chemistry are usually divided into two groups: specialized screens and general screening bioassays. The screening methods are different for pharmaceutical industry and university research groups. In any bioassay method, high capacity, sensitive, low cost, and quiet solutions take preference. Generally, two types of screening are available: 1) the vertical screening mostly used in industry shows high selectivity and narrow results (1:10,000-1:20,000); it is used in combination with high throughput techniques. 2) The horizontal screening, which exhibits low selectivity, however, a higher responds rate (1:3-1:100) and gives therefore a quick overview. In our research group, the screening of the crude extract is performed by using the agar diffusion test with bacteria (Gram-positive, Gram-negative), fungi, plant pathogens and higher organisms for cytotoxicity: The crude extracts are tested against *Escherichia coli*, *Bacillus subtilis*, *Mucor miehei*, *Candida albicans*, *Streptomyces viridochromogenes* (Tü 57), and *Staphylococcus aureus* as well as the microalgae *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus subspicatus*.

Cellular cytotoxicity can be tested using the brine shrimp assay with larvae of *Artemia salina*. The brine shrimp test is a good indicator for potential anticancer activity and has a parallelism of about 95% with cellular systems.

Further pharmacological tests in our group are carried out by the collaborative industries, for example Oncotest (Freiburg) or BASF (Ludwigshafen). Both the results from chemical and biological screening complement each other and allow us to choose suitable strains for further scale-up.

3.5 Cultivation and scale-up

After a primary screening, the cultivation and scale-up steps are carried out. In order to choose the best medium for highest activity, an optimisation of the culture conditions may sometimes be done to improve the yield or the metabolite pattern. Either the fermentation in shaking flask or in a fermentor is used for the culture of bacteria. For the fermentor, a pre-culture of 2 or 5 L is used for the inoculation.

After harvesting of the selected strain, the culture broth is mixed with Celite and filtered under vacuum. After separation of the mycelium, the water phase can be extracted with a suitable organic solvent like ethyl acetate. However, it is highly recommend using a solid phase extraction with XAD resin as it is not harmful and reduces considerably the volume of solvents used and can adsorb more polar com-
pounds effectively. The mycelium is extracted with ethyl acetate and acetone. The organic extracts are evaporated to dryness and the crude extract obtained is used for separations.

### 3.6 Isolation methods

Polarity or molecular weight of the compounds of interest play the most important role in the separation but the amount of the crude extract is always a factor in choosing an appropriate method. Fat is produced by every strain and makes the purification sometimes difficult. Therefore, the crude extract is first defatted using cyclohexane, followed by silica gel chromatography using a gradient of increasing polarity with various solvent systems (CH$_2$Cl$_2$/MeOH or cyclohexane/ethyl acetate etc.). Another commonly used and effective method is size-exclusion chromatography (Sephadex LH-20), which offers the advantage of a high recovery rate and minimizes the destruction of compounds. It is used preferentially when the amount of the crude extract is relatively less and already partially purified by silica column chromatography. Some further methods like RP-18 chromatography, PTLC and HPLC are also used for final purification.

### 3.7 Partial identification and dereplication

Researchers are still trying to find more effective methods for the discovery of unexplored bioactive compounds and to minimize the re-isolation of known metabolites. This steadily increases the challenge for the natural product chemist. To meet this challenge a number of methods have already been developed to identify known metabolites at the earliest stages of the purification or from a partially purified mixture of metabolites. The complimentary processes for the rapid identification of already reported compounds or the elucidation of a partial structure of an unknown compound is called “dereplication”.$^{47}$ In the recent years, several databases became available to identify known or relevant compounds from NMR derived sub-structures or physical and chemical properties by using computers.$^{48}$ To date, the most effective and comprehensive tools for the detection of microbial and marine natural products are the data collections AntiBase$^{49}$ and AntiMarin. For the detection of natural compounds from any other source the “Dictionary of Natural Products (DNP)”$^{50}$ is also a good choice. These databases are also important tools in the identification of new metabolites with respect to compounds classes and chromophors. Presently, dereplication is being done with liquid chromatography in combination with NMR spectroscopy (HPLC-NMR) as well as tandem mass spectroscopy (HPLC-MS/MS) using refer-
ences. The advantage of using HPLC-NMR or HPLC-MS/MS is that both methods require minimum amounts of material to obtain information about known constituents from biological matrices e.g. extracts from marine microorganisms\(^5\) or plants\(^6\). Most of the isolated natural compounds of interest are thermally labile. Therefore, HPLC-ESIMS/MS method has made it possible to identify known metabolites from multi-component mixtures with high selectivity and sensitivity.\(^5\) Due to the achiral character of mass techniques and NMR spectroscopy, the information concerning the full absolute three-dimensional structures can not be obtained by HPLC NMR or HPLC-MS/MS techniques. Two further methods, CD (circular dichroism) and ORD (optical dichroism) spectroscopy are widely used for the attribution of the absolute configuration by comparing the experimental data with those of structurally closely related compounds or quantum-chemical calculations.
3.8 Description of Strain Codes

Bacterial strains and other samples were obtained from various sources:

1) Strains with the prefix ADM were isolated by Prof. H. Anke (Kaiserslautern) from soil samples provided by Professor Armin de Meijere from our institute.

2) Strains with the prefix B are marine *Streptomyces*, collected and identified by Dr. E. Helmke, Alfred-Wagner Institute (AWI) for Polar Marine Research, Bremen, Germany; Act strains are marine actinomycetes from the same origin.

3) Strains with the prefix GT are terrestrial *Streptomyces* sp., which were received from the Hans Knöll Institute, Germany, in June 2006.

4) The terrestrial actinomycete N 435 was obtained from Prof. Wolf, one of our research collaborators. The goal in selecting this strain was to find active compounds against plant pathogenic fungi.

5) Strains with the prefix GW are terrestrial *Streptomyces* sp., which were obtained from the strain collections of bioLeads, Heidelberg, Germany.

6) A fungal metabolite was sent by Prof. Dr. H. Anke, Institute for Biotechnology and Drug Research, Kaiserslautern, Germany.

7) The plant samples from *Tephrosia calophylla* were obtained from Professor Seru Ganapaty from The Pharmacognosy and Photochemistry Division, Andhra University, India.

4 Investigation of selected marine and terrestrial *Streptomyces*

4.1 Terrestrial *Streptomyces* sp. ADM 14

The crude extract of the terrestrial *Streptomyces* sp. ADM 14 showed moderate activity in the biological screening against the test organisms *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, and a good activity against *Streptomyces viridochromogenes*. In the TLC screening, the extract showed nonpolar yellow zones, and medium to high polar grey-violet bands, after spraying with anisaldehyde/sulphuric acid. In addition, numerous UV absorbing and fluorescent zones were observed, which were coloured orange to violet when sprayed with anisaldehyde/sulphuric acid, or pink to blue with Ehrlich’s reagent. All major compounds showed medium to high polarity on TLC.
Well-grown agar plates of the strain ADM 14 were used to inoculate 20 litres of M2-medium and cultivated as shaker culture for 5 days. The obtained broth was filtered over Celite, the water phase was extracted with XAD-16, and the resin eluted with methanol; the biomass was extracted with ethyl acetate. The combined extracts were evaporated to dryness under vacuum to afford a brown crude extract. The extract was defatted with cyclohexane and the methanolic part was pre-separated by column chromatography into five fractions. Further purification on Sephadex LH-20 followed by PTLC and silica gel column chromatography afforded 14 compounds, mostly primary metabolites. These compounds were 1-hydroxy-4-methoxy-naphthalene-2-carboxylic acid (43), 5'-methyl-thioadenosine (44), 5,7,9,11-tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid (45), uridine, adenine, uracil, indole-3-acetic acid, indole-3-carboxylic acid, tryptophol, tyrosol, phenylacetic acid, anthranilic acid and a new compound. Description of trivial compounds like uracil, adenine, indole-3-acetic acid, indole-3-carboxylic acid, tryptophol, and tyrosol are not included here, as they have been isolated frequently in our group.

![Diagram of isolation process](image)

**Figure 2:** Working-up scheme for terrestrial *Streptomyces* sp. ADM 14.
4.1.1 1-Hydroxy-4-methoxynapththalene-2-carboxylic acid

From fraction 2, a grey coloured UV active solid was obtained, which gave a molecular weight of $m/z$ 218 by (-)-ESIMS spectrometry. The $^1$H NMR spectrum showed a methoxy signal at $\delta$ 3.96, but no further signal in the aliphatic region. Five protons were observed in the aromatic region, an ABCD system and a singlet at $\delta$ 7.29 of a proton, which was obviously present in another ring. With the help of ESI mass spectrometry and $^1$H NMR measurements, the compound was tentatively identified as 1-hydroxy-4-methoxy-napthalene-2-carboxylic acid. The $^1$H NMR data were, however, not completely identical with the published values. To confirm the structure of the natural product, a sample was synthesized for comparison.

1-Hydroxy-4-methoxynaphthol (42) and freshly heated K$_2$CO$_3$ were mixed homogeneously. It was then taken into a 250 ml autoclave and dry ice was added. The reaction mixture was heated in the sealed autoclave to 150 °C for 6 hours under a pressure of 70-80 bar to produce 1-hydroxy-4-methoxynaphtoic acid (43).

![Figure 3](image_url): Synthesis of 1-hydroxy-4-methoxy-napthalene-2-carboxylic acid (43).

The synthetic compound also showed substantial deviations from the values of the natural acid. The isomeric 4-hydroxy-1-methoxy-2-naphtoic acid was definitely excluded due to the chelated 1-OH signal in natural and synthetic samples. As also no further alternative came into consideration, natural and synthetic product were finally mixed. The $^1$H NMR spectrum of this mixture showed only a single data set, so that impurities or concentration effects must explain the previous differences.
4.1.2 5'-Methyl-thioadenosine

Fraction 5 contained a colourless UV absorbing band of a polar component, which was coloured to brown after spraying with anisaldehyde/sulphuric acid and heating. The constituent was isolated as a colourless solid by silica gel column chromatography followed by slow evaporation of the sample.

The $^1$H NMR spectrum showed two 1H singlets in the aromatic region at $\delta$ 8.35 and 8.15, and a broad 2H singlet at $\delta$ 7.23 (H/D exchangeable), which is characteristic for an adenine moiety. In the aliphatic region, it displayed a doublet at $\delta$ 5.92 ($^3J = 6.1$ Hz) of an anomeric proton, a broad singlet of 2 OH groups at $\delta$ 5.65, in addition to three oxygenated methine protons at $\delta$ 4.73 (t, $^3J = 6.3$ Hz), 4.16 (m), and 4.07 (m). This resembled the spectrum of adenosine, however, the signal between $\delta$ 3.64-3.57 of the oxygenated methylene group in adenosine had disappeared and was replaced up-
field by an ABX 2H signal between $\delta$ 2.90-2.75 (dd, $^3J = 14.1$ Hz, 6 Hz). In addition, a 3H singlet at $\delta$ 2.05 was interpreted as a methyl group attached to an $sp^2$ carbon moiety or as a thiomethyl group.

In the aliphatic region, the $^1H$ NMR data were characteristic for a ribose moiety except that the oxygenated methylene group at 5'-position (displayed normally between $\delta$ 3.5-3.7) was exchanged against an ABX signal of a methylene group at $\delta$ 2.90-2.75.

**Figure 5:** $^1H$ NMR spectrum ([D$_6$]DMSO, 300 MHz) of 5'-methyl-thioadenosine (44).

The (+)-ESI mass spectrometry showed three quasi-molecular ions at $m/z$ 298 ([M + H]$^+$), 320 ([M + Na]$^+$), and 616 ([2M + Na]$^+$). (-)-ESIMS showed two quasi-molecular ion peaks at 296 ([M - H$^-$]), and 593 ([2M - H$^-$]) pointing to 297 as the molecular mass of the compound.

A search in AntiBase using the above spectral data resulted in 5'-methyl-thioadenosine, which was further confirmed by direct comparison with an authentic spectrum as well as with literature data. 5'-Methyl-thioadenosine is a common bacterial metabolite and was isolated previously in our group.
4.1.3 5,7,9,11-Tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid

5,7,9,11-Tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid (45) was obtained as a colourless liquid, which showed a violet colour reaction with anisaldehyde/sulphuric acid. In the $^1\text{H}$ NMR spectrum, there were two protons observed, which were attached with $sp^2$ carbon. The $^1\text{H}$ NMR spectrum showed several methyl groups between $\delta$ 0.98-0.78. There were two $sp^2$ protons at $\delta$ 6.32 and 5.86 and four oxygenated methine protons at $\delta$ 3.74-3.41.

The $^{13}\text{C}$ NMR spectrum delivered 17 signals as expected for the molecular formula, a carbonyl at $\delta$ 171.7 of an ester or acid, and two $sp^2$ carbon signals at $\delta$ 150.4 and 126.4 which seemed to be adjacent to each other. There were four oxygenated methine carbons observed at $\delta$ 80.3, 79.2, 76.4, and 74.6. Moreover, five methyl and four methine carbons were also present in the spectra.

Figure 6: $^1\text{H}$ NMR spectrum (CDCl$_3$, 300 MHz) of 5,7,9,11-tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid (45).
The H-H COSY spectrum showed that the four methyl groups appeared as doublets because of adjacent methine protons. A methyl triplet indicated a methylene group as next neighbour. The spectra showed further a long aliphatic chain.

In the HMBC spectrum, the $sp^2$ protons showed correlations with the carbonyl signal. The structure of 5,7,9,11-tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid resulted taking all further correlations into account. The structure was confirmed by
comparing the spectroscopic data with those of the related 10-deoxymethynolide (46).56

![Chemical structures of compounds 45 and 46](image)

**Figure 9:** HMBC spectrum (CDCl$_3$, 600 MHz) of 5,7,9,11-tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid (45).

Compound 45 is part of macrolides like erythromycin and may be a biosynthetic intermediate of the macrolides. Compound 46 showed significant antibiotic activity.57

![HMBC correlation of compound 45](image)

**Figure 10:** HMBC correlation of compound 45
4.2 *Streptomyces sp. B7880*

The ethyl acetate extract of the marine-derived streptomycete isolate B7880 drew our attention due to the presence of several pink to red coloured, UV absorbing zones. The strain exhibited high biological activity against *Mucor miehei* (Tü284), *Candida albicans*, and the alga *Chlorella sorokiniana*.

The strain B7880 was cultivated on a 20-liter scale for 7 days at 28 °C as shaker culture (110 rpm). After filtration, the dark red broth was filtered to separate the water phase and mycelium. The water phase was extracted by XAD-16 and the mycelium was extracted with ethyl acetate. The extracts from both phases were combined, as TLC did not show much difference. For isolation and purification of the metabolites, the resulting product was chromatographed on silica gel column using a chloroform-methanol gradient. Further separation of the fractions containing the reddish-orange peri-hydroxyquinones delivered four cinerubin derivatives: ζ-pyrromycinone (47), cinerubin B (49), cinerubin A (51), and cinerubin X (52).

![Figure 11: Working-up scheme for strain B 7880](image-url)

4.2.1 ζ-Pyrromycinone; Galirubinone C

Through the further isolation and purification of the fast moving fraction 2, compound was isolated as low polar main product from an orange fluorescent zone (366 nm),
which turned to violet by treating with sodium hydroxide, thus indicating a peri-hydroxyquinone. It delivered a red-orange solid on further PTLC and chromatography on Sephadex LH-20. The $^1$H NMR spectrum showed three $^1$H singlets at $\delta$ 13.02, 12.61, and 12.28, which are characteristic for peri-hydroxy groups in a quinone moiety. In the aromatic region, one singlet of $^1$H at $\delta$ 7.69, and an AB system $\delta$ 7.29 and 7.28 was detected, as for ortho-coupled protons in a 1,2,3,4-tetrasubstituted benzene ring. In addition, a $^1$H singlet was observed at $\delta$ 3.96, which could be assigned as oxymethine or as methine group flanked by two $sp^2$ carbon atoms.

![Figure 12: $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of galirubinone C (47).](image)

One methoxy group was observed at $\delta$ 3.74 (s). Four multiplets, each of $^1$H of two vicinal methylene groups were displayed at $\delta$ 3.07 (7-H$_A$), 2.86 (7-H$_B$), 2.30 (8-H$_A$) and 1.95 (8-H$_B$). The first methylene group ($\delta$ 3.07 and 2.86) should be next to an $sp^2$ carbon, while the other one ($\delta$ 2.30 and 1.95) should be next to an oxygenated quaternary $sp^3$ carbon. Furthermore, a third methylene group was giving two multiplets between $\delta$ 1.70-1.58, which could be adjacent to a methyl group, observed as triplet at $\delta$ 1.09, to constitute an ethyl group. The (−)-ESI mass spectrometry exhibited two quasi-molecular ions at $m/z$ 845 ([2M + Na −2H]−) and 411 ([M - H]−), establishing the molecular weight as 412 Dalton. It was further confirmed by a CI mass spectrometry, which exhibited an ion peak at $m/z$ 430 [M + NH$_4$]$^+$]. Based on the above chromatographic and spectroscopic data and searching in AntiBase, one structure was suggested, ζ-pyromycinone$^{58}$ (47). The compound was further confirmed by direct comparison of the 1D NMR and MS data with authentic values.
4.2.2  Cinerubin B; Ryemycin B

Cinerubin B was isolated as an UV fluorescent major product from fraction 3 as red-orange solid. By using sodium hydroxide, it showed a colour change to violet, indicating a peri-hydroxyquinone. The $^1$H NMR spectrum showed a close similarity with $\zeta$-pyrromycinone. Three peri-hydroxy groups of the pyrromycinone moiety were observed at $\delta$ 12.97, 12.83 and 12.25. In addition, the 1H singlet of an aromatic proton at $\delta$ 7.73 (11-H), and two AB protons were observed at $\delta$ 7.32 and 7.28 (2,3-H), as part of the pyrromycinone skeleton. Also, the ester methoxy group and the 10-H singlet were present at $\delta$ 3.70 and 4.13, respectively. Moreover, an ethyl group was established due to a triplet at $\delta$ 1.09 and a methylene group at $\delta$ 1.87 and 1.52. Besides, 8-H$_2$ protons were observed between $\delta$ 2.48-2.32. This pointed to the same pyrromycinone skeleton, substituted at position 7. In the sugar region, it displayed four oxymethine at $\delta$ 5.51 (s br), 5.28 (m), 5.19 (d) and 5.13 (m). The proton displayed at $\delta$ 5.28 is characteristic for 7-H in pyrromycinone. In addition, three methyl groups were observed as three doublets at $\delta$ 1.37, 1.30 and 1.25. This pointed to the presence of three sugar moieties in this compound. In addition, a 6H singlet of two equivalent N methyl groups was displayed at $\delta$ 2.16. Furthermore, seven hydroxymethines were displayed between $\delta$ 4.81 and 3.72, in addition to a methylene doublet at $\delta$ 2.60 linked most likely to $sp^2$ carbon. A further multiplet of a methylene group was exhibited at $\delta$ 2.09 (2''-H$_2$).
In accordance, this compound had a pyrromycinone chromophore with three sugar moieties attached at 7-position, which could be rhodosamine and 2-deoxyfucose. The ESI mass spectrometry fixed its molecular weight as 825 Dalton. A search in Anti-Base with the help of MS and $^1$H NMR data resulted in three structures: cinerubin B (49), spartanamicin A (48) and pyrraculomycin (50). However, the $^1$H NMR spectra exhibited no olefinic double bond, and hence pyrraculomycin (50) was excluded. Spartanamicin A (48) and cinerubin B (49) are configurational isomers. They differ only in the stereochemistry of the methyl group at 5'''-position, which is equatorial (H-5'''axial at $\delta$ 4.37) in cinerubin B (49) and axial in spartanamicin A (48) (H-5''' equatorial at $\delta$ 4.62). By comparing $^1$H NMR and mass spectra of both compounds 49 and 48 with the present values, the compound was elucidated as cinerubin B (49). Cinerubin B (49) is characterised by its pronounced activity against Gram-positive bacteria, viruses and tumours. Cinerubin B (49) was isolated and identified previously from different *Streptomyces* sp. and described by Ettlinger et al. as red/orange antibiotic. The aglycone of the cinerubins named previously by Brockmann et al. as η-pyrromycinone. Cinerubin B$^{59}$ (49) was isolated frequently from bacteria e.g. *Streptomyces antibioticus, Streptomyces griseorubiginosus, and Streptomyces galilaeus.*
4.2.3 Cinerubin A/ Spartanamicin B

A further red compound was separated from fraction 4. The mass was 827 Dalton confirmed by ESIMS with $m/z$ 828 [M + H]$^+$, 826 (M - H)$^-$, which was only 2 Dalton more than that of cinerubin B. The $^1$H NMR spectrum showed similarity with that of the previously described compound 49. A difference in the $^1$H NMR spectrum was observed in the sugar region. There was one proton signal less at $\delta$ 4-5. By searching with mass and $^1$H NMR data in AntiBase, the compound was identified as cinerubin A. The structure of cinerubin A$^{60}$ (51) was confirmed by comparing the $^1$H NMR and MS data of an authentic sample.
Cinerubin X (52) had the same colour and appearance as cinerubin A (51) but a different $R_f$ value, and the N,N-dimethyl signal at $\delta$ 2.20 in cinerubin A was not observed in $^1$H NMR spectrum for cinerubin X (52). ESI mass spectrometry showed $m/z$ 783 (M - H)$^-$, which was 43 less than for cinerubin A (51). This could be explained by the loss of the N,N-dimethyl group from the first sugar. A search in AntiBase resulted in cinerubin X (52). The assumption was found to be correct and the $^1$H NMR spectrum corresponded with that of the authentic sample.\textsuperscript{61}
The ethyl acetate extract of the terrestrial *Streptomyces* sp. isolate ACT 7619 was found to be highly active against bacteria and fungi. Some colourless weakly UV absorbing bands gave violet colouration with anisaldehyde/sulphuric acid. The 25 L fermentation of the strain using M₂ medium was performed as a shaker culture during five days, followed by extraction with ethyl acetate and XAD-16. This strain provided dinactin (53), bonactin (54), 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (56), Nβ-acetyltryptamine, tyrosol and maltol.
Figure 16: Working-up scheme of terrestrial strain ACT 7619.

4.3.1 Dinactin

A light yellow coloured liquid was obtained from fraction 3 after fractionation by column chromatography. The TLC chromatography showed a green-grey colour reaction with anisaldehyde/ sulphuric acid. $^1$H NMR spectrum showed the absence of aromatic protons. There were two protons observed at $\delta$ 4.93. At $\delta$ 2.52, a 2H multiplet was present, whose shift was due to the connection with an $sp^2$ carbon. Seven methylene multiplets were also observed in the range of $\delta$ 2.01-1.40. Three 3H doublets and one 3H triplet were present at $\delta$ 1.23, 1.10, 1.08, and 0.87 respectively. The ESIMS showed the mass as $m/z$ 788 [M + Na]$^+$. With the help of $^1$H NMR spectrum and mass a search in AntiBase detected a possible candidate. The structure was confirmed by comparing the mass and $^1$H NMR spectrum with the published literature as dinactin (53)$^{62}$.

Dinactin (53) is a member of the macrotetrolide complex produced by a number of Streptomyces species. It is a monovalent cation ionophore with high selectivity for
ammonium and potassium. It also inhibited T-cell proliferation induced by IL-2 and cytokine production at nanomolar levels for IL-2, IL-4, IL-5 and interferon-γ.63

![Diagram of compound 53]

**Figure 17:** $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of dinactin (53).

### 4.3.2 Bonactin

The $^1$H NMR spectrum displayed close similarity with those of dinactin (53). Dinactin is a dimer of two molecules of bonactin. The ESIMS showed a molecular ion peak at $m/z$ 423 ([M + Na]$^+$) affording a molecular weight of 400 Dalton. Searching in AntiBase using the above $^1$H NMR data and the molecular mass as well as the 'Rule of 13' afforded the molecular formula C$_{21}$H$_{36}$O$_7$ resulting in bonactin (54), which was further confirmed by direct comparison with the literature.64
Lee and co-worker succeeded in synthesizing bonactin (54) and its C-3\textsuperscript{\textprime} epimer which was previously published with a wrong structure as feigrisolide C (55).\textsuperscript{65} Bonactin (54) showed moderate inhibition of the 3\textalpha-hydroxysteroid dehydrogenase, moderate cytotoxicity against L-929, K562 and HeLa cell lines, and 50\% inhibition of Coxackie virus B3 at 25 \textmu g/mL.\textsuperscript{66}

4.3.3 4,10-Dihydroxy-10-methyl-dodec-2-en-1,4-olide

Purification of fraction 1 delivered a colourless oil, which showed no UV absorbance on TLC and turned to violet-red colour with anisaldehyde/sulphuric acid. In the \textsuperscript{1}H NMR spectrum, the three signals at \(\delta 7.44\) (dd), 6.12 (dd) and 5.04 (m) are typical for monosubstituted butenolides. In the aliphatic region, one methyl triplet at \(\delta 0.90\), one
methyl singlet at 1.12 and multiplet of some methylene groups between 1.43 and 1.70 were observed.

![Figure 19](image)

**Figure 19:** $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (56).

By searching in AntiBase using the above $^1$H NMR data as well as comparison with authentic spectra, the isolated compound was assigned as 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (56).$^{67,68}$

4.4 **Terrestrial Streptomycete GW 6311**

The culture filtrate of the terrestrial streptomycete GW6311 was antibiotically active against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Streptomyces viridochromogenes* (Tü57), *Mucor miehei* (Tü284), and *Candida albicans*. In the chemical screening, it exhibited two low-polar red-orange bands, which turned to blue-violet by treatment with sodium hydroxide, pointing to peri-hydroxyquinones. Additionally, an UV absorbing band was present, which was stained to yellow-orange by Ehrlich’s reagent.

4.4.1 **β-Indomycinone**

Compound (57) was isolated as a yellow amorphous compound from fraction 2. The $^1$H NMR spectrum showed singlets at δ 12.85 (OH) and δ 8.08 (H-6), three ortho-
coupled aromatic protons at $\delta$ 7.83, 7.70 and 7.38, a 1H singlet at $\delta$ 6.56, and the singlet of a peri-methyl group at $\delta$ 3.02. In addition, two multiplets of an olefinic double bond were detected at $\delta$ 5.74 and 5.38. In the aliphatic region, two methylene protons revealing an ABX system were detected at $\delta$ 2.91 and 2.78. Finally, two methyl groups linked to $sp^2$ carbons were displayed at $\delta$ 1.72 and 1.64, of which the first gave a singlet, and the second a doublet. The molecular weight of this compound was established as 404 Dalton by ESIMS. By using $^1$H NMR and MS data the structure was found as $\beta$-indomycinone (57). The structure was further confirmed by comparing with an authentic sample.$^{69,70}$

![Image](image.png)

### 4.4.2 Saptomycin A

Compound 58 was another peri-hydroxyquinone obtained as a yellow solid from the same fraction, from which $\beta$-indomycinone (57) was isolated. The $^1$H NMR spectrum showed nearly the same signal pattern as in $\beta$-indomycinone (57). A singlet of a peri-hydroxy group at $\delta$ 12.64 was exhibited. In the aromatic region, a 1H singlet at $\delta$ 8.09, three aromatic protons at $\delta$ 7.83, 7.69 and 7.36 of a 1,2,3-trisubstituted aromatic ring, a singlet at $\delta$ 6.28 (3-H), in addition to the singlet of a peri-methyl group at $\delta$ 3.02 (13-H$_3$). In addition, two multiplets of an olefinic double bond were detected at $\delta$ 5.65 and 5.50. Moreover, two methine protons were displayed at $\delta$ 5.01 and 2.98, of which the first could be oxygenated, while the second was a methine flanked most likely by two $sp^2$ carbons. Finally, two methyl doublets were detected at $\delta$ 1.71 and 1.45, of which the first could be attached to a $sp^2$ carbon. Based on the revealed spectral data and the molecular formula, a search in AntiBase directed to saptomycin A. This compound was further confirmed by comparing the data with those in the literature.$^{71}$
Figure 20: $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of mixture of $\beta$-indomycinone (57) and saptomycin A (58).

$\beta$-indomycinone (57) and saptomycin A (58) are composed of an anthraquinone $\gamma$-pyrone nucleus, but bear a different side chain. Both of them belong to pluramycin group and showed antimicrobial and potent antitumor activities against human or murine tumor cell lines in vitro and Meth A fibrosarcoma in vivo.$^{72}$

4.5 Terrestrial Streptomyces sp. GT 2005/079

The biological screening of the crude extract of the terrestrial Streptomyces sp. GT 2005/079 exhibited pronounced antibacterial activity against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli. The TLC screening revealed several polar green fluorescent zones as well as some middle polar zones, which were not observable under UV at 254 or 366 nm but gave a dark green colour reaction on TLC with anisaldehyde/sulphuric acid. The strain was cultivated on M$_2$ medium in a 20 L scale during seven days. The grey culture broth was mixed with Celite and filtered through a filter press. Both water and mycelium phases were extracted with ethyl acetate and
the organic phase brought to dryness. The dark oily crude extract was defatted using cyclohexane and submitted to column chromatography on silica gel using a CH$_2$Cl$_2$/MeOH gradient to get 6 new compounds: four new antifungal amides 59-62 together with two new oxazole antibiotics. The structures of these compounds were deduced by interpretation of high resolution mass, 1D and 2D NMR spectra.

The crude extract was chromatographed on a silica gel column eluting with a gradient system of CH$_2$Cl$_2$: MeOH (100:0 to 90:10) to give five main fractions. Fraction 1 contained mostly lipids, whereas fraction 2 showed three main spots on TLC. This fraction was further purified on a silica gel and on Sephadex LH-20 followed by a RP-8 column eluted with 50-60 % aqueous methanol to get compound 59 and compound 60. Fraction 3 was subjected to silica gel and Sephadex LH-20 followed by RP-8 column chromatography yielding compound 61. Fraction 4 was subjected to RP-18 column chromatography with 30 % aqueous methanol to give two sub-fractions. Further purification with methanol on Sephadex LH-20 yielded 62. Fraction 5 contained a highly fluorescent polar compound. It was separated on Sephadex LH-20 into two sub-fractions. The solubility of these compounds was low in dichloromethane or methanol or even in a mixture of both solvents. Therefore, the solids were washed several times with a mixture of dichloromethane and methanol to obtain compound 63 and 64.
Figure 21: Work-up scheme of the strain *Streptomyces* sp. GT 2005/079.
4.5.1 *N-(7-Hydroxy-6-methyl-octyl)-acetamide*

On ESIMS, *N-(7-hydroxy-6-methyl-octyl)-acetamide* (59) showed a pseudo molecular ion at *m/z* 224 [M + Na]^+. (+)-HRESIMS established the formula as C_{11}H_{23}NO_{2} with 1 double bond equivalent (DBE). The $^1$H NMR spectrum displayed a broad signal at $\delta$ 5.70, which could be due to NH/OH, as in the HSQC spectrum there was no cross peak with a carbon signal visible. The quartet of a methylene group at $\delta$ 3.22 showed an H-H COSY correlation with this acidic proton at $\delta$ 5.70 as well as with another methylene group at $\delta$ 3.22. There were 3 methyl signals observed at $\delta$ 1.97 (s), 1.15 (d) and 0.85 (d). From the value of the methyl singlet at $\delta$ 1.97, it could be assumed that it was attached to an $sp^2$ carbon or near a carbonyl group or a nitrogen atom. The other two methyl groups are neighbours of two CH groups as they were doublets. There were overlapping signals of four methylene groups in the range of $\delta$ 1.2-1.4. A CH quartet at $\delta$ 3.65 depicted that it was attached to a methyl group and a hetero atom.

![Figure 22: $^1$H NMR spectrum (CDCl3, 300 MHz) of *N-(7-hydroxy-6-methyl-octyl)-acetamide* (59).](image)

The $^{13}$C NMR spectrum showed a carbon signal at $\delta$ 170.1, which suggested the presence of an ester, acid or amide group. The methine carbon at $\delta$ 71.6 depicted an attachment of oxygen, while the methylene carbons at $\delta$ 43.3 and 39.3 were either attached to a nitrogen atom or a $sp^2$ carbon. There were seven more methylene carbons observed between $\delta$ 30-14.
Figure 23: $^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) of $N$-(7-hydroxy-6-methyl-octyl)-acetamide (59).

In the H-H COSY spectrum, the acidic proton at $\delta$ 5.70 showed a correlation with the methylene at $\delta$ 3.15.

Figure 24: H-H COSY spectrum (CDCl$_3$, 600 MHz) of $N$-(7-hydroxy-6-methyl-octyl)-acetamide (59).

From HMBC spectrum the methyl group at $\delta$ 1.97 showed a $^2J$ coupling with the carbonyl at $\delta$ 170.6. The carbonyl also displayed correlation with the acidic proton, which is probably an NH, as only two oxygen atoms are present and one was already used for the carbonyl. The other one was attached to the CH at $\delta$ 71.5, so the substructures A and B could be drawn.
Figure 25: HMBC spectrum (CDCl$_3$, 600 MHz) of $N$-(7-Hydroxy-6-methyl-octyl)-acetamide (59).

![Figure 25](image)

Figure 26: Substructure A and B of $N$-(7-Hydroxy-6-methyl-octyl)-acetamide (59).

Using all the 1D and 2D NMR correlations, the structure of compound 59 was established.$^{73}$

![Figure 26](image)

Figure 27: H-H COSY (↔) and HMBC (→) correlation of (7-hydroxy-6-methyl-octyl)-acetamide (59).

4.5.2 $N$-(7-Hydroxy-octyl)-acetamide

$N$-(7-Hydroxy-octyl)-acetamide (60) showed similarity with $N$-(7-hydroxy-6-methyl-octyl)-acetamide (60) in the $^1$H NMR spectrum and physical properties. From the HRESIMS the molecular formula was established as C$_{10}$H$_{21}$NO$_2$, which was 14 Daltons less than that of compound 60. The $^1$H NMR pattern depicted a difference of a methylene group. The methyl signal at $\delta$ 0.85 was observed as doublet, while a
number of methylene groups appeared between $\delta$ 1.40-1.15. The acetyl methyl group appeared at $\delta$ 1.97 as in 59.

**Figure 28:** $^1$H NMR spectrum (CDCl$_3$, 300 MHz) spectra of N-(7-hydroxy-octyl)-acetamide (60).

In the $^{13}$C NMR spectrum of 60, there was a carbonyl signal at $\delta$ 171.3 observed which could be that of an amide as in 59. There was an oxymethylene group at $\delta$ 68.2 along with the methylene near nitrogen at $\delta$ 39.6. The methyl signal of the acetyl group was at $\delta$ 23.4, while another methyl was seen at $\delta$ 16.0.

**Figure 29:** $^{13}$C NMR spectrum (CDCl$_3$, 150 MHz) of N-(7-hydroxy-octyl)-acetamide (60).

In the H-H COSY spectrum the methyl doublet showed a correlation with a methine proton, and the NH group showed a correlation with a methylene group. All other methylene signals appeared nearly in the same position as in 59. From the pattern it was clear that they were attached to each other.
Figure 30: H-H COSY spectrum (CDCl$_3$, 600 MHz) spectra of N-(7-hydroxy-octyl)-acetamide (60).

Based on the correlations as described above and by comparing with the data of published compounds structure 60 was derived.$^{74,75}$

Figure 31: HMBC spectrum (CDCl$_3$, 600 MHz) of N-(7-hydroxy-octyl)-acetamide (60).

Figure 32: H-H COSY (↔) correlations and HMBC (→) correlations.
4.5.3 *N-(6-Hydroxy-6-methyl-heptyl)-acetamide*

*N-(6-Hydroxy-6-methyl-heptyl)-acetamide* (61) was a further acetamide isolated from the same strain. The molecular formula C_{10}H_{21}NO_{2} was derived from the HRESI mass spectrum. The molecular formula was same as for compound 60. There were two methyl groups, which appeared as a singlet in this compound at δ 1.19. So, it was one possibility that the methyls were present in an isopropyl group, while the methine proton of that isopropyl group was substituted with oxygen, as the shift of the respective carbon atom was observed at δ 70.5. The NH proton and the adjacent methylene group at δ 3.20 showed the same shifts as for 59 and 60.

![Figure 33: 1H NMR spectrum (CDCl3, 300 MHz) spectrum of N-(6-hydroxy-6-methyl-heptyl)-acetamide (61).](image)

In the ^13^C NMR spectrum, 10 carbon signals appeared. The acetamide carbonyl signal appeared at δ 170.4 and a quaternary oxygenated \(sp^3\) carbon was observed at δ 70.5. The other carbons showed values in the same range as for the previously described amides.
Figure 34: $^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) spectra of $N$-(6-hydroxy-6-methyl-heptyl)-acetamide (61).

In the H-H COSY spectrum the methylene at $\delta$ 3.20 showed correlations with the NH signal and another methylene. The other correlations were similar to those of 59 and 60, except for the methyl groups, which gave a singlet.

Figure 35: H-H COSY spectrum (CDCl$_3$, 600 MHz) of $N$-(6-hydroxy-6-methyl-heptyl)-acetamide (61).

In the HMBC spectrum the methylene at $\delta$ 3.20 and the nearby NH group showed correlation with the carbonyl at $\delta$ 170.4. The isopropyl group showed correlation with the
Terrestrial Streptomyces sp. GT 2005/079

A quaternary carbon at $\delta$ 70.4. The other correlations were the same as in $N$-(7-hydroxy-octyl)-acetamide (59) and $N$-(7-hydroxy-6-methyl-octyl)-acetamide (60).

**Figure 36:** HMBC Spectrum (CDCl$_3$, 600 MHz) of $N$-(6-hydroxy-6-methyl-heptyl)-acetamide (61).

Based on the 1D and 2D NMR data and comparing with related compounds (e.g. 65), structure 61 was confirmed.$^{76}$

![Structure 61](image)

**Figure 37:** $R' = (\text{CH}_2)_n$ with $n = 3, 4, 5, 6$ and $R'' = \text{Me}$ or $\text{Pr}$.

Amides are interesting compounds due to the natural occurrence of their derivatives, e.g., peptides. There is considerable interest of amides of carboxylic acid derivatives in the formation of peptides and lactams.$^{77}$ Bellenamine (66) is an antibiotic which inhibits the growth of Gram-positive bacteria and has activity against the human immunodeficiency virus and enhances both delayed-type hypersensitivity to sheep red blood cells and antibody formation in the mouse spleen.$^{78}$
4.5.4 Homononactic acid amide

The $^1$H NMR spectrum of compound 62b showed similarity with that of homononactic acid (62a). ESIMS showed a quasi molecular ion at $m/z$ 216 $[M + H]^+$. The odd mass number was an indication of an odd number of nitrogen atoms in the molecule. HRMS established the molecular formula as C$_{11}$H$_{22}$NO$_3$ and confirmed the presence of one nitrogen atom in this compound.

![Figure 38: $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of homononactic acid amide (62).](image)

The $^1$H NMR spectrum showed a methyl triplet and another 3H doublet at $\delta$ 0.94 and 1.17, respectively. There were three protons present at $\delta$ 4.18 (m), 3.90 (q) and 3.80 (m) which were a hint for attached hetero atoms. There were two H/D exchangeable protons observed as broad singlets at $\delta$ 6.27 and 5.50.
Figure 39: $^{13}$C NMR spectrum (CDCl$_3$, 150 MHz) of homononactic acid amide (62).

In $^{13}$C NMR spectrum there were ten carbon signals observed. The carbon signal at $\delta$ 176.9 can be attributed to the carbonyl of an ester, acid or amide. There were three methine carbons at $\delta$ 81.1, 77.0 and 70.6, which were attached to oxygen. Another methine appeared at $\delta$ 46.0, and four methylene signals at $\delta$ 41.1, 30.6, 30.2 and 29.6. There were two methyl signals observed at $\delta$ 14.2, 14.2.

Figure 40: H-H COSY spectrum (CDCl$_3$, 600 MHz) of homononactic acid amide (62).

In the H-H COSY spectrum, one of the methyl signals showed correlations with a methylene and another one with a methine. The three oxygenated methines showed correlations with methylene groups.
In the HMBC spectrum, the methyl group at $\delta$ 1.17 showed correlation with the carbonyl at $\delta$ 176.9 and with the methine (CH) carbon at $\delta$ 81.1, which was probably attached to oxygen. There was another methyl triplet at $\delta$ 0.94, which showed a long-range correlation with one proton at position $\delta$ 3.80, which was also at a methine carbon attached to oxygen. Protons at $\delta$ 5.44 and 6.25 did not show any correlation in the HSQC spectrum and were obviously H/D exchangeable. The HRESIMS ($C_{11}H_{21}NO_{3}$) showed two double bond equivalents in compound 62b. The $^1$H and $^{13}$C NMR as well as H-H COSY spectra suggested the structure of homononactic acid (62a). But the HRESIMS as well as two H/D protons at $\delta$ 5.44 and 6.25 were different from that of homononactic acid (62a). The carbonyl at $\delta$ 176.9 presented the value as ester, amide or acid.

![Figure 41: HMBC Spectrum (CDCl₃, 600 MHz) of homononactic acid amide (62).](image)

62a: Homononactic acid, $R = $ OH.
62b: Homononactic acid amide, $R = $ NH₂.

On the basis of 1D and 2D NMR data and in comparison with the data of homononactic acid (62a)⁷⁹ the structure of homononactic acid amide (62) was confirmed.
Figure 42: HMBC correlations of homononactic acid amide (62).

4.5.5 2-Phenylbenzoxazole-4-carboxylic acid

2-Phenylbenzoxazole-4-carboxylic (63) acid was isolated as a polar, green fluorescent pale yellow solid. In the $^1$H NMR spectrum, 9 protons were found in the $sp^2$ region, but there were no aliphatic proton signals. Two of the protons were acidic and appeared as a broad signal at $\delta$ 11.5.

Figure 43: $^1$H NMR spectrum ([D$_6$]DMSO, 300 MHz) of 2-phenylbenzoxazole-4-carboxylic acid (63).

HRESIMS afforded the molecular formula C$_{14}$H$_{10}$NO$_4$. From the H-H COSY spectrum two aromatic rings were confirmed with two substructures A and B.

Figure 44: Substructures A and B of compound 63.
Figure 45: $^{13}$C NMR spectrum ([D6]DMSO, 125 MHz) of 2-phenylbenzoxazole-4-carboxylic acid (63).

From the $^{13}$C NMR spectrum there was a quaternary carbon signal observed at δ 165.5, which could be a carbonyl of an ester, amide or an acid. As the presence of an acidic proton was already described, a carboxylic acid or an amide was more likely.

Figure 46: H-H COSY spectrum ([D$_6$]DMSO, 500 MHz) of phenylbenzoxazole-4-carboxylic acid (63).

In the HMBC spectrum, the proton at δ 7.96 showed a $^3J$ correlation with the carbon at δ 163.4, where another proton at δ 7.99 showed correlation with the carbonyl at δ 165.5. In AntiBase, with the molecular formula and the substructures A and B along with $^{13}$C NMR values, no respective compound was found, which indicated a new secondary metabolite. A search in the Chemical Abstract delivered, however, compound 63. The $^1$H NMR, $^{13}$C NMR and mass spectrometry as well as the colour of the compound were identical to 2-phenylbenzoxazole-4-carboxylic acid (63), which is now reported from a natural source for the first time.
Compound 63 showed activity as 5-HT inhibitor and exhibited central activity in treating chemotherapy–induced nausea and vomiting (CINV) as well as diarrhoea and irritable bowel syndrome (IBS-D).

4.5.6 2-(3-Chloro-2-hydroxy phenyl) benzoxazole-4-carboxylic acid

2-(3-Chloro-2-hydroxyphenyl)-benzoxazole-4-carboxylic acid (64) is a derivative of 63 and was also isolated from the same fraction. It was obvious from ESI mass spectrometry that this compound contained chlorine. In ESIMS one signal was observed at \( m/z \) 288 [M-H]+, followed by another signal at \( m/z \) 290 Dalton which was 1/3 of the first signal at \( m/z \) 288. The same pattern was also observed at \( m/z \) 244 and \( m/z \) 246 Dalton. The signal at \( m/z \) 244 was the decarboxylation product of the parent signal at 288 Dalton ([M-H]+). HRESIMS afforded the molecular formula \( \text{C}_{14}\text{H}_8\text{NO}_4\text{Cl} \). Compound 64 showed 7 protons at \( \delta \) 7-8, while compound 63 showed only 6 protons in the same region. One of them was obviously replaced by a chlorine atom.
In 63, there were two protons (3' and 5') at δ 7.10, but in compound 64 there was only one proton at δ 7.10. So, either the 3'- or 5'-position of this compound was occupied by chlorine. The H-H COSY spectrum showed correlations between the protons H-4', 5' and 6'. Therefore, the chlorine was assigned to position 3'. Based on mass, ¹H NMR and H-H COSY data, compound 64 was identified as 2-(3-chloro-2-hydroxy phenyl)-benzoxazole-4-carboxylic acid.

Synthesized UK-1 (67) and the analogue 68 showed cytotoxicity against human carcinoma cell lines. Synthetic 2-hydroxylpropylvalpromide (69) was useful for the treatment of neural psychotic, epilepsy, pain and migraine. Amides derived from ferulic acid showed stimulatory effects on insulin secretion. The synthetic azole 1H-pyrazole-4-carboxamide (70) showed potential antifungal and herbicidal activity.
4.6 Terrestrial Actinomycete N 435

The goal in selecting this strain was to find active compounds against plant pathogenic fungi. N 435 showed moderate antifungal activity, and on TLC of the crude extract, several UV absorbing zones were visible; spraying with anisaldehyde/sulphuric acid showed a pink colour reaction. This behaviour, the UV absorbance together with the antifungal activity of the strain drew our attention for further investigation.

The strain was cultivated on a 20-liter scale shaker culture (110 rpm) at 28 °C for 72 hours. After filtration using Celite the water phase was extracted with XAD-16 and the bio-mass with ethyl acetate. The brown extract was chromatographed using silica gel column chromatography and eluted with dichloromethane and methanol with a gradient of increasing polarity. As a result, seven fractions were obtained. Working up and purification of the fractions led to the isolation of many diketopiperazines along with a new secondary metabolite, ramthacin B (78).
Figure 49: Work-up scheme for terrestrial Actinomycete N 435.

4.6.1 Cyclo(dehydroalanyl-isoleucyl)

Compound 71 was isolated from fraction 2 as a white colourless solid after fractionating by silica column chromatography followed by Sephadex LH-20 column chromatography. Compound 71 crystallized from dichloromethane/methanol as thin needles.
EI mass spectrum established the molecular weight as $m/z$ 170. The $^1$H NMR spectrum showed two acidic proton signals at $\delta$ 10.43 and 8.40 as broad singlets. There was an $sp^2$ methylene with two 1H singlets at $\delta$ 5.20 and 4.78, as well as an isobutyl group [at $\delta$ 1.80 (m, 1H, CH), 1.77 (dd, 2H)] and 2 methyl groups as a doublet at $\delta$ 0.85. Another methine proton at $\delta$ 3.97 was observed as a multiplet. Based on $^1$H NMR spectrum mass, a search in AntiBase identified this compound as cyclo(dehydroalanyl-isoleucyl) (71); this was confirmed by comparison with an authentic spectrum.

4.6.2 Cis-Cyclo(leucyl-tyrosyl)

Compound 72 was isolated as colourless solid from fraction 3. Beside the $R_f$ value, it showed the same physical properties as compound 71. In the $^1$H NMR spectrum there were two H/D exchangeable protons at $\delta$ 9.15 and 8.00. A para-substituted benzene ring was observed at $\delta$ 6.91 and 6.65, and two methine protons were visible at $\delta$ 4.06 and 3.46 suggesting their attachment to hetero atoms. There was a methylene observed at $\delta$ 3.01 and 2.73 near a chiral centre, an isopropyl group at $\delta$ 0.66 (2 methyl) and 1.45 (methine multiplet) as well as another methylene at $\delta$ 0.80 and 0.23.
**Figure 51:** $^1$H NMR spectrum ([D$_6$]DMSO, 300 MHz) of *cis-cyclo*(leucyl-tyrosyl) (72).

HRESIMS established the mass as $m/z$ 275.1403 ([M-H]$^-$ for C$_{15}$H$_{19}$N$_2$O$_3$, indicating that there were seven double bond equivalents present.

**Figure 52:** $^{13}$C NMR spectrum ([D$_6$]DMSO, 75 MHz) of *cis-cyclo*(leucyl-tyrosyl) (72).

In the $^{13}$C NMR spectrum there were two carbonyl signals of an ester, acid or amide system at $\delta$ 167.4 and 166.2. There was an oxygenated $sp^2$ carbon observed at $\delta$ 156.3 as well as five carbon signals at $\delta$ 131.1 (2 CH), 125.9 and 114.8 (2 CH) for the rest of an aromatic system. Seven additional carbon signals were observed between $\delta$ 55.6-21.3.
Figure 53: H-H COSY spectrum ([D₆]DMSO, 300 MHz) of cis-cyclo(leucyl-tyrosyl) (72).

From the H-H COSY, an isobutyl system was determined and the substructures A and B resulted from the observed correlations.

![Substructures A and B](image)

Figure 54: Fragments (A and B) from the H-H COSY spectrum of cis-cyclo(leucyl-tyrosyl) (72).

Figure 55: H-H COSY spectrum ([D₆]DMSO, 300 MHz) of cis-cyclo(leucyl-tyrosyl) (72).
The methylene at δ 0.80 and 0.23 showed correlation with the phenol moiety, which indicated that they were directly attached. This methylene also showed correlation with another methine. Additionally, the methine showed correlation with the carbonyl of an amide. The same type of correlation was observed for the isobutyl group. The EI mass spectrum showed the following fragmentation (Figure 56), which supported a further diketopiperazine. From the HMBC correlations and published data\textsuperscript{86} the structure was confirmed as \textit{cis-cyclo}(leucyl-tyrosyl).

![Figure 56: EI-MS fragmentation of \textit{cis-cyclo}(leucyl-tyrosyl) (72).](image)

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**Figure 57: HMBC (\(\rightarrow\)) and H-H COSY (\(\leftarrow\)) correlations of \textit{cis-cyclo}(leucyl-tyrosyl) (72).**

![Cyclo(phenylalanyl-prolyl) (73) and Albonoursin (74)](image)
Diketopiperazines are the smallest cyclic peptides known, commonly biosynthesised from amino acids by different organisms, including mammals, and are considered to be secondary functional metabolites or side products of terminal peptide cleavage. Cyclic dipeptides are extensively obtained by extraction from natural sources, but may be easily synthesized. They are generally biosynthesised from proteinogenic L-α-amino acids having a cis-configuration and possess a wide spectrum of biological activities, which include: antitumor, antiviral, antifungal, antibacterial and antihyperglycaemic. In addition, they have been found to inhibit plasminogen activator inhibitor and alter cardiovascular and blood-clotting functions. Diketopiperazines are also described as signalling compounds however the exact role that they play in bacterial cell-to-cell communication has not been ascertained. In particular some diketopiperazines containing tyrosine have been found to bind μ-opioid receptors while cyclo(leucyl-prolyl) inhibited the growth of Mycobacterium marinum, an experimental model for M. tuberculosis. It is quite unusual for such a variety of diketopiperazines to be isolated from one source.

4.6.3 Ramthacin B

Colourless oil with middle polarity was isolated from sub-fraction 3. It was UV absorbing at 254 nm and fluorescent at 366 nm. After spraying the TLC plate with anisaldehyde/sulphuric acid, it gave a dark green colour. The molecular weight was established by EIMS to be m/z 367. In the aromatic region it showed three 1H signals, two of which were doublets of doublets at δ 7.79 and δ 6.59 indicating two ortho-coupled protons; the third proton at δ 7.77 showed a meta-coupling. In the aliphatic region three methyl groups were found as a singlet at δ 1.63. The chemical shift for these three methyl groups indicated the attachment of \( sp^2 \) carbon atoms or influence inductive effect. At δ 4.91, 4.60 and 3.67, three methine protons were observed which were attached with hetero atoms. At δ 3.48 and 3.11, one methylene was observed which was obviously near a chiral centre. The 1H NMR spectrum showed substructures A, B, C. With the help of ESIMS and 1H NMR spectrum the compound was searched in AntiBase resulted no hit. The substructures from the 1H NMR spectrum leaded to a high similarity to virantmycin (77). 1H NMR spectrum was found same
with compound 78 which was recently isolated and the structure has already been established by 1D and 2D NMR (publication in process) from marine Streptomyces sp. B7801.92 The 1H NMR spectrum and mass were identical to that of the compound 78. Compound 78 has been named as ramthacin B, which is a new secondary metabolite.

Figure 58: Substructures of compound 78 derived from $^1$H NMR spectrum.

Figure 59: $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of ramthacin B (78).
Virantmycin (77) was isolated from *Streptomyces* sp. by Japanese coworkers in 1980 which showed potential antiviral activity.\(^93\) Compound 78 was not stable enough to check antiviral activity. The benzastatin family and virantmycin (77) are a novel class of indoline and tetrahydroquinoline alkaloids isolated from *Streptomyces nitrososporous*.\(^94\) Benzastatins show inhibitory activity against glutamate toxicity and lipid peroxidation in rat liver microsomes that can be used to prevent brain ischemia injury, and consists of indoline alkaloids such as benzastatin E (80), and tetrahydroquinoline alkaloids such as benzastatin C (79), which are structurally related to virantmycin. Virantmycin, a potent inhibitor towards RNA and DNA viruses, is a unique 2,2-disubstituted tetrahydroquinoline alkaloid with contiguous quaternary and tertiary stereo centres. To date, several research groups have reported the total syntheses of virantmycin.\(^97\)\(^,\)\(^98\)

![Chemical structures of virantmycin (77) and benzastatins (79, 80)](image)

### 4.7 *Streptomyces* sp. B7857

The chemical investigation of the marine-derived *Streptomyces* sp. B 7857 delivered four new oxygenated α-gorgonene sesquiterpenes named bacteriogorgonene A (81), (82), bacteriogorgonene B (83) and bacteriogorgonene C (84) and a few trivial compounds as well as ten known secondary metabolites, namely homononactic acid, 1-acetyl-β-carbolin, polypropylen glycol, nonactic acid, uracil, thymidine, deoxy-
uridine, and 2-hydroxy-1-(1H-indol-3-yl)-ethanone. The structures of the new constituents were elucidated by chemical and spectroscopic studies.

The strain *Streptomyces* sp. B 7857 was selected due to its moderate to high biological activity against *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Chlorella vulgaris*, and *Artemia salina*. It also showed several UV absorbing zones. Spraying with anisaldehyde/sulphuric acid on TLC showed a dark pink colour reaction, turned to green with time.

The strain was incubated using M2 medium on a 20 L scale. After 7 days, the well-grown culture broth was mixed with Celite, and filtered under pressure. The filtrate was extracted with XAD-16 column and the resin was washed with demineralised water and eluted with methanol to get a dark brown extract.

On TLC the biomass and XAD extract did not show much difference and hence were combined. The combined crude extract was chromatographed on a silica gel column eluting with a dichloromethane/methanol gradient to give 4 fractions. Fraction 1 consisted mostly fats and lipids along with polypropylene glycol, whereas fraction 2 showed two main spots on TLC. Thus this fraction 2 was further purified by using Sephadex LH-20, silica column chromatography, PTLC and finally by RP-18 open column chromatography eluting with 50-60 % aqueous methanol to give compound 81. PTLC was most effective in separating the fractions as several compounds overlapped in column chromatography. After PTLC further purification was done by RP-18 column chromatography. Using the same method, another two new terpenes, compounds 83 and 84 were isolated from the same fraction. Fraction 5 was further purified to give seven trivial compounds, namely 1-acetyl-β-carbolin, 3-(2-hydroxyethyl)indole, uracil, thymidine, deoxyuridine, and 2-hydroxy-1-(1H-indol-3-yl)-ethanone.
Figure 60: Working scheme of *Streptomyces* sp. B 7857.

### 4.7.1 Bacteriogorgonene A

Compound **81** was isolated as very unpolar colourless oil, which was difficult to separate from the fat fraction. PTLC was done by multiple developments, followed by RP-18 and Sephadex LH-20 column chromatography to isolate compound **81**. In the $^1$H NMR spectrum there were no protons observed in the aromatic region. Four methyl groups were visible between $\delta$ 1.00-2.00. The methyl singlet at $\delta$ 0.94 was easily determined as being attached to a quaternary carbon atom. Two further methyl groups appeared in the same place at $\delta$ 1.31 as singlet, which indicated both the methyl groups were attached to a quaternary carbon. The remaining methyl at $\delta$ 1.95 showed a very small coupling ($J = 1.4$ Hz) indicating a $^4J$ distance. The olefinic proton at $\delta$ 5.90 also showed the $^4J$ coupling with the methyl and was near an electron-withdrawing group. This proton was in the $\alpha$-position of a carbonyl group, indicating the $\beta$-position was substituted with the methyl group. From the spectra, the presence of at least 3 methylene groups was clear. Two of the methylene groups (at $\delta$ 2.25 and 1.37) showed ABX systems, indicating the presence of neighbouring chiral centres.
Figure 61: $^1$H NMR spectrum (300 MHz, CDCl$_3$) of bacteriogorgonene A (81).

Figure 62: Substructures of bacteriogorgonene A (81).

The $^{13}$C NMR spectrum revealed 15 carbon signals, among them 12 signals were observed in the $sp^3$ and 2 in the $sp^2$ region. One carbon signal at $\delta$ 200.1 was the carbonyl of a ketone or aldehyde, while the carbon at $\delta$ 74.4 was attached to an oxygen atom.

Figure 63: $^{13}$C NMR spectrum (CDCl$_3$, 150 MHz) of bacteriogorgonene A (81).
Figure 64: H-H COSY correlations of fragment D in bacteriogorgonene A (81).

In the HMBC spectrum, the proton at $\delta$ 5.90 made a correlation with carbon signals at $\delta$ 41.5 (CH) and 54.7 (CH$_2$). This proton also showed a $^2J$ correlation with the carbonyl at $\delta$ 200.1 which meant that this proton was located in $\alpha$-position to the carbonyl. The methyl group at $\delta$ 1.90 showed a small correlation with the signal at $\delta$ 165.0 as well as correlations with $\delta$ 42.9 (CH) and $\delta$ 126.3. This indicated that the methyl at $\delta$ 1.90 is attached to the carbon at $\delta$ 165.0.

Figure 65: H-H COSY spectrum (CDCl$_3$, 600 MHz) of bacteriogorgonene A (81).

The methylene proton at $\delta$ 54.7 showed many correlations to the carbon signals at $\delta$ 200.1, 126.3, 42.9, 36.9 (C$_q$), 36.9 (CH$_2$) and 17.8 (CH$_3$). The methyl at $\delta$ 17.8 made correlation with $\delta$ 36.9 (C$_q$), $\delta$ 36.9 (CH$_2$), 42.9 and 54.7. Therefore, it was deduced that the methyl was directly attached to 36.9 (C$_q$) as this was a singlet and $\delta$ 36.9 (CH$_2$) and 42.9 (CH) were adjacent to this quaternary carbon at $\delta$ 36.9. So, a six-membered ring is confirmed. The methyl at 21.9 also showed a correlation with $\delta$ 42.9 (CH). The two methyls at $\delta$ 1.25 made correlation with each other as well as with the oxygenated quaternary carbon at $\delta$ 74.4.
The (+)-HRESIMS measurement delivered the formula C_{15}H_{24}O_{2}, pointing to a sesquiterpene. By comparing the spectroscopic data with a similar structure (85), structure **81** was established.99-100

There were three stereo centres present at position 4a, 5 and 8a. In the NOESY experiment of compound **81**, the proton at position 4a showed a correlation with the proton at position 5, which confirmed that these two protons were on the same side. However, the methyl at position 4a did not show any correlation with the methyl protons at position 8a, which agrees better with the assumption that both were in a *trans* position than in a *cis* orientation.
Figure 67: NOESY spectrum (CDCl₃, 300 MHz) of bacteriogorgonene A (81).

![NOESY spectrum](image)

Figure 68: The H H COSY(→) and HMBC (→→) correlations of bacteriogorgonene A (81).

ESIMS showed two masses at $m/z$ 236 and 218 Dalton, and HRMS established the molecular formula as C₁₅H₂₃O for compound 82, which was one molecule of water less than compound 81. In the $^1$H NMR spectrum, one proton was observed at $\delta$ 5.90, together with another peak at $\delta$ 5.95, where the integration was, however, not fitting; this suggested a mixture with another compound. This was established indeed by the $^{13}$C NMR spectrum where two $sp^2$ carbon signals were detected at $\delta$ 99.1 and 113.5 which were not part of compound 81. The second compound had obviously one double bond more than 81. Since only one hydroxyl group was present in compound 81 at position 9, there were two possibilities to loose a water molecule. If the proton was taken from position 10 it would create a terminal alkene which would be less stable than that with the double bond in position 5. Therefore, the most plausible structure for the compound with $m/z$ 218 was 82.
4.7.2 Bacteriogorgonene B

Another similar sesquiterpene was isolated from subfraction 2b, which was obviously closely related with the previous compounds. There was an additional oxygenated methine proton observed in the $^1$H NMR spectrum of compound 83 at $\delta$ 4.21, which was not visible in 81. In the $^{13}$C NMR spectrum there were three quaternary $sp^3$ carbon signals appearing at $\delta$ 84.6, 81.1 and 67.7, whereas in compound 81, two carbon signals were observed in this region. This fact was also supported by the $^1$H NMR spectrum, which showed an additional methine attached to oxygen. HRESIMS established the molecular formula as C$_{15}$H$_{22}$O$_3$.

Figure 69: $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of bacteriogorgonene B (83).

The methine carbon signal at $\delta$ 42.9 in compound 81 was replaced by a quaternary carbon at 84.6. At $\delta$ 67.7 there was another quaternary $sp^3$ carbon signal observed which could also be connected with oxygen. In compound 81 there were 4 double bonds equivalents whereas the molecular formula C$_{15}$H$_{22}$O$_3$ of compound 83 showed
five equivalents. Therefore, one oxygen atom was attached to both of the carbon atoms at $\delta$ 84.6 and 67.7. One carbonyl at $\delta$ 199.3 and an oxygenated methine at $\delta$ 81.1 were also present in $^{13}$C NMR spectrum of 83 which were also observed in the similar area in compound 81.

![13C NMR spectrum of bacteriogorgonene B (83).](image1)

**Figure 70:** $^{13}$C NMR (CDCl$_3$, 125 MHz) spectrum of bacteriogorgonene B (83).

![H-H COSY spectrum of bacteriogorgonene B (83).](image2)

**Figure 71:** H-H COSY spectrum (CDCl$_3$, 600 MHz) of bacteriogorgonene B (83).

In the HMBC spectrum, the proton attached at carbon 6 showed a $^3J$ coupling with the carbon at $\delta$ 84.6. This proton also showed a COSY correlation with the methylene attached with carbon 7 in compound 83, while the methylene at carbon 7 showed correlations with carbon 4a, 9 and 11. These correlations were similar to those observed in compound 81.
Figure 72: HMBC spectrum (CDCl$_3$, 600 MHz) of bacteriogorgonene B (83).

Figure 73: NOESY spectrum (CDCl$_3$, 300 MHz) of bacteriogorgonene B (83).

With the help of 1D and 2D NMR as well as the correlation of 81, structure 83 was established.
4.7.3 Bacteriogorgonene C

Compound 84 was isolated as a colourless oil and separated from the same fraction 2D as 83. The mass was determined by CIMS as $m/z$ 270 [M + NH$_4$]$^+$. The $^1$H NMR spectrum had similarity to the other three sesquiterpenes, which already have been described. However, no oxymethine was observed in compound 84. HRESIMS delivered the molecular formula C$_{15}$H$_{24}$O$_3$.

This compound presented one more oxygen atom than the compound 81. The $^1$H NMR spectrum showed no proton signals in the region between $\delta$ 2.90-5.90 as in compound 82. The $^{13}$C NMR spectrum showed a carbon signal at $\delta$ 82.2 along with the quaternary carbon at $\delta$ 74.4 as in compound 81.
Figure 76: $^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) of bacteriogorgonene C (84).

H-H COSY spectrum showed similar correlations as found in compound 81, but in compound 84, C-4a was a quaternary carbon, while it was a methine carbon in compound 81.

Figure 77: H-H COSY spectrum (CDCl$_3$, 600 MHz) of bacteriogorgonene C (84).

In the HMBC spectrum the methyl at $\delta$ 1.90 showed a $^3J$ coupling with the oxygenated quaternary carbon at $\delta$ 82.2. The other HMBC correlations were similar to compound 81. With the help of 1D and 2D spectra the structure 84 was elucidated.$^{101-102}$
Gorgonene terpenes are a rare class of sesquiterpenes in comparison to other terpenes such as selinane, cadinane, calamane, eudesmane, amorphane etc. Gorgonene terpenes possess an isopropyl group in the unusual C-6 position and are apparently biosynthesised from two tail-to-tail fused isoprene units. The few known gorgonene terpenes were isolated from plant sources. This is the first report of bacteria producing gorgonene sesquiterpenes.

During the last two decades, sesquiterpenoids and their biological activities have been the focus of numerous phytochemical, pharmacological and synthetic studies. Because sesquiterpenes exhibit a wide range of biological activities, such as plant growth regulators, insect antifeedant, antifungal, anti-tumour and antibacterial activities, there has been much interest in relating structure and the relation between oxy-
genation pattern and function. Sesquiterpenes are common metabolites from plant or fungi, and only a few have been isolated from microorganism.

Fraction 3 and 4 constituted the major portion of this strain, which consisted of homononactic acid (86) and nonactic acid (87). Therefore, it could be concluded that the high biological activity of this strain was due to homononactic acid and its derivatives.

4.8 Terrestrial Streptomycete GW5127

This strain was cultivated on M2 medium using tap water. A 25 litre shaker culture of the terrestrial streptomycete strain GW5127 was to incubate a 28 °C using M2 medium. The fermentor broth was harvested after 8 days, mixed with Celite, and then filtered. The filtrate and mycelia were subjected to extraction separately using XAD-2 for the water phase, followed by elution with MeOH/H2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times). The extracts yielded three new metabolites, i.e. 9-ethyl-6,11-dihydroxy-4-methoxy-naphthacene-5,12-dione (88), 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (90), and N-[2-(3-hydroxy-4-methoxy-phenyl)-ethyl]-acetamide (93) along with three known compounds, 4-hydroxy-3-methoxy-tyrosol (94), β-rubromycin (95) and γ-rubromycin (96).

Silica gel column chromatography of the mycelial fraction followed by PTLC and size exclusion chromatography yielded β-rubromycin and γ-rubromycin as red solids. The extract from the water phase was separated into four fractions on silica gel. PTLC of fraction II followed by Sephadex LH-20 delivered compound 88 as a red solid. The middle polar fraction III (0.25 g) yielded on Sephadex LH-20 compound 90 and 3-hydroxy-4-methoxy-β-phenylethyl alcohol (94) as colourless solids. The polar fraction IV yielded on Sephadex LH-20 N-[2-(3-hydroxy-4-methoxy-phenyl)-ethyl]-acetamide (93) as colourless oil.
4.8.1 13-Deoxy-1,3-dihydrodaunomycinone

EIMS of compound 88 showed a molecular ion at \( m/z \) 348, and HREIMS revealed the molecular formula as \( \text{C}_{21}\text{H}_{16}\text{O}_{5} \) corresponding to 14 double bond equivalents. The UV spectra (MeOH) of 88 displayed four strong bands at \( \lambda_{\text{max}} \) 249, 310, 542 and 577 nm in neutral solution. Under basic methanol conditions, the latter band showed a bathochromic shift to \( \lambda_{\text{max}} \) 621 nm.

The \(^1\text{H}\) NMR spectrum of 88 exhibited two singlets of chelated hydroxyl groups at \( \delta \) 16.12 and 15.30. In the aromatic region, two spin systems were observed, the signals of a 1,2,3-trisubstituted benzene ring at \( \delta \) 8.13, 7.74 and 7.29, and a second pattern of a 1,2,4-trisubstituted aromatic system at \( \delta \) 8.39, 7.63, and 8.26. In the aliphatic region, the signals of a methoxy group (\( \delta \) 4.08) and an ethyl group (\( \delta \) 2.86, q; 1.38, t) were also detected.

The \(^{13}\text{C}\)/APT experiment afforded 21 carbon signals, which were classified into 12 quaternary carbons, 6 methines, 1 methylene and 2 methyl carbons. These data were comparable with those reported for a tetracycline, 1,3-dihydrodaunomycinone (88)\(^{9-10}\) with only one exceptions. In the reference compound, C-13 contained a hydroxyl
group, which was missing in compound 88 and therefore, the combination of C-13 and C-14 composed an ethyl group.

**Figure 81:** $^{13}$C NMR spectrum (CDCl$_3$, 300 MHz) of 13-deoxy-1,3-hydrodaunomyacinone (88).

The downfield shift of the carbon and HMBC couplings with aromatic carbon signals indicated the direct linkage to an aromatic system.

**Figure 82:** H-H COSY spectrum (CDCl$_3$, 500 MHz) of 13-deoxy-1,3-dihydrodaunomycinone (88).

These data and the $^{13}$C NMR spectrum indicated clearly that 88 was a homologue of 6,11-dihydroxy-4-methoxy-9-methyl-naphthacene-5,12-dione (89), a quinone isolated recently from *Streptomyces* sp. GW10/1811. Structure 88 was confirmed by further HMBC data. A number of similar compounds have been reported from bacterial sources, and some of which are reported to show potent antitumor and antibiotic activities. Tetracenequinones can be formed as artefacts from corresponding anthracyclines, which are dehydrated easily during work-up, and so both types are often iso-
lated together. It is worth to mention therefore, that neither feudomycines, nor feudomycinones or other anthracyclines were detectable here.

Figure 83: HMBC spectrum (CDCl₃, 500 MHz) of 13-deoxy-1,3-dihydrodaunomycinone (88)

Figure 84: The HMBC correlations of 13-deoxy-1,3-dihydrodaunomycinone (88).

4.8.2 4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one

The UV spectra of a second compound C₈H₇N₃O₂ (HREIMS) showed two maxima at 262 and 316 nm of an aromatic system. The ¹H NMR spectrum displayed only three proton signals at δ 8.26 (d, J = 5.1 Hz, 1H), 7.23 (d, J = 5.1 Hz, 1H) and δ 2.68 (s, Me).
In the $^{13}$C NMR spectrum, two aromatic methine signals were found at $\delta$ 142.7 and 108.8; a methyl at $\delta$ 25.9 and 5 quaternary carbon signals appeared at $\delta$ 201.4, 157.6, 139.6, 135.0, and 128.5.

The presence of a benzene ring was ruled out on the basis of the low coupling constants (5.1 Hz) of two methine doublets; also because of the downfield shifts, a pyridine system was more plausible. An acetyl moiety was established on the basis of the methyl singlet in the $^1$H NMR spectrum and the corresponding carbon signals at $\delta$ 25.9 as well as a carbonyl signal at $\delta$ 201.4. The shift of the remaining carbon atom ($\delta$ 157.6) suggested a carbonyl group, which would form a 1,3-dihydro-imidazo[4,5-c]pyridin-2-one (90) or a 1,3-dihydro-imidazo[4,5-b]pyridin-2-one (91) unit with the residual two NH fragments and the pyridine ring.
Both alternatives explained the CO shift and also the additional IR CO signals at 1685 ($\alpha$,$\beta$-unsaturated CO) and 1619 cm$^{-1}$ (NH-CO-NH). The data are comparable to the values of urea, which gives intense absorption bands at $\nu \sim$1680 and 1620 cm$^{-1}$.

One of the two $\alpha$-coupling protons was connected with a downfield-shifted carbon atom ($\delta$ 8.26, d, $J$ = 5.1 Hz) and must therefore be placed in $\alpha$-position with respect to the pyridine nitrogen. This suggested that the acetyl group must be attached to C-4 or to the other $\alpha$-position. The resulting isomers 90/91 were, however, indistinguishable on the basis of spectroscopic data, and only an indirect argument was favouring a 2-acetyl-pyridine, that is the missing HMBC coupling between the aromatic protons and the acetyl-CO signal. The structure was confirmed on the basis of syntheses, which finally excluded structure 91. Compound 90 was later obtained also from the marine *Streptomyces* isolate B7967.106
A few simple dihydroimidazolones have been found linked to sugar moieties, e.g. in ureidobalhimycin, a glycopeptide antibiotic. Anellated imidazolones are rare in nature, and not more than \( \text{ca.} \) 40 derivatives have been reported so far, well-known purine derivatives like uric acid or guanin-8-ones being the predominant examples. The closest similarity with \( \text{90} \) is shown by the azobactin chromophor \( \text{92} \).

Many imidazolones exhibit activity against gram-positive and gram-negative bacteria, are herbicidal or act as biotin antimetabolites and are antifungal agents. In our tests, \( \text{90} \) was inactive against microorganisms (\textit{Staphylococcus aureus}, \textit{Escherichia coli}, \textit{Bacillus subtilis}, \textit{Streptomyces viridochromogenes}, \textit{Candida albicans}, \textit{Mucor miehei}), and showed no cytotoxicity (brine shrimp test).

### 4.8.3 \( N-[2-(3-Hydroxy-4-methoxy-phenyl)-ethyl]-acetamide \)

The \(^1\text{H}\) NMR data of compound \( \text{93} \) resembled those of N-acetyl-tyramine and showed broad singlets of \( \text{D}_2\text{O}\)-exchangeable protons at \( \delta \) 6.16 (OH) and 5.75 (NH), an acetyl group and an ethanediyl fragment. The signal pattern of a 1,2,4-trisubstituted benzene ring and an additional methoxy signal suggested 3-methoxy-N-acetylttyramine or the isomeric \( N-[2-(3-hydroxy-4-methoxy-phenyl)-ethyl]-acetamide \) (\( \text{93} \)), in accordance with the molecular formula by EIHRMS.
Figure 89: $^1$H NMR (CD$_3$OD, 300 MHz) spectrum of 3 $N$-[2-(3-hydroxy-4-methoxyphenyl)-ethyl]-acetamide (93).

The $^{13}$C/APT NMR spectrum of 93 afforded carbon signals which were identified as four quaternary carbons, three methines, 2 methylenes and two methyls.

Figure 90: $^{13}$C NMR (CD$_3$OD, 125 MHz) spectrum of $N$-[2-(3-hydroxy-4-methoxyphenyl)-ethyl]-acetamide (93).

Structure 93 was fully confirmed by the 2D NMR spectra. It is a synthetically known neuroprotectant and a metabolite of L-DOPA in rats, but this is the first report from a natural source.107
4.8.4 4-(2-Hydroxyethyl)-2-methoxy-phenol

The colourless UV active compound 94 showed a molecular weight of \(m/z\) 168 by EIMS. The \(^1\)H NMR spectrum showed three protons attached to \(sp^2\) carbon atoms at \(\delta\) 6.80 (2H) and 6.72 (1H) of an aromatic ring. There was a singlet of methoxy protons at \(\delta\) 3.90. Two methylene triplets appeared at \(\delta\) 3.83 and 2.75, and an acidic proton (H/D exchangeable) gave a broad singlet at \(\delta\) 5.60. With the help of EIMS and \(^1\)H NMR data, a search in AntiBase identified this compound as 4-(2-hydroxyethyl)-2-methoxy-phenol (94).
TLC of the mycelium phase showed a major red compound, which did not change colour on spraying with anisaldehyde/sulphuric acid. On purification by PTLC followed by Sephadex LH-20, the metabolite was obtained as a red powder. The (+)-ESI mass spectrum indicated an [M +Na]$^+$ ion at $m/z$ 559. In the $^1$H NMR spectrum of this compound, two broad singlets of chelated hydroxyl groups were observed at $\delta$ 12.50 and 10.66, three aromatic $1H$ singlets were found at $\delta$ 7.60, 7.22 and 7.04, three singlets in the range of $\delta$ 4.00-3.80 were attributed to three methoxy groups, three methylene groups were seen in the aliphatic region. A search with this information in AntiBase led to the identification as $\beta$-rubromycin, which was confirmed by comparison with authentic spectra from our collection as well as published data from the literature.\textsuperscript{108}
The red antibiotic $\beta$-rubromycin (95) was isolated as a mixture with $\gamma$-rubromycin by Brockmann et al. from *Streptomyces collinus*. Its structure was determined by degradation reactions, and the two carbonyl groups were initially placed in *ortho* position. Further structural and biosynthetic investigations led to the revision of its structure into 95.109

**Figure 94:** $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of $\beta$-rubromycin (95).

**Figure 95:** $^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) of $\beta$-rubromycin (95)
4.8.6  γ-Rubromycin

A second quinone 96 was also obtained as a red powder from the same mycelium phase, which exhibited similar characteristics as 95. The EI mass spectrum indicated a molecular weight of \( m/z \) 522 and the mass difference of \( \Delta m = 15 \) indicated the loss of one methyl group. The \(^1\)H NMR spectrum was also very similar to that of β-rubromycin, but in addition to the two chelated hydroxyl groups, a further one appeared at \( \delta \) 13.14. In the aliphatic region the major difference was the presence of only two methoxy signals. The compound was identified as γ-rubromycin (96), which always occurs in mixture with β-rubromycin (95).

\[
\begin{align*}
&\text{6'} &\text{9'} &\text{1'} &\text{3'} \\
&\text{H}_2\text{C} &\text{O} &\text{O} &\text{H} \\
&\text{O} &\text{O} &\text{OH} &\text{OH} \\
&\text{1} &\text{3} &\text{5} &\text{10} \\
&\text{HO} &\text{HO} &\text{H}_2\text{C} &
\end{align*}
\]

Figure 96: \(^1\)H NMR spectrum (CDCl₃, 300 MHz) of γ-rubromycin (96).

\(\beta\)-Rubromycin (95) and γ-rubromycin (96) have been reported to show a marked growth inhibition of Bacillus subtilis, Staphylococcus aureus, Staphylococcus albus, Escherichia coli and to be active against other Gram positive germs.
Figure 97: $^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) of $\gamma$-rubromycin (96).

4.9 Terrestrial Streptomyces sp. GW 18/1811

The crude extract of the terrestrial Streptomyces sp. GW 18/1811 obtained from a 1 L culture exhibited biological activity against Streptomyces viridochromogenes (Tü 57), Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Chlorella vulgaris, Chlorella sorokiniana, Candida albicans and Mucor miehei. TLC of the crude extract showed two relatively non-polar orange spots, however, on spraying with anisaldehyde/sulphuric acid, several colourless zones without UV absorption became visible as brown and violet spots. A 20 shaker culture in M$_2$ medium became brown-yellow after four days of growth and delivered on work-up 1.8 g of a dark brown crude extract, which after defatting was chromatographed on silica gel and pre-separated into four fractions by TLC.
4.9.1 Komodoquinone B

Compound 97 was isolated as orange-red solid, which showed UV-absorbance at 254 nm and orange fluorescence at 366 nm. Addition of sodium hydroxide on the TLC plate gave a colour change to violet pointing a peri-hydroxyquinone, while addition of anisaldehyde/sulphuric acid did not produce remarkable colour change. ESIMS showed a quas-molecular ion peak at m/z 379 [M +Na]+. The 1H NMR spectrum indicated the presence of one methyl group at δ 1.36. There were two chelated H/D exchangeable protons at δ 13.40 and 12.20 as broad singlets and two methylene groups near two chiral centres at δ 3.02, 2.53, 2.05 and 1.84. Also one oxygenated methine at δ 4.94 and a hydroxyl at δ 5.28 were observed. The 1H NMR spectrum led us to construct the partial structures assignable to the A-ring and D-ring parts of an anthracycline.

Figure 99: Partial structures A and D of compound 97.
Figure 100: $^1$H NMR spectrum ([D$_6$]DMSO, 300MHz) of Komodoquinone B (97).

With the help of the mass and $^1$H NMR data, a search in AntiBase led to komodoquinone B (97), which was confirmed with the data of the published literature. Komodoquinone B (97) was isolated for the first time in 2003 as novel neuritogenic anthracycline from a marine Streptomyces sp. KS3. Komodoquinones belong to the rather rare class of 1,10-unsubstituted 9-methyl-anthracyclines.

4.9.2 Feudomycinone C

Compound 98 was isolated as an orange solid, which showed UV absorbance at 254 nm and an orange fluorescence at 366 nm. Addition of sodium hydroxide on the TLC plate changed the orange colour to violet pointing a peri-hydroxyquinone. Compound 98 was also isolated from the same fraction as Komodoquinone B (97) but showed a difference in the polarity on TLC. ESIMS showed an ion peak at m/z 393 [M + Na]$^+$. The $^1$H NMR spectra showed similarity with the $^1$H NMR of komodoquinone B (97) except for the presence of one methoxy group at $\delta$ 4.11 for compound 98. There were two chelated hydroxyl singlets at $\delta$ 14.10 and 13.32. $^1$H NMR led to the partial structures assignable to the A-ring and D-ring parts of anthracycline. With the help of the mass and $^1$H NMR data, a search in AntiBase led to feudomycinone C (98). The structure was confirmed by comparison of data with those in the literature.
Figure 101: $^1$H NMR spectrum ([D$_6$]DMSO, 300MHz) of Feudomycinone C (98).

4.9.3 $\alpha$-Hydroxyacetovanillone

Compound 99 was isolated as colourless UV absorbing oil with an $R_f$ value of 0.43 (CH$_2$Cl$_2$/5% MeOH). EIMS spectrum showed the mass as 182. From the $^1$H NMR spectrum, the presence of an ABX system ($\delta$ 7.52, 7.43, and 6.96) was observed. There was also one oxygenated methylene at $\delta$ 4.81 and one methoxy group at $\delta$ 3.95.
In the $^{13}$C NMR spectrum, there were ten carbon signals visible, one carbonyl at $\delta$ 196.7, two oxygenated $sp^2$ carbons at $\delta$ 151.4 and $\delta$ 146.9 as well as further 4 $sp^2$ carbons. Additionally, one methoxy group at $\delta$ 56.2 and a methylene carbon at $\delta$ 64.9 were present.
Figure 104: EIMS fragmentation pattern of α-hydroxyacetovanillone (99).

The fragments from EIMS were helpful in determining the structure of compound 99. The molecular peak appeared at m/z 182 Dalton. At m/z 151, the peak was [M - OCH₃]⁺, m/z 136 [M – OH - OCH₃]⁺, m/z 123 [M - COCH₂OH]⁺ (Figure 104). By using the NMR data and mass spectrum, the compound was searched in the Dictionary of Natural Products, which resulted in compound 99. The 1D and 2D correlations confirmed the structure as 3-methoxy-4-hydroxy-acetophenone (α-hydroxyacetovanillone, 99). Compound 99 was previously isolated from plants¹¹³ but there was no report from bacterial sources.
Figure 105: H-H COSY spectrum (CDCl₃, 500 MHz) of α-hydroxyacetovanillone (99).

α-Hydroxyacetovanillone derivatives, e.g. cis-N-p-coumaroyloctopamine (100), are known as wound inducible compounds, which activate virulence genes of Agrobacterium tumefaciens, especially in species within the Solanaceae family.
4.9.4  *N*-Acetylhomocysteine thiolactone

Compound 101 was isolated as non-UV absorbing colourless needles from fraction 4. ESIMS showed the mass as *m/z* 159. $^1$H NMR spectrum displayed one H/D exchangeable proton as a broad singlet at $\delta$ 6.10. The methyl at $\delta$ 2.05 was assumed to be a part of an acetyl group or to be attached to an $sp^2$ carbon atom or a nitrogen atom. The proton at $\delta$ 4.55 indicated its attachment to a hetero atom. The pattern of the protons at $\delta$ 1.90 and 2.95 suggested a methylene group near a chiral centre. Another methylene group appeared at $\delta$ 3.30 as a multiplet and was obviously not beside a chiral centre.
The $^{13}$C NMR spectrum showed only 6 carbon signals. There was a carbonyl signal of an aliphatic ketone observed at $\delta$ 205.8, a carbonyl signal of an ester, amide or acid at $\delta$ 170.8 as well as a carbon at $\delta$ 59.4, which was near a hetero atom, and three additional carbon signals at $\delta$ 31.7, 27.5 and 23.0.

Based on NMR and mass data, a search in AntiBase gave a thiolactone structure 101. $N$-Acetylhomocysteine thiolactone (101) is commercially available and is included in some medical preparations. It is also used as a reagent for insolubilizing antibodies.
4.9.5 Lysolipin I

Compound 102 was isolated as UV absorbing bright yellow needles from the third fraction after silica column chromatography followed by Sephadex LH-20. (+)-ESIMS showed the molecular peaks at m/z 597 and 599 in the mass spectrum. The height of the signal at 599 Dalton was 33% in comparison to 597 in the mass spectrum which was a clear indication that the compound contained chlorine. The $^1$H NMR spectrum showed two chelated hydroxyl group at $\delta$ 13.13 and 12.93, as well as three aromatic protons at $\delta$ 7.94, 7.37 and 7.09. There were two protons observed at $\delta$ 5.63 and 5.40 and four methine protons at $\delta$ 5.03, 5.02, 4.70 and 4.46, which were either attached with $sp^2$ carbon or with heteroatoms. Also three methyl signals were seen at $\delta$ 4.18, 3.55, and 3.37 (6H).

![Figure 110: $^1$H NMR (CDCl$_3$, 300 MHz) of lysolipin (102).](Image)

From $^{13}$C NMR spectrum there were 29 carbons: two carbonyls at $\delta$ 181.6 and 168.2, seven oxygenated quaternary $sp^2$ carbon signals were shown at $\delta$ 158.9, 151.6, 149.9, 145.1, 143.6, 139.6, and 139.0 and eleven more in the $sp^2$ region. From the data a search in AntiBase led to the identification as lysolipin I (102). The spectroscopic data were identical with the those previously published.$^{118,119}$
Figure 111: $^{13}$C NMR (CDCl$_3$, 125 MHz) of lysolipin (102).
5 Fungal metabolites

5.1 Bikaverin

Compound 103 was isolated as a deep red solid from a crude sample sent by Prof. Dr. H. Anke, Institute for Biotechnology and Drug Research, Kaiserslautern. It was insoluble in methanol, chloroform, acetone, pyridine and even in DMSO. The $^1$H NMR spectrum was measured in a mixture of chloroform and trifluoroacetic acid. The $^1$H NMR spectrum showed three olefinic protons at $\delta$ 7.39, 7.35 and 6.80. There were two methoxy and one methyl group at $\delta$ 4.16 and $\delta$ 2.97, respectively.

![Figure 112: $^1$H NMR spectrum (300 MHz, 50% CDCl$_3$ + 50% TFA) of bikaverin (103).](image)

In the $^{13}$C NMR spectrum there were 20 carbons visible. There was one methyl signal at $\delta$ 23.4, two methoxy at $\delta$ 57.5 and 58.0, which were also obvious from the $^1$H NMR spectrum. All other carbon signals were in the $sp^2$ region. There were probably 4 carbonyls at $\delta$ 179.5 178.2, 172.0 and 166.1. The $^1$H NMR and $^{13}$C NMR spectra and mass data showed identity with the literature values for bikaverin (103).
Figure 113: $^{13}$C NMR spectrum (75 MHz, CDCl$_3$ + 50% TFA) of Bikaverin (103).

Bikaverin (103) was previously isolated from Fusarium oxysporum.$^{120}$ Its benzoxanthone ring system is very rare among natural products. Due to its high bioactivity it has been synthesized and the way of biosynthesis has also been investigated by sev-
eral research groups.\textsuperscript{121} McInnes A. G. \textit{et al.}\textsuperscript{122} have found that bikaverin is an acetogenin and postulated a biosynthetic pathway.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{bikaverin_pathway.png}
\caption{Suggested biosynthetic pathway of bikaverin (103) according to McInnes.\textsuperscript{122} Bold lines are indicating the acetate units.}
\end{figure}
6 Plant Metabolites from *Tephrosia* sp.

Twenty five samples from the plant *Tephrosia calophylla* were obtained from one of our research collaborator, Professor Seru Ganapaty from the Pharmacognosy and Phytochemistry Division, Andhra University, India. Twentyfive different compounds were confirmed in these samples; 21 of them were known and 4 were new compounds including the new benzil derivative calophione A (104).

*Tephrosia* is a tropical and subtropical genus of *Leguminosae* in the *Fabaceae* family, which forms perennial woody shrubs. Between 300 and 400 species are known, of which 35 occur in India, 30 are native to South America, 70 are found in South Africa and 50 in equatorial Africa. *Tephrosia calophylla* is a perennial undershrub found widely in Andhra Pradesh, south India. Many species in the *Tephrosia* genus are poisonous, particularly to fish, for their high concentration of rotenone. *Tephrosia* species have historically been used by many indigenous cultures as fish poisons. In the last century, several *Tephrosia* species have been studied in connection with the use of rotenone as an insecticide and pesticide. The plants are also used traditionally in folk medicine. According to Ayurveda, the plant is useful as an anthelmintic, antipyretic and as well as an alexiteric drug. It is also active against leprosy, ulcers, and used as alternative cures for diseases of the liver, spleen, heart and blood. According to the Unani system of medicine, the root is diuretic, allays thirst, enriches blood, cures diarrhoea, is useful in bronchitis, inflammations, boils and pimples. Leaves are tonic to intestines and a promising appetizer. The plants prevent the soil erosion and helps in fixing nitrogen. Their leaves are used as fodder and the seeds can be used as substitute for coffee.

6.1 Spinoflavanone B

Compound 103 was obtained as a yellow solid. The molecular weight was deduced from the (+)-ESI mass spectrum, which indicated a pseudomolecular peak at \( m/z \) 393 \([M + H]^+\); HRESIMS established the molecular formula \( \text{C}_{25}\text{H}_{28}\text{O}_4 \). The \(^1\text{H}\) NMR spectrum showed the resonances typical of a saturated flavanone ring \([7, 8]\). A set of peaks characteristic of two C-3-methylbut-2-enyl groups \([4, 5]\) was also observed in its spectrum. The \(^1\text{H}\) NMR spectrum showed two singlets, one at \( \delta \) 12.35 attributed to a chelated phenolic hydroxyl and another at \( \delta \) 6.39 for a free OH group. A broad singlet at \( \delta \) 7.44 integrating for five protons in the \(^1\text{H}\) NMR spectrum was attributed to a monosubstituted benzene ring.
Figure 116: $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of spinoflavanone B (103).

In $^{13}$C NMR spectrum, 17 $sp^2$ carbon signals were observed; one of them at $\delta$ 196.2 indicated the presence of a ketone or aldehyde and three appeared at $\delta$ 162.3, 159.3 and 157.6 indicating the attachment of oxygen. There was one methine carbon at $\delta$ 78.8, which is also obviously attached with oxygen. Three methylene carbons were observed at $\delta$ 43.4 (CH$_2$-3), 21.9 (CH$_2$-1", 1'") as well as 4 methyl carbons.

Figure 117: $^{13}$C NMR spectrum (CDCl$_3$, 75 MHz) of spinoflavanone B (103).
In the H-H COSY spectrum, two isoprenyl units could be confirmed from the correlation along with other fragments (A-C).

![H-H COSY spectrum (300 MHz) of spinoflavanone B (103) in CDCl₃.](image)

**Figure 118:** H-H COSY spectrum (300 MHz) of spinoflavanone B (103) in CDCl₃.

From HMBC spectrum, the methylene protons at δ 43.4 showed correlation with the only carbonyl group and with the phenyl group. The OH group δ 12.35 was likely to be acid or chelated with the carbonyl group. All information derived from ¹H NMR, ¹³C NMR, ESIMS and 2D spectroscopy supported the spinoflavanone B structure. The published spectroscopic and spectrometric data for spinoflavanone B¹²⁵ were identical with that of the obtained structure 103.
Figure 119: HMBC spectrum (300 MHz) of spinoflavanone (103) in CDCl₃.

Figure 120: The HMBC (→) and H-H COSY (▬) correlations of spinoflavone B (103).

6.2 Caliphione A

The molecular formula of compound 104 was obtained as white amorphous powder. HRESIMS 369.09699 [M + H]+ established the molecular formula as C₂₀H₁₇O₇. The ¹H NMR spectrum displayed resonances for two hydrogen-bonded hydroxyls at δ_H 12.24 (OH-6') and δ_H 12.06 (OH-6''), a singlet resonance for a methylenedioxy group at δ_H 6.00 (H-2') and four well-separated 1H singlets for aromatic protons, δ_H 7.23 (H-4''), δ_H 6.82 (H-4'), δ_H 6.53 (H-7') and δ_H 6.45 (H-7'') clearly indicating that this compound consisted of two tetra-substituted rings.
In addition, the $^1$H NMR spectrum exhibited an ABX system for a tri-substituted dihydrofuran ring comprising of an oxymethine at $\delta$ 5.30 coupled to the gem-methylene protons at $\delta$ 3.30 and signals for an exomethylene, $\delta$ 4.98 and 5.10 as singlets and a methyl singlet at $\delta$ 1.72 constituting the presence of an isopropenyl unit as part of the side chain.

The $^{13}$C NMR spectrum displayed resonances for two carbonyl signals at $\delta$ 194.0 and 193.5, together with signals corresponding to an oxymethine carbon ($\delta$ 88.3), an up-field methylene ($\delta$ 32.6), eight quartenary carbons and a down-field $sp^3$ carbon at $\delta$ 102.4, confirming to be associated with a benzil moiety, a benzofuran and a benzodioxo functionality.

From the H-H COSY spectrum the following substructure of the compound was elucidated.
In HMBC spectrum the methylenedioxy protons showed correlation with the carbon C-3'a, 7a ($\delta$ 141.2 and 156.4). The protons H-4', 7' ($\delta$ 6.82 and $\delta$ 6.53) attached at C-4', 7' ($\delta$ 98.9 and 108.0) showed correlation with both carbons at C-3'a, 7a ($\delta$ 141.2 and 156.4), indicating that they are in the same aromatic ring. The proton at H-4' ($\delta$ 6.82) also showed correlation with one oxygenated carbon at C-6' ($\delta$ 167.6) and C_q-5' ($\delta$ 109.4), while the proton H-7' ($\delta$ 6.53) showed correlation with one of the carbonyl at $\delta$ 194.0. From the value of the OH at $\delta$ 12.2 it was clear that it was chelated.

The HMBC spectrum showed another part (substructure C) with a similar pattern, which was connected with the substructures A and B already obtained from the H-H COSY spectrum.
Figure 124: HMBC spectrum (600 MHz) of caliphione A (104) in CDCl₃.

R = H or CH₃
From the 1D and 2D NMR data and comparison with those of published compound 106,126 structure 104 was confirmed.

### 6.3 Tephcalostan

Tephcalostan (107) was obtained as colourless needles and showed a *quasi*molecular ion peak at $m/z$ 363.08641 [M + H]$^+$ for C$_{21}$H$_{15}$O$_6$. The $^1$H NMR spectrum exhibited four aromatic singlet signals at $\delta$ 7.67, 7.43, 7.06, and 6.87, a methylenedioxy group at $\delta$6.05 and an isopropenyl dihydrofuran moiety, visible from two diastereotopic proton signals at $\delta$ 3.45, 3.13 (ABX), an oxygenated methine proton signal at $\delta$ 5.33 (ABX), two olefinic protons at $\delta$ 5.12 (7'-H$_a$), 4.94 (7'-H$_b$), and a methyl signal at $\delta$ 1.80 (8'-CH$_3$).

![Figure 125: $^1$H NMR spectrum (300 MHz) of tephcalostan (107) in CDCl$_3$.](image1)

![Figure 126: $^{13}$C NMR spectrum (75 MHz) of tephcalostan (107) in CDCl$_3$.](image2)

The $^{13}$C NMR spectrum showed 21 carbon signals, of which 19 were placed in the $sp^2$ region. Since there was a methylenedioxy group ($\delta$ 6.05), this carbon signal was also
observed in the \(sp^2\) region. Based on the \(^1\)H and \(^{13}\)C NMR spectra and the substructures in addition to the molecular formula the compound was searched in Dictionary of Natural Products and Chemical Abstracts. The 1D and 2D NMR data was identical to the published values of tephlocalostan.\(^{127}\)

![Chemical structure of Tephcalostan A](image)

\[107\]

### 6.4 Tephcalostan A

A further new tephcalostan derivative \(^{108}\) was obtained as colourless needles, which showed a \([M + H]^+\) peak at \(m/z\) 363.08641 for \(C_{21}H_{15}O_6\). The \(^1\)H NMR spectrum exhibited signals for four aromatic protons at \(\delta\) 7.80, 7.43, 7.28 and 6.96, a methylenedioxy group at \(\delta\) 6.13 and an allyl dihydrofuran moiety, differentiable from two diastereotopic proton signals \(\delta\) 3.58, 3.13 (\(ABX\)), an oxygenated methane proton signal \(\delta\) 5.43 (\(ABX\)) and two olefinic protons at \(\delta\) 5.45 (7'-\(H_a\)) and 5.28 (7'-\(H_b\)).

The \(^{13}\)C NMR spectrum showed 21 carbon signals, among which 19 were observed in the \(sp^2\) region. As the methylenedioxy group (\(\delta\) 6.05) is present, the respective carbon also appeared in the \(sp^2\) region (\(\delta\) 101.6).

![NMR spectrum](image)

**Figure 127:** \(^1\)H NMR spectrum (300 MHz) of tephcalostan A (\(^{108}\)) in [\(D_6\)]DMSO.
Further confirmation was obtained from HMBC correlations between the exocyclic methylene protons at $\delta$ 5.28 and 7'-H ($\delta$ 5.45) and the olefinic carbon C-6' ($\delta$ 136.5) and a $^2J$ coupling between the oxymethine, H-5' ($\delta$ 5.43) of the dihydrofuran ring and the $sp^2$ carbon at $\delta$ 136.5.
According to Chemical Abstracts this was a new compound. Tephlocastan A (108) was confirmed by comparing the 1D and 2D data with the related tephlocastan (109) from the same plant. NMR data are closely related to those of 109, which has one methyl group more in 6'-position.

Figure 131: HMBC spectrum (600 MHz) of tephcalostan A (108) in [D₆]DMSO.
6.5 Tephcalostan B and C

Tephcalostan B (111) and C (112) were white solids with poor solubility in all common organic solvents including DMSO. However, at 100 °C the compound was dissolved in DMSO. From the ESIMS spectrum, two masses were found at $m/z$ 379 ([M - H]$^-$) and 377 ([M - H]$^-$). HRESIMS delivered the formulas C$_{21}$H$_{15}$O$_7$ and C$_{21}$H$_{17}$O$_7$. In the $^1$H NMR spectrum from the integrations a mixture of two closely related compounds was assumed. From the mass difference and the $^1$H NMR spectrum it was revealed that the two compounds differed only by one double bond.

![Figure 132: 1H NMR spectrum (125 MHz) of tephcalostan B (111) and C (112) in [D6]DMSO.](image)

Also from the $^{13}$C NMR spectrum the evidence of two different compounds was clear. Due to the poor solubility and the similarity in the mass we did not try to separate them but the two compounds could be easily distinguished from the spectra. A search using HRESIMS and $^1$H NMR in the Dictionary of Natural Products and the Chemical Abstracts did not yield hits.

![Figure 133: $^{13}$C NMR spectrum (300 MHz) of Tephcalostan B (111) and C (112) in [D$_6$]DMSO.](image)
In $^1$H NMR and $^{13}$C NMR spectrum the intensity of the signals were not proportional indicating a mixture of compounds. From the HSQC and H-H COSY it was clear that this a mixture of two closely related compounds.

Figure 134: HSQC spectrum (600 MHz) of Tephcalostan B (111) and C (112) in [D$_6$]DMSO.

From the HMBC spectrum, a quaternary carbon at δ 157.2 showed no correlation at all. The methylenedioxy at δ 6.10 showed correlation with C-6 and C-7. Additionally, the protons at H-5 and H-8 showed correlations with C-6 and C-7. The proton at H-5 showed two more correlations with C-3 and C-4. The two methyl groups at δ 1.36 showed correlation with carbon signal at C-2". The olefinic proton at H-5" also showed correlation with C-2". Considering all the HMBC correlations and with the help of COSY unit A was drawn. Unit B showed the similar correlations except the olefinic proton δ 6.02. This bond was saturated. Taking all the substructures, 1D and 2D NMR compounds 111 and 112 were drawn. They were further confirmed by comparison with the related isoglycyrol (110).$^{128,129}$

Figure 135: Fragment of tephcalostan B (Unit A).
**Figure 136:** Fragment of tephcalostan C (Unit B).

**Figure 137:** HMBC spectrum (600 MHz) of tephcalostan B (111) and C (112) in [D$_6$]DMSO.
6.6 Milletone

Compound 113 was obtained as yellow needles, showed a [M + H]$^+$ peak at 379.11766 [M + H]$^+$ established the molecular formula C$_{21}$H$_{19}$O$_6$ by HRESIMS. The $^1$H NMR spectrum exhibited signals for six aromatic protons, while a methylenedioxy group was found at δ 5.85. From the molecular formula there were 13 double bond equivalents calculated. The shift of two methine protons at δ 4.90 and 3.79 indicated the attachment of oxygen. There were 2 methyl singlets and one methylene at δ 4.62 and 4.17 also observed in $^1$H NMR spectrum.
Figure 138: $^1$H NMR spectrum (300 MHz) of milletone (113) in CDCl$_3$.

In the $^{13}$C NMR spectrum there was a carbonyl at $\delta$ 189.1, which is typical for flavones. There were five $sp^2$ carbons, which seemed to be attached to oxygen. There were also 3 $sp^3$ carbon signals at $\delta$ 77.7 (C$_q$), 72.1 (CH), and 66.2 (CH$_2$).

Figure 139: $^{13}$C NMR spectrum (125 MHz) of milletone (113) in CDCl$_3$.

Five fragments (A-E) were derived from the H-H COSY spectrum. Substructures A and B were two pairs $o$-coupled olefinic protons, substructure C and D were also olefinic but without neighbouring proton.
In the HSQC spectrum a methylenedioxy group ($\delta$ 5.85) was observed. However, it was displayed as a doublet in $^1$H NMR spectrum, which was assumed to be due to 2 singlets of two different conformers in the ratio 1:1.

In the HMBC spectrum the methylenedioxy group at $\delta$ 5.85 showed correlations with signals at C-3 ($\delta$ 147.7) C-2 ($\delta$ 142.2). Two aromatic protons at H-1 ($\delta$ 6.75) and H-4 ($\delta$ 6.41) also showed correlation with the same carbons indicating that they were in the same ring. As a result, the substructure A was derived from the HMBC spectrum.
By using the HRMS, H-H COSY and HMBC spectra and the substructure A, the compound was searched in Dictionary of Natural Products and Chemical Abstract. The structure was confirmed as milletone after comparing with spectroscopic data published in the literature.\textsuperscript{130}

![Diagram showing the structure of Glabranine](image)

**Figure 142:** HMBC spectrum (600 MHz) of milletone (\textbf{113}) in CDCl\textsubscript{3}.

### 6.7 Glabranine

Glabranine (\textbf{114}) was isolated as a colourless solid. The $^1$H NMR spectrum showed the resonances typical for the saturated ring of a flavanone. A set of peaks characteristic of a C-3-methylbut-2-enyl subunit was observed at $\delta$ 5.22 (t), $\delta$ 3.32 (d, CH\textsubscript{2}) and $\delta$ 1.71 (CH\textsubscript{3})\textsubscript{2}). The $^1$H NMR spectrum showed two singlets, one at $\delta$ 12.00 attributed to a chelated phenolic hydroxyl and another at $\delta$ 6.55 attributed to a phenolic hydroxyl. A broad singlet at $\delta$ 7.45 integrating for five protons was attributed to the unsubstituted B-ring.
In the $^{13}$C NMR spectrum 17 $sp^2$ carbons were observed, among which that at $\delta$ 196.3 indicated the presence of a ketone or aldehyde and three at $\delta$ 163.8, 162.2, and 159.7 indicated the attachment of oxygen. There was also one methine carbon at $\delta$ 78.9, which is also obviously attached to oxygen. Two methylene carbons were observed at $\delta$ 43.2, and 21.8 (two) as well as 2 methyl carbons at $\delta$ 25.8 and 17.8. The compound was identified as glabranine (114) by comparing the 1D and 2D NMR data with the literature.\textsuperscript{131}
6.8 Betulinic acid and Betulinic acid methyl ester

The mass spectrum of compound 115 showed a molecular ion at $m/z$ 456 by EIMS, which was in agreement with the molecular formula $C_{30}H_{48}O_3$. Compound 116 was prepared by methylation of 115 with diazomethane. The $^1$H and $^{13}$C NMR data suggested that this compound could be a triterpene. This fact was confirmed by the similarity of the $^1$H and $^{13}$C NMR spectra of compound 115 with those of lupeol or betulinic acid (115). The $^1$H NMR spectrum of compound 115 revealed the presence of six tertiary methyl groups at $\delta$ 0.65, 0.77, 0.98, 0.87, 0.93 and an isopropenyl group at $\delta$ 1.65, 4.70 and 4.56. The $^{13}$C NMR spectrum showed the typical pattern $^{132}$ ($\delta$ 177.7, 150.8, 110.9, and 77.3) of a 3-hydroxy lupeol or betulinic acid derivative. The other carbon signals were found in the $sp^3$ region. To determine the position of the carboxylic acid, the compound was methylated with diazomethane. In the proton spectrum, the expected methoxy group was visible, but did not show any NOESY correlations. The spectra were, however, identical with those of spectra and published values of authentic betulinic acid (115), so that also identity of their structures was assumed.$^{133,134}$

Figure 145: $^1$H NMR (300 MHz) spectrum of betulinic acid (115) in [D$_6$]DMSO.
Figure 146: $^{13}$C NMR (125 MHz) spectrum of betulinic acid (115) in [D$_6$]DMSO.

Figure 147: The isoprenyl system was confirmed by from the H-H COSY spectrum.

Figure 148: H-H COSY spectrum (600 MHz) of betulinic acid (115) in [D$_6$]DMSO.
Stigmasterol and γ-Sitosterol

Stigmasterol (117) and γ-sitosterol (118) were isolated as a white crystalline mixture. From EIMS, the molecular weight was determined as \( m/z \) 414 and 412. It was thought that one of the compounds was present as impurity. In the \( ^1H \) NMR spectrum the multiplet at \( \delta \) 3.50 is typical for steroids with hydroxyl group at 3-position; at \( \delta \) 5.00-5.40 there was evidence of 3 \( sp^2 \) protons. Using the mass and \( ^1H \) NMR data, 3 olefinic protons were observed.
Figure 150: $^1$H NMR (300 MHz) spectrum of stigmasterol (117) and $\gamma$-sitosterol (118) in CDCl$_3$.

In $^{13}$C NMR spectrum, four carbon signals were appeared in the $sp^2$ region at $\delta$ 102-142, which indicated that there were at least two double bonds. There was the signal of an oxygenated methine carbon at $\delta$ 77.0. In $sp^3$ region, overlapping carbon signals appeared.

Figure 151: $^{13}$C NMR (125 MHz) spectrum of stigmasterol (117) and $\gamma$-sitosterol (118) in CDCl$_3$.

The NMR data were identical with those of a previously obtained stigmasterol (117) / $\gamma$-sitosterol (118) mixture.
6.10 2-Methoxymaackiain

Compound 122 was isolated as a yellow powder. In the $^1$H NMR spectrum showed signals at $\delta$ 3.45 (m) of one methane attached with a hetero atom and at $\delta$ 3.58 (t, $J = 10.8$ Hz), 4.20 (dd, $J = 5.4, 4.9$ Hz) one methylene also attached with one hetero atom. At $\delta$ 5.45 (d, $J = 6.9$ Hz), one proton was also observed which was either olefinic or attached to a hetero atom. The spectrum further showed the presence of a hydroxyl ($\delta$ 5.72), a methylenedioxy ($\delta$ 5.91) and a methoxy group ($\delta$ 3.91), and four aromatic $^1$H singlets ($\delta$ 6.43, 6.53, 6.71 and 6.92).

Figure 152: $^1$H NMR (300 MHz) spectrum of 2-methoxymaackiain (122) in CDCl$_3$.

In the $^{13}$C NMR spectrum, there were 17 signals visible. Thirteen of them appeared in the $sp^2$ region, and six of them were observed between $\delta$ 140-155 and could be connected with oxygen.
Figure 153: $^{13}$C NMR (125 MHz) spectrum of 2-methoxymaackiain (122) in CDCl$_3$.

One aromatic proton, a singlet at $\delta 6.92$, was assignable to H-1 after comparison with the proton signals of maackiain (119), 2-hydroxypterocarpin (120) and 2-methoxypterocarpin (121).
Figure 154: From the H-H COSY spectrum and based on $^{13}$C NMR values substructure A was derived.

Figure 155: H-H COSY spectrum (600 MHz) of 2-methoxymaackiain (122) in CDCl$_3$.

The methoxy protons at C-2 ($\delta$ 3.90) showed only one correlation with the carbon signal at C-2 ($\delta$ 142.1). Therefore, the methoxy group was attached at position C-2. The methylenedioxy protons showed correlations with carbon signals at C-8 and C-) ($\delta$ 148.1, 141.7).

Figure 156: HMBC spectrum (600 MHz) of 2-methoxymaackiain (122) in CDCl$_3$. 
The methoxy group showed only one correlation with C-3. The hydroxyl group showed correlation with C-4. The value of carbon signal C-2 was $\delta 142.1$, which is also attached to oxygen as there was no other hetero atom observed from HRESIMS. So, the hydroxyl group was attached with C-2. Using all the 1D and 2D NMR data, the compound was searched in the Dictionary of Natural Products and a pterocarpan, 2-methoxymaackiain was found identical. 2-methoxymaackiain was published$^{135,136}$ in the year 1990.

![Figure 157: The H-H COSY (→) and HMBC (→→) correlations of 122.](image)

### 6.11 Tephrosol (2-methoxymedicagol)

2-Methoxymedicagol (123) was isolated as amorphous powder, had NMR data similar to 2-methoxymaackiain. There were four aromatic protons at $\delta 7.41, 7.23, 7.16, 6.89$ and a methylenedioxy group at $\delta 6.12$ as well as a methoxy signal at $\delta 3.90$. HRESIMS gave the formula $C_{17}H_{11}O_7$ for $[M + H]^+$.

![Figure 158: $^1$H NMR (300 MHz) spectrum of 2-methoxymedicagol (123) in CDCl$_3$.](image)

$^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) showed 13 carbons signals. Among them, only one carbon signal was observed in the aliphatic region ($\delta 56.0$) for a methoxy group.
and a methylenedioxy group at δ 102.0. Four aromatic methine carbon signals were at δ 116.3, 103.6, 102.9 and 102.4 visible. The remaining carbon signals were due to quaternary \( sp^2 \) carbons.

**Figure 159:** \(^{13}\)C NMR (125 MHz) spectrum of 2-methoxymedicagol (123) in CDCl\(_3\).

In the HMBC spectrum, the methylenedioxy protons showed correlation with the carbon signal at C-8 and C-9. The only methoxy showed correlation with carbon signal C-4 and hydroxyl group showed correlation with carbon signal at C-4, which means the hydroxyl group is attached with carbon C-3 (δ 151.1). Compound 123 showed similarity in the 2D correlations with already described 2-methoxymaackiaien (122). But in compound 122 one methylene group is substituted by a carbonyl group to form a lactone. Compound 122 was confirmed as 2-methoxymedicagol (122).\(^{137,138}\)

**Figure 160:** HMBC spectrum (500 MHz) of 2-methoxymedicagol (123) in CDCl\(_3\).
6.12 Didehydrovillosin (mixture of two stereoisomers at C-6)

Didehydrovillosin (124) was obtained as pale yellow pellets. (+)-ESIMS and HRMS resulted in C_{23}H_{21}O_8 for [M + H]^+. The $^1$H NMR spectrum exhibited signals due to a chelated hydroxyl group (δ 13.20), three aromatic protons at δ 8.33, 6.75, and 6.44, an isopropenylidihydrofuran ring, two methoxy groups at δ 3.81 and 3.78 and a hemiacetal proton at δ 6.20. The $^1$H NMR data of this compound were similar to villinol, a rotenoid, which exhibits a methoxy group at δ 3.65, assigned to C-6. From the NMR data, it was clear that it was a mixture of two stereoisomers.
Didehydrovillosin (mixture of two stereoisomers at C-6)

The $^{13}$C NMR spectrum showed a signal at $\delta$ 88.5, which could be attributed to a hemiacetal carbon (C-6). All carbon signals appeared as twins, due to the presence of two similar isomers. In the $sp^3$ region, there were two methoxy signals at $\delta$ 56.0 and one methyl carbon signal at $\delta$ 56.0, a methylene and a methyl signal. All remaining carbon signals were present in the $sp^2$ region.

**Figure 161:** $^1$H NMR (300 MHz) spectrum of dehydrovillosin (124) in [D$_6$]DMSO.

**Figure 162:** $^{13}$C NMR (125 MHz) spectrum of dehydrovillosin (124) in [D$_6$]DMSO.

**Figure 163:** From H-H COSY spectrum substructure A was derived.
Figure 164: H-H COSY spectrum (600 MHz) of dehydrovillosin (124) in [D$_6$]DMSO.

The methoxy protons showed correlation with carbon signals C-2 and C-3. With the same carbon signals, the aromatic protons at H-1 and H-4 were correlating, but these two protons did not show COSY correlation. Therefore, they were in one benzene ring and the protons were in para-position as shown in substructure B. With the help of COSY and HMBC spectra, substructure C was developed.
Figure 165: HMBC spectrum (600 MHz) of dehydrovillosin (124) in [D$_6$]DMSO.

The hydroxyl at carbon 6 showed correlation with 4a and 12b. The proton at carbon 10 showed correlation with 8, 9 and 11a. The hydroxyl signal showed correlation with 10, 11 and 11a. The 1D and 2D NMR data were compared with the published values for to confirm the structure as didehydrovillosin (124).$^{139}$

6.13 Dehydrorotenone

Compound 126 was only sufficiently soluble in pyridine and showed (+)-ESIMS ions at m/z 807 ([2 M + Na]$^+$, 100) and 393 ([M + H]$^+$). The $^1$H NMR spectrum of this compound showed similarity with the phenolic rotenoids. In the $^1$H NMR spectrum there were four $sp^2$ signals at $\delta$ 9.32, 8.74, 7.41 and 7.21. There was also an oxygenated isoprenyl system at $\delta$ 5.85, 5.61 and 5.38, an oxygenated methylene at $\delta$ 5.50 (s, CH$_2$) and two methoxy groups at $\delta$ 4.30 and 4.17. There was another methylene at $\delta$ 3.83, 3.56 which was possibly near a chiral centre and a hetero atom. There was only one methyl at $\delta$ 1.87.
Figure 166: $^1$H NMR spectrum (300 MHz) spectrum of dehydrorotenone (126) in [D$_5$]pyridine.

In the $^{13}$C NMR spectrum there was a carbonyl of an ester or acid at $\delta$ 174.1, six oxygenated quaternary $sp^2$ carbons and 9 $sp^2$ carbons attached with hydrogen were observed. Two methoxy signals were also present $\delta$ 56.7 and 56.0.

Figure 167: $^{13}$C NMR (125 MHz) spectrum of dehydrorotenone (126) in [D$_5$]pyridine

From the H-H COSY spectrum, substructure A was derived.
Figure 168: H-H COSY spectrum (600 MHz) of dehydrorotenone (126) in [D₅]pyridine.

Based on the molecular formula and the other spectroscopic data as well as the similarity with phenolic rotenoids, the compound was searched in the Dictionary of Natural Products and confirmed as dehydrorotenone (126).¹⁴⁰,¹⁴¹

Figure 169: HMBC spectrum (600 MHz) of dehydrorotenone (126) in [D₅]pyridine.
6.14 Substituted Dichromen-7-one

The rotenoid 127 was isolated as a yellow solid which showed m/z 423 ([M-H]⁻ (ESIMS) and HRESIMS [M + H]⁺ for C₂₃H₂₀O₈. The ¹H NMR spectrum showed one chelated hydroxy group at δ 12.90 (s); 7 further signals of protons which could be attached to sp² carbon atoms were present at δ 8.30 (s), 8.00 (s, OH) 6.75, 6.70, 6.28, 6.20, 5.80. There were two methoxy and two methyl groups observed.

Figure 170:¹H NMR (300 MHz) spectrum of 13-Hydroxy-9,10-dimethoxy-3,3-dimethyl-3H,13H-pyrano[2,3-c;6,5-f]dichromen-7-one (127) in [D₆]DMSO.

The molecular formula C₂₃H₂₁O₈ for rotenoid 127 provided fourteen double bond equivalents.
Substituted Dichromen-7-one

Figure 171: $^{13}$C NMR (300 MHz) spectrum of 13-Hydroxy-9,10-dimethoxy-3,3-dimethyl-3H,13H-pyrano[2,3-c;6,5-f']dichromen-7-one (127) in [D$_6$]DMSO.

From the H-H COSY spectrum there was a correlation between the proton at $\delta$ 6.20 and the hydroxyl group at $\delta$ 8.00. Another COSY correlation was also observed between the protons at $\delta$ 6.70 and 5.80.

Figure 172: H-H COSY spectrum (600 MHz) of 13-Hydroxy-9,10-dimethoxy-3,3-dimethyl-3H,13H-pyrano[2,3-c;6,5-f']dichromen-7-one (127) in [D$_6$]DMSO.

In the HMBC spectrum, correlations of H-1 to C-12a, H-6 to C-4a and C-6a, OH-11 to C-11a, H-4' to C-2' and C-3', H-1' to C-8, 9 and 7a as well as H-10 to C-11a and C-8 revealed the presence of a rotenoid skeleton. Furthermore, two methoxy groups were found to be attached to C-2 and C-3, respectively, and the two methyl groups were attached to C-3'. Compound 127 was established by comparing the 1D and 2D
NMR data with the published data. Rotenoids are known for their insecticidal and pesticidal activities. Their toxicity is caused by the inhibition of NADH oxidation in the respiratory chain. A known rotenoid derrisin was found highly active against *Helicobacter pylori*.

![Figure 173: HMBC spectrum (600 MHz) of 13-Hydroxy-9,10-dimethoxy-3,3-dimethyl-3H,13H-pyrano[2,3-c;6,5-f']dichromen-7-one (127) in [D$_6$]DMSO.](image)

**Figure 173:** HMBC spectrum (600 MHz) of 13-Hydroxy-9,10-dimethoxy-3,3-dimethyl-3H,13H-pyrano[2,3-c;6,5-f']dichromen-7-one (127) in [D$_6$]DMSO.

### 6.15 Obovatin methyl ether

The salient feature in the $^1$H NMR spectrum of obovatin methyl ether (128) was the ABX system, diagnostic for the C-2 and C-3 protons of a flavanone. The C-2 proton (X), appeared as a double doublet at $\delta$ 5.40 (ABX), while the AB signal of the C-3 protons appeared at $\delta$ 2.79 (ABX). The aromatic region was defined by a sharp multiplet at $\delta$ 7.45 (5H) indicating the unsubstituted B-ring; a singlet at $\delta$ 6.10 (1H) could
be assigned to either the C-6 or the C-8 proton. There was a methoxy signal at $\delta$ 3.89 and two methyl singlets at $\delta$ 1.46 and 1.44, respectively.

Figure 174: $^1$H NMR (300 MHz) spectrum of obovatin methyl ether (128) in CDCl$_3$.

The $^{13}$C NMR spectrum showed 21 carbon signals. Among them 3 aliphatic carbons were observed at $\delta$ 45.6 (CH$_2$), 28.4 (CH$_3$) and 28.1 (CH$_3$); one methoxy carbon was at $\delta$ 56.1 and two oxygenated carbon signals at $\delta$ 78.9, 77.9.

Figure 175: $^{13}$C NMR (125 MHz) spectrum of obovatin methyl ether (128) in CDCl$_3$.

The H-H COSY spectrum confirmed the described ABX system along with two ortho-coupled protons, which were connected with $sp^2$ carbon atoms. These are shown as substructure A and B.
Figure 176: H-H COSY spectrum (600 MHz) of obovatin methyl ether (128) in CDCl₃.

From the HMBC spectrum the proton at H-6 showed $^2J$ correlation with the signal at C-5; with the same carbon, the methoxy protons at δ 3.89 showed a correlation, and therefore this methoxy group was attached here. Two methyl groups, which appeared as a singlet at C-2", showed correlations with C-3" and C-7. The olefinic proton C-4" showed correlation with C-2", C-7, C-8 and C-8a. The methylene at H-3 showed correlation with carbon signal at C-1'.

Figure 177: HMBC spectrum (600 MHz) of obovatin methyl ether (128) in CDCl₃.

By using the 1D and 2D NMR data, the compound was searched in the Dictionary of Natural Products. Obovatin methyl ether (128) was found identical with respect to 1D and 2D NMR data.145,146
6.16 Obovatin

Obovatin (129) showed similarity in 1D and 2D NMR with that of obovatin methyl ether (128), which was previously described and elucidated. In obovatin, the 5-OCH₃ group is replaced by an OH group. All the other correlations were the same.

Figure 178: ¹H NMR (300 MHz) spectrum of obovatin (129) in CDCl₃.

Figure 179: ¹³C NMR (125 MHz) spectrum of obovatin (129) in CDCl₃.
Figure 180: H-H COSY spectrum (600 MHz) of obovatín (129) in CDCl₃.

Figure 181: HMBC spectrum (600 MHz) of obovatín (129) in CDCl₃.

6.17 7-Methylglabranin

The molecular formula of 7-methylglabranin (130) was established as C₂₁H₂₂O₄ by HRESIMS. The ¹H NMR spectrum showed the presence of one methoxy and one chelated hydroxyl group by singlets at δ 3.84 (3H) and δ 12.14 respectively and also the presence of a dimethylallyl side chain by a triplet at 5.15 (1H), a doublet at δ 3.22 and
a sharp singlet at $\delta$ 1.62 (6H). The aromatic region was dominated by a multiplet at $\delta$ 7.44 (5H) indicating the unsubstituted B-ring and a singlet at $\delta$ 6.09 (1H) which could be assigned to either the C-6 or the C-8 proton. The salient feature of the high resolution $^1$H NMR spectrum was the ABX system, diagnostic for the C-2 and C-3 protons of a flavanone. The C-2 proton (X partial signal) appeared at $\delta$ 5.41, while the C-3 protons (AB) appeared at $\delta$ 3.05 and $\delta$ 2.85 ($ABX$, 2H, $J_{AB} = 17.1$, $J_{AX} = 12.6$, $J_{BX} = 3.2$ Hz, 3-CH$_2$). The value of $J_{AX} = 12.6$ Hz) for the coupling constant $J_{AX}$ was indicative of an axial coupling. Therefore, the C-2 hydrogen was axial and ring B was equatorial. The position of the hydroxyl group followed from the low-field phenolic proton signal in the $^1$H NMR spectrum.

**Figure 182:** $^1$H NMR (300 MHz) spectrum of 7-methylglabranin (130) in CDCl$_3$.

In the $^{13}$C NMR spectrum a ketone signal was present at $\delta$ 196.2. The monosubstituted phenyl ring appeared at C-2', C-6' ($\delta$ 128.6), C-3', C-5' ($\delta$ 125.9) and C-4' ($\delta$ 128.4). There was one methoxy signal at $\delta$ 55.8.

**Figure 183:** $^1$H NMR (125 MHz) spectrum of 7-methylglabranin (130) in CDCl$_3$. 
The $^1$H NMR and $^{13}$C NMR values were identical with the published values of 7-methylglabranin (130), which was also isolated from *Tephrosia sp.*

![Structure of Candidone](image)

### 6.18 Candidone

Candidone (131) was obtained as a white solid. The molecular formula was established as C$_{22}$H$_{24}$O$_4$ by HRESIMS. The $^1$H NMR spectrum showed the presence of two methoxy groups by singlets at $\delta$ 3.92 and 3.87, respectively and also the presence of a dimethylallyl side chain was indicated by a triplet at $\delta$ 5.15 (1H), a doublet at $\delta$ 3.22 and two singlets at $\delta$ 1.67 and 1.62. The aromatic region was defined by a multiplet at $\delta$ 7.41 (5H) indicating the unsubstituted B-ring, and a singlet at $\delta$ 6.13 (1H), which could be assigned to either the C-6 or the C-8 proton. The salient feature of the $^1$H NMR spectrum was the $ABX$ system, diagnostic for the C-2 and C-3 protons of a flavanone. The C-2 proton, the X part, appeared as a double doublet at $\delta$ 5.40, while the C-3 protons (AB) appeared at $\delta$ 2.98, 2.85 ($ABX$, $J_{AB} = 17.1$, $J_{AX} = 12.8$, $J_{BX} = 3.2$ Hz, CH$_2$). The $^1$H NMR spectrum showed similarity to 130, which was previously described.

![NMR Spectrum](image)

**Figure 184:** $^1$H NMR spectrum (300 MHz) of candidone (131) in CDCl$_3$.

The $^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) showed similarity with the previously described 7-methylglabranin (130). In compound 131 there were, however, two methoxy
units instead of one methoxy unit as in 7-methylglabranin (130). The $^1$H NMR and $^{13}$C NMR spectra showed values identical with the published data of candidone (131) which was also isolated from Tephrosia sp. 148,149

Figure 185: $^1$H NMR spectrum (300 MHz) of candidone (131) in CDCl$_3$.

**6.19 Ovalichalcone**

Ovalichalcone (132) showed a molecular formula of C$_{21}$H$_{20}$O$_5$ from HRESIMS. In the $^1$H NMR spectrum there were two chelated hydroxy groups at $\delta$ 15.47 and 13.66 visible; additionally, six protons attached to $sp^2$ carbon atoms along with one methoxy protons and two methyl groups were observed in the $^1$H NMR spectrum.
In the $^{13}$C NMR spectrum there was many more carbon signals found than expected from the formula. The sample seemed very pure from $^1$H NMR spectrum but $^{13}$C NMR spectrum gave a different picture. After searching in Dictionary of Natural Products with the help of the $^1$H NMR spectrum and the molecular formula, ovalichalcone (132) was found. Structure 132 was confirmed by comparing the data with published compounds.150

During $^1$H NMR spectrum measurement, a portion of the enol was obviously converted into the keto form which was visible in the $^{13}$C NMR spectrum (3 CO groups), but not in the $^1$H spectrum. The spectral data are similar to the published data, where also this keto-enol tautomerism is mentioned.

**Figure 186:** $^1$H NMR (300 MHz) spectrum of ovalichalcone (132) in CDCl$_3$.

**Figure 187:** $^{13}$C NMR spectrum (300 MHz) spectrum of ovalichalcone (132) and its diketo tautomer (133) in CDCl$_3$. 
6.20 Praecansone B

Compound 134 was another chalcone derivative which showed the molecular formula C_{22}H_{22}O_{4}. There were 12 double bond equivalents calculated from the molecular formula. From the coupling constant of an olefinic double bond at δ 7.40 and 7.00 (d, J = 16.1 Hz) the existence of an α,β-unsaturated carbonyl system was clear; additionally, a mono substituted benzene ring was found. At δ 6.26 there was a singlet as well as further proton signals observed at H-6 (δ 6.54) and H-3''-H (δ 5.54), two methoxy signals at δ 3.74, 3.73 and two methyls as a singlet δ 1.46.

![Figure 188: 1H NMR (300 MHz) spectrum of praecansone B (134) in CDCl₃.](image)

In the ¹³C NMR spectrum there was a carbonyl of ketone or aldehyde at δ 194.1. There were six sp² quaternary carbon signals and ten methine carbons observed. There was an oxygenated methine at δ 76.8, two methoxy groups at δ 63.3 and 55.8 as well as two methyls at δ 27.9.
Figure 189: $^{13}$C NMR (125 MHz) spectrum of praecansone B (134) in CDCl$_3$.

From the H-H COSY spectrum substructures A-C were confirmed.

![Diagram showing substructures A, B, and C.]

Figure 190: H-H COSY spectrum (600 MHz) of praecansone B (134) in CDCl$_3$.

In the HMBC spectrum, the proton at H-6 ($\delta$ 6.26) showed correlation with two oxygenated $sp^2$ carbons at C-5, 7 ($\delta$ 158.1, 156.0) as well as two more quaternary $sp^2$ carbons at $\delta$ 116.2, 107.9. Two methyl groups at $\delta$ 1.46 showed correlation with an oxygenated methine at $\delta$ 76.8. The proton H-4" at $\delta$ 6.54 showed correlation with C-7, 8 and 9. The olefinic protons H-1 and H-2 at $\delta$ 7.40 and 7.00 respectively showed correlation with C$_q$-1' ($\delta$ 134.8) which is a $sp^2$ carbon from the monosubstituted phenyl group. These two protons also showed correlations with carbonyl at $\delta$ 194.1. By using the HMBC correlation drawn above as well as HRESIMS the compound was searched
in the Dictionary of Natural Products. The structure of compound 134 was confirmed by comparing with the published data.151

Figure 191: HMBC spectrum (600 MHz) of praecansone B (134) in CDCl3.

Praecansone B (134) was cytotoxic and showed activity against protozoa, e.g. Leishmania parasites and against leukaemia in cell cultures.152
7 Summary

An increasing number of infectious diseases is still not curable as many organisms are showing antibiotic resistance which urges the development of new medicaments to combat them. Natural products are playing an important role for this purpose. Even though the research on secondary bioactive metabolites is comparatively old, there are still unexplored areas. Especially in marine natural products research, microorganisms are considered to be a productive and successful focus. For about two decades, microbial marine natural products have been the subject of chemical and pharmacological interest and have established themselves as a group of biomedical importance. To date, more than 2700 microbial compounds have been reported from marine sources (AntiBase).

In the research work, six terrestrial Streptomyces sp. and three marine-derived bacteria strains were selected and based on their chemical and biological screening, fermented under standard conditions. One fungal strain and extracts from a plant, a tropical Tephrosia sp. were additionally analysed.

After a pre-screening to select strains with interesting properties, the bacteria were cultured in the usual way and extracted with solvents or adsorber resins. A sequence of chromatographic steps delivered the individual compounds, which underwent a dereplication step to sort out known metabolites. The structures of new compounds were elucidated by mass data and 1D and 2D NMR spectra.

7.1 Terrestrial Streptomyces sp. ADM 14

The terrestrial Streptomyces sp. ADM 14 was found to inhibit the growth of Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Streptomyces viridochromogenes, and the fungus Candida albicans in the agar diffusion test. This strain produced many trivial compounds, among them uridine, 5’-methyl-thioadenosine, adenine, uracil, indole-3-acetic acid, indole-3-carboxylic acid, tryptophol, tyrosol, 1-hydroxy-4-methoxy-naphthalene-2-carboxylic acid, phenyl acetic acid, and anthranilic acid. 5’-Methyl-thioadenosine has anticancer activity, 1-hydroxy-4-methoxy-naphthalene-2-carboxylic acid (43) is a herbicidal compound firstly isolated from Streptosporangium cinnabarinum, but is also known from synthesis.54 5,7,9,11-Tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid (45) was the only new metabolite of this streptomycete. Compound 45 is a part of macrolides like erythromycin and may be a biosynthetic intermediate of the macrolides. A relevant compound named 10-deoxymethylnolide (46) showed significant antibiotic activity.57
7.2 Marine Streptomycete B7880

The marine-derived streptomycete isolate B7880 is a producer of ζ-pyromycinone (47) cinerubin B A (49), cinerubin (51), and cinerubin X (52). Cinerubins are known antibiotics with a common galirubinone (47) aglycone, but differ in the sugar residues.
7.3 Terrestrial Streptomyces sp. ACT 7619

The terrestrial Streptomyces sp. isolate ACT 7619 was found to be highly active against bacteria and fungi. This strain produced mainly homononactic acid and its derivatives like dinantin. A known butenoloide, 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide, (56) was also isolated. The activity of this strain was due to the presence of dinactin (53).

7.4 Terrestrial Streptomycete GW6311

The extract from the terrestrial streptomycete GW6311 exhibited two low-polar red-orange bands, which turned to blue/violet by treatment with sodium hydroxide, pointing to peri-hydroxyquinones. Further purification provided two bioactive quinones, namely β-indomycinone (57) and saptomycin A (58).

7.5 Terrestrial Streptomyces sp. GT 2005/079

The crude extract of the terrestrial Streptomyces sp. GT 2005/079 exhibited in the biological screening pronounced antibacterial activity against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli. Chromatography delivered six new compounds: Three of them were straight-chain amides (59-62), one was a homononactic acid amide and two new natural oxazole antibiotics (63, 64) were isolated. The straight-chain amides were moderately active against Mucor miehei. Compounds 63
and 64 were active against *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus* and *Escherichia coli*.

Fairfax *et al.* published the use of benzoxazolecarboxamides, their preparation and pharmaceutical compositions for treating chemotherapy-induced nausea and vomiting (CINV) and Irritable Bowel Syndrome (IBS-D).<sup>153</sup> Most of them are synthetic, but a few are known also from natural sources. Taniguchi *et al.* isolated the bis(benzoxazole) UK-1 (135) from the mycelial cake of an actinomycete strain with potential activity against B16, HeLa and P338 cells.<sup>154</sup> UK-1 analogues show catalytic inhibition of human topoisomerase II.<sup>155</sup>

Homononactic acid amide is a close derivative of homononactic acid which is known for its antifungal and pesticidal activity<sup>156</sup> and is a building block for other anticancer compounds.<sup>157</sup> Yoshihito Tanouchi *et al.* showed the immunosuppressive effects of oligonactins (tetranactin, trinactin and dinactin) on experimental autoimmune uveoretinitis in rats.<sup>158</sup> Shelby P Umland *et al.* showed the effects of cyclosporin A and dinactin on T-cell proliferation, interleukin-5 production, and murine pulmonary inflammation.<sup>159</sup>
7.6 Terrestrial Actinomyces sp. N 435

The terrestrial Actinomyces sp. N 435 was investigated with the goal to find active compounds against plant-pathogenic fungi, as the crude extracts showed moderate antifungal activity. Working up and purification of the fractions led to the isolation of diketopiperazines along with a new secondary metabolite, ramthacin B (78), which is structurally closely related to the antiviral natural compound virantmycin (77). Ramthacin B (78) was not so stable. After $^1$H NMR and MS spectrum, this compound was degraded in the NMR tube. So, the bioactivity could not be measured.

![Chemical structures of 77 and 78](image)

7.7 Marine-derived Streptomyces sp. B 7857

The chemical investigation of the marine-derived Streptomyces sp. B 7857 delivered four new oxygenated $\alpha$-gorgonene sesquiterpenes derivatives named bacteriogorgonene (A-D) (81-84) as well as ten known secondary metabolites, namely homononactic acid, 1-acetyl-$\beta$-carbolin, 3-(2-hydroxyethyl)indole, polypropyleneglycole, nonactic acid, uracil, thymidine, deoxyuridine, and 2-hydroxy-1-(indol-3-yl)-ethanone.

![Chemical structures of 81-84](image)

Gorgonene terpenes are a rare class of sesquiterpenes, which possess an isopropyl group in the unusual C-6 position, an indication that they apparently contain two tail-to-tail fused isoprene units. This is the first report that bacteria contain oxygenated gorgonene sequiterpenes. Until recently; only 50 sesquiterpenoids had been isolated from micro organisms.
7.8 Terrestrial streptomycete GW5127

The terrestrial streptomycete GW5127 yielded three new metabolites, i.e. 9-ethyl-6,11-dihydroxy-4-methoxy-naphthacene-5,12-dione (88), 4-acetyl-1,3-dihydroimidazo[4,5-b]pyridin-2-one (90), 5 and N-[2-(3-hydroxy-4-methoxy-phenyl)-ethyl]-acetamide (93), along with three known compounds, 4-hydroxy-3-methoxy-tyrosol, β-rubromycin (95) and γ-rubromycin (96).

7.9 Terrestrial Streptomyces sp. GW 18/1811

The terrestrial Streptomyces sp. GW 18/1811 exhibited good biological activity against bacteria, fungi and algae. This strain produced komodoquinone B (97), feudomycinone C (98), a new bacterial metabolite α-hydroxyacetovanillone (99), N-acetylhomocysteine thiolactone (99) and a chlorinated known antibiotic lysolipin I (102).
The antitumor-active komodoquinone B showed reduced side effects of neuropathy and they are useful for controlling multiple drug resistant tumors.\textsuperscript{160} Lysolipin I is active against gram-pos. and gram-neg. bacteria.\textsuperscript{118}

### 7.10 Fungal metabolite

The fungal antibiotic bikaverin (103) was received from Prof. Dr. H. Anke, Institute for Biotechnology and Drug Research, Kaiserslautern, Germany.

Bikaverin inhibited the incorporation of purine bases into nucleic acids and acid soluble nucleotides in tumour cells and inhibits ATP formation at the level of the oxidative phosphorylation.\textsuperscript{161} Bikaverin showed inhibition of mitochondrial functions also an efficient hemolytic agent.\textsuperscript{162}
7.11 Plant metabolites from *Tephrosia calophylla*

Twenty-eight samples from the plant source (*Tephrosia calophylla*) were obtained from one of our research collaborators, Professor Seru Ganapaty from Andhra University, India. Twenty-five different compounds were confirmed in those samples. A novel benzil derivative calophione A (104) together with three new coumestan derivatives, tephcalostan A (108), C and D (111, 112) were isolated from the roots of *Tephrosia calophylla*. The all compounds were evaluated for their cytotoxic activity against RAW and HT-29 cancer cell lines and antiprotozoal activity against various parasitic protozoa. Calophione A exhibited significant cytotoxicity with IC$_{50}$ values of 5.00 (RAW) and 2.90 $\mu$M (HT-29) respectively.

![Chemical structures of compounds 104, 108, 111, and 112](image)

In this thesis 9 terrestrial and marine-derived streptomycetes, one plant and one fungal extracts were studied. From the terrestrial and marine strains, 47 compounds were isolated, from which 14 compounds can be considered as new secondary metabolites. From the plant *Tephrosia* sp., 25 compounds were isolated, from which 4 compounds can be considered as new secondary metabolites. The altogether 73 metabolites contained different groups such as peptides, quinones, macrolides, and indoles.

Table 3: Total number of isolated compounds from bacteria in this thesis.

<table>
<thead>
<tr>
<th>Source</th>
<th>No of Strains or plant sp.</th>
<th>No of compounds</th>
<th>No of new compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine and terrestrial streptomycetes</td>
<td>10</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>Fungal metabolites</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Plant metabolites</td>
<td>1</td>
<td>25</td>
<td>4</td>
</tr>
</tbody>
</table>
8 Experimental Part

8.1 General

IR spectra: Perkin-Elmer 1600 Series FT–IR; Perkin-Elmer 297 Infrared Spectrophotometer; Beckman DU-640; Shimadzu FT-IR; (KBr tablet and film). – UV/VIS spectra: Perkin-Elmer Lambda 15 UV/VIS spectrometer. – Optical rotations: Polarimeter (Perkin-Elmer, model 241). – $^1$H NMR spectra: Varian Unity 300 (300.542 MHz), Bruker AMX 300 (300.542 MHz), Varian Inova 500 (499.8 MHz). Coupling constants ($J$) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. – $^{13}$C NMR spectra: Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical shifts were measured relatively to tetramethylsilane as internal standard. Abbreviations: APT (Attached Proton Test): CH/CH$_3$ up and C$_d$/CH$_2$ down. - 2D NMR spectra: H,H COSY spectra (H,H-Correlated Spectroscopy), HMBC spectra (Heteronuclear Multiple Bond Connectivity), HMQC spectra (Heteronuclear Multiple Quantum Coherence) and NOESY spectra (Nuclear Overhauser Effect Spectroscopy). - Mass spectra: EIMS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perfluorokerosene as standard. ESIMS with Quattro Triple Quadrupole mass spectrometer Finigan MAT-Incos 50, ESIMS LCQ (Finnigan).

8.2 Materials

Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV$_{254}$ (Macherey-Nagel & Co.). – Glass plates for chemical screening: Merck silica gel 60 F254, (10 × 20 cm). - Preparative thin layer chromatography (PTLC): 55 g silica gel P/UV$_{254}$ (Macherey-Nagel & Co.) is added to 120 ml of demineralised water with continuous stirring for 15 minutes. 60 ml of the homogenous suspension is poured on a horizontal held (20 × 20 cm) glass plate and the unfilled spaces are covered by distributing the suspension. The plates are air dried for 24 hours and activated by heating for 3 hours at 130 °C. - Column chromatography (CC): MN silica gel 60: 0.05-0.2 mm, 70-270 mesh (Macherey-Nagel & Co). Sephadex LH-20 (Pharmacia) was used for size exclusion chromatography.

8.3 Spray reagents

Anisaldehyde/sulphuric acid: 1 ml anisaldehyde was added to 100 ml of a stock solution containing 85 ml methanol, 14 ml acetic acid and 1 ml sulphuric acid. – Ehrlich’s
reagent: 1 g 4-dimethylaminobenzaldehyde was dissolved in a mixture of 25 ml hydrochloric acid (37%) and 75 ml methanol; it gives a red to violet colouration with indoles, a yellow colour with anthranilic acid derivatives and a grey colour reaction with some 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 gives a blue to a violet colouration with amino acids, peptides and polypeptides with free amino groups.

Chlorine/o-dianisidin reaction: The reagent was prepared from 100 ml o-dianisidin (0.032%) in 1 N acetic acid, 1.5 g Na2WO4 2 H2O 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 KClO3 + 2 ml conc. HCl) and then dried for ca. 1 h, till the excess of chlorine was evaporated and then dipped into the reagent. The reagent is specific for peptides as universal spraying reagent.

NaOH or KOH: 2 N NaOH or KOH solutions are used to identify peri-hydroxyquinones by deepening of the colour from orange to violet or blue.

8.4 Microbiological materials

8.5 Recipes

All culture media were autoclaved at 1.2 bar and 120 °C. Sterilisation time for 1 L shaker culture: 33 min.

Artificial sea water

Iron(III) citrate 2 g (powder)
NaCl 389 g
MgCl₂ · 6 H₂O 176 g
Na₂SO₄ 68.8 g
CaCl₂ 36.0 g
Na₂HPO₄ 0.16 g
SiO₂ 0.30 g
Trace element stock soln. 20 mL
Stock soln. 200 mL
tap water add 20 L

Trace element stock solution

H₃BO₃ 0.611 g
MnCl₂ 0.389 g
CuSO₄ 0.056 g
ZnSO₄ · 7 H₂O 0.056 g
Al₂(SO₄)₃ · 18 H₂O 0.056 g
NiSO₄ · 6 H₂O 0.056 g
CO (NO₃)₃ · 6 H₂O 0.056 g
TiO₂ 0.056 g
(NH₄)₆Mo₇O₂₄ · 4 H₂O 0.056 g
LiCl 0.028 g
SnCl₂ 0.028 g
KI 0.028 g
tap water ad 1 L

Stock solution

KCl 110 g
NaHCO₃ 32 g
KBr 16 g
SrCl₂ · 6H₂O 6.8 g (dissolved separately)
H₃BO₃ 4.4 g
NaF 0.48 g
NH₄NO₃ 0.32 g
tap water ad 2 L

8.5.1 Nutrients

M₂ medium (without sea water)
Malt extract 10 g
Glucose 4 g
Yeast extract 4 g
Tap water ad 1 L
The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar

M₂⁺ medium (M₂ medium with sea water)
Malt extract 10 g
Glucose 4 g
Yeast extract 4 g
Artificial seawater 500 mL
Tap water 500 mL
The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.
Luria-Bertani-Medium (LB)

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

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

Soja-Mannitol Medium

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

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

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
CaCO₃ 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
KNO₃ 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 FeSO₄ · 7 H₂O 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:

MnSO₄ · H₂O 16.9 mg
Na₂MoO₄ · 2H₂O 13.0 mg
Co \((\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}\) 10.0 mg

Salts are dissolved in 10 ml of demineralised water.

Solution B:

\[
\begin{align*}
\text{CuSO}_4 \cdot 5\text{H}_2\text{O} & \quad 5.0 \text{ mg} \\
\text{H}_3\text{BO}_3 & \quad 10.0 \text{ mg} \\
\text{ZnSO}_4 \cdot 7\text{H}_2\text{O} & \quad 10.0 \text{ mg}
\end{align*}
\]

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 \textit{Chlorella vulgaris}, \textit{Chlorella sorokiniana} and \textit{Scenedesmus subspicatus}.

\[
\begin{align*}
\text{NaNO}_3 & \quad 0.250 \text{ g} \\
\text{KH}_2\text{PO}_4 & \quad 0.175 \text{ g} \\
\text{K}_2\text{HPO}_4 & \quad 0.075 \text{ g} \\
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & \quad 0.075 \text{ g} \\
\text{NaCl} & \quad 0.025 \text{ g} \\
\text{CaCl}_2 \cdot 2\text{H}_2\text{O} & \quad 0.025 \text{ g} \\
\text{Fe-EDTA} & \quad 1.0 \text{ ml} \\
\text{Trace element solution II} & \quad 0.1 \text{ ml}
\end{align*}
\]

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

8.6.1 Storage of Strains

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

8.6.2 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 M2 or (for marine strains) M2+ 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 μg/ml.

8.6.3 Biological screening

The crude extract was dissolved in CHCl3/10% MeOH (concentration 50 μg/mL), in which the paper disks were dipped, dried under sterile conditions (flow box) and put on agar plates inoculated with Bacillus subtilis (ATCC6051), Staphylococcus aureus, Streptomyces viridochromogenes (Tü 57), Escherichia coli, Chlorella vulgaris, Chlorella sorokiniana, Scenedesmus subspicatus, Candida albicans and Mucor miehei (Tü 284).

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

8.6.4 Chemical and pharmacological screening

Samples of the extracts were separated on silica gel glass plates (10 × 20 cm) with two solvent systems CHCl3/5% MeOH and CHCl3/10% MeOH. After drying, the plates were photographed under UV light at 254 nm and marked at 366 nm, and subsequently stained by anisaldehyde and Ehrlich’s reagent. Finally, the plates were scanned for documentation. For the pharmacological investigations, approximately 25 mg of the crude extract was sent to industrial partners.
8.6.5 Brine shrimp microwell cytotoxicity assay

To a 500 ml separating funnel, filled with 400 ml of artificial seawater, 1 g of dried eggs of *Artemia salina* 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:

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

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

8.6.6 Primary screening

Antibiotic screening (disk diffusion test): The test is performed using paper discs with a diameter of 8 mm under standardized conditions. 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 (+++).

8.7 Terrestrial Streptomyces sp. ADM 14

8.7.1 Primary Screening

The *semi*quantitative results of antibacterial, antifungal and phytotoxic tests are listed in the Table 4. The TLC of the crude extract exhibited a light yellow spot and three colourless UV inactive bands, which became first brown and later turned to violet with anisaldehyde/sulphuric acid.

Table 4: Biological activity of the crude extract of the strain ADM 14.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (Ø mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25</td>
</tr>
<tr>
<td><em>Streptomyces viridochromogenes</em> (Tü 57)</td>
<td>15</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Scenedesmus subspicatus</em></td>
<td>22</td>
</tr>
</tbody>
</table>

8.7.2 Cultivation, work-up and isolation

For the cultivation, well grown agar plates of the strain ADM 14 were used to inoculate 20 litres of M₂ medium, which was cultivated as shaker culture for 5 days at 28 °C. The obtained broth was filtered over Celite, the water phase was extracted with XAD-16, and the resin eluted with methanol; the biomass was extracted with ethyl acetate. The combined extracts were evaporated to dryness under vacuum to afford a brown crude extract (2.1 g). The extract was defatted with cyclohexane and the methanolic part was pre-separated by column chromatography into five fractions. Further purification on Sephadex LH-20 followed by PTLC and silica gel column chromatography afforded 14 compounds: 1-hydroxy-4-methoxy-naphthalene-2-carboxylic
acid (43, 2.5 mg), 5'-methyl-thioadenosine (44, 75 mg), and 5,7,9,11-tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid (45, 6.7 mg).

1-Hydroxy-4-methoxynaphthoic acid (43): light grey coloured solid, $R_f = 0.66$ (CH$_2$Cl$_2$/5% MeOH), blue UV fluorescent. – 1H NMR (CD$_3$OD, 300 MHz) $\delta$ 8.27 (dd, 1H, $^3J = 8.0$, $^4J = 1.4$ Hz, 8-H), 8.12 (dd, 1H, $^3J = 8.0$, $^4J = 1.4$ Hz, 8-H), 7.50 (m, 2H, 7, 8-H), 7.29 (s, 1H, 3-H), 3.96 (s, 3H, 4-OCH$_3$); – (-)-ESIMS $m/z$ (217 [M-H]$^-$, 100), (217 [2M-2H+ Na]$^-$, 55).

5'-Methyl-thioadenosine (44): Colourless solid, $R_f = 0.41$ (CHCl$_3$/10% MeOH), UV absorbing, dark grey with anisaldehyde/sulphuric acid. – 1H NMR ([D$_6$]DMSO, 300 MHz) $\delta$ 8.38 (s, 1H, 2-H), 8.17 (s, 1H, 6-H), 7.23 (br s, H/D exchangeable, 2H, NH$_2$), 5.92 (d, $^3J = 6$ Hz, 1H, 1'-H), 5.82-5.55 (br s, H/D exchangeable, 2H, OH), 4.73 (t, $^3J = 6.3$ Hz, 1H, 2'-H), 4.16 (m, 1H, 3'-H), 4.03 (ABX, 1H, 4'-H), 2.90 (ABX, 1H, 5'-Ha), 2.75 (ABX, 1H, 5'-Hb), 2.07 (s, 3H, 5'-SCH$_3$). – (+)-ESIMS $m/z$ 617 ([2M +Na]$^+$, 100), 320 ([M + Na]$^+$, 93), 298 ([M + H]$^+$, 10); – (-)-ESIMS $m/z$ 593 ([2M-H]$^-$, 100), 296 ([M-H]$^-$, 10); – EIMS (70 eV) $m/z$ 297 ([M]$^+$, 12), 250 (6), 194 (28), 166 (8), 164 (100), 138 (8), 136 (100), 61 (54).

5,7,9,11-Tetrahydroxy-4,6,8,10-tetramethyl-tridec-2-enoic acid (45): Grey solid, $R_f = 0.38$ (CHCl$_3$/7% MeOH), UV absorbing, violet with anisaldehyde/sulphuric acid. – 1H NMR (CD$_3$OD, 300 MHz) $\delta$ 6.32 (dd, 1H, $^3J = 16.0$, 9.5 Hz, 3-H), 5.86 (d, 1H, $^3J = 15.7$ Hz, 2-H), 3.41-3.74 (m, 4H, 5,7,9,11-H), 2.43 (m, 1H, 4-H), 1.86 (m, 1H, 8-H), 1.84 (m, 1H, 6-H), 1.77 (m, 1H, 10-H), 1.58 (m, 2H, 12-CH$_2$a), 1.32 (m, 2H, 12-CH$_2$b), 0.98 (t, 3H, $^3J = 7.0$ Hz , 12-CH$_3$), 0.98 (t, 3H, $^3J = 7.0$ Hz, 4-CH$_3$), 0.92 (d, 3H, $^3J = 6.9$ Hz, 8-CH$_3$), 0.85 (d, 3H, $^3J = 7.0$ Hz, 6-CH$_3$), 0.78 (d, 3H, $^3J = 6.9$ Hz, 10-CH$_3$). – $^{13}$C NMR (CD$_3$OD, 125 MHz) $\delta$ 171.7 (1-CO), 150.4 (2-CH), 126.4 (3-CH), 80.3 (7-CH), 79.2 (9-CH), 76.4 (5-CH), 74.6 (11-CH), 42.6 (4-CH), 41.7 (10-CH), 37.9 (8-CH), 36.8 (6-CH), 26.2 (12-CH$_2$), 16.9 (4-CH$_3$), 11.8 (8-CH$_3$), 10.5 (6-CH$_3$), 9.7 (10-CH$_3$), 5.9 (12-CH$_3$). – (+)-ESIMS $m/z$ 333 ([M + H]$^+$); – (+)-HRESIMS $m/z$ 333.20610 [M + H]$^+$, (calcd for C$_{17}$H$_{33}$O$_6$, 333.20603).

8.7.3 Synthesis of 1-hydroxy-4-methoxynaphthoic acid (43)

1-Hydroxy-4-methoxynaphthol (42) (8.7 g, 0.05 M) and freshly heated K$_2$CO$_3$ (17.2 g, 0.125 M, preheated on a Bunsen burner to about 500 °C for 45 minutes) were mixed homogeneously. It was then taken in a 250 ml autoclave and dry ice (~50 g) was added. The reaction mixture was heated in the sealed autoclave to 150 °C for 6 hours under the resulting pressure of 70-80 bar. After cooling to r.t., the product was mixed with 50 ml water and air was passed through the solution for 15 minutes, to
oxidize unreacted naphthol. The deep blue precipitation formed was separated by filtration and discarded. The filtrate was acidified with 6N HCl, whereby a light blue precipitate was formed. The solid was separated again by filtration and the filtrate was discarded. The product (1.4 g) was purified by crystallization from methanol/dichloromethane. The NMR data were identical with those of the natural product (see theoretical part).

8.8 Marine Streptomyces sp. B 7880

8.8.1 Primary screening

The ethyl acetate extract of the streptomycete isolate B8969 showed the presence of several pink to red coloured, UV absorbing zones. The strain exhibited high biological activity against *Mucor miehei* (Tü284), *Candida albicans*, and the alga *Chlorella sorokiniana*.

Table 5: Biological activity of the crude extract of the strain B 7880

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (Ø mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Streptomyces viridochromogenes</em> (Tü 57)</td>
<td>11</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>12</td>
</tr>
<tr>
<td><em>Mucor miehei</em></td>
<td>14</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Chlorella sorokiniana</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Scenedesmus subspicatus</em></td>
<td>0</td>
</tr>
</tbody>
</table>

8.8.2 Isolation and cultivation

The strain B7880 was cultivated on a 20-litre scale for 7 days at 28 °C as shaker culture (110 rpm). After filtration, the dark red broth was filtered to separate the water phase and mycelium. The water phase was extracted by XAD-16 and the mycelium was extracted with ethyl acetate. The extracts from both phases (2.58 g) were combined, as TLC did not show much difference. For isolation and purification of the metabolites, the resulting product was chromatographed on silica gel column using a chloroform-methanol gradient. After fractionation of the crude extract through silica gel column chromatography, fraction 2 was further purified by PTLC and Sephadex
LH-20 to isolate ζ-pyrromycinone (47, 1.6 mg) and cinerubin B (49, 1.8 mg) as low polar, orange fluorescent (366 nm), which turned to violet by treating with sodium hydroxide. Following the same method cinerubin X (51, 2.1 mg) and cinerubin A (52, 1.1 mg) were separated from fraction 4.

ζ-Pyrromycinone (47): Low polar orange-red solid, $R_f = 0.57$ (CHCl₃/5% MeOH), violet with NaOH. – ¹H NMR (CDCl₃, 300 MHz) δ 13.02 (s, 1H, OH), 12.61 (s, 1H, OH), 12.28 (s, 1H, OH), 7.69 (s, 1H, 11-H), 7.29, 7.25 (AB, $^3J = 9.4$ Hz, 2H, 2,3-H), 3.96 (s, 1H, 10-H), 3.74 (s, 3H, 15-OCH₃), 3.07, 2.86 (ABX, $J_{AB} = 14.1$, $J_{AX} = 2.2$ Hz, $J_{AX} = 2.4$ Hz, 2H, 7α,7β-H), 2.33 (m, 1H, 8-Heq), 1.95 (m, 1H, 8-Heq), 1.72, 1.62 (m, 2H, 12-CH₂), 1.09 (t, $^3J = 7.6$ Hz, 3H, 13-CH₃); – (+)-ESIMS $m/z$ 845 ([2 M + Na]⁺, 100), – (-)-ESIMS $m/z$ 411 ([M-H]⁻, 100).

Cinerubin B (49): Middle polar red amorphous solid, $R_f = 0.60$ (CHCl₃/10% MeOH), orange UV fluorescent, turned to blue-violet with 2 N NaOH. – ¹H NMR (CDCl₃, 300 MHz) δ 12.97 (br s, 1H, OH), 12.80 (br s, 1H, OH), 12.21 (s, br, 1H, OH), 7.73 (s, 1H, 11-H), 7.32, 7.28 (AB, $^3J = 9.4$ Hz, 2H, 2,3-H), 5.51 (d, $^3J = 2.4$ Hz, 1'-H), 5.20 (d, $^3J = 2.4$ Hz, 1H, 1''-H), 5.17 (d, $^3J = 2.1$ Hz, 1'''-H), 4.81 (q, $^3J = 5.2$ Hz, 1H, 5''-H), 4.68 (q, $^3J = 5.2$ Hz, 1H, 5'''-H), 4.38 (m, 3H, 3',4'',5'''-H), 4.13 (s, 1H, 1-OH), 4.04 (m, 2H, 5''-2''-H), 3.83 (s, 1H, 4'-H), 3.72 (s, 15-OCH₃), 2.60 (d, $^3J = 3.4$ Hz, 2H, 3''-CH₂), 2.48 (m, 2H, 8-Heq, 2''-Heq), 2.38-2.17 [m, 7H, 8-H, NMe₂], 1.75 (m, 1H, 13-H), 1.53 (m, 1H, 13-H), 1.37 (d, $^3J = 7.2$, 6''-CH₃), 1.30 (d, $^3J = 7.2$ Hz, 3H, 6'-CH₃), 1.23 (d, $^3J = 7.3$ Hz, 3H, 6''-H), 1.09 (t, $^3J = 7.4$ Hz, 3H, 14-CH₃); – (+)-ESIMS $m/z$ 826 ([M + H]⁺, 100); – (-)-ESIMS $m/z$ 1671 ([2 M + Na – 2 H]⁺, 100), 824 ([M - H]⁻, 75), 411 (90).

Cinerubin A (51): Red amorphous, middle polar substance, $R_f = 0.54$ (CHCl₃/10% MeOH), UV orange fluorescence, turned to blue-violet with 2 N NaOH. – ¹H NMR (CDCl₃, 300 MHz) δ 12.92 (s, 1H, 1-OH), 12.76 (s, 1H, 4-OH), 12.17 (s, 1H, 6-OH), 7.69 (s, 1H, 11-H), 7.29 (d, $^3J = 9.4$ Hz, 1H, 3-H), 7.27 (d, $^3J = 9.4$ Hz, 1H, 2-H), 5.56 (br s, 1H, 1''-H), 5.30 (br d, $^3J = 4.2$ Hz, 1H, 7-H), 5.12 (t, $^3J = 6.4$ Hz, 1'''-H), 5.08 (d, $^3J = 3.5$ Hz, 1''-H), 4.62 (q, $^3J = 6.4$ Hz, 5''-H), 4.54 (q, 1H, $J = 6.2$ Hz, 5''-H), 4.19 (q, $J = 6.8$ Hz, 5'''-H), 4.16 (m, 1H, 3''-H), 4.11 (s, 1H, 10-H), 3.78 (m, 1H, 3'-H), 3.76 (s, 1H, 4''-H), 3.69 (s, 3H, 10-COOCH₃), 3.62 (br s, 4''-H), 2.40-2.60 (m, 2H 2''-CH₂b, 3''-H), 2.40 (d, 1H, $J = 15.0$ Hz, 8-CH₂a), 2.35 (dd, 1H, $J = 15.0$, 4.2 Hz, 8-CH₂b), 2.20 [s, 6H, N (CH₃)₂], 2.16 (m, 2''-CH₂b), 2.15 (m, 1H, 2''-CH₂b), 2.10 (m, 1H, 2''-CH₂a), 1.95 (m, 1H, 2'-CH₂b), 1.81 (m, 1H, 2'-CH₂b), 1.75 (m, 1H, 13-CH₂b), 1.51 (m, 1H, 13-CH₂a), 1.38 (d, $J = 6.7$ Hz, 6''-H), 1.34 (d, $J = 6.4$ Hz, 6'''-H), 1.22 (d, $J = 6.4$Hz, 6''-H), 1.15 (t, $J = 7.3$ Hz, 14-CH₃). – (+)-ESIMS $m/z$ 828 [M + H]⁺; – (-)-ESIMS $m/z$ 826 [M - H].
Cinerubin X (52): Red amorphous, middle polar substance $R_f = 0.48$ (CHCl$_3$/10% MeOH), an orange UV fluorescence, turned to blue-violet with 2 N NaOH. – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 12.92 (s, 1H, 1-OH), 12.76 (s, 1H, 4-OH), 12.24 (s, 1H, 6-OH), 7.72 (s, 1H,11-H), 7.29 (d, $J = 9.4$ Hz, 1H, 9.4, 3), 7.27 (d, $J = 9.4$ Hz, 1H, 2-H), 5.56 ($br$ s, 1H, 1'-H), 5.30 ($br$ d, $J = 4.2$ Hz, 1H,7-H), 5.12 (t, $J = 6.4$ Hz, 1'''-H), 5.08 (d, $J = 3.5$ Hz, 1'''-H), 4.62 (q, $J = 6.4$ Hz, 5'''-H), 4.54 (q, 1H, $J = 6.2$ Hz, 5'-H), 4.19 (q, $J = 6.8$ Hz, 5'''-H), 4.16 (m, 1H, 3'''-H), 4.11 (s, 1H, 10-H), 3.78 (m, 1H, 3'-H), 3.76 (s, 1H, 4''-H), 3.69 (s, 3H, 10-COOCH$_3$), 3.62 ($br$ s, 4'-H), 2.40-2.60 (m, 2H 2'''-CH$_2$b, 3'''-H), 2.40 (d, 1H, $J = 15.0$ Hz, 8-CH$_2$a), 2.35 (dd, 1H, $J = 15.0$, 4.2 Hz, 8-CH$_2$b), 2.16 (m, 2'''-CH$_2$a), 2.15 (m, 1H, 2'''-CH$_2$b), 2.10 (m, 1H, 2'''-CH$_2$a), 1.95 (m, 1H, 2'-CH$_2$b), 1.81 (m, 1H, 2'-CH$_2$a), 1.75 (m, 1H, 13-CH$_2$b), 1.51 (m, 1H, 13-CH$_2$a), 1.38 (d, $J = 6.7$ Hz, 6''''-H), 1.34 (d, $J = 6.4$ Hz, 6'-H), 1.22 (d, $J = 6.4$Hz, 6'''-H), 1.15 (t, $J = 7.3$ Hz, 14-CH$_3$). – (+)-ESIMS $m/z$ 807 ([M + Na]$^+$, 100), 1591 ([2M + Na]$^+$, 70); – (-)-ESIMS $m/z$ 783 ([M - H]$^-$, 100).

8.9 Terrestrial Streptomyces sp. ACT 7619

8.9.1 Primary Screening

The ethyl acetate extract of the terrestrial Streptomyces sp. isolate ACT 7619 showed high activity against bacteria and fungi. Some colourless weakly UV absorbing band gave violet colouration with anisaldehyde/sulphuric acid.

Table 6: Biological activity of the crude extract of the strain Act 7619

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (Ø mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>11</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12</td>
</tr>
<tr>
<td>Streptomyces viridochromogenes (Tü 57)</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>11</td>
</tr>
<tr>
<td>Mucor miehei</td>
<td>11</td>
</tr>
</tbody>
</table>

8.9.2 Fermentation and isolation

The 25 L fermentation of the strain ACT 7619 was performed as a shaker culture using M$_2$ medium during five days at 28 °C on a linear shaker. The brown mycelium of the growth culture was separated by filtration with the aid of Celite into mycelia cake and filtrate. Both were extracted with ethyl acetate and combined after the analysis of
the TLC to give a dark oily crude extract (3.23 g). The crude extract was subjected to silica column chromatography with a dichloromethane-methanol gradient to separate four fractions. Fraction 2 was further purified by Sephadex LH-20 and silica gel column chromatography to find bonactin (54, 3.0 mg). Fraction 3 was further purified in the same way as 54 to obtain nonactic acid (86, 36 mg), homononactic acid (87, 120 mg), and dinactin (53, 15 mg). After passing fraction 4 through Sephadex LH-20, 4,10-dihydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olid (56, 1.0 mg) was obtained.

**Dinactin (53):** Colourless oil, $R_f = 0.27$ (CHCl$_3$/ 10% MeOH), violet band on spraying with anisaldehyde/sulphuric acid. – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 4.93 (m, 2H, 17-H, 8-H), 4.02 (br q, 2H, 3-H, 12-H), 3.87 (m, 2H, 6-H, 15-H), 2.52 (m, 2H, 2-H, 11-H), 2.01 (m, 2H, 5-CH$_2$, 14-CH$_2$), 1.92 (m, 2H, 4-H$_2$, 13-CH$_2$), 1.67 (m, 2H, 7-CH$_2$), 1.56 (m, 2H, 4-CH$_2$, 13-CH$_2$), 1.56 (m, 2H, 18-CH$_2$), 1.49 (m, 2H, 5-H$_2$, 14-CH$_2$), 1.23 (d, $^3J = 6.2$ Hz, 3H, 8-CH$_3$), 1.10 (d, $^3J = 7.0$ Hz, 3H, 11-CH$_3$), 1.08 (d, $^3J = 7.0$ Hz, 3H, 2-CH$_3$), 0.87 (t, $^3J = 7.4$ Hz, 3H, 19-CH$_3$); – (+)-ESIMS $m/z$ 787 ([M + Na]$^+$, 100).

**Bonactin (54):** Colourless oil, $R_f = 0.2$ (CH$_2$Cl$_2$/5 % MeOH), turns first brown and then violet with anisaldehyde/sulphuric acid. – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 6.10 (br s, 2H, 2 OH), 5.02 (m, 1H, 8-H), 4.18 (m, 1H, 6’-H), 4.00 (m, 3H, 3-H, 3’-H, 6-H), 3.78 (m, 1H, 8’-H), 2.50 (dq, $^3J = 8.3$, 7.0 Hz, 2H, 2-H, 2’-H), 2.05-1.90 (m, 4H), 1.40-1.80 (m, 10H), 1.22 (d, $^3J = 6.3$ Hz, 3H, 9-CH$_3$), 1.10 (d, $^3J = 7.0$ Hz, 3H, 2’-CH$_3$), 1.15 (d, $^3J = 7.0$ Hz, 3H, 2-CH$_3$), 0.93 (t, $^3J = 7.4$ Hz, 3H, 10-CH$_3$). – (+)-ESIMS $m/z$ 423 ([M + Na]$^+$, 48); – (-)-ESIMS $m/z$ 399 ([M - H]$^-$, 45).

**4,10-Dihydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (56):** Colourless solid, $R_f = 0.33$ (CH$_2$Cl$_2$/5% MeOH), blue colouration with anisaldehyde/sulphuric acid. – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.43 (dd, $^3J = 5.6$ Hz, $^4J = 1.5$ Hz, 1H, 3-H), 6.11 (dd, $^3J = 5.6$ Hz, $^4J = 1.9$ Hz, 1H, 2-H), 5.03 (m, 1H, 4-H), 3.84 (s, 1H, 10-OH), 2.20 (s, 3H, 12-CH$_3$), 1.78-1.30 (m, 10H, 5,6,7,8,9-CH$_2$), 1.36 (s, 3H, 10-CH$_3$).

8.10 Terrestrial Streptomyces sp. GW 6311

8.10.1 Primary Screening

The pale yellow crude extract exhibited a moderate activity only against micro algae. TLC using CH$_2$Cl$_2$/5% MeOH and CH$_2$Cl$_2$/10% MeOH indicated the presence of yellow bands, which changed to red with diluted sodium hydroxide indicating peri-
hydroxy-quinones. Further purification of the crude extract provided \(\beta\)-indomycinone (57) and saptomycinone A (58).

### Table 7: Biological activity of the crude extract of the strain GW 6311.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone ((\varnothing) mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0</td>
</tr>
<tr>
<td>Streptomyces viridochromogenes (Tü 57)</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0</td>
</tr>
<tr>
<td>Mucor miehei</td>
<td>0</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>15</td>
</tr>
<tr>
<td>Chlorella sorokiniana</td>
<td>15</td>
</tr>
<tr>
<td>Scenedesmus subspicatus</td>
<td>16</td>
</tr>
</tbody>
</table>

#### 8.10.2 Cultivation and isolation

The fermentation was performed on a 20 l scale at 28 °C for 5 days using M2 medium as linear shaker culture. In each 1 l Erlenmeyer flask was inoculated with 1 cm\(^2\) pieces from a well grown agar plate. After 5 days the culture was pink-yellow and was separated by filtration with the aid of Celite into mycelial cake and filtrate. Both were extracted with ethyl acetate and combined after the analysis by TLC to give 1.57 g crude oily extract. The crude extract was subjected to silica gel column chromatography using dichloromethane/methanol gradient to find 2 fractions. Fraction 2 was further separated by Sephadex LH-20 and PTLC into a mixture of \(\beta\)-indomycinone (57) and saptomycin A (58, 3.2 mg).

**\(\beta\)-Indomycinone (57):** Yellowish-orange amorphous powder, \(R_f = 0.78\) (CHCl\(_3\)/5 % MeOH), orange UV fluorescent, colour change to red with NaOH, brown with sulphuric. – \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 12.85 (br s, 1H, OH), 8.08 (s, 1H, 6-H), 7.83 (dd, \(^3\)J = 7.6, 1.3 Hz, 1H, 8-H), 7.68 (t, \(^3\)J = 8.0 Hz, 1H, 9-H), 7.38 (dd, \(^3\)J = 8.4, 1.3 Hz, 10-H), 6.56 (s, 1H, 3-H), 5.74 (m, 1H, 18-H), 5.39 (m, 1H, 17-H), 3.02 (s, 3H, 13-CH\(_3\)), 2.91 (dd, \(^3\)J = 14.4, \(^3\)J = 8.5 Hz, 1H, 16-H\(_A\)), 2.78 (dd, \(^3\)J = 14.4, \(^3\)J = 7.2 Hz,1H, 16-H\(_B\)), 1.68 (s, 3H, 15-CH\(_3\)), 1.64 (d, \(^3\)J = 6.8 Hz, 19-CH\(_3\)). – (+)-ESIMS \(m/z\) 405 ([M + H]\(^+\), 8), 405 ([2 M + Na]\(^+\), 100); – (-)-ESIMS \(m/z\) 403 ([M - H]\(^-\), 100).

**Saptomycin A (58):** Yellow amorphous powder, \(R_f = 0.70\) (CHCl\(_3\)/5 % MeOH), orange UV fluorescent on TLC, red by treatment with NaOH, brown by sulphuric acid. – \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 12.64 (br s, 1H, OH), 8.09 (s, 1H, 6-H), 7.83 (dd, \(^3\)J = 7.6 Hz, 1.3 Hz, 1H, 8-H), 7.69 (t, \(^3\)J = 8.0 Hz, 1H, 9-H), 7.38 (dd, \(^3\)J = 8.4,
1.3 Hz, 10-H), 6.28 (s, 1H, 3-H), 5.65 (m, 1H, 18-H), 5.53 (td, \(^3J = 10.5, ^3J = 6.8\) Hz, 1H, 17-H), 5.01 (dd, \(^3J = 8.5, ^3J = 3.8\) Hz, 1H, 16-H), 3.02 (s, 3H, 13-CH\(_3\)), 2.99 (dq, \(^3J = 3.8, ^3J = 7.5\) Hz, 1H, 14-H), 1.71 (d, \(^3J = 7.0\) Hz, 3H, 19-CH\(_3\)). - (+)-ESIMS m/z 405 ([M + H]\(^+\), 8), 427 ([2 M + Na]\(^+\), 100); - (-)-ESIMS m/z 403 ([M - H]\(^-\), 100).

**8.11 Streptomyces sp. GT 2005/079**

**8.11.1 Primary screening**

The crude extract of the terrestrial *Streptomyces* sp. GT 2005/079 showed antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. The TLC screening revealed several non-polar non-UV absorbing compounds, which changed to green with anisaldehyde/sulphuric acid.

Table 8: Biological activity of the crude extract of *Streptomyces* sp. 2005/079.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (Ø mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>11</td>
</tr>
<tr>
<td>Streptomyces viridochromogenes (Tü 57)</td>
<td>17</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
</tr>
<tr>
<td>Candida albicans</td>
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<tr>
<td>Mucor miehei</td>
<td>14</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>0</td>
</tr>
<tr>
<td>Chlorella sorokiniana</td>
<td>0</td>
</tr>
<tr>
<td>Scenedesmus subspicatus</td>
<td>0</td>
</tr>
<tr>
<td>Artemia salina</td>
<td>100 %</td>
</tr>
</tbody>
</table>

**8.11.2 Cultivation and isolation**

20.0 L of M\(_2\) medium was prepared and transferred to 1.0 L Erlenmeyer flasks (250 ml each) and inoculated by the pre-cultured terrestrial streptomycete GT 2005/079. The flasks were then incubated on a linear shaker (110 rpm) at 28 °C for 8 days until the broth attained dark brown colour. The culture broth was mixed with Celite and filtered off under pressure. The filtrate was extracted with XAD-16 column and the resin eluted with methanol to get dark brown extract. The biomass was extracted with ethyl acetate (3 times). Both extracts were combined due to their identical chromatograms to give an oily crude extract (4.25 g). Chromatography on a silica gel (col-
umn, CH₂Cl₂/0-50 % MeOH gradient) gave four main fractions. Fraction 1 consisted mostly of fats and lipids, whereas fraction 2 showed three non UV absorbing spots which showed green colour with anisaldehyde/sulphuric acid on TLC. Fraction 2 was further purified on silica gel column chromatography, Sephadex LH-20 and followed by RP-8 open column eluting with 50-60 % aqueous methanol to get 59 (15 mg) which gave bright green colour with anisaldehyde-sulphuric acid. Fraction 3 was separated on silica gel and Sephadex LH-20 into 3 subfractions. The subfractions afforded on an RP-8 column eluting with 40% aqueous methanol 60 (6 mg) and 61 (16 mg). Fraction 3 from the main column was subjected to a RP-phenyl column (12 × 1 cm) and eluted with 30 % aqueous methanol to get three sub-fractions. Further purification with methanol on Sephadex LH-20 yielded 62 (1.2 mg). Fraction 4 contained a highly fluorescent polar compound. It was separated on Sephadex LH-20 into two sub-fractions. F₄a and F₄b were sparingly soluble in dichloromethane or methanol, and even in a mixture of dichloromethane and methanol. The fractions were washed several times with a mixture of dichloromethane and methanol to obtain 63 (14 mg) and 64 (3 mg).

N-(7-Hydroxy-6-methyl-octyl)-acetamide (59): Colourless oil, Rᵢ = 0.65 (CHCl₃/5 % MeOH), green colouration with anisaldehyde/sulphuric acid. – ¹H NMR (CDCl₃, 300 MHz) δ 5.63 (br s, 1H, 1-NH), 3.66 (quint, 1H, J = 6.7 Hz, 7-CH), 3.22 (q, 2H, 3J = 6.2 Hz, 1-CH₂), 1.97 (s, 3H, COCH₃), 1.25-1.38 (m, 8H, 2,3,4,5-CH₂), 1.18 (s, 6H, 6-(CH₃)₂). – ¹³C NMR (CDCl₃, 125 MHz) δ 170.1 (CO-1), 71.6 (Cq-6), 39.9 (CH₂-5), 39.6 (CH₂-1), 32.3 (CH₂-4), 29.5 (CH₂-3), 27.1 (CH₃-6), 26.7 (CH₂-2), 23.3 (CH₂-5), 19.4 (-COCH₃), 14.5 (7-CH₃). – (+)-ESIMS m/z 202 [M + H]+, 224 [M + Na]⁺, 425 [2 M + Na]⁺; – (+)-ESI-HRMS m/z 224.16218 [M + Na]⁺ (calcd for C₁₁H₂₃NO₂Na, 224.16210).

N-(7-Hydroxy-octyl)-acetamide (60): Colourless oil, Rᵢ = 0.60 (CHCl₃/5 % MeOH), green colouration with anisaldehyde/sulphuric acid. – ¹H NMR (CDCl₃, 300 MHz) δ 5.49 (br s, 1H, 1-NH), 3.66 (q, 1H, J = 6.2 Hz, 7-CH), 3.06 (q, 2H, 3J = 6.2 Hz, 1-CH₂), 1.96 (s, 3H, COCH₃), 1.25-1.38 (m, 10H, 2,3,4,5,6-CH₂), 1.13 (d, 3H, 3J = 6.38 Hz, COCH₃), 0.86 (t, 3H, 3J = 6.74 Hz, 7-CH₃). – ¹³C NMR (CDCl₃, 125 MHz) δ 170.1 (CO), 68.2 (7-CH), 41.0 (6-CH₂), 39.6 (1-CH₂), 29.2 (3-CH₂), 27.2 (2-CH₂), 23.7 (5-CH₂), 23.4 (-COCH₃), 16.6 (8-CH₃). – (+)-ESIMS m/z 201 [M + Na]⁺; – (+)-HRESIMS m/z 210.14653 [M + Na]⁺ (calcd for C₁₀H₂₁NO₂Na, 210.14645).

N-(6-Hydroxy-6-methyl-heptyl)-acetamide (61): Colourless oil, Rᵢ = 0.55 (CHCl₃/5 % MeOH), green colouration with anisaldehyde/sulphuric acid. – ¹H NMR (CDCl₃, 300 MHz) δ 6.66 (br s, 1H, 1-NH), 5.33 (s, 1H, 6-OH), 3.20 (q, 3J = 6.2 Hz, 2H, 1-CH₂), 1.97 (s, 3H, COCH₃), 1.25-1.38 (m, 8H, 2,3,4,5-CH₂), 1.18 [s, 6H, 6-(CH₃)₂]. –
$^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 170.4 (CO), 70.5 (C$_q$-6), 43.4 (CH$_2$-5), 39.3 (CH$_2$-1), 29.2 (CH$_2$-3), 28.9 ((CH$_3$)$_2$-6), 27.2 (CH$_2$-2), 23.7 (CH$_2$-5), 22.9 (COCH$_3$). – (+)-ESIMS m/z 210 ([M + Na]$^+$, 100); – (+)-ESI-HRMS m/z 210. 14653 [M + Na]$^+$ (calcd 210. 14645 for C$_{10}$H$_{21}$NO$_2$Na).

Homononactic acid amide (62): Colourless oil, $R_f$ = 0.47 (CHCl$_3$/5 % MeOH), violet colouration with anisaldehyde/sulphuric acid – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 6.27 (br s, 1H, NH/OH), 5.50 (br s, 1H, NH/OH), 4.18 (m, 1H, 5-H), 3.90 (q, $J$ = 7.7 Hz, 2-H), 3.80 (m, 1H, 9-H), 2.71 (br s, 1H, NH/OH), 2.36 (quint, $^3J$ = 7.2 Hz, 9-H), 2.00 (m, 2H, 3,4-H), 1.71 (m, 2H, 8-H$_2$). 1.62 (m, 2H, 3,4-H), 1.50 (m, 2H, 10-H$_2$), 1.17 (d, 3H, $^3J$ = 7.0 Hz, 6-CH$_3$), 0.94 (t, 3H, $^3J$ = 7.4 Hz, 10-CH$_3$). – $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 176.9 (CO-7), 81.1 (CH-2), 77.0 (CH-5), 70.6 (CH-9), 46.0 (CH-6), 41.1 (CH$_2$-8), 30.6 (CH$_2$-4), 30.2 (CH$_2$-10), 29.6 (CH$_2$-3), 14.2 (6-CH$_3$), 14.2 (10-CH$_3$). – (+)-ESIMS m/z 216 ([M + H]$^+$, 25), 238 ([M + Na]$^+$, 75). – (+)-HRESIMS m/z 216.15946 [M + H]$^+$ (calcd for C$_{11}$H$_{22}$NO$_3$, 216.15942).

2-(2'-Hydroxy-phenyl)-benzoxazole-4-carboxylic acid (63) Pale yellow solid, $R_f$ = 0.34 (CHCl$_3$/15 % MeOH), green fluorescent. – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 12.03 (br s, 1H, 8-OH), 7.96-8.08 (m, 3H, 5,7,6'-H), 7.55 (br s, 2H, 4',6'-H), 7.06-7.15 (m, 2H, 3',5'-H). – $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 165.5 (CO-8), 163.4 (2-C$_q$), 158.3 (2'-C$_q$), 149.4 (7a-C$_q$), 138.6 (3a-C$_q$), 134.3 (4-CH), 127.3 (6'-CH), 127.1 (5-CH), 125.2 (6-CH), 122.4 (4-C$_q$), 119.8 (5'-CH), 117.2 (3'-CH), 115.0 (7-CH), 109.7 (1'-C$_q$). – (+)-ESIMS m/z 278 ([M + Na]$^+$, 58). – (+)-HRESIMS m/z 278.04238 ([M + Na]$^+$) (calcd for C$_{14}$H$_9$NO$_4$Na, 278.04239).

2-(2'-Hydroxy-4'-chloro-phenyl)benzoxazole-4-carboxylic acid (64) Pale yellow solid, green fluorescent. $R_f$ = 0.30 (CHCl$_3$/15 % MeOH). – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 12.03 (br s, 1H, 8-OH), 7.96-8.08 (m, 3H, 5,7,6'-H), 7.55 (br s, 2H, 4',6'-H), 7.06-7.15 (m, 2H, 3',5'-H). – $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 165.5 (8-CO), 163.4 (2-C$_q$), 158.3 (2'-C$_q$), 149.4 (7a-C$_q$), 138.6 (3a-C$_q$), 134.3 (4'-CH), 127.3 (6'-CH), 127.1 (5-CH), 125.2 (6-CH), 122.4 (4-C$_q$), 119.8 (5'-CH), 117.2 (3'-CH), 115.0 (7-CH), 109.7 (1'-C$_q$). – (-)-ESIMS m/z 288 ([M - H]$^-$, 100), 290 ([M - H]$^-$, 30). – (-)-HRESIMS m/z 288.00691 ([M - H]$^-$) (calcd for C$_{14}$H$_8$NO$_4$Cl, 288.00636).
8.12 Terrestrial Actinomycete N 435

8.12.1 Primary Screening

TLC of the crude extract of the terrestrial actinomycete N 435 showed several UV absorbing zones. Anisaldehyde/sulphuric acid on TLC showed a pink colour reaction.

Table 9: Biological activity of the crude extract of the strain N 435.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (∅ mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0</td>
</tr>
<tr>
<td>Streptomyces viridochromogenes (Tü 57)</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0</td>
</tr>
<tr>
<td>Mucor miehei</td>
<td>0</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>20</td>
</tr>
<tr>
<td>Chlorella sorokiniana</td>
<td>20</td>
</tr>
<tr>
<td>Scenedesmus subspicatus</td>
<td>0</td>
</tr>
</tbody>
</table>

8.12.2 Fermentation and Isolation

The strain was cultivated on a 20-liter scale shaker culture (110 rpm) at 28 °C for 72 hours. After filtration using Celite, the water phase was extracted with XAD-16 and the bio-mass with ethyl acetate. Both extracts were combined according to the TLC analysis, to deliver an oily brown crude extract (3.25 g). The extract was chromatographed using silica gel column chromatography and eluted with dichloromethane and methanol with a gradient of increasing polarity. As a result, seven fractions were obtained. Working up and purification of the fractions led to the isolation of diketopiperazines (71, 6.5 mg; 72, 12 mg) along with ramthacin B (78, 1.50 mg).

Cyclo(dehydroalanyl-isoleucyl) (71): Colourless solid, $R_f = 0.53$ (CHCl$_3$/5% MeOH), with anisaldehyde/sulphuric acid orange. – $^1$H NMR ([D$_6$]DMSO, 300 MHz) $\delta$ 10.43 (s, 1H, NH), 8.40 (s, 1H, NH), 5.20 (s, 1H, 3-H$_{2a}$), 4.78 (s, 1H, 3-H$_{2b}$), 3.97 (m, 1H, 6-H), 1.80 (m, 1H, 8-H), 1.77 (dd, 2H, $J = 8.4$, 3.3 Hz, 7-H$_2$) 0.85 [d, 6H, $J = 10.3$ Hz, 9,10- (CH$_3$)$_2$]. – EIMS (70 eV) m/z 170 ([M]$^+$, 100), 114 (M-isobutyl, 25).

cis-Cyclo(leucyl-tyrosyl) (72): Colourless solid, $R_f = 0.60$ (CHCl$_3$/10% MeOH), with anisaldehyde/sulphuric acid orange. – $^1$H NMR ([D$_6$]DMSO, 300 MHz) $\delta$ 9.14 (s, 1H, NH), 7.97 (s, 1H, NH), 6.91 (d, 2H, $J = 8.5$ Hz, 3’, 5’-H), 6.65 (d, 2H, $J = 8.5$ Hz, 3’, 5’-H), 4.06 (quint, $J = 4.0$ Hz, 3-H), 3.03 (dd, 1H, $J = 13.7$, 4.0 Hz, 7-CH$_2$)), 2.71 (dd,
1H, J = 13.7, 4.0 Hz, 7-CH₂(b), 1.45 (m, 1H, 11-H₂a), 0.66 (dd, 6H, J = 4.9, 1.7 Hz, 9,10-CH₃), 0.23 (m, 1H, 11-H₂a). – ¹³C NMR ([D₆]DMSO, 300 MHz) δ 167.4 (C_q, 2-CO),166.2 (C_q, 5-CO), 156.3 (C_q, 4'-OH), 131.1 (2CH, 3',5'-H), 125.9 (C_q-1'), 114.8 (2CH, 2',6'-H), 55.6 (CH, 6-H), 52.3 (CH, 3-H), 43.6 (CH₂,11-CH₂), 37.7 (CH₂,7-H₂), 22.9 (CH, 8-H), 22.7 (CH₂, 9-H₂), 21.3 (CH₃, 10-H₃). – EIMS m/z 276 ([M]+, 15), 170 (100), 114 (36), 107 (98) 44 (52). – (-)-ESIMS m/z 275 ([M - H]-, 47), 551 ([2 M - H]⁻, 100); – (-)-HRESIMS 275.13974 (calcd for C₁₅H₂₀N₂O₃, 275.13956).

**Ramthacin B (78):** UV absorbing, colourless oil, R₇ = 0.73 (cyclohexane/60% ethylacetate), dark green colouration with anisaldehyde/sulphuric acid. – ¹H NMR (CDCl₃, 300 MHz) δ 7.79 (dd, 3J = 9.1, 4J = 1.8 Hz, 1H, 7-H), 7.77 (d, 4J = 1.8 Hz, 1H, 5-H), 6.59 (d, 3J = 9.1 Hz,1H, 8-H), 4.91 (dd, 3J = 10.0, 3J = 2.4 Hz 1H, 12-H), 4.60 (t, 3J = 11.1 Hz, 1H, 3-H), 3.67 (s, 2H, 17-H), 3.48 (dd, 3J = 17.5, 2J = 4.9 Hz, 1H, 4-Hₐ), 3.42 (s, 1H, 3-H 17-OCH₃), 3.11 (dd, 3J = 17.3, 2J = 6.2 Hz, 1H, 4-Hₐ),, 2.05 (m, 1H, 11-Hₐ), 1.74 (dd, 3J = 14.4, 2J = 2.4 Hz, 1H, 11-Hₐ), 1.63 (s, 9H, 3 CH₃, 15-CH₃, 18-CH₃, 19-CH₃). – EIMS m/z 367 ([M]+, 10), 322 (12), 304 (40), 258 (100), 224 (68), 99 (34). – (+)-ESIMS m/z 801 ([2 M – 2 H + 3 Na]+, 50), 412 [M - H + 2 Na]+, 100). – (-)-ESIMS m/z 755 ([2 M – 2 H + Na]⁻, 100), 733 ([2 M - H]⁻, 70), 366 ([M - H]⁻, 10), – HREIMS m/z (calcd for C₁₉H₂₆NO₄Cl, 367.15500).

### 8.13 Marine *Streptomyces* sp. B 7857

#### 8.13.1 Primary screening

*Streptomyces* sp. B 7857 showed moderate biological activity (Table 9). It also showed several UV fluorescent zones on TLC. Some non-UV absorbing spots showed green colour with spraying with anisaldehyde/sulphuric acid. The crude extract showed 34% activity against *Artemia salina*.

**Table 10:** Biological activity of the crude extract of the strain B7857.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (∅ mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Streptomyces viridochromogenes</em> (Tü 57)</td>
<td>14</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Mucor miehei</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>11</td>
</tr>
</tbody>
</table>
8.13.2 Fermentation and Isolation

The terrestrial *Streptomyces* sp. B 7857 was cultivated using M 2 medium in Erlenmeyer flasks (250 ml each) at a 20 l scale on a linear shaker (110 rpm) at 28 °C for 7 days. The culture broth was mixed with Celite, and filtered off under pressure. The filtrate was extracted with XAD-16 column and the resin was eluted with methanol to get a dark brown extract. The biomass was extracted with ethyl acetate (3 times). From the TLC the biomass and XAD extract did not show much difference and were mixed together. The total crude extract (2.57 g) was chromatographed on a silica gel column eluting with a CH₂Cl₂/MeOH gradient to get four fractions. Fraction 1 was further purified by using Sephadex LH-20, silica column chromatography, PTLC and finally by an RP-8 gravity column eluting with 50-60 % aqueous methanol to get compound **81** (15.8 mg) and **83** (9 mg), which gave first bright pink colour and turned to green with anisaldehyde/sulphuric acid. In the same way compound **84** (1.1 mg) was isolated from fraction 3. Fraction 3 and 4 contained the major components of this strain, which were homononactic acid (**87**, 175 mg) and nonactic acid (**86**, 23 mg).

**Bacteriogorgonene A (81):** Colourless oil, \( R_f = 0.40 \) (CHCl₃/5 % MeOH). \( ^1\text{H} \) NMR (CDCl₃, 300 MHz) \( \delta \) 5.86 (s, 1H, NH), 2.25 (s, 2H, 1-CH₂), 2.25 (t, \( ^4\text{J} = 1.1 \) Hz, 1H, 3-H), 2.84, 2.89 (m, 1H, 5-H), 1.37 (m, 1H, 6-H), 2.14, 2.21 (m, 2H, 7-CH₂), 1.37 (m, 4H, 8,9-(CH₂)₂), 1.31 (s, 6H, 12,13-Me) 0.94 (s, 3H, 14-CH₃),1.95 (t, \( ^4\text{J} = 1.4 \), 15-CH₃). \( ^{13}\text{C} \) NMR (CDCl₃, 150 MHz) \( \delta \) 200.1 (2-Cq), 165.0 (4-Cq), 126.3 (3-CH), 74.4 (11-Cq), 42.9 (5-CH), 41.5 (6-CH), 36.9 (9-CH₂), 36.9 (10-Cq), 29.3 (12, 13-(CH₃)₂), 23.3 (7-CH₂), 21.9 (15-CH₃), 20.6 (8-CH₂), 17.8 (14-CH₃); – (+)-ESIMS \( m/z \) 237 [M + H]+. – (+)- HRESIMS \( m/z \) 237.18510 (calcd. for C₁₅H₂₅O₂, 237.18510).

**82**- (+)-ESIMS \( m/z \) 219 [M + H]+ – (+)- HRESIMS \( m/z \) 219.17447 (219.17444 calcd. for C₁₅H₂₅O₂).

**Bacteriogorgonene B (83):** Colourless oil, \( R_f = 0.50 \) (CHCl₃/5 % MeOH). \( ^1\text{H} \) NMR (CDCl₃, 300 MHz) \( \delta \) 5.93 (t, \( J = 1.1 \) Hz, 1H, 3-H), 4.21 (br s, 1H, 8-H), 2.72 (d, 1H, \( J = 15.6 \) Hz, 1-CH₂b), 2.52 (t, \( ^3\text{J} = 11.3 \) Hz, 1H, 6-H), 2.21 (d, \( J = 2.2 \) Hz, 1H, 1-CH₂a), 2.21 (m, 1H, 7-CH₂b), 1H, 1-CH₂b), 2.00 (s, 3H, 15-CH₃), 1.97 (m, 2H, 8-CH₂), 1.96 (m, 1H, 7-CH₂b), 1.56 (dd, \( J = 15.0 \), 2.5 Hz, 2H, 9-CH₂), 1.38 (s, 3H, 13-CH₃), 1.34 (s, 3H, 12-CH₃), 1.23 (s, 3H, 14-CH₃). \( ^{13}\text{C} \) NMR (CDCl₃, 150 MHz) \( \delta \) 199.3 (2-Cq), 156.2 (4-Cq), 128.9 (3-CH), 84.6 (5-Cq), 81.1 (11-Cq), 67.7 (8-CH₂), 51.4 (6-CH),
49.2 (1-CH₂), 41.7 (9-CH₂), 40.4 (10-C₉), 27.4 (7-CH₂), 24.8 (14-CH₃), 22.6 (12-CH₃), 20.3 (13-CH₃), 19.7 (15-CH). – CIMS (NH₃) m/z 518 ([2 M + NH₄]⁺, 24), 285 ([M + NH₃ + NH₄]⁺, 24), 268 ([M + NH₄]⁺, 100); – (+)-HRESIMS m/z 273.14640 (calcd for C₁₅H₂₂O₃Na, 273.14642).

**Bacteriogorgonene C (84):** Colourless oil, _R_f_ = 0.35 (CHCl₃/5 % MeOH). – ¹H NMR (CDCl₃, 300 MHz) _δ_ 5.80 (t, _J_ = 1.1 Hz, 1H, 3-H), 2.82 (1H, 1-CH₂b), 1.90 (1H, 1-CH₂a), 2.25 (1H, 7-CH₂b), 2.05 (1H, 9-CH₂b), 1.95 (1H, 8-CH₂b), 1.90 (s, 3H, 14-CH₃), 1.85 (1H, 7-CH₂a), 1.80 (2H, 6-CH₂), 1.80 (1H, 8-CH₂a), 1.40 (s, 6H, 12,13-(CH₃)₂), 1.15 (1H, 9-CH₂a), 1.02 (s, 3H, 15-CH₃). – ¹³C NMR (CDCl₃, 150 MHz) _δ_ 199.9 (2-C₉), 161.2 (4-C₉), 127.6 (3-CH), 74.4 (11-C₉), 71.9 (5-CH), 48.4 (1-CH₂), 40.6 (6-CH), 39.8, (10-C₉), 31.9 (9-CH₂), 28.5 (7-CH₂), 28.5 (13-CH₃), 28.3 (12-CH₃), 22.4 (15-CH₃), 19.6 (8-CH₂), 19.2 (14-CH₃). – DCIMS m/z 270 ([2 M + NH₄]⁺, 42); – (+)-HRESIMS m/z 275.16176 (calcd for C₁₅H₂₄O₃Na, 275.16177).

### 8.14 Terrestrial Streptomyces sp. GW 5127

#### 8.14.1 Primary screening

The crude extract of terrestrial Streptomyces sp. GW 5127 was found to inhibit the growth of Gram-positive and Gram-negative bacteria, fungi and algae. TLC of the crude extract exhibited several red spots, which turned to violet with anisaldehyde/sulphuric acid and some colourless UV inactive bands, which became first brown and later turned to violet with anisaldehyde/sulphuric acid.

**Table 11:** Biological activity of the crude extract of the strain GW 5127.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (Ø mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Streptomyces viridochromogenes</em> (Tü 57)</td>
<td>13</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>10.5</td>
</tr>
<tr>
<td><em>Mucor miehei</em></td>
<td>12</td>
</tr>
</tbody>
</table>

#### 8.14.2 Fermentation and isolation

A sub-culture of the terrestrial strain *Streptomyces* GW5127 was used to incubate a 25 l shaker culture using M₂ medium at 28°C in a linear shaker culture. The culture
broth was harvested after 8 days, mixed with Celite, and filtered off. The filtrate and mycelia were separately extracted using XAD-16 for the water phase, followed by elution with MeOH-H₂O. The methanolic extract was evaporated and the water residue was again extracted with ethyl acetate. The biomass was extracted with ethyl acetate (3 times).

Silica gel column chromatography of the mycelial fraction (0.91 g) followed by PTLC and size exclusion chromatography yielded β-rubromycin (95, 25 mg) and γ-rubromycin (96, 20 mg) as red solids. The extract from the water phase (1.84 g) was separated into four fractions on silica gel (column, CH₂Cl₂-MeOH gradient). PTLC of fraction 2 (0.31 g) followed by Sephadex LH-20 (CH₂Cl₂-MeOH 6:4 and MeOH) 90 (1.5 mg) and 3-hydroxy-4-methoxy-β-phenylethyl alcohol (94, 7.2 mg) as colourless solids. The polar fraction 4 (0.21 g) yielded on Sephadex LH-20 (MeOH) N-[2-(3-hydroxy-4-methoxy-phenyl)-ethyl]-acetamide (93, 3.7 mg) as colourless oil.

**8-Ethyl-6,11-diydroxy-1-methoxy-naphthacene-5,12-dione (88):** Red amorphous solid, Rf = 0.50 (CH₂Cl₂), pink UV fluorescent; UV (MeOH): ²max 310 (4.07), 510 sh (3.38), 542 (3.58), 577 (3.53); ²max (MeOH/HCl) nm (log ε): 310 (4.07), 510 (3.43), 542 (3.64), 578 (3.57); ²max (MeOH/NaOH) nm (log ε): 313 (4.03), 621 (3.52). – IR (KBr): νmax 3440, 2924, 1635, 1557, 1419, 1384, 1271, 1042 cm⁻¹. – ¹H NMR (CDCl₃, 600 MHz) δ 16.10 (1H, s, 6-OH), 15.31 (1H, s, 11-OH), 8.39 (1H, d, J = 8.2 Hz, 7-H), 8.26 (1H, d, J = 1.8 Hz, 10-H), 8.13 (dd, J = 7.8, 1.0 Hz, 1-H), 7.74 (1H, t, J = 8.1 Hz, 2-H), 7.63 (1H, dd, J = 8.2, 1.8 Hz, 8-H), 7.29 (1H, d, J = 8.1 Hz, 3-H), 4.08 (3H, s, 4-OCH₃), 2.86 (2H, q, J = 7.7 Hz, 13-H), 1.34 (3H, t, J = 7.7 Hz, 14-H). – ¹³C NMR (CDCl₃, 150 MHz) δ 176.0 (5-CO), 174.0 (12-CO), 171.4 (11-Cq), 170.5 (6-Cq), 160.0 (4-Cq), 149.4 (9-Cq), 134.9 (12a-Cq), 133.9 (2-CH), 133.0 (8-CH), 131.5 (10a-Cq), 130.5 (6a-Cq), 126.2 (7-CH), 124.5 (10-CH), 121.1 (4a-Cq), 118.7 (1-CH), 115.4 (3-CH), 108.2 (11a-Cq), 107.1 (5a-Cq), 56.6 (4-OCH₃), 29.3 (13-CH₂), 15.2 (14-CH₃). – (+)-ESI MS: m/z (%) 349 ([M + H]⁺, 35), 719 ([2M + Na]⁺, 100). – EI MS (70 eV) m/z 348 ([M]⁺, 100), 330 ([M - H₂O]⁺, 65), 302 ([M - (CO+ H₂O)]⁺, 12), 262 (6), 205 (4), 189 (5), 165 (5); – HREIMS m/z [M]⁺ (calcd for C₂₁H₁₆O₅, 348.09977; found: 348.0998).

**4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (90):** White amorphous powder, Rf = 0.66 (CH₂Cl₂-10% MeOH): UV ²max (MeOH) nm (log ε): 264 (3.86), 349 (3.32), 379 (3.15); ²max (MeOH/HCl) nm (log ε): 265 (4.05), 327 (3.56), 373 (2.97); ²max (MeOH/NaOH) nm (log ε): 272 (3.73), 341 (3.38), 369 (3.33), 379 (3.36). – IR νmax (KBr): 3437 (NH), 2967 (CH), 1685 (C=O), 1619, 1460, 1383, 1268, 1160, 638
N-[2-(3-Hydroxy-4-methoxy-phenyl)-ethyl]-acetamide (93): Colourless oil, Rf = 0.30 (CH2Cl2-5% MeOH). – IR (KBr): νmax 3441 (OH), 2932, 1651 (amide), 1513, 1440, 1383, 1276, 1132, 1027 cm⁻¹. – UV λmax (MeOH) nm (log ε): 325 (3.71); λmax (MeOH/HCl) nm (log ε): 325 (3.73); λmax (MeOH/NaOH) nm (log ε): 334 (3.72). –1H NMR (CDCl3, 300 MHz) δ 6.78 (1 H, d, J = 8.1 Hz, 5-H), 6.77 (1H, d, J = 2.1 Hz, 6-H), 6.65 (1H, dd, J = 8.1, 2.1 Hz, 6-H), 6.16 (br s, 1H, 3-OH), 5.75 (br s, 1H, 1H, 3'-NH), 3.84 (3H, s, 4-OCH3), 3.44 (2H, q, J = 6.8 Hz, 2'-H), 2.68 (2H, t, J = 6.8 Hz, 1'-H), 1.92 (3H, s, 5'-H3). – 13C NMR (CDCl3, 150 MHz) δ 170.4 (4'-Cq), 145.7 (3-Cq), 145.4 (4-C q), 131.9 (1-C q), 120.0 (6-CH), 114.9 (2-CH), 110.9 (5-CH), 55.9 (4-OCH3), 40.7 (1'-CH2), 34.8 (2'-CH2), 23.2 (5'-CH3). EIMS (70 eV) m/z 209 [M]+ (18), 150 (100), 137 (39), 135 (20), – HREIMS m/z [M]+ (calcd for C11H15NO3, 209.1052; found: 209.1052).

4-(2-Hydroxy ethyl)-2-methoxy-phenol (94): colourless oil, Rf = 0.430 (CH2Cl2-10% MeOH) – 1H NMR (CD3OD, 300 MHz): δ 6.82 (dd, J = 8.1, 1.8 Hz, 1H, 5-H), 6.81 (d, J = 1.8 Hz, 1H, 2-H), 6.73 (dd, J = 8.1, 1.9 Hz, 1H, 6-H), 3.90 (s, 3H, 3-OCH3), 3.86 (t, J = 7.4 Hz, 2H, 8-CH2), 2.78 (t, J = 7.4 Hz, 2H, 7-CH2).

β-Rubromycin (95): Red powder, Rf = 0.60 (CH2Cl2/10% MeOH). – 1H NMR (CDCl3, 300.0 MHz) δ 12.50 (br s, 1H, OH), 10.64 (br s, 1H, OH), 7.61 (s, 1H, 6-H), 7.22 (s, 1H, 5-H), 7.04 (s, 1H, 6'-H), 3.98 (s, 3H, OCH3), 3.90 (s, 3H, OCH3), 3.86 (s, 3H, OCH3), 3.40, 3.38 (AB, J = 18.0 Hz, 1H, 3'-H_{a,b}), 3.20-2.98 (m, 2H, 4-H2), 2.55, 2.30 (2 m, 2H, 3-H2). – 13C NMR (125 MHz, CDCl3) δ 181.1 (9'-Cq), 177.9 (4'-Cq), 163.4 (9-Cq), 159.7 (7-Cq), 155.3 (9'a-Cq), 154.7 (5'-CH), 153.9 (7'-Cq), 148.8 (10-Cq), 148.4 (8'-Cq), 140.4 (10a-Cq), 140.3 (7-Cq), 131.5 (4a-Cq), 127.2 (3'a-Cq), 127.0 (5a-Cq), 118.6 (5-CH), 114.1 (8'a-Cq), 113.2 (6-CH), 111.2 (2-Cq), 109.5 (6'-Cq), 106.2 (9a-Cq), 105.2 (6'-Cq), 56.7 (7'-OCH3), 56.3 (5'-OCH3), 52.4 (7-CO2CH3), 38.7 (3'-CH2), 28.2 (3-CH2), 21.4 (4-CH2). – (+)-ESIMS m/z 1632 [(3 M + Na)+, 68], 1095 [(2M + Na)+, 100], 559 [(M + Na)+, 10]; – (-)-ESIMS m/z 535 [(M - H)+].

γ-Rubromycin (96): Red powder, Rf = 0.63 (CH2Cl2/10% MeOH). – 1H NMR (CDCl3, 300.0 MHz) δ 13.07 (s, 1H, OH), 12.28 (s, 1H, OH), 11.05 (s, 1H, OH), 7.63 (s, 1H, 6-H), 7.22 (s, 1H, 5-H), 6.37 (s, 1H, 6'-H), 3.85 (s, 6H, 7'-OCH3, 7-CO2CH3), 3.60-3.00 (m, 4H, CH2), 2.40-2.20 (m, 2H, CH2). – 13C NMR (125 MHz, CDCl3) δ
8.15 Terrestrial *Streptomyces* sp. GW 18/1811

8.15.1 Primary screening

The crude extract of the terrestrial *Streptomyces* sp. GW 18/1811 obtained from a 1 L culture exhibited biological activity against *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Candida albicans*, and *Mucor miehei*.

**Table 12:** Biological activity of the crude extract of the strain GW 18/1811.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition zone (Ø mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>14</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Streptomyces viridochromogenes</em> (Tü 57)</td>
<td>11</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>28</td>
</tr>
<tr>
<td><em>Chlorella sorokiniana</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Scenedesmus subspicatus</em></td>
<td>10</td>
</tr>
</tbody>
</table>

8.15.2 Cultivation and isolation

With a well grown agar culture of GW 18/1811, 100 of 1 L Erlenmeyer flasks each containing 250 ml of M2 medium were inoculated and incubated for 6 days at 28 °C on a linear shaker (110 rpm). The culture broth was mixed with Celite, and filtered off. The filtrate and mycelia were separately extracted using XAD-16 for the water phase, followed by elution with MeOH-H2O. The methanolic extract was evaporated and the water residue was again extracted with ethyl acetate. The biomass was extracted with ethyl acetate (3 times). Evaporation of the organic solvent gave 1.5 g of a brown oily extract. The crude extract was subjected to silica column chromatography using CH2Cl2/MeOH gradient to separate four fractions. Fraction 1 contained fat and fatty acids. After purifying fraction 2 through Sephadex LH-20 and PTLC, feudomyycinone (98, 3.5 mg) and komodoquinone B (97, 7 mg) were isolated. Separation of
fraction 3 on Sephadex LH-20 provided α-hydroxyacetovanillone (99, 3.0 mg) and lysolipin I (102, 2.5 mg). PTLC and Sephadex LH-20 of fraction 5 gave N-acetylhomocysteine thiolactone (101, 30 mg).

**Komodoquinone B (97):** Red solid, UV absorbing (254 nm) and orange fluorescent (366 nm), \( R_f = 0.42 \) (CH\(_2\)Cl\(_2\)/5%MeOH). – \(^1\)H NMR ([\(\text{D}_6\)]DMSO, 300 MHz) \( \delta \) 13.40 (br s, 1H, 11-OH), 12.20 (br s, 1H, 4-OH), 7.81 (dd, \(^3\)\( J = 7.7 \) Hz, 7.5 Hz, 1H, 2-H), 7.75 (dd, \(^3\)\( J = 7.5 \) Hz, \(^4\)\( J = 1.5 \) Hz, 1H, 1-H), 7.37 (dd, \(^3\)\( J = 7.7 \) Hz, \(^4\)\( J = 1.5 \) Hz, 1H, 3-H), 5.28 (s, 1H, OH), 4.94 (br s, 1H, 7-H), 3.02 (d, \(^2\)\( J = 18.5 \) Hz, 1H, 10-H\(_A\)), 2.53 (d, \(^2\)\( J = 18.5 \) Hz, 1H, 10-H\(_B\)), 2.05 (dd, \(^2\)\( J = 13.7 \) Hz, 1H, 8-H\(_A\)), 1.84 (dd, \(^2\)\( J = 13.7 \) Hz, 1H, 8-H\(_B\)), 1.36 (s, 3H, 13-H). – (+)-ESIMS \( m/z \) 1091 [\(3 \text{ M} + \text{Na}\)] \(^+\) (45), 735 ([\(2 \text{M} + \text{Na}\)]\(^+\), 100), 379 ([M + Na]\(^+\), 7). – (-)-ESIMS \( m/z \) 733 ([\(2 \text{M} + \text{Na} – 2 \text{H}\)]\(^+\), 100), 355 ([M - H]\(^-\)], 52).

**Feudomycinone C (98):** Red solid, \( R_f = 0.48 \) (CH\(_2\)Cl\(_2\)/5%MeOH), UV absorbing (254 nm) and orange fluorescent (366 nm). – \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 14.10 (br s, 1H, 11-OH), 13.32 (s, 1H, 6-OH), 8.05 (dd, \(^3\)\( J = 7.7 \) Hz, 1H, 1-H), 7.78 (t, \(^3\)\( J = 7.7 \) Hz, 1H, 2-H), 7.40 (d, \(^3\)\( J = 7.7 \) Hz, 1H, 3-H), 5.28 (s, 1H, OH), 4.30 (t, 1H, \(^3\)\( J = 7.7 \) Hz, 7-CH), 4.07 (s, 3H, 4-OCH\(_3\)), 3.02 (d, \(^2\)\( J = 18.5 \) Hz, 1H, 10-H\(_A\)), 2.53 (d, \(^2\)\( J = 18.5 \) Hz, 1H, 10-H\(_B\)), 2.05 (dd, \(^2\)\( J = 13.7 \) Hz, 1H, 8-H\(_A\)), 1.84 (dd, \(^2\)\( J = 13.7 \) Hz, 1H, 8-H\(_B\)), 1.36 (s, 3H, 13-H). – (+)-ESIMS \( m/z \) 763 ([\(2 \text{ M} + \text{Na}\)]\(^+\), 100), 393 ([M + Na]\(^+\), 40); – (-)-ESIMS \( m/z \) 761 ([\(2 \text{ M} + \text{Na} – 2 \text{H}\)]\(^+\), 30), 369 ([M - H]\(^-\)], 100).

**α-Hydroxyacetovanillone (99):** Colourless oil, \( R_f = 0.43 \) (CH\(_2\)Cl\(_2\)/5%MeOH), UV absorbing at 254 nm. – \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 7.52 (d, 1H, \(^1\)J = 1.8 Hz, 2-H), 7.43 (dd, \(^1\)J = 8.3, 1.8 Hz, 1H, 6-H), 6.96 (d, \(^1\)J = 8.3 Hz, 1H, 5-H), 4.81 (s, 2H, COCH\(_3\)OH), 3.95 (s, 3H, 3-OCH\(_3\)). – \(^13\)C NMR (CDCl\(_3\), 150 MHz) \( \delta \) 196.7 (1'-CO), 151.4 (4-Cq), 146.9 (3-Cq), 126.2 (1-Cq), 122.9 (6-CH), 114.3 (5-CH), 109.5 (2-CH), 64.9 (2’-CH\(_2\)), 56.2 (3-OCH\(_3\)). – EIMS (70 eV) \( m/z \) 182 (18), 151 (100), 136 (8), 123 (20).

**N-Acetylhomocysteine thiolactone (101):** White solid, \( R_f = 0.57 \) (CH\(_2\)Cl\(_2\)/7%MeOH) \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 6.10 (br s, 1H, NH), 4.55 (m, 1H, 3-H), 3.20-3.40 (m, 2H, 5-CH\(_2\)), 2.95 (m, 1H, 4-CH\(_2\_2\)), 2.05 (s, 3H, COCH\(_3\)) and 1.90 (m, 1H, 4-CH\(_2\_2\)). – \(^13\)C NMR (CDCl\(_3\), 150 MHz) \( \delta \) 205.8 (CO-2), 170.8 (1-CO), \( \delta \) 59.4 (CH-3), 31.7 (4-CH\(_2\)), 27.5 (5-CH\(_2\)), 23.0 (1-COCH\(_3\)). – (+)-ESIMS \( m/z \) 341 ([\(2 \text{ M} + \text{Na}\)]\(^+\),100), 182 ([M + Na]\(^+\), 68).

**Lysolipin I (102):** Yellow solid, UV absorbing, \( R_f = 0.65 \) (CH\(_2\)Cl\(_2\)/10%MeOH), on TLC pink with NaOH – \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 13.13 (s, 1H, 11-OH), 12.93 (s, 1H, 19-OH), 7.94 (d, \( J = 8.0 \) Hz, 1H, 3-CH), 7.37 (d, \( J = 8.0 \) Hz, 1H, 2-CH), 7.09
(s, 1H, 22-CH), 5.63 (d, J = 5.4 Hz, 1H, 28-CH2a), 5.03 (m, 1H, 23-CH), 5.02 (d, J = 3.0 Hz, 1H, 16-CH), 4.70 (d, J = 4.1 Hz, 1H, 24-CH), 4.46 (d, J = 3.0 Hz, 1H, 15-CH), 4.18 (s, 3H, 6-OCH3), 3.55 (s, 3H, 24-OCH3), 3.37 (s, 6H, 16-OCH3, NCH3). – 13C NMR (CDCl3, 150 MHz) δ 181.6 (10-CO), 168.2 (26-CO), 158.9 (6-Cq), 151.6 (14-Cq), 149.9 (8-Cq), 145.1 (13-Cq), 143.6 (5-Cq), 139.6 (19-Cq), 139.0 (1-Cq), 134.4 (20-Cq), 133.3 (9-Cq), 127.5 (4-Cq), 125.6 (2-CH), 120.8 (3-CH), 120.7 (21-Cq), 118.8 (17-Cq), 116.2 (22-CH2), 110.9 (13-Cq), 110.4 (18-Cq), 108.9 (12-Cq), 92.0 (28-CH2), 91.1 (24-CH), 78.8 (15-CH), 75.4 (16-CH), 67.8 (23-CH), 61.7 (6-OCH3), 58.1 (24-OCH3), 57.7 (23-OCH3), 36.6 (N-CH3). – (+)-ESIMS m/z 620 [M + Na]+, 1217 [2 M + Na]+, – (-)-ESIMS m/z 596 [M - H]-.

8.16 Fungal metabolite bikaverin

Bikaverin (103). Prof. H. Anke had sent a dark red sparingly soluble material for further identification. The sample (75 mg) was suspended in CHCl3 and methanol and centrifuged (2000 rpm, 10 min) to separate easily soluble impurities. – 1H NMR (300 MHz, [CHCl3+TFA]) δ 7.39 (br s, 1H, 4-H), 7.31 (br s, 1H, 2-H), 6.80 (br s, 1H, 9-H), 4.16 (br s, 6H, 8-(OCH3)2), 2.97 (br s, 3H, 1-CH3). – 13C NMR (75 MHz, CHCl3+TFA) δ 179.5 (12-CO), 178.2 (11-Cq), 172.0 (3-Cq), 166.8 (6-Cq), 166.1 (7-Cq), 163.4 (8-Cq), 162.4 (4a-Cq), 157.1 (5a-Cq), 146.3 (1-Cq), 124.0 (2-CH), 113.4 (6a-Cq), 112.4 (11a-Cq), 112.1 (12a-Cq), 109.3 (9-CH), 103.7 (10a-Cq), 100.1 (4-CH), 58.0 (3-OCH3), 57.5 (8-OCH3), 23.4 (1-CH3). – EIMS (70 eV) m/z 382 ([M]+, 100), 368 (56), 352 (84), 324 (36).

8.17 Plant metabolites

The root parts of Tephrosia calophylla were collected from Khailasa hills, Visakhapatnam, India, during February 2006. Air-dried and finely powdered roots (1.5 kg) of T. calophylla were macerated at room temperature for 72 h with chloroform (3 × 4.5 L). The crude extracts were subjected to column chromatography on silica gel.

Spinoflavanone B (103): Yellow solid. – 1H NMR (CDCl3, 300 MHz) δ 12.35 (s, 1H, 5-OH), 7.44 (m, 5H, phe), 6.39 (s, 1H, 7-OH), 5.39 (ABX, 1H, 2-H), 5.22, 5.20 (2 t, J = 7.2 Hz, each 1H, 2", 2"'-H), 3.35 (t, J = 7 Hz, 4H, 1", 1"'-CH2), 1.82, 1.75 (2 s, each 3 H, 2 Me), 1.70 (s, 6H, 2 Me) – 13C NMR (CDCl3, 125 MHz) δ 196.2 (4-CO), 162.3 (5-Cq), 159.3 (7-Cq), 157.6 (8a-Cq), 138.9 (1'-Cq), 134.7 (3"-Cq), 133.9 (3"'-Cq), 128.7 (2", 6'-CH), 128.5 (4'-CH), 125.9 (3', 5'-CH), 122.0/121.7 (2", 2"'-CH), 107.3 (6-CH), 106.5 (8-CH), 102.8 (4a-Cq), 78.8 (2-CH), 43.4 (3-CH2), 25.82/25.80 (3"'-CH2, 2Me), 21.9
Calophione A (104): White amorphous powder. – 1H NMR (CDCl3, 300 MHz) δ 12.24 (s, 1H, 6'-OH), 12.06 (s, 1H, 6''-OH), 7.23 (br s, 1H, 4''-H), 6.82 (s, 1H, 4'-H), 6.53 (br s, 1H, 7'-H), 6.45 (s, 1H, 7''-H), 6.00 (s, 2H, 2'-CH2), 5.50 (ABX, 1H, 2''-H), 5.10 (br s, 1H, 2''-H), 3.30, 2.90 (ABX, JAB = 15.6, JAX = 10.6, JBX = 8.7 Hz; 2H, 3''-CH2), 1.72 (s, 3H, Me). – 13C NMR (CDCl3, 125 MHz) δ 194.0 (1-CO), 193.5 (2-CO), 168.5 (6''-Cq), 167.6 (6'-Cq), 164.3 (7''a-Cq), 156.4 (7'a-Cq), 142.7 (1''-Cq), 141.2 (3'a-Cq), 128.0 (4''-CH), 120.3 (3''-Cq), 113.2 (2''-CH2), 110.6 (5''-Cq), 109.4 (5'-Cq), 108.0 (4''-CH), 98.9 (7''-CH), 98.5 (7''-CH), 88.3 (2''-CH), 32.6 (3''-CH2), 16.9 (3''-CH3). – (+)-ESIMS m/z 391 ([M + Na]+); – (+)-HRESIMS m/z 369.09699 [M + H]+, (calcd for C20H17O7, 369.09688).

Tephcalostan (107): Colourless needles. – 1H NMR (CDCl3, 300 MHz) δ 7.67 (s, 1H, 1-H), 7.43 (s, 1H, 7-H), 7.06 (s, 1H, 10-H), 6.87 (s, 1H, 4-H), 6.05 (s, 2H, O-CH2-O), 5.33 (ABX, 1H, J = 8.6 Hz, 1H, 5'-H), 5.12 (br s, 1H, 7''-Ha), 4.94 (br s, 1H, 7''-Hb), 3.45, 3.13 (ABX, JAB = 16.2, JAX = 9.3, JBX = 7.8 Hz, 2H, 4''-H2), 1.80 (s, 3H, 8'-CH3). – 13C NMR (CDCl3, 125 MHz) δ 162.9 (3-Cq), 160.2 (11a-Cq), 158.5 (6-CO), 154.6 (4a-Cq), 150.3 (10a-Cq), 147.2 (9-Cq), 146.0 (8-Cq), 142.9 (6'-CH), 124.9 (2-CH), 117.0 (6b-Cq), 116.8 (8-CH), 112.9 (7-CH2), 106.0 (11b-Cq), 103.2 (6a-Cq), 101.9 (O-CH2-O), 100.0 (7-Cq), 98.5 (4-CH), 93.9 (10-CH), 87.5 (5'-CH), 33.6 (4'-Cq). – (+)-ESIMS m/z 747 ([2 M + Na]+); – (+)-HRESIMS m/z 363.08641 [M + H]+, (calcd for C21H15O6).

Tephcalostan A (108): Colourless powder. – 1H NMR ([D6]DMSO, 300 MHz, 100 °C) δ 7.80 (s, 1H, 1-H), 7.43 (s, 1H, 10-H), 7.28 (s, 1H, 7-H), 6.96 (s, 1H, 4-H), 6.13 (s, 2H, -O-CH2-O-), 6.08 (m, 1H, 6'-H), 5.45 (m, 1H, 7'-CH2a), 5.43 (m, 1H, 5'-H), 5.28 (m, 1H, 7'-CH2b), 3.58, 3.13 (ABX, JAB = 15.1, JAX = 9.2, JBX = 7.3 Hz, 2H, 4''-H2). – 13C NMR ([D6]DMSO, 125 MHz, 100 °C) δ 162.1 (3-Cq), 159.4 (11a-Cq), 156.8 (6-CO), 153.8 (4a-Cq), 149.6 (10a-Cq), 146.7 (9-Cq), 145.5 (8-Cq), 136.5 (6'-CH), 125.0 (2-CH), 116.7 (1-CH), 116.5 (7'-CH2), 116.0 (6b-Cq), 105.0 (6a-Cq), 101.6 (O-CH2-O), 101.5 (11b-Cq), 98.3 (10-CH), 97.6 (4-CH), 94.0 (7-CH), 84.5 (5'-CH), 33.6 (4'-Cq). – (+)-ESIMS m/z 719 ([2 M + Na]+); – (+)-HRESIMS m/z 349.07073 [M + H]+, (calcd for C20H13O6).

Tephcalostan B (111): Colourless solid. – 1H NMR (DMSO, 300 MHz) δ 7.37 (s, 1H, 5-H), 7.20 (s, 1H, 6'-H), 6.77 (s, 1H, 8-H), 6.10 (s, 2H, 9-CH2), 3.25 (t, J = 6.8 Hz, 2H, 6''-CH2), 1.90 (t, J = 6.8 Hz, 2H, 5''-CH2), 1.36 (s, 6H, 2''-CH3). – 13C NMR (CDCl3, 125 MHz) δ 160.6 (2-Cq), 157.3 (3a-CO), 150.4 (5'-Cq), 149.4 (8a-Cq) 148.0
Tephcalostan C (112): Colourless solid. – $^1$H NMR ([D$_6$]DMSO, 300 MHz) $\delta$ 7.44 (s, 1H, 5-H), 7.32 (d, $J = 10.1$ Hz, 1H, 6''-H), 7.21 (s, 1H, 6'-H), 6.82 (s, 1H, 8-H), 6.11 (s, 2H, 9-CH$_2$), 6.02 (d, $J = 10.1$ Hz, 1H, 5''-H), 1.46 (s, 6H, 2''-CH$_3$). – $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 159.8 (4-C$_q$), 157.2 (3a-CO), 149.7 (5'-C$_q$), 149.6 (8a-C$_q$), 148.2 (3'-C$_q$), 146.8 (7-C$_q$), 145.7 (6-C$_q$), 137.7 (2'-C$_q$), 133.3 (5''-CH), 118.9 (6''-CH), 115.7 (1'C$_q$), 103.6 (3-C$_q$), 103.2 (3a-C$_q$), 101.9 (9-CH$_2$), 98.8 (6'-C), 98.3 (8-CH), 94.3 (5-CH), 94.3 (4'-C$_q$), 75.7 (2''-C$_q$), 26.8 (3'',4''-CH$_3$). – (-)-ESIMS m/z 377 ([M - H] -, 70); – (+)-HRESIMS m/z 379.08113 [M + H]$^+$, (calcd for C$_{21}$H$_{17}$O$_7$, 379.08177).

Milletone (113): Yellow needles. – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.74 (d, $J = 8.7$ Hz, 1H, 11-H), 6.75 (s, 1H, 1-H), 6.64 (d, $J = 10.1$ Hz, 1H, 4''-H), 6.45 (d, $J = 8.7$ Hz, 1H, 10-H), 6.41 (s, 1H, 4-H), 5.85 ("d", $J = 14.4$ Hz, 2H, OCH$_2$O), 5.56 (d, $J = 10.1$ Hz, 1H, 3''-H), 4.90 (m, 1H, 6a-H), 4.62 (dd, $J = 12.1$, $J = 3.2$ Hz, 1H, 5''-H), 3.79 (d, 1H, $J = 4.1$ Hz, 12a-H), 1.45, 1.39 (2s, each 3H, 2 2''-CH$_3$). – $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 189.1 (12-CO), 160.0 (9-C$_q$), 156.8 (7a-C$_q$), 148.3 (4a-C$_q$), 147.7 (3-CH), 142.2 (2-C$_q$), 128.7 (11-CH), 128.5 (3''-CH), 115.7 (4''-CH), 112.6 (11a-CH), 111.4 (10-CH), 109.1 (8-CH), 107.0 (1-CH), 105.7 (12b-C$_q$), 101.1 (O-CH$_2$-O), 98.7 (4-CH), 77.7 (2''-C$_q$), 72.1 (6a-C$_q$), 66.2 (6-C$_q$), 44.6 (12a-C), 28.4 (2''-CH$_3$), 28.1 (2'-CH$_3$). – (-)-ESIMS m/z 377 ([M - H]$^+$), 70; – (+)-HRESIMS m/z 379.08113 [M + H]$^+$, (calcd 379.08177 for C$_{21}$H$_{19}$O$_6$).

Glabranine (114): Colourless solid. – $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 12.00 (s, 1H, 5-OH), 7.45 (m, 5H, Phe), 6.55 (s, 1H, 7-OH), 6.04 (s, 1H, 6-H), 5.43 (ABX, 1H, 1), 5.22 (t, $J = 7.3$ Hz, 1H, 2''-H), 3.32 (d, $J = 7.3$, 2H, 1''-CH$_2$), 3.08, 2.85 (ABX, $J_{AB} = 17.1$, $J_{AX} = 12.8$, $J_{BX} = 3.2$ Hz, 3-CH$_2$), 1.71 (s, 6H, 4''-5''-CH$_3$). – $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 196.3 (3-CO), 163.8 (7-C$_q$), 162.2 (8a-C$_q$), 159.7 (5-C$_q$), 138.7 (1'-CH), 134.6 (4'-C$_q$), 128.8 (2',6'-CH$_2$), 128.6 (4'-CH), 125.9 (3',5'-CH), 121.6 (2''-CH), 106.5 (8-CH), 103.1 (4a-C$_q$), 96.9 (6-CH), 78.9 (2-C), 43.2 (1''-CH$_2$), 25.8 (4''-CH$_3$), 21.8 (CH$_2$-3), 17.8 (5''-CH$_3$); " assignment may be exchanged. – (+)-ESIMS m/z 325 ([2 M + H]$^+$). – (+)-HRESIMS m/z 325.14350 [M + H]$^+$, (calcd for C$_{20}$H$_{21}$O$_4$, 325.14351).

Betulinic acid (115): White needles. – $^1$H NMR ([D$_6$]DMSO, 300 MHz) $\delta$ 12.0 (bs, 1H, 28-OH), 4.70 (s, 1H, 30-Ha), 4.56 (s, 1H, 30-Hb), 4.20 (d, $J = 4.9$ Hz, 3-OH), 2.97 (m, 1H, 3-H), 2.22 (dt, $J = 14.1$, 3.7 Hz, 1H, 19-H), 1.80 (m, 2H, 16-H), 1.65 (s, 3H, 30-CH$_3$), 0.65-1.85 (m, 1,2,6,7, 11, 12, 15, 21, 22-CH$_2$, 5-H), 0.93 (s, 3H, 23-
CH₃), 0.87 (s, 6H, 26, 27- (CH₃)₂), 0.77 (s, 3H, -CH₃), 0.65 (s, 3H, 23-CH₃). — ¹³C NMR ([D₆]DMSO, 125 MHz) δ 177.7, 150.8, 110.9, 77.3, 56.0, 55.4, 50.5, 49.1, 47.1, 42.5, 40.8, 39.0, 38.8, 38.2, 37.3, 36.9, 34.5, 32.3, 30.7, 29.7, 28.6, 27.7, 25.6, 21.0, 19.5, 18.5, 16.5, 16.29, 16.27, 14.9.—EIMS m/z 456 ([M]+, 98), 438 (24), 248 (75), 220 (28), 207 (65), 189 (100), 175 (23), 147 (20), 135 (32), 119 (26), 95 (32), 81 (28), 69 (24) – (-)-ESIMS m/z 911 ([2M -H]+); – (+)-HREIMS 457.36757 (calcd for C₃₀H₄₈O₃, 457.36761).

Betulinic acid methyl ester (116): White solid. – ¹H NMR (CDCl₃, 300 MHz) δ 4.78 (bs, 1H, 30-Ha), 4.60 (bs, 1H, 30-Hb), 3.86 (s, 3H, 28-O CH₃), 3.20 (m, 1H, 3-H), 3.00 (m, 1H, 19-H), 2.20 (m, 2H, 16-H), 1.70 (s, 3H, 30-CH₃), 0.65-1.85 (m, 9 CH₂,-1,2,6,7, 11, 12, 15, 21, 22 and 1 CH, 5-H), 0.97 (s, 6H, 26, 27- (CH₃)₂), 0.93 (s, 3H, 23-CH₃), 0.82 (s, 3H, -CH₃), 0.73 (s, 3H, 23-CH₃).

Stigmasterol (117): White crystaline solid.– ¹H NMR (CDCl₃, 300 MHz) δ 5.35 (br d, J = 5.6 Hz, 1H, 6-H), 3.53 (1H, m, 3-H), 1.01 (s, 3H, 19-H3), 0.93 (d, J = 6.4 Hz, 3H, 21-H3), 0.85 (d, J = 6.0 Hz, 3H, 29-H3), 0.83 (d, J = 7.6 Hz, 3H, 27-H3), 0.81 (d, J = 6.8 Hz, 3H, 26-H3), 0.68 (s, 3H, 18-H3). – ¹³CNMR (CDCl₃, 125 MHz) δ 140.7 (5-C), 121.7 (6-C), 71.7 (3-C), 56.8 (14-C), 55.9 (17-C), 50.1 (9-C), 45.8 (24-C), 42.30 (13-C), 42.26 (4-C), 39.8 (12-C), 37.3 (1-C), 36.5 (10-C), 36.3 (20-C), 33.9 (22-C), 31.90 (8-C), 31.91 (7-C), 31.7 (2-C), 28.9 (25-C), 28.2 (16-C), 26.4 (23-C), 24.3 (15-C), 21.1 (11-C), 19.6 (26-C), 19.4 (19-C), 19.0 (27-C), 18.8 (21-C), 12.2 (29-C), 11.9 (18-C). – EIMS (70 eV) m/z 414 ([M]+, 100), 396 (32), 255 (55). – HREIMS 414.3862 (calcd for C₂₉H₅₀O, 414.3857).

2-Methoxymaackiain (122): Yellow powder. – ¹H NMR (CDCl₃, 300 MHz) δ 6.94 (s,1H,1-H), 6.72 (s,1H, 7-H), 6.54 (s,1H,10-H), 6.44 (s,1H, 4-H), 5.91 (d, CH₂, 2H, 12-CH₂), 5.79 (s, 1 OH, 3-OH), 5.45 (d, J = 6.9 Hz, 11a-CH), 4.20 (dd, J = 5.4, 4.9 Hz, 1H, 12-CH₂a), 3.90 (s, 3H, 2-OCH₃), 3.58 (t, J = 10.8 Hz, 1H, 6-CH₂b), 3.45 (m, 1H, 6a-CH). – ¹³C NMR (CDCl₃, 125 MHz) δ 154.1 (10a-CO), 150.2 (6-CO), 148.4 (9-Cq), 147.2 (3-Cq) 142.1 (2-Cq), 141.7 (8-Cq),118.0 (6b-Cq), 111.6 (1-C), 110.6 (11b-Cq), 104.8 (7-CH), 103.5 (4-CH), 101.3 (12-CH₂) 93.7 (10-CH), 78.8 (11a-CH), 66.5 (6-CH₂), 56.4 (2-OCH₃), 40.3 (6a-CH).

Tephrosol (2-methoxymedicagol) (123): Amorphous powder – ¹H NMR ([D₅]pyridine, 300 MHz) δ 7.41 (s, 1H, 10-H), 7.23 (s, 1H, 1-H), 7.16 (s, 1H, 7-H), 6.89 (s, 1H, 4-H), 6.12 (s, 2H, 12-CH₂), 3.90 (s, C2-OCH₃). – ¹³C NMR (CDCl₃, 125 MHz) δ 159.6 (11a-Cq), 157.4 (6-CO), 151.1 (3-Cq), 149.6 (10a-Cq) 148.4 (4a-C), 146.8 (9-Cq), 145.9 (8-Cq), 145.7 (2-C), 116.3 (6b-CH), 103.6 (4-CH), 102.9 (11b-Cq), 102.4 (1-CH), 102.0 (12-CH₂), 102.0 (6a-Cq), 98.5 (7-CH), 94.4 (10-CH), 56.0
(2-OCH₃). – (-)-ESIMS m/z 325 ([M -H]⁺, 100); – (+)-HRESIMS m/z 327.049844 [M + H]⁺, (calcd for C₁₇H₁₁O₇, 327.04994).

**Didehydrovillosin (124):** Yellow pellets. – ¹H NMR ([D₆]DMSO, 300 MHz) δ 13.05 (s, 1H, 11-OH), 8.30/8.27, (s, 1H, 1-H), 7.97 (s,1H, 6-OH), 6.70 (s,1H, 4-H), 6.36/6.35 (s, 1H, 10-H), 6.10 (d, J = 8.7 Hz,1H, 6-H; instead of a double by coupling with OH, the signal could also be explained by two singlets of the individual isomers.), 5.42/5.40 (q, J = 8.5 Hz, 2'-H; quartet due to overlapping of two triplets from the two isomers), 5.10 (s, CH, 4-CH₂a), 4.98 (s, CH, 4'-CH₂b), 3.79 (s, 3H, 3-OCH₃), 3.77/3.75 (s, 3H, 2-OCH₃), 3.40 (m, CH, 1'-CH₂a), 3.02 (m, CH, 1'-CH₂b), 1.74 (d, J =1.7 Hz, 3H, 5'-CH₃). – ¹³C NMR (CDCl₃, 125 MHz) δ 179.72/179.65 (12-C), 165.93/165.89 (9-C), 162.77/162.75 (11-Cq), 155.5/155.4 (6a-C), 151.0/150.9 (7a-Cq), 149.43/149.37 (3-Cq), 143.53/143.50 (2-Cq), 143.3/143.2 (4a-Cq), 142.84/142.81 (CH-3'), 112.47/112.45 (CH-4'), 109.9/109.7 (CH-10), 108.5/108.4 (1-Cq), 107.48/107.45 (12b-Cq), 104.90/104.87 (12a-C), 103.3/103.2 (11a-Cq), 101.81/101.75 (4-C), 93.99/93.97 (10-CH), 88.0 (6-CH), 87.56/87.54 (2'-CH), 56.07/56.00 (3-OCH₃), 55.6 (2-OCH₃), 29.91/29.87 (1'-CH₂), 16.78/16.73 (5'-CH₃). – (-)-ESIMS m/z 423 ([M - H]⁻, 80); – (+)-HRESIMS m/z 425.12307 [M + H]⁺, (calcd for C₂₃H₂₁O₈, 425.12310).

**Dehydrorotenone (126):** Pale yellow solid. – ¹H NMR ([D₅]pyridine, 300 MHz) δ 9.32 (s,1H, 1-H), 8.74 (d, J = 8.6 Hz, 1H, 11-H), 7.41 (d, J = 8.6 Hz, 1H, 10-H), 7.21 (s, 1H, 4-H), 5.85 (ABX, 1H, 2'-H), 5.61 (s, 1H, 4'-H), 5.50 (s, 6-CH₂), 5.38 (s, 1H, 4'-H), 4.30 (s, 3H, 3-OCH₃), 4.17 (s, 3H, 2-OCH₃), 3.83 (ABX, Jₐₕ = 15.8, Jₓₓ = 9.9 Hz, 1H, 1'-CH₂a), 3.56 (1H, Jₐₕ = 15.8, Jₓₓ = 7.9 Hz, 1H, 1'-CH₂b), 1.87 (s, 3H, 5'-CH₃). – ¹³C NMR ([D₅]pyridine, 125 MHz) δ 174.1 (12-CO), 165.1 (9-Cq), 156.7 (6a-Cq), 152.6 (7a-C), 150.2 (3-Cq),147.4 (4a-Cq), 145.9 (2-Cq), 143.7 (3'-Cq), 128.1 (11-CH), 119.5 (11a-Cq), 113.8 (8-CH), 112.7 (4'-CH₂), 112.2 (1-CH), 111.8 (12b-Cq), 111.5 (12a-CH), 108.8 (10-CH), 101.8 (4-CH), 88.2 (2'-CH), 65.1 (6-CH₂), 56.7 (3-OCH₃), 56.0 (2-OCH₃), 31.5 (1'-CH₂), 17.2 (5'-CH₃). – (+)-ESIMS m/z 807 ([2 M + Na]⁺, 100), 393 ([M + H]⁺, 48).

**13-Hydroxy-9,10-dimethoxy-3,3-dimethyl-3H,13H-pyran[2,3-c:6,5-f']dichromen-7-one (127):** – ¹H NMR ([D₆]DMSO, 300 MHz) δ 12.90 (s, 1H, 11-OH), 8.30 (s, 1H, 1-H), 8.00 (s, 1H, 6-OH), 6.75 (s, 1H, 4-H), 6.70 (d, J = 7.8 Hz, 1H, 1'-H), 6.28 (s, 1H, 10-H), 6.20 (s, 1H, 6-H), 5.80 (d, J = 7.8 Hz, 1H, 2'-H), 3.80 (s, 3H, 2-OCH₃), 3.77 (s, 3H, 3-OCH₃), 1.45 (s, 3H, 4'-CH₃), 1.43 (s, 3H, 5'-CH₃). – ¹³C NMR (CDCl₃, 125 MHz) δ 179.9 (12-CO), 161.4 (11-Cq), 158.9 (9-Cq), 155.9 (6a-Cq), 150.3 (7a-Cq), 149.5 (3-Cq), 143.6 (2-Cq), 143.3 (4a-Cq), 128.2 (2'-CH), 113.6 (1'-CH), 109.8 (1-CH), 108.7 (12a-Cq), 107.4 (12b-C), 105.2 (11a-Cq), 101.9 (4-CH), 110.7 (8-Cq), 99.7 (10-CH), 88.0 (6-CH), 78.3 (3'-Cq), 56.1 (3-OCH₃), 55.6 (2-OCH₃), 27.8 (4'-CH₃),
27.6 (5'-CH₃). – (-)-ESIMS m/z 423 ([M - H]⁺, 100); – (+)-HRESIMS m/z 425.12307 [M + H]⁺, (calcd for C₂₃H₂₁O₈, 425.12310).

**Obovatin methyl ether (128):** White soild. – ¹H NMR (CDCl₃, 300 MHz) δ 7.45 (m, 5H, Phe), 6.60 (d, J = 9.6 Hz, 1H, 4''-H), 6.10 (s, 1H, 6-H), 5.46 (d, J = 10.0 Hz, 1H, 3''-H), 5.40 (ABX, 1H, 2-H), 3.89 (s, 3H, 5-OCH₃), 2.99 (ABX, J₅B = 16.5, Jₓₓ = 12.9 Hz, 1H, 3-H₂a), 2.79 (ABX, J₅B = 16.5, Jₓₓ = 3.2 Hz, 1H, 3-H₂b), 1.46 (s, 3H, 2''-CH₃), 1.44 (s, 3H, 2''-CH₃). – ¹³C NMR (CDCl₃, 125 MHz) δ 189.1 (4-CO), 162.1 (5-Cq), 159.9 (7-C₁q), 158.7 (8a-C₁q), 138.9 (1'-CH), 128.6 (3',5'-CH), 128.4 (4'-CH), 126.2 (4''-CH), 125.9 (2',6'-CH), 115.9 (3''-CH), 105.6 (4a-C₁q), 102.8 (8-C₁q), 93.7 (6-CH), 78.9 (2-CH), 77.9 (2''-C₁q), 56.1 (5-OCH₃), 45.6 (CH₂-3), 28.4 (2''-CH₃), 28.1 (2''-CH₃) – (+)-ESIMS m/z 695 ([2 M + Na]⁺, 100), 337 ([M + H]⁺, 15). – (+)-HRESIMS m/z 695.14357 [M + Na]⁺, (calcd for C₂₁H₂₁O₄, 695.14344).

**Obovatin (129):** White soild. – ¹H NMR (CDCl₃, 300 MHz) δ 12.10 (s, OH, 5-OH), 7.45 (m, 5H, Phe), 6.55 (d, J = 10.1 Hz, 1H, 4''-H), 6.00 (s, 1H, 6-H), 5.45 (d, J = 10.1 Hz, 1H, 3''-H), 5.40 (dd, J = 9.8, 3.1 Hz, 1H, 2-H), 3.05 (ABX, J₅B = 17.1, Jₓₓ = 3.2 Hz, 1H, 3-H₂a), 2.82 (ABX, J₅B = 17.1, Jₓₓ = 3.2 Hz, 1H, 3-H₂b), 1.44 (s, 3H, 2''-CH₃), 1.42 (s, 3H, 2''-CH₃). – ¹³C NMR (CDCl₃, 125 MHz) δ 195.6 (4-CO), 163.7 (5-Cq), 162.2 (7-C₁q), 156.7 (8a-C₁q), 138.5 (1'-CH), 128.8 (3',5'-CH), 128.4 (4'-CH), 126.4 (4''-CH), 125.9 (2',6'-CH), 115.9 (3''-CH), 105.6 (4a-C₁q), 102.8 (8-C₁q), 93.7 (6-CH), 78.9 (2-CH), 77.9 (2''-C₁q), 56.1 (5-OCH₃), 45.6 (CH₂-3), 28.4 (2''-CH₃), 28.1 (2''-CH₃) – (-)-ESIMS m/z 665 ([2M -2H+ Na]⁺, 100), 321 ([M -H]⁺, 50). – (+)-HRESIMS m/z 665.15913 [M + H]⁺, (calcd for C₂₀H₁₉O₄, 665.15991).
(CDCl₃, 125 MHz) δ 189.8 (4-CO), 163.2 (8a-C), 160.9 (7-C), 160.6 (5-C), 139.2
(3''-CH), 131.2 (1'-C), 128.5 (2',6'-CH), 128.2 (4'-CH), 125.8 (3',5'-CH), 122.4 (2''-
CH-2), 110.2 (8-C), 105.9 (4a-CH), 88.6 (6-CH), 78.5 (2-CH), 56.0 (7-OMe), 55.6
(5-OMe), 45.6 (1''-CH₂), 25.7 (4''*-CH₃), 21.8 (3-CH₂), 17.6 (5''*-CH₃); * assignment

**Ovalichalcone (β-hydroxychalcone) and its diketo tautomer (132):** Yellow solid. –
¹H NMR (CHCl₃, 300 MHz) δ 15.47 (s, 1H, β-OH), 13.66 (s, 1H, 6'-OH), 7.85 (dd, J =
7.8, 1.4 Hz; 2H, 2,6-H), 7.45 (m, 3H, 3,4,5-H), 7.31 (s, 1H, α-H), 6.68 (d, J = 10 Hz,
1H, 3''-H), 5.93 (s, 1H, 3'-H), 5.45 (d, J = 10 Hz, 1H, 4''-H), 3.90 (s, 3H, 2''-OMe),
1.45 (s, 6H, 2''-(CH₃)₂). – (+)-ESIMS m/z 727 ([2 M + Na]⁺, 47); – (+)-HRESIMS m/z 353.13846 [M + H]⁺, (calcd for C₂₁H₂₁O₅, 363.13834).

**Praecansone B (134):** Pale yellow solid. – ¹H NMR (CDCl₃, 300 MHz) δ 7.52 (m,
2H, 2',6'-H), 7.40 (d, 1H, J = 16.1, 1-H), 7.38 (m, 3H, 3',4',5'-H), 7.00 (d, 1H, J =
16.1, 2-H), 6.54 (d, 1H, J = 9.2, 4''-H), 6.26 (s, 1H, 6-H), 5.54 (d, 1H, J = 9.2 Hz, 3''-
H), 3.74, 3.73 (2 s, 6H, 5,9-OMe), 1.46 (s, 6H, 5'',6''-CH₃). – ¹³C NMR (CDCl₃, 125
MHz) δ 194.1 (3-CO), 158.1 (5-C), 156.0 (7-C), 154.7 (9-C), 144.7 (1-CH), 134.8
(1'-C), 130.3 (4'-CH), 128.8 (2',6'-CH), 128.7 (2-CH), 128.4 (3',5'-CH), 127.7 (3''-
CH), 116.5 (4''-CH), 116.2 (4-C), 107.9 (8-C), 96.1 (6-CH), 76.8 (2''-C), 63.3 (9-
OCH₃), 55.8 (5-OCH₃), 27.9 (5'',6''-CH₃). – (+)-ESIMS m/z 723 ([2 M + Na]⁺); – (+)-
HRESIMS m/z 351.15919 [M + H]⁺, (calcd for C₂₂H₂₃O₄, 351.15910).
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Curriculum Vitae

I was born in 2nd December, 1974 in Munshipara, Satkhira, Bangladesh as the second son of my parents Mr. Md. Fazlur Rahman and Mrs. Fazilatunessa. From 1980 to 1984 I attended the Sultanpur primary school, Satkhira. From 1985 to 1989, I attended the Satkhira Government High School where I completed my secondary school certificate. From 1989 to 1991, I was in Satkhira Government College where I completed my higher secondary certificate. From 1993 to 1997 I completed my BSc (Honours) in Applied Chemistry and Chemical Technology from University of Dhaka, Bangladesh. In 1999 I received my MSc (research) from the same department as my BSc. The thesis title of my MSc was “Preparation of solar selective surface”. From 1999 to 2003, I worked as an environmental analytical chemist in International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). From April 2003 to October 2008, I completed my MSc equivalence in the Faculty of Chemistry, University of Goettingen, Germany. From 1st October to 31st October 2007, I was in the National Centre for Biotechnology, Madrid, Spain for a research project with DAAD scholarship. In 7th December, 2007 I married the gynaecologist Dr. Kulsum Ara. From 2004 to October 2008, I worked as a PhD student in the Institute of Organic and Biomolecular Chemistry, University of Goettingen, Germany under the direct supervision of Professor Dr. Hartmut Laatsch.