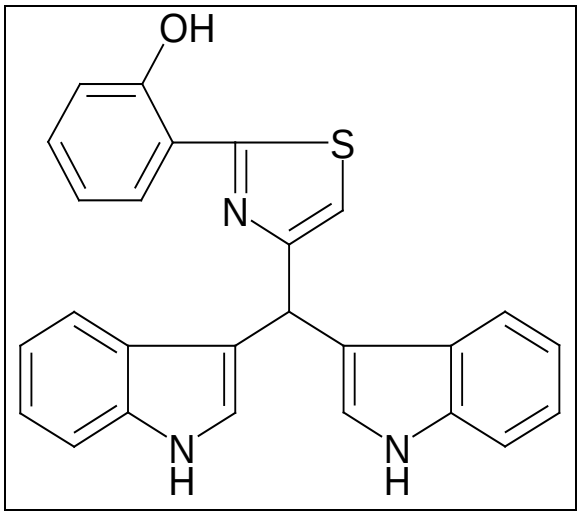
ZENDAH EL EUCH

Isolation, Purification and Structure Elucidation of New Secondary Metabolites from Terrestrial, Marine, and Ruminal Microorganisms



Dissertation

Isolation, Purification and Structure Elucidation of New Secondary Metabolites from Terrestrial, Marine and Ruminal Microorganisms

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultäten

der Georg-August-Universität zu Göttingen

vorgelegt von

Imène ZENDAH EL EUCH

aus

Tunesien

Göttingen 2012

D7

Referent: Prof. Dr. H. Laatsch

Referent: Prof. Aly Raies

Korreferent: Prof. Dr. A. Zeeck

Tag der mündlichen Prüfung: 13. Juli 2012

Die vorliegende Arbeit wurde in der Zeit von Oktober 2005 bis März 2007 in der Faculté des Sciences de Tunis (Laboratoire des Microorganismes et des Biomolécules Actives) unter der Leitung von Herrn Prof. RAIES Aly und von April 2007 bis Juli 2012 im Institut für Organische und Biomolekulare Chemie der Georg-August-Universität zu Göttingen unter der Leitung von Herrn Prof. Dr. H. Laatsch angefertigt.

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

Für meine Eltern, meine Geschwister

und meinen Ehemann

TABLE OF CONTENTS

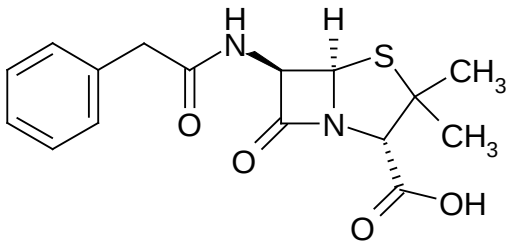
1 Introduction

1.1 Nature as a source of natural products

Nature has proven to be the most reliable and unique source of new and biologically active antimicrobial agents and an example of molecular diversity with recognized potential in drug discovery and development. [1,2] An impressive number of novel and clinically important drugs can be traced back to natural products or habitats. [3]

Natural products are chemical compounds derived from living organisms such as plants, animals, and microorganisms. [4,5] They are usually having a pharmacological or biological activity and may be of interest for use in pharmaceutical drug discovery or drug design. As chemicals, natural products include many classes of compounds such as terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, nucleobases and other heterocycles, and so forth. In particular, some of these compounds are important in the treatment of life-threatening diseases. [3,6] Natural products with antibiotic activity show inhibition of the growth of pathogens (e.g. bacteria, fungi, viruses) at low concentration, and subsequently can be used to cure infectious diseases. [7,8] Not all natural products serve as antibiotics; others may inhibit higher organisms (e.g. tumour cells) or function as signalling substances between microorganisms such as γ-butyrolactones, which are responsible for the formation of mycelium and pigments. [9]

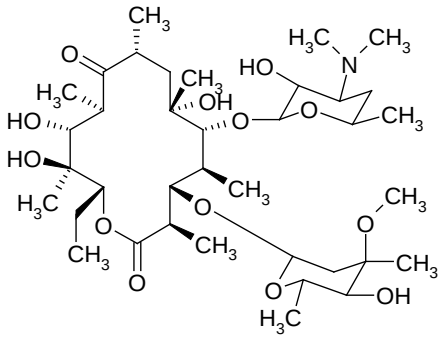
Microorganisms such as bacteria and fungi have been invaluable for discovering drugs and lead compounds. These microorganisms produce a large variety of antimicrobial agents, which may have evolved to give their hosts an advantage over their competitors in the microbiological world. The screening of microorganisms became highly popular after the discovery of penicillin (**1**) in 1928. [10,11]



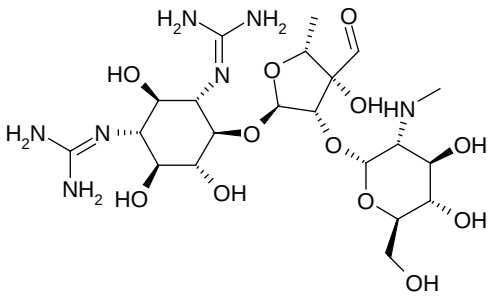
1

Based on traditional medicine, secondary metabolites isolated from nature are used by humans mainly to treat health disorders like infections or other illnesses. [12]

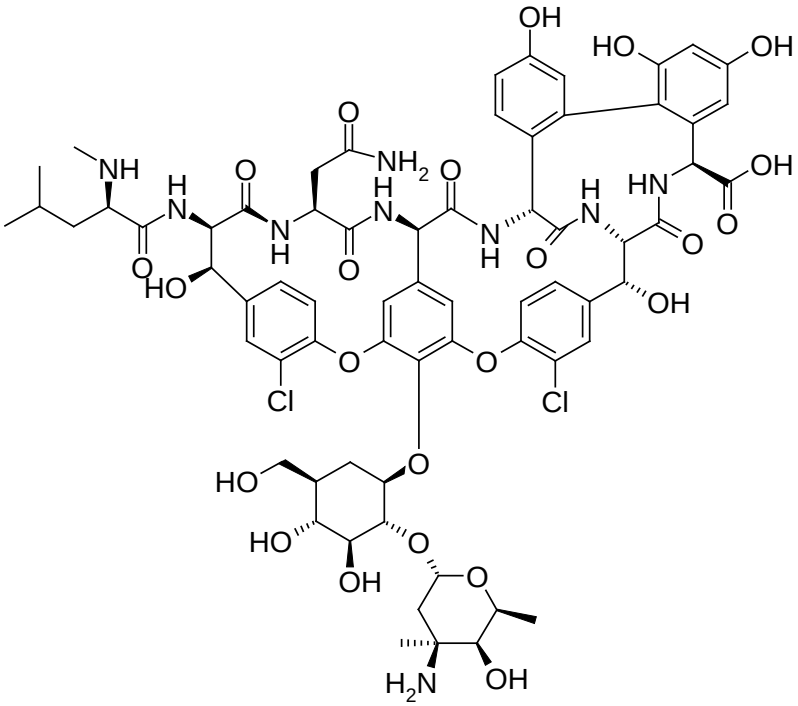
Important microbial metabolites used as antibacterials are e.g erythromycin A (**2**), streptomycin (**3**), vancomycin (**4**)]. Antifungal drugs are amphotericin B (**5**), imidazoles (**6**), or griseofulvin (**7**); others are antiviral [acyclovir (**8**)] or antineoplastic agents [e.g., mitomycin (**9**)], [13] immunosuppressive factors [e.g., rapamycin (**10**)], [14] hypocholesterolemic agents [e.g., pravastatin (**11**)], [15] enzyme inhibitors [e.g., desferal (**12**)], [16] antimigraine agents [e.g., ergot alkaloids], [17] herbicides [e.g., bialaphos, phosphinothricin-alanyl-alanine (**13**)], [18] antiparasitic agents [e.g., salinomycin (**14**)], bioinsecticides [e.g., tetranactin] and ruminant growth promoters [e.g., monensin (**15**)]. [19] A number of other natural products have been reported in the literature to be of value in the treatment of Epstein-Barr virus infection, leukemia, thrombosis and coagulopathy, malaria, anaemia, and bone marrow diseases. [20]



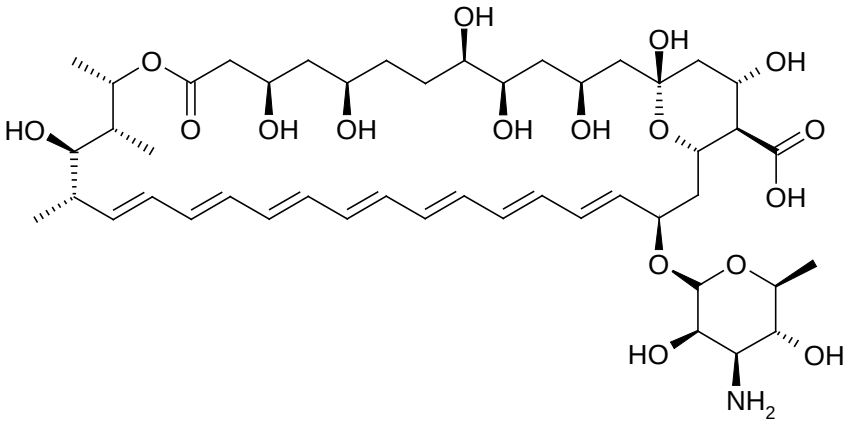
2



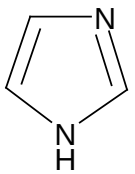
3



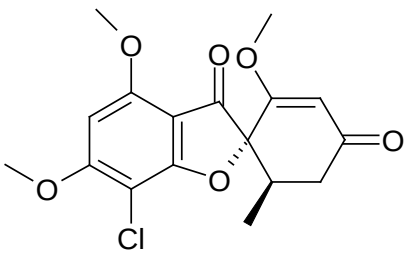
4



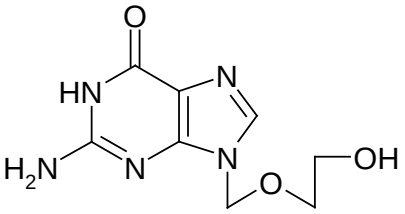
5



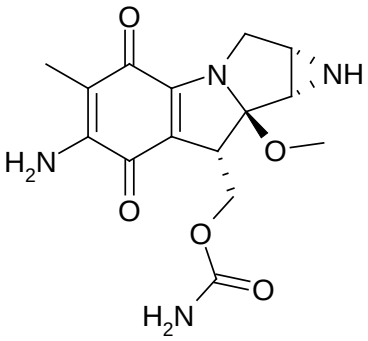
6



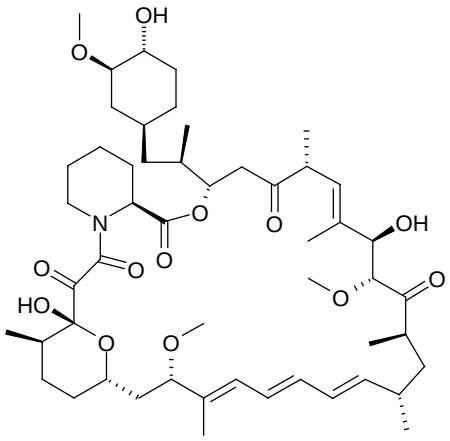
7



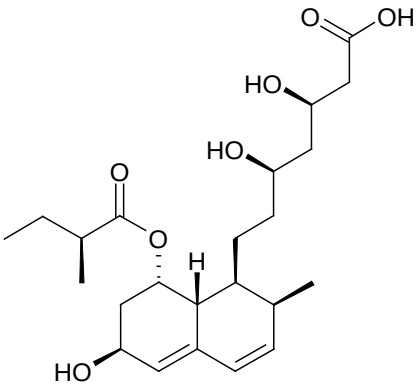
8



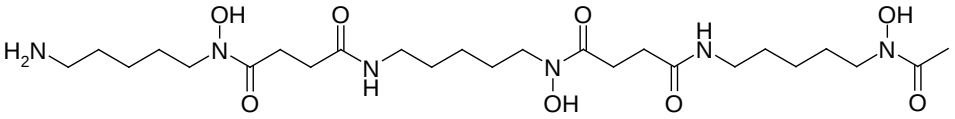
9



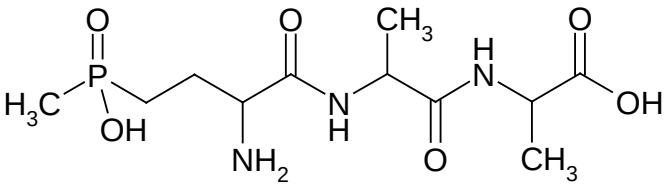
10



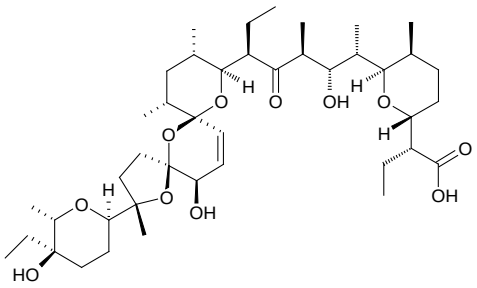
11



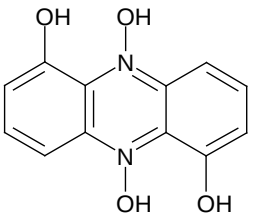
12



13

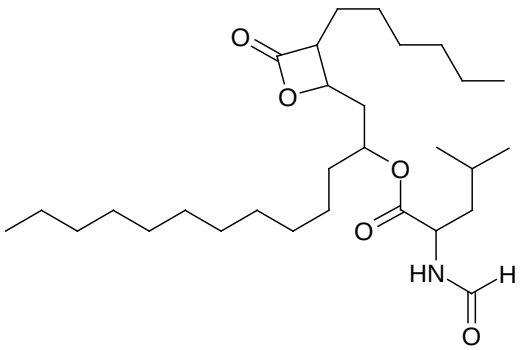


14

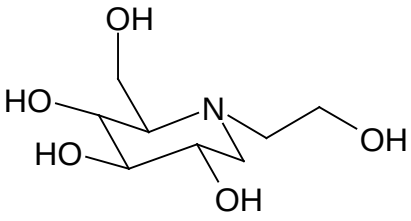


15

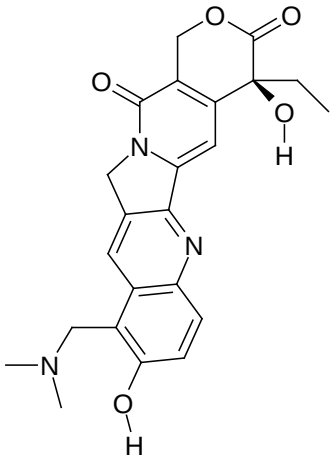
A few examples of some natural products with a high medicinal and commercial potential as modern drugs are: orlistat (**16**) (obesity), miglitol (**17**) (antidiabetic), topotecan (**18**) (antineoplastic), docetaxel (**19**) (antimitotic), tacrolimus (**20**) (immunosuppressant), paclitaxel (**21**) (antineoplastic), manoalide (**22**) (anti-inflammatory); staurosporine (**23**), dolastatin 10 (**24**), epothilones A (**25**) and B (**26**) (antineoplastic), calanolide A (**27**) and B (**28**) (immunodeficiency syndrome, AIDS), or huperzine A (**29**) (Alzheimer's disease). [21]



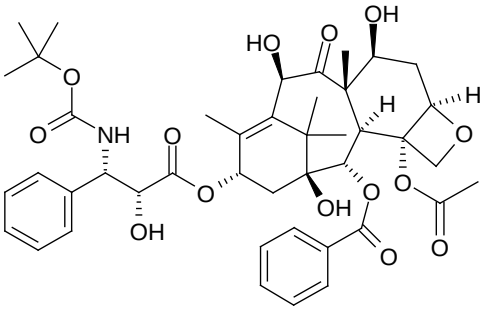
16



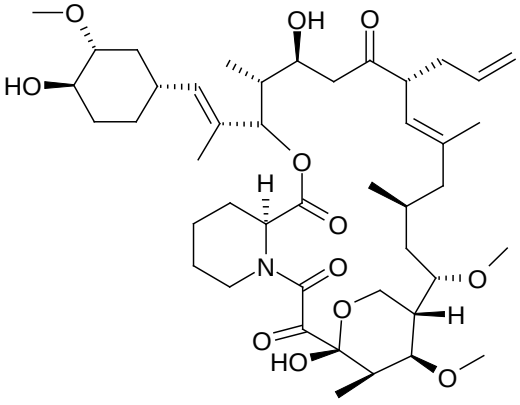
17



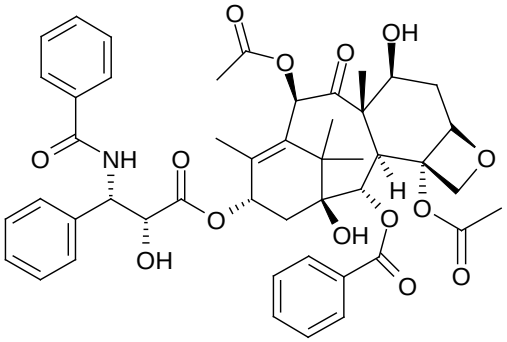
18



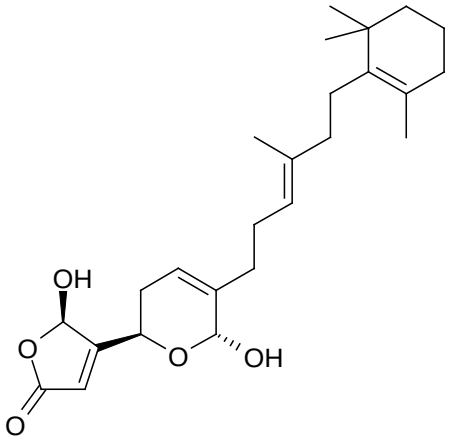
19



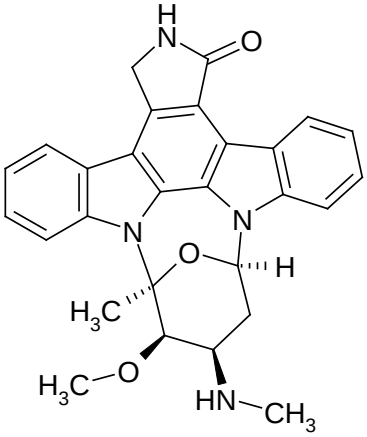
20



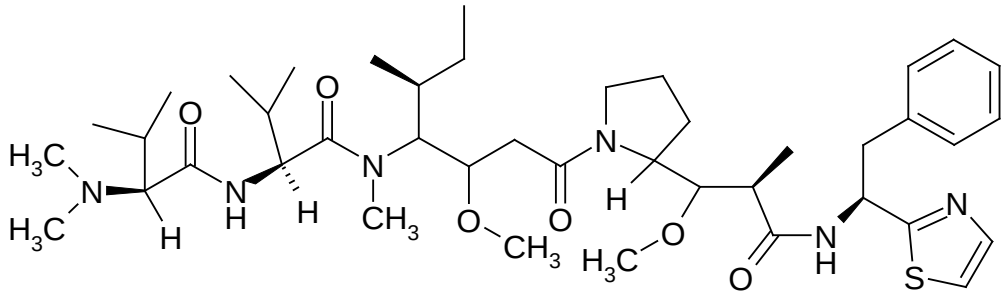
21



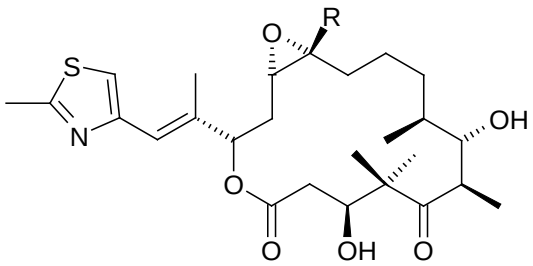
22



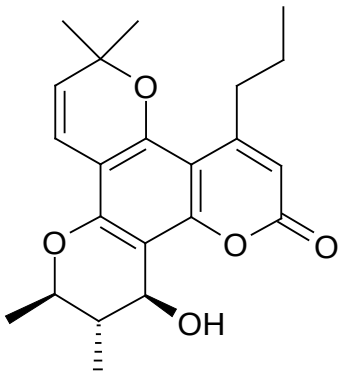
23



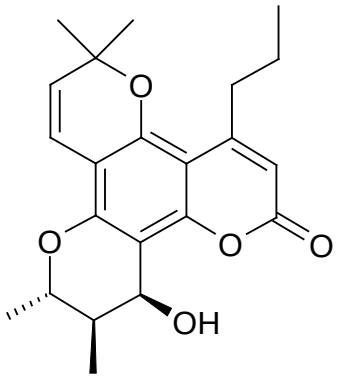
24



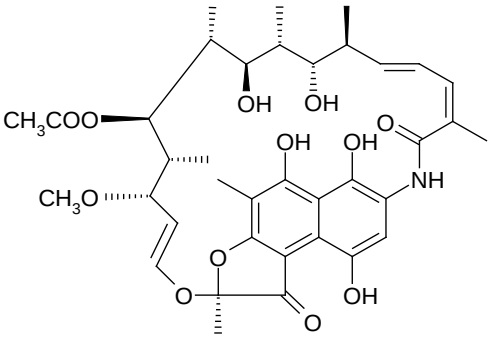
25 R = H
26 R = CH_3



27



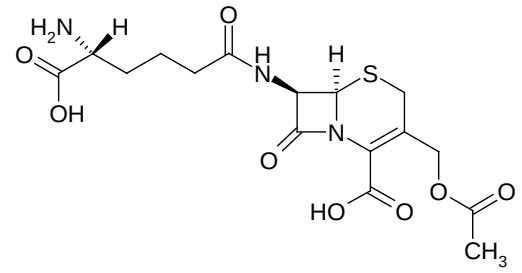
28



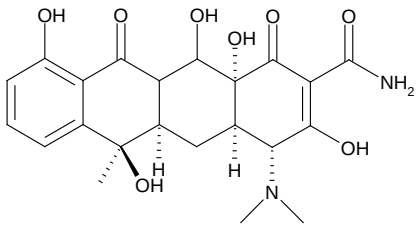
29

Microorganisms are a rich source of structurally unique and pharmaceutically important bioactive substances leading to an impressive arsenal of antibacterial agents such as the cephalosporins (eg. cephalosporin C, **30**), tetracyclines (**31**), rifamycins,

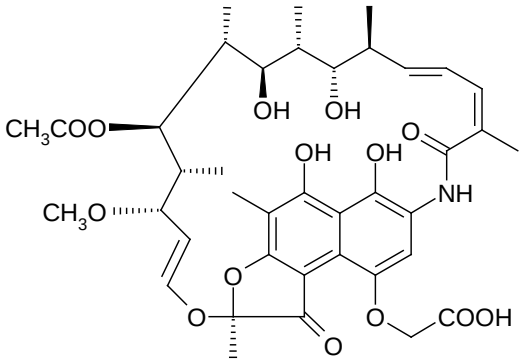
and chloramphenicol. Seven rifamycins were discovered, named rifamycin A, B (**32**), C, D, E, S and SV (**33**).



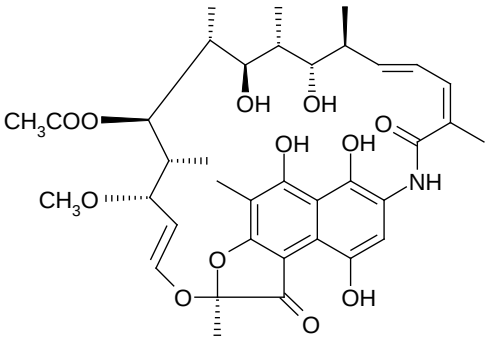
30



31



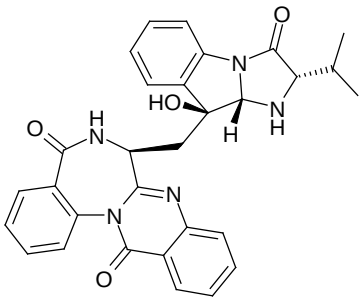
32



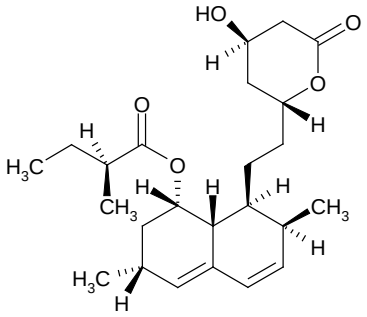
33

Although most of the drugs derived from microorganisms are used in antibacterial therapy, some microbial metabolites have provided lead compounds in other fields of medicine. For example, asperlicin (**34**) (isolated from *Aspergillus alliaceus*) is a novel antagonist of a peptide hormone called cholecystokinin (CCK), which is involved in the control of appetite. [22] CCK also acts as a neurotransmitter in the brain and is thought to be involved in panic attacks. [23] Analogues of asperlicin may therefore have potential in treating anxiety. Other examples include the fungal metabolite lovastatin (**35**), which was the lead compound for a series of drugs that lower cholesterol levels, [24] and another fungal metabolite called cyclosporin (**36**) is used to suppress the immune response after transplantation operations. [25]

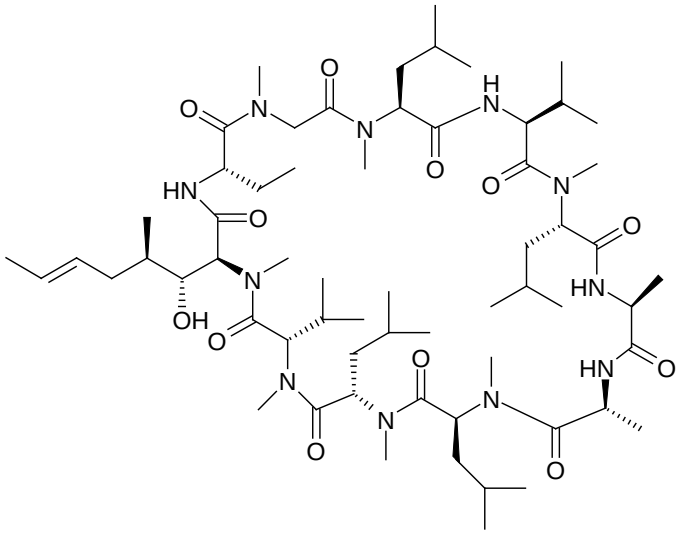
Alamethicin, a polypeptide produced by *Trichoderma viride*, has demonstrated ion-gating activity. [26] Some fungal ribotoxins such as mitogillin have been found to act as specific ribonucleases. [27]



34



35



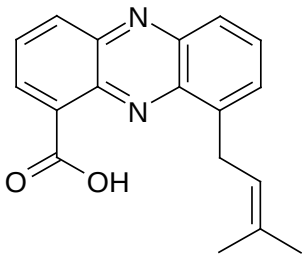
36

Bacteriocins are ribosomally produced antibiotic peptides and proteins produced by bacteria and that can be subdivided into different categories, lantibiotics, and microcins. Lantibiotics are produced by Gram-positive bacteria and microcins are produced by Gram-negative bacteria. Both lantibiotics and microcins possess the ability to form pores or punch holes in membranes of susceptible microorganisms. This property is of interest to the food industry, as bacteriocins are produced by *Lactococcus* spp., which are used in the preservation of various foodstuffs. [28]

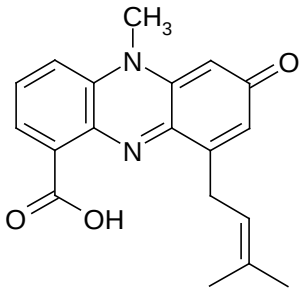
Compounds isolated from *Streptomyces platensis* may be useful in the treatment of thrombocytopenia. [29]

Streptomyces hygroscopicus ascomyceticus manufactures a macrolide that has been reported to have immunosuppressant activity and may prove to be beneficial in preventing transplant rejection in humans. [29]

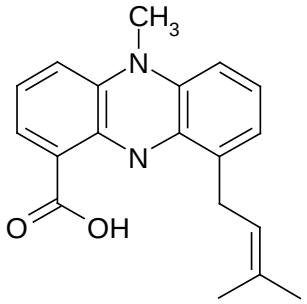
Streptomyces anulatus, a symbiotic actinomyces living in the intestines of millipedes, beetles, and wood lice, produced *C*-isoprenylated endophenazines A-C (**37** - **39**) along with endophenazine D (**40**) and phenazine carboxylic acid. [30,31]



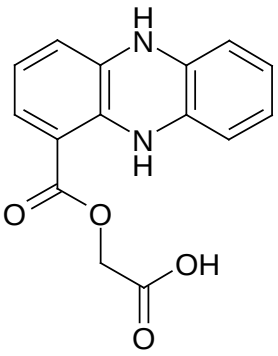
37



38



39

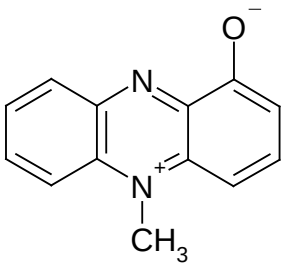


40

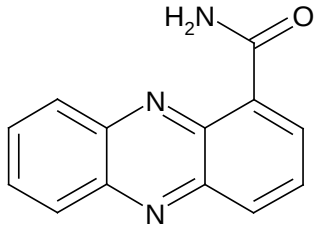
1.2 Natural Products as Pharmaceutical Drugs from Terrestrial bacteria

Natural phenazine, for example, were isolated as secondary metabolites primarily from *Pseudomonas*, [32] *Streptomyces*, [33] and a few other genera from soil. *Pseudomonas aeruginosa*, a common gram-negative soil bacterium and an opportunistic human pathogen, was know as the first and for several years the only producer of phenazine pigments. It was also well known for its ability to produce a blue phenazine, called pyocyanin (**41**), which is toxic to numerous bacteria and fungi and damages mammalian cells. [34-35] *P. aeruginosa* culture supernatants could also contain

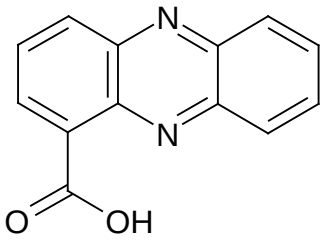
phenazine-1-carboxamide (**42**), phenazine-1-carboxylic acid (**43**) and 1-hydroxyphenazine (**44**).



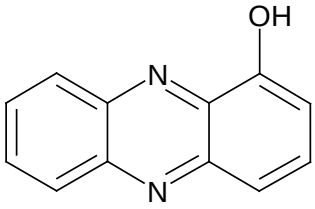
41



42

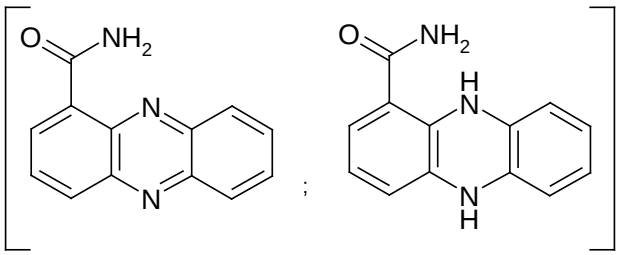


43

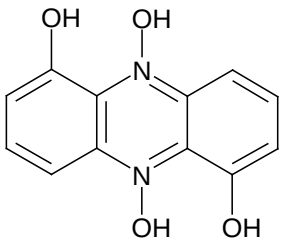


44

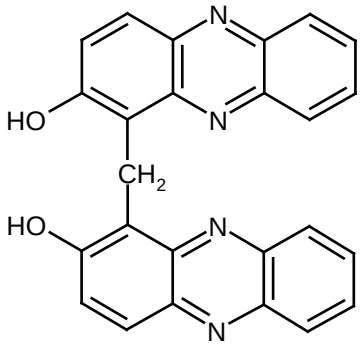
Other phenazines derivatives were produced by *Pseudomonas* sp. such as chlororaphine (1:1 complex) (**45**), iodinin (**46**) and di-(2-hydroxy-1-phenazinyl) methane (**47**). [36]



45



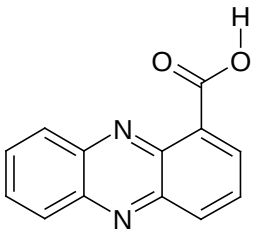
46



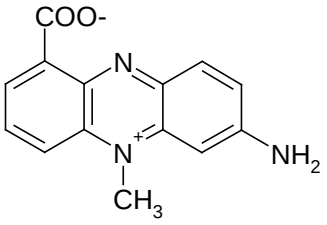
47

Phenazine-1-carboxylic acid (**48**), for example, secreted by *Pseudomonas fluorescens* contributes to biocontrol activity against fungal phytopathogens such as *Gaeumannomyces graminis*, [37,38] and phenazine-1-carboxamide (**42**) produced by *Pseudomonas chlororaphis* PCL1391 is essential for inhibition of the fungus *Fusarium oxysporum*, which causes tomato root rot. [39] Many toxic effects have been reported for different phenazines, and much of their toxicity depends on their redox activity and their ability to generate reactive oxygen species. [40-41]

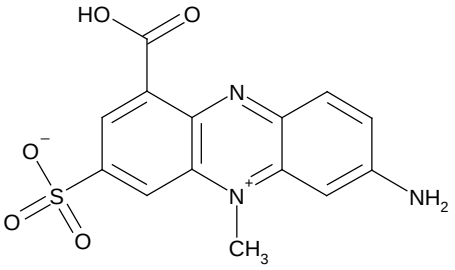
In addition, *Pseudomonas aeruginosa* can produce two red pigments, aeruginosins A (**49**) and B (**50**) (5-methyl-7-amino-1-carboxymethylphenazinium betaine and 5-methyl-7-amino-1-carboxy-3-sulfo-methylphenazinium betaine, respectively), after prolonged incubation. Unlike the other phenazines produced by *P. aeruginosa*, aeruginosins A and B are highly water soluble, and their biological activities are much less characterized. [42,43]



48



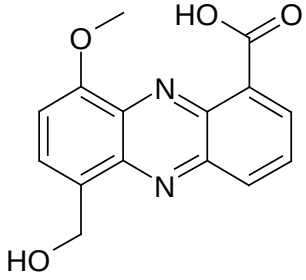
49



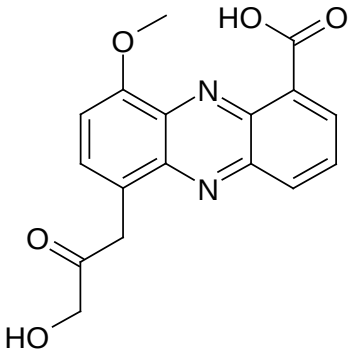
50

The first phenazine derivative isolated from streptomycetes was the antibiotic griseolutein. [44] Since then, an increasing number of phenazine derivatives with different activities have been isolated from different *Streptomyces* species (e.g. griseolutein which was isolated from *S. luteogriseus*, others were isolated from *S. antiboticus,* and *S. prunicolor*). [52]

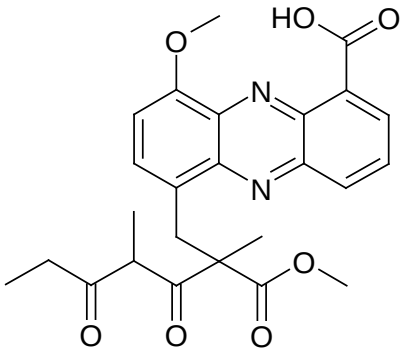
Streptomyces sp. ICBB8198, for example, delivered the phenazines antibiotics, griseoluteic acid (**51**), griseolutein A (**52**) together with two other phenazines derivatives **53** and **54**. [45]



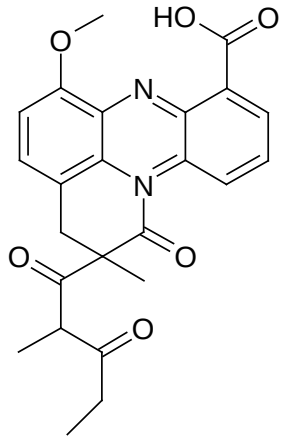
51



52



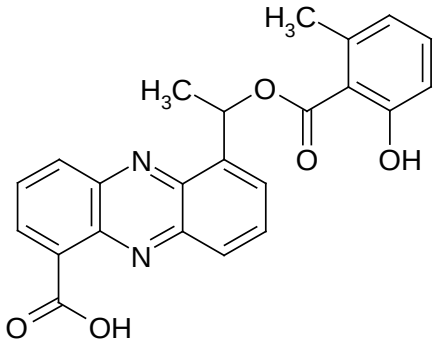
53



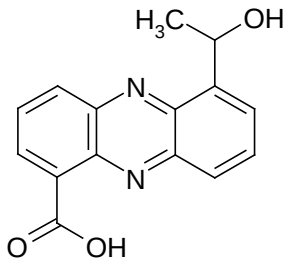
54

Streptomyces antibioticus Tü 2706, produced six yellow phenazines: saphenamycin (**55**), saphenic acid (**56**), saphenic acid methyl ether (**57**), 6-acetylphenazine-1-carboxylic acid (**58**), phenazine (**59**) and phenazine-1-caboxylic acid (**43**). [33]

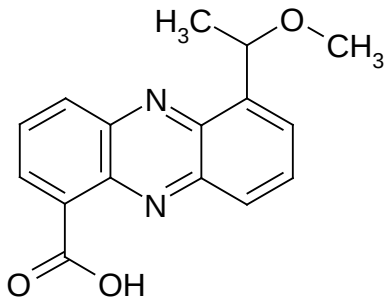
Saphenamycin **55** is known to improve feed efficiency in ruminants when administered in 0.5-1 mg/kg body weight of the animal and acts as a mosquito larvacide. [33, 46,47]



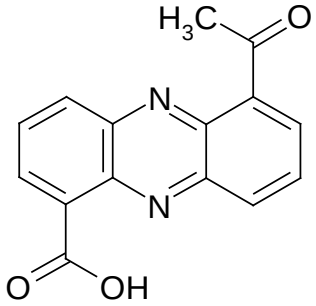
55



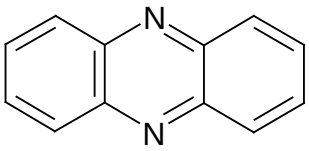
56



57

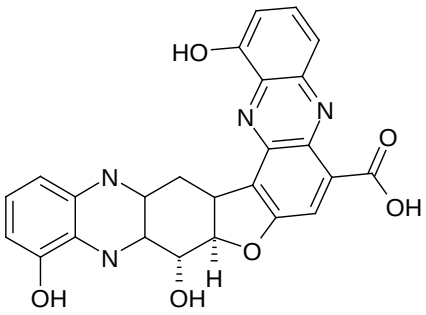


58

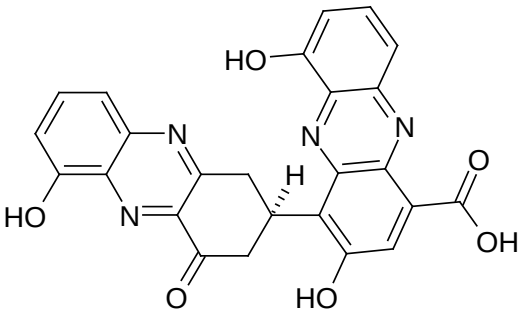


59

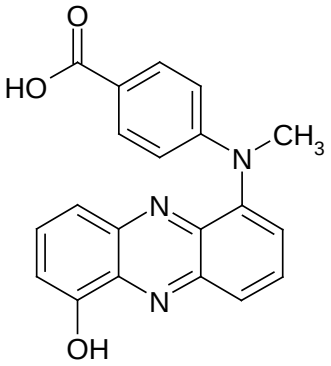
The *Streptomyces sp.* IFM 11204 produced four phenazines derivatives, named izumiphenazines A-C (**60**, **61**, **62**). [48] From the same strain Abdelfattah et *al.* isolated further phenazines derivatives named izumiphenazine D [49] (**63**) together with phenazine-1-carboxylic acid (**43**), 1-hydroxyphenazine (**44**), phenazine-1,6-dicarboxylic acid (**64**) and 6-hydroxyphenazine-1-carboxylic acid (**65**).



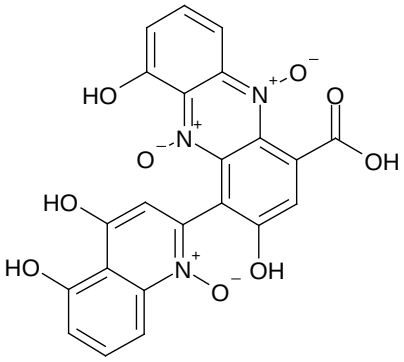
60



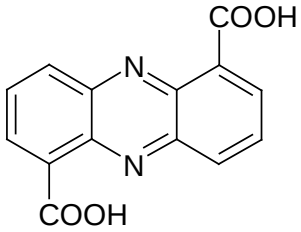
61



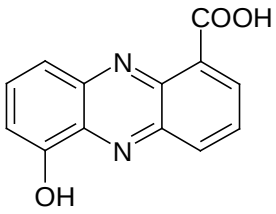
62



63



64



65

Phenazine antibiotics are also known from a variety of other microorganisms, including *Pelagiobacter variabilis,* [50] *Pantoea agglomerans,* [51] and *Vibrio* sp. [52]

The biological properties of this class of natural products include cytotoxicity, antibacterial, antitumor, antimalarial, and antiparasitic activities. [48] The role of phenazine pigments as antibiotics and virulence factors has been briefly reviewed recently, [53] and the biochemistry of phenazine production was reviewed in 1986. [54]

Phenazines have antibiotic properties toward bacterial and eukaryotic species, and the side chain substituents on the phenazine backbone contribute to the biological activities of specific compounds. [55]

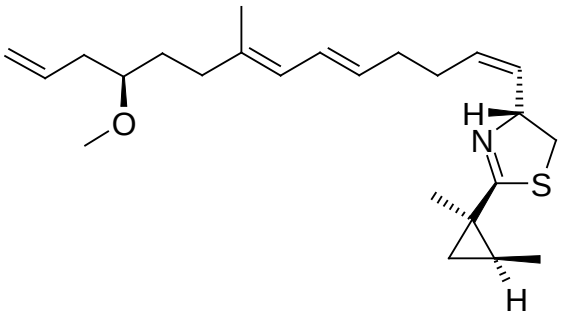
1.3 Marine Natural Products as Pharmaceutical Drugs

Marine microorganisms have a wealth of biologically potent chemicals with interesting inflammatory, antiviral, and anticancer activity. In recent years, there has been a great interest in finding lead compounds from marine sources. [56-57]

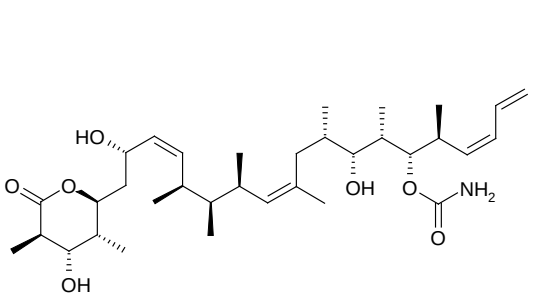
The cyanobacterium *Nostoc ellipsosporum*, for example, has been found to produce a novel protein cyanovirin-N (CV-N), which has generated interest because of its viricidal activity and apparent potential as an anti-HIV therapeutic agent. The antiviral activity of this chemical is reported to be mediated through specific interactions with the HIV envelope glycoproteins gp120 and possibly gp41. It has further revealed that cyanovirin-N (CV-N) is a new class of antiviral agent because of its unique interaction with envelope glycoproteins. [58]

Another example to mention is curacin A [59] (**66**), which was obtained from a marine cyanobacterium *Lyngbya majuscula* and showed potent antitumor activity.

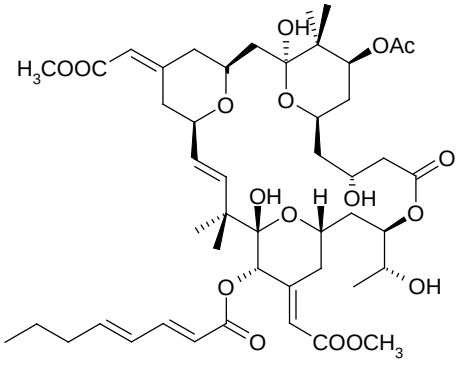
Other antitumor agents derived from marine sources include discodermolide [60,61] (**67**), bryostatins, [62] and cephalostatins. [63]



66



67

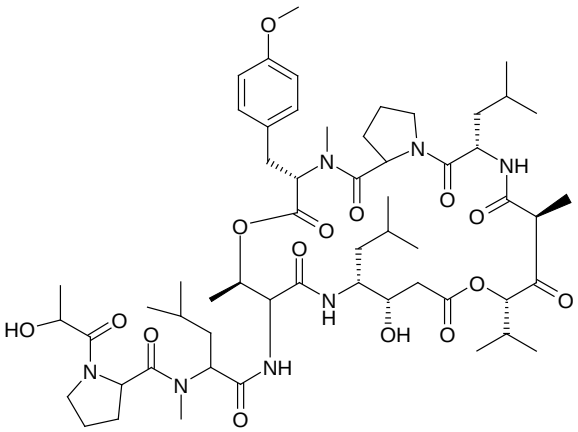


68

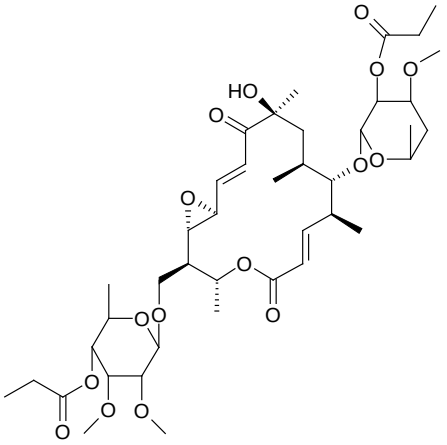
Bryostatin 1, (**68**), is a macrocyclic metabolite, which was isolated from the bryozoans *Bugula neritina* and from *Amathia convulata;* it blocks the protein kinase C or the synthesis of macromolecules. It is currently in phase II clinical trials.

Didemnin B (**69**) isolated from the Caribbean tunicate *Tridemnum solidum,* [64] inhibits the synthesis of RNA, DNA and proteins in various cancer cell lines. It showed antiviral and immunosuppressive activities as well as being an effective agent in treatment of leukaemia and melanoma. However, due to its toxicity, it was withdrawn from phase II clinical trials. [64,65]

The culture broth of the marine isolate *Streptomyces* sp. B7064 was a source for the new macrolide, chalcomycin B (**70**) in our research group. Compound **70** exhibited strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* with MIC values of 0.39 µg/ml, >50 µg/ml, and 6.25 µg/ml, respectively. [66]

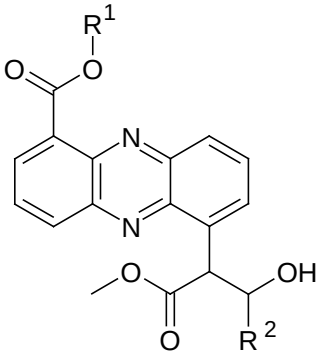


69



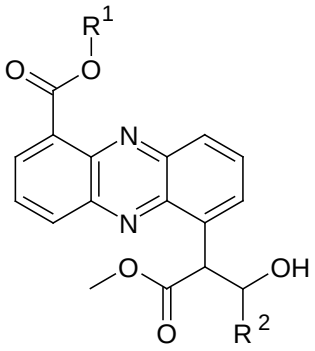
70

Chandrananimycins A-C (**70-73**), novel anticancer and antibacterial agents, were isolated in our research group from *Actinomadura* sp. [67]



71	$R^1 = COCH_3$, $R^2 = OH$
72	$R^1 = COCH_2OH$, $R^2 = H$
73	$R^1 = R^2 = CH_3$

Recent studies showed interesting results, which were obtained with a sponge-derived marine *Streptomyces* isolate producing new streptophenazines A-H (**74-81**), (Figure 1).[68]



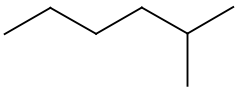
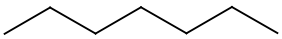
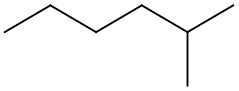
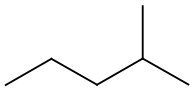
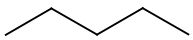
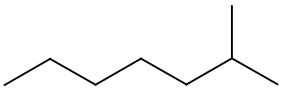
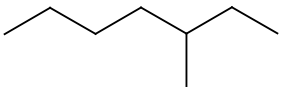
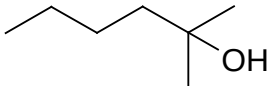
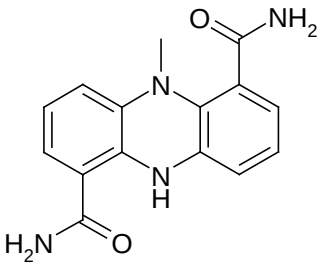
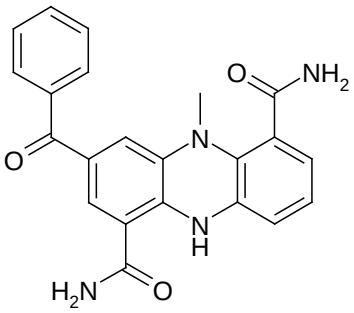
	R^1	R^2
74	CH_3	
75	CH_3	
76	H	
77	CH_3	
78	CH_3	
79	CH_3	
80	CH_3	
81	CH_3	

Figure 1: Streptophenazines A-H (**74**-**81**) as examples of phenazine derivatives isolated from marine *Streptomyces* isolate. [79]

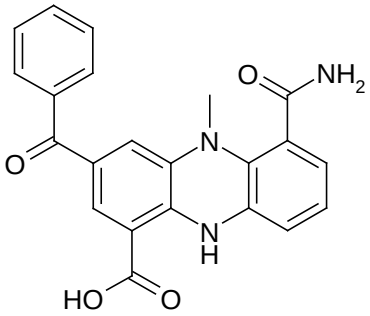
Seven new oxidized and reduced phenazine-type pigments, named dermacozines A–G (**82**-**88**), have been isolated from the actinomycetes *Dermacoccus abyssi* sp. nov., strains MT1.1 and MT1.2 together with the known phenazine-1-carboxylic acid (**43**) and phenazine-1,6-dicarboxylic acid (**64**). [69]



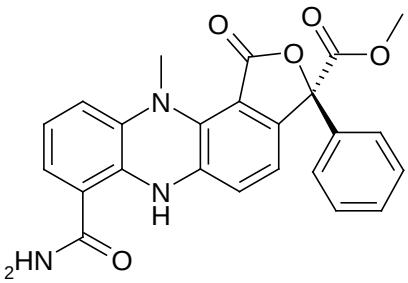
82



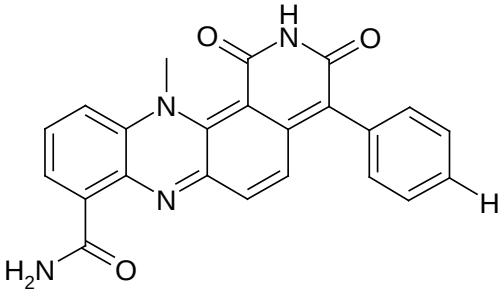
83



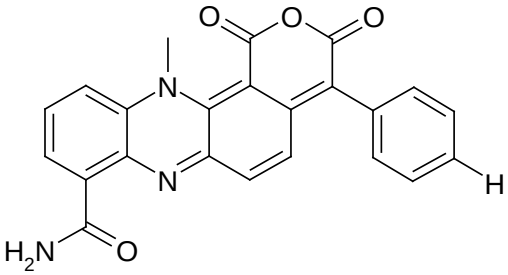
84



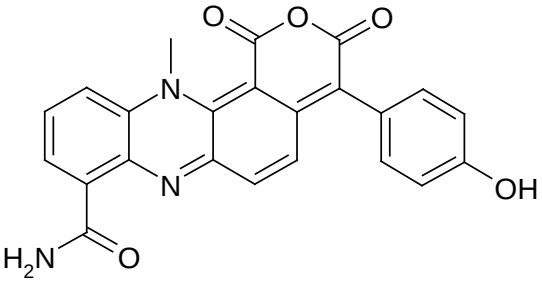
85



86



87



88

Table 1: Examples of new marine drugs [70]

Chemical name	Source	Chemical class	Chemical target	Therapeutic Indication
AM336 (AM-RAD)	cone snail	peptide	ion channels	chronic pain
GTS21 (Taiho)	nemertine worm	anabaseine-derivative	ion channels	Alzheimer's disease and schizophrenia
LAF389 (Novartis)	sponge	amino acid derivative	Methionic aminopeptidase inhibitor	Cancer
OAS1000 (OsteoArthritis Sciences)	soft coral	diterpene-pentoseglycoside	PLA2 inhibitor	wound healing and inflammation
ILX651 (Ilex Oncology)	sea slug	peptide	microtubule-interference	Cancer
Cematodin (Knoll)	sea slug	peptide	microtubule interference	Cancer
Yondelis,	sea squirt	isoquinolone	DNA-interactive agent	Cancer
Alipidin	sea	squirt	cyclic depsipeptide	oxidative stress inducer cancer
Kahalalide F	sea slug/alga	cyclic depsipeptide	lysosomotropic compound	Cancer
KRN7000 (Kirin)	sponge	-galactosylceramide	immunostimulatory agent	Cancer
squalamine lactate	shark	aminosteroid	calcium-binding protein antagonist	Cancer
IPL512602 Inflazyme/Aventis	sponge	steroid	unknown	inflammation

1.4 Ruminal bacteria as new sources of potential antimicrobials

Animal digestive tracts are complex ecosystems with a large degree of microbial diversity. [71] Bacteria colonizing the gut are usually involved in metabolic transformations of substrates and occasionally are considered as obligate symbionts of ruminant hosts. [82,72] Consequently, numerous interactions (synergism, cross feeding, antagonism) are expected between ruminal bacteria. [73-74]

Research on antimicrobials produced by ruminal bacteria allowed identification of various substances with bactericidal or bacteriostatic characteristics including organic acids, [75] hydrogen peroxide, [76] and bacteriocins. [77] These findings encouraged the isolation of novel ruminal bacteria with potential antagonism against pathogens.

Gratia observed that it exists an antagonism between *Escherichia coli* strains and they could inhibit each other in the rumen. [78] Later it was reported that ruminal lactococci were able to produce antibacterial substances. [79] Whitehead then demonstrated that the lactococcal factor was proteinaceous. [80] Mattick and Hirsh, tested the inhibitory substance against pathogenic streptococci, [81] and Taylor et *al.* attempted to use the same inhibitory factor to treat bovine mastitis. [82] Lactococcal strains produce a variety of antibacterial substances, [83] called bacteriocins, which are ribosomally synthesized peptides and are different from classical antibiotics. [84] The classical definition of bacteriocins was largely based on colicins, [85] and bacteriocins have been recently re-defined. [86]

Mattick and Hirsh called nisin the group N inhibitory substance of *Lactococcus lactis*, [87] and nisin has been the most studied and best understood bacteriocin. [88] It is a relatively short peptide (34 amino acids) with five unusual sulfur-containing (lanthionine) rings and a number of dehydrogenated residues. [89,90] Nisin molecules appear to assemble in the cell membrane to form a barrel-like structure that facilitates the loss of intracellular solutes. [91]

Odenyo et *al.* observed that *Ruminococcus albus* 8 produced a heat stable protein factor that inhibited the growth of *Ruminococcus flavefaciens* FD1 lawns, [92] and further work indicated that other *Ruminococcus albus* strains produced bacteriocin-

like compounds that could inhibit *Ruminococcus flavefaciens* strains and *Butyrivibrio fibrisolvens*. [93] Teather et *al.* noted that many strains of *Butyrivibrio fibrisolvens* produced bacteriocins that could inhibit other butyrivibrios. [94] They purified two butyrivibriocins, AR 10 butyrivibriocin and OR79 butyrivibriocin; both of them had relatively wide spectra of activity and were able to inhibit a variety of Gram-positive ruminal bacteria.

Wells and *al.* showed that many of the lactobacilli produced a substance that could inhibit the growth of laboratory *Streptococcus bovis* strains. [95] They identified the most active strain as *Lactobacillus fermentum*, and this species was previously reported to produce a bacteriocin. [96]

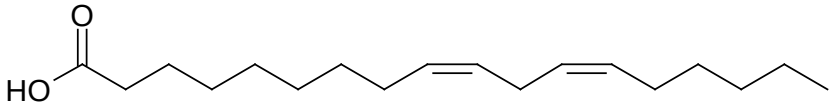
Because some ruminal bacteria can produce bacteriocins, Teather and Forster speculated that these compounds might provide effective alternatives to antibiotics as feed supplements. In order for ruminal bacteriocins to be effective, the peptide would need to be relatively stable, to have a broad spectrum of activity, and to remain active (not subject to resistance development). [97]

Lauková and Czikková (1998) noted that *Enterococcus faecium* is not a predominant ruminal bacterium, but bacteriocin-producing *Enterococcus faecium* strains have been isolated from the rumen. [98] *Enterococcus faecium* CCM4231 and BC25 both inhibited *Streptococcus bovis*, but the bacteriocin BC25 appears to have a bacteriostatic rather than bactericidal mode of action.

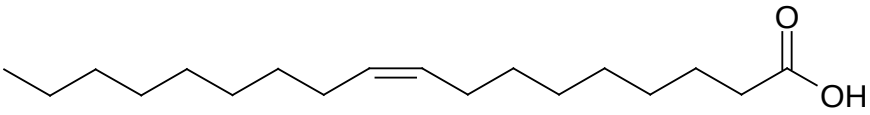
Chan and Dehority (1999) noted that inhibitory activity of *Ruminococcus albus* strains was decreased or completely destroyed by the proteolytic activity of *Butyrivibrio fibrisolvens* H15c. [106]

Dehority and Tirabasso (2000) indicated that ruminal bacteria produced a bacteriocin-like substance that inhibited ruminal fungi. [84] More recent work indicates that *Streptococcus bovis* strains can also produce bacteriocins. [99,100] Whitford et *al.* (2001) purified a bacteriocin from *Streptococcus gallolyticus* LRC0255, [112] and Russell et *al.* (2002) demonstrated also that a variety of ruminal bacteria produce bacteriocins. [101] Koppová et *al*. (2006) demonstrated the strong growth-inhibitory activity of linoleic (**89**) and oleic acids (**90**) in some rumen bacteria. [88] Linoleic acid

produced by *Pseudobutyrivibrio ruminis* inhibited the bacterial growth of *Pseudobutyrivibrio ruminis* B 24. Linoleic acid was also produced by the ruminal bacteria *Pseudobutyrivibrio xylanivorans* and *Butyrivibrio hungatei*. [88]



89



90

In the present work, we isolated and identified ruminal bacteria from different species of domestic livestock in Tunisia. These ruminal bacteria exhibiting activity against human pathogenic strains and phytopathogenic fungi were analysed for their inhibitory substances.

2 Aim of the present work

Natural products continue to play a highly significant role in the drug discovery and development process. [3] The main goal of the present work was the isolation, purification and structure elucidation of new and preferably biologically active secondary bacterial and fungal metabolites isolated from special habitats like the rumen, rare terrestrial locations and selected aquatic biotopes. Special attention will be focused on bacteria from the stomach of ruminal animals.

- ✓ Part one of the present work was focussed on the isolation, purification and structure elucidation of compounds produced by selected Tunisian ruminal bacteria. The idea behind this work was to explore a new natural origin and to find out that ruminal microorganisms could produce inhibitory substances with negative effect on pathogenic microorganisms, as it was reported, that in the rumen (*in vivo*) there is an antagonism between ruminal microorganisms (protozoa, fungi and bacteria).

- ✓ Part two of this work was to highlight attention on new structures from terrestrial bacteria (*Streptomyces* sp, *Pseudomonas*, etc.), by using modern techniques of separation and structure elucidation.

- ✓ Part three of this work was an investigation of the ability of marine bacteria to produce secondary metabolites.

- ✓ Part four was a study of secondary metabolites produced by two fungi: *Aspergillus flavus* and *Aspergillus niger*.

- ✓ Part five was to check different activities of these isolated metabolites against bacteria, fungi, algae, oomycetes (zoosporicidal activity test), brine shrimps (cytotoxicity test), and cancer (selective cellular toxicity).

  To achieve this work several chromatographic (i.e. HPLC, Sephadex LH-20, silica gel, RP-18 column chromatography, PTLC, etc.), spectroscopic (NMR) and mass spectrometry methods were carried out, supported by database guidance using AntiBase, [102] the Dictionary of Natural Products [103] and Chemical Abstracts. [104]

- ✓ The use of such naturally occurring compounds may reduce the need for synthetic insecticides and herbicides in agriculture. It could be also a contribution for pharmaceutical industry and drug discovery in the future.

- ✓ Also, many food industries use preservatives to prevent the spoilage by bacteria, and antimicrobial natural products have already shown this effectiveness in such fields. Secondary metabolites may also be used in veterinary industries to make better use of the feed (nutritional effect). We hope to make a contribution to one of these fields.

3 General Techniques

3.1 Collection of strains

The strains of this work were obtained via cooperations with various microbiological groups:

- The collection of ruminal bacteria was obtained during the Master Thesis of the author, Z.I. The collected organisms were isolated from the rumen of Tunisian ruminals and identified with help of Prof. Fethi Ben Hassine and his group at the Policlinic CNSS de Bizerte, Tunisia. In the case of the bacterium ZIO, the taxonomy was determined with help from E. Helmke, Alfred-Wegener-Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany.

- All terrestrial *Streptomyces* spp. Ank strains were isolated and identified by H. Anke, IBWF. [105] The strains were cultivated on M_2 medium; the taxonomy was determined with help of Anja Schüffler, IBWF (Institute for Biotechnology and Drug Research, Erwin-Schrödinger-Str. 56, D-67663 Kaiserslautern, Germany).

- The marine *Streptomyces* spp. (B strains) were isolated and taxonomically identified by E. Helmke from the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. They were cultivated on M_2^+ medium (sea water is added to the M_2 medium).

- The terrestrial WO strains belong to a project with Prof. Wolf on the search for compounds with activity against plant pathogenic fungi.

- The *Bacillus* sp. ZIR, *Bacillus pumilus* ZIBP1, and *Pseudomonas* sp. ZIPS were isolated by the author and identified with help of Prof. Fethy Ben Hassine and his group at the Policlinic CNSS of Bizerte, Tunisia.

- The bacteria will be described at the beginning of each chapter on the basis of morphological characteristics (colour, shape, etc.). In some cases, the taxonomy was fully determined on the RNA level.

- The fungi *Aspergillus flavus*, *Aspergillus niger*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium oxysporum* and *Botrytis cinerea* belong also to the collection prepared by the author.

3.2 Work up procedure for selected bacterial strains

The general work up procedure of the investigated strains can be summarized in the following steps (Figure 2). Firstly, the strains were evaluated based on their chemical and biological screening. Then the cultivation of the interesting strains is scaled up. After isolation of the metabolites, structure elucidation is performed. Finally, the activity tests for the isolated compounds are carried out.

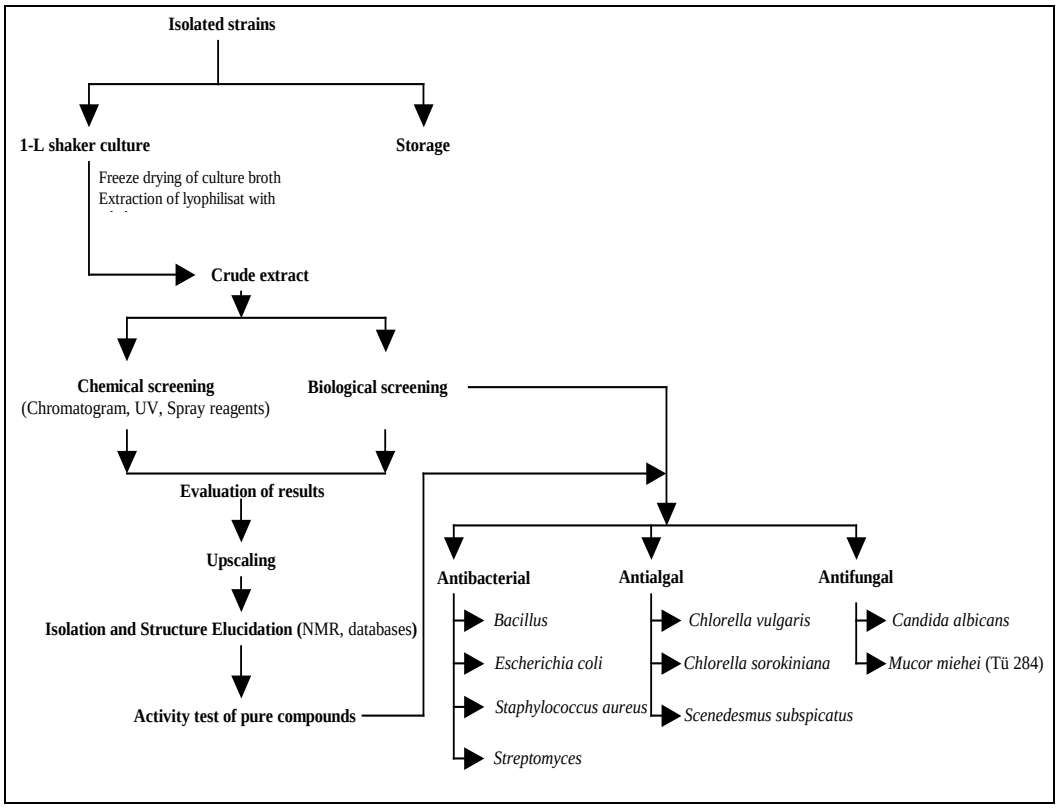


Figure 2: General screening of the selected strains

3.3 Pre-screening

Among the ruminal isolated strains, around 25% were able to produce metabolites with bioactivity or further interesting properties. These strains were selected on the basis of activity test and also characterized. A so-called pre-screening for biological

or chemical interactions of metabolites with test systems was performed for *Streptomyces* strains. In this method, the strains were selected by a number of suitable qualitative or quantitative criteria.

The strains were sub-cultured on agar plates and microscopically examined for contaminations. Small pieces of the agar culture were then used to inoculate 1 L Erlenmeyer flasks with inflections containing 250 ml of medium, followed by incubation on a rotary shaker at 37 °C for ruminal bacteria or at 28 °C for *Streptomyces* and *Bacillus* bacteria. The culture broth was then lyophilized and the dried residue extracted with ethyl acetate. The obtained crude extract was used for biological, chemical and pharmacological screenings and also for HPLC-MS.

3.3.1 Biological screening

In biological screening, the extracts were subjected to agar diffusion tests using *Escherichia coli*, *Streptomyces viridochromogenes* (Tü 57), *Bacillus subtilis*, *Staphylococcus aureus*, the fungi *Mucor miehei* (Tü 284) and *Candida albicans*, and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus* as test organisms. In parallel, the cytotoxic activity was evaluated against brine shrimps (*Artemia salina*).

3.3.2 Chemical screening

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

The TLC (Thin Layer Chromatography) is one of the cheapest and simplest methods used for the detection of bacterial constituents in the crude extract. Compared with other methods like HPLC it is easy to perform, quick, requires simple equipment and is sufficiently reproducible. A spot of the crude extract is developed on a TLC card with a CH_2Cl_2/MeOH solvent system. The developed TLC plate is inspected under UV light, and interesting zones are further localized by exposure to spray reagents.

Many sprays reagents are available for the detection, some specific, and other universal. In our group, only the following spray reagents are used routineously:

• Anisaldehyde/sulphuric acid gives different colour reactions with many structurally diverse compounds.

• Ehrlich's reagent is a specific reagent used to determine indoles and some other nitrogen containing compounds; indoles turn pink, blue or violet, pyrroles and furans become brown, anthranilic acid derivatives change to yellow.

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

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

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

3.3.3 Pharmacological and biological assays

It is evident that in order to screen a crude extract for bioactive substances, an appropriate test is need. Many screening programs have been developed in natural product chemistry, and are usually divided into two groups: general screening bioassays and specialized screens. These screening programs will be different, whether they were organized by a pharmaceutical company, or university research groups. In both cases, all bioassays should have high capacity, sensitive, low cost, and must give rapid answers. There are two types of screening: the *vertical screening* mostly used in industry shows high selectivity and narrow results (1:10.000-1:20.000). [106] The *horizontal screening* used in our group exhibits low selectivity, however, broad results (1:3-1:100) and gives therefore a quick overview. In our group the crude extract is screened using the agar diffusion test with bacteria (Gram-positive, Gram-negative), fungi, plants and higher organisms, the latter for cytotoxicity. Our crude extracts were tested against *Escherichia coli*, *Bacillus subtilis*, *Mucor miehei*, *Candida albicans*, *Streptomyces viridochromogenes* (Tü 57), and *Staphylococcus aureus* as well as the microalgae *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus subspicatus*.

The brine shrimp toxicity has a strong correlation with cellular cytotoxicity and is therefore a good indicator for potential anticancer activity. The bio-autography on TLC gives simultaneously more information about an unknown bioactive component in the crude extract. This is readily seen with antimicrobial compounds. The pharmacological tests in our group were carried out at Oncotest, BASF, or other companies. Chemical and biological screening complements each other and allows us to choose suitable strains for the scale-up.

3.4 Cultivation and scale-up

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

There were two possibilities available for the culture of bacteria: the fermentation in shaking flaks or in a fermenter. For the latter, a pre-culture of 2 or 5 L is to be used for the inoculation. After harvesting, the culture broth is mixed with Celite (diatomaceous earth) and filtered under pressure. The water phase can be submitted to extraction with ethyl acetate, but it is highly recommended to use a solid phase extraction with XAD-16 resin due to the fact that the latter extracts also more polar compounds, is not harmful and reduces considerably the costs for solvents. The mycelium is extracted with ethyl acetate and acetone. The organic phases are evaporated to dryness and the remaining crude extracts are used for separations.

3.5 Isolation methods

The separation methods depend on the amount of the crude extract and the polarity of the compounds of interest. Generally, the crude extract is first defatted using cyclohexane, than subjected to silica gel chromatography using a gradient of increasing polarity with various solvent systems (CH_2Cl_2/MeOH or cyclohexane/ethyl acetate etc.). Size-exclusion chromatography (Sephadex LH-20) offers the advantage of a higher recovery rate and minimizes the destruction of compounds. It is used preferentially when the amount of the crude extract is less than 50 mg. Further methods

like RP-18, PTLC and HPLC are also used for some final purification. All steps were guided by monitoring on TLC.

3.6 Partial identification and dereplication

Nowadays, more than 170 000 compounds have been discovered as natural products and about 700 new compounds are added annually from microorganisms. It is a loss of time and money if certain compounds are re-isolated again and again.

It is therefore a great challenge for the natural product chemists to face the steadily increasing problem of how to optimise the discovery of new compounds and to minimize the re-isolation of known metabolites. Methods of dereplication have been developed to recognize known compounds at the earliest stages of the purification or from a partially purified mixture; these complementary processes for rapid identification of known compounds or the elucidation of a partial structure of an unknown compound to prioritise or perform an isolation have been termed “dereplication”. [107]

Nowadays there are database available where NMR derived sub-structures or physico-chemical properties can be searched using computers. [108] The most useful and comprehensive tools for our purpose are the data collection AntiBase (Wiley-VCH) [115] and the Dictionary of Natural Products (DNP, Chapman & Hall). [116] The latter allows also the dereplication of plant metabolites but has limited spectral information, which are the weak features of DNP.

AntiBase, [115] containing a full range of structure and substructure search capabilities, is much easier to use. AntiBase offers rapid identification of known compounds using a combination of structure fragments, high resolution mass spectra, ^{13}C chemical shifts and other data. Presently, there are over 39,000 microbial compounds in AntiBase, so that related structures of new compounds can also be directly compared. AntiBase offers either experimental or SpecInfo-calculated ^{13}C NMR data of almost all metabolites, which demonstrates more significant characteristics than other natural product databases. In addition thousands of original NMR spectra are available for direct comparison.

A search in the Chemical Abstracts, which is the most comprehensive worldwide collection of compounds, accomplished the final confirmation of a compound. These databases are also important tools in the identification of new metabolites with respect to compound classes and chromophores.

Dereplication is also done with new methods like the combination of liquid chromatography and detection methods such as NMR spectroscopy (HPLC-NMR) and the tandem mass spectrometry (HPLC-MS/MS), by which biological matrices, e.g. extracts from marine microorganisms, [109] extracts from plants [110] are screened to obtain most comprehensive information about known constituents with minimum amount of material. Because most compounds of interest are thermally labile, HPLC-ESI-MS/MS is the method of choice to identify known molecules from multi-component mixtures with high selectivity and sensitivity. [111] Due to the non-chiral character of NMR spectroscopy and mass techniques, no information concerning the full absolute 3-dimentional structure is available by HPLC NMR or HPLC-MS/MS techniques. The CD (circular dichroism) spectroscopy [112] is widely used for the attribution of the absolute configuration by comparison of the experimental data from structurally related compounds, if available.

4 Investigation of selected bacterial and fungal strains: Ruminal bacteria

4.1 Ruminal *Pseudomonas aeruginosa* ZIO

The ruminal bacterium *Pseudomonas aeruginosa* was selected due to its biological activity against human pathogenic bacteria and fungi (Figure 3) and phytopathogenic fungi. The strain formed separated colonies on LB medium after incubation 24 hours at 37 °C.

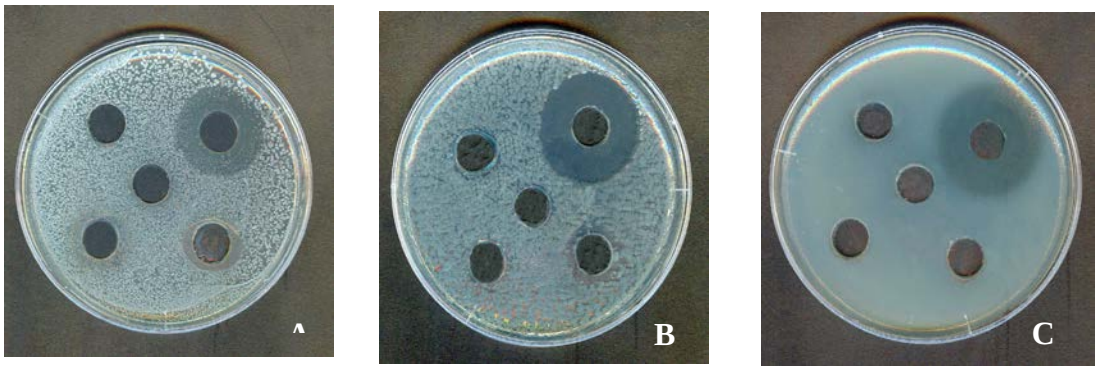


Figure 3: Activity of *Pseudomonas aeruginosa* against: (A): *Staphylococcus aureus*, (B): *Escherichia coli*, (C): *Klebsiella pneumoniae*

Pieces of well-grown agar plates were used to inoculate 120 of 1L Erlenmeyer flasks, each containing 250 ml of LB medium (LB medium: A solution of 10 g peptone extract, 5 g yeast extract, 5 g NaCl and 5 g glucose in 1 l of tap water was set to pH 7.8 with 2 N NaOH and sterilized for 30 min at 121°C). The fermentation was carried out at 180 rpm on a rotary shaker for 3 days at 37 °C. The brown culture broth was harvested and filtered by a filter press (Figure 4) to separate the mycelium, which was extracted with ethyl acetate, while the culture filtrate was passed through Amberlite XAD-16.

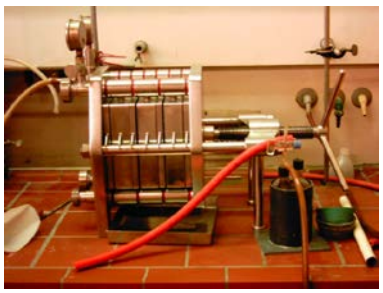


Figure 4: Filter press

The column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluate was concentrated under reduced pressure (Figure 5) and finally extraction of the residue was done with ethyl acetate.

Figure 5: Rotary evaporator

On the other hand the Celite phase was extracted with ethyl acetate (3 times) and acetone (2 times); the organic phases were then concentrated to dryness. Both crude extracts were combined based on TLC yielding a greenish-brown crude extract. The TLC showed several UV absorbing bands and with anisaldehyde/sulphuric acid some compounds gave a pink colour.

Chromatography of the crude extract (10.84 g) on silica gel column using a CH_2Cl_2-MeOH gradient resulted in four fractions I-IV on monitoring by TLC. They were further chromatographed in different ways to isolate the individual compounds in pure form (Figure 6).

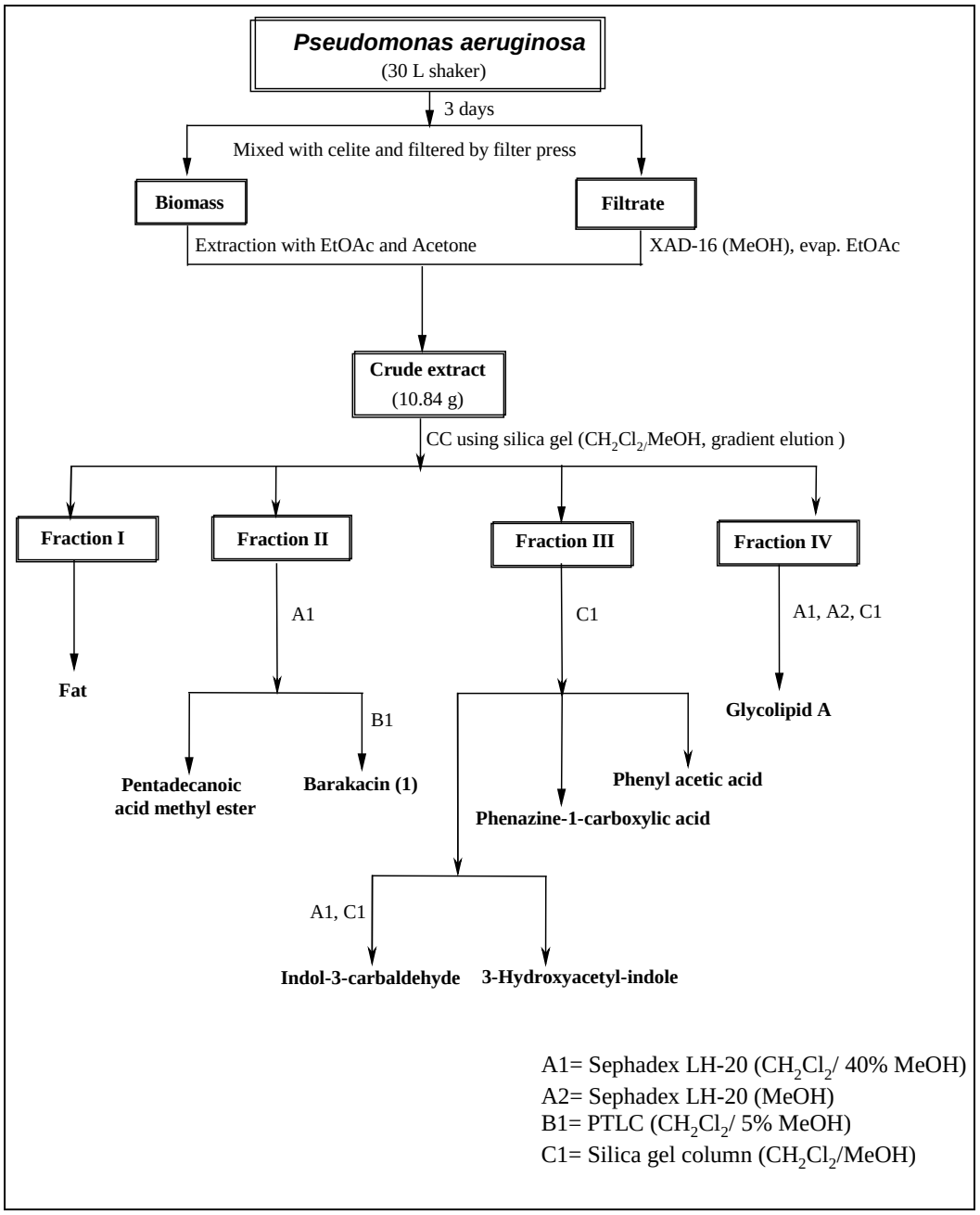


Figure 6: Work-up scheme of the ruminal bacterium ZIO

4.1.1 1*H*-Quinazoline-2,4-dione

Compound **91** was isolated from fraction I as colourless solid. It was UV absorbing under UV at 254 nm, but did not give a colour reaction with anisaldehyde/sulphuric acid. It showed a ^{1}H NMR spectrum with downfield signals at δ 7.97 (dd, 1H), δ 7.32 (t, 1H), δ 7.06 (dd, 1H), δ 6.94 (t, 1H), δ 8.12 (s, NH), δ 11.8 (s br, NH).

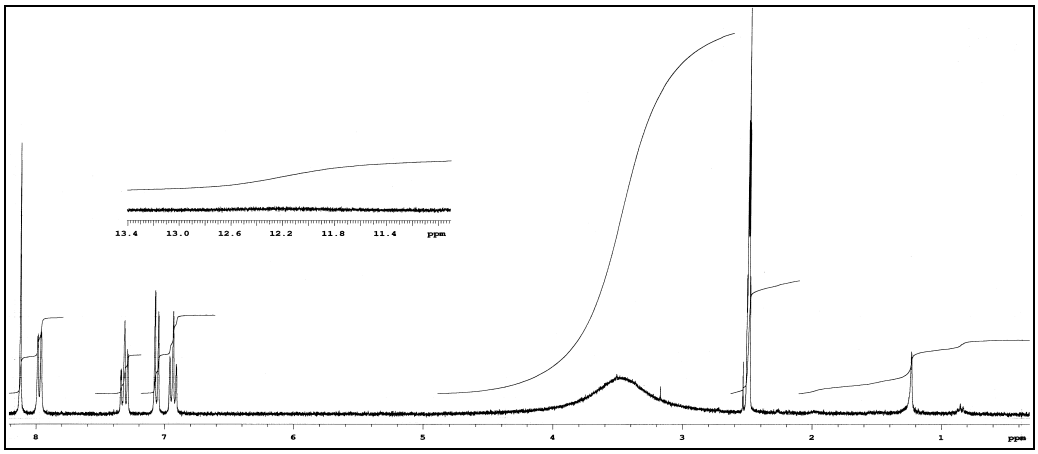


Figure 7: ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of 1*H*-quinazoline-2,4-dione (**91**)

The ^{13}C NMR spectrum displayed two carbonyl signals at δ 163.8 (C-4) and 162.6 (C-2), six aromatic carbon signals, four methines, and two quaternary carbon atoms (Figure 8).

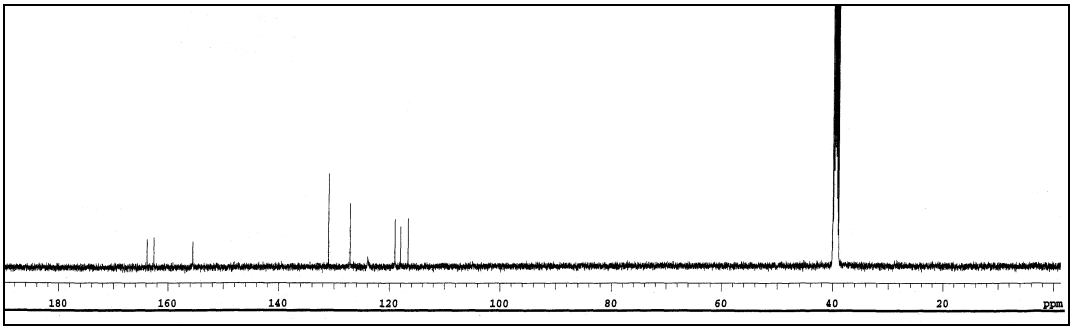


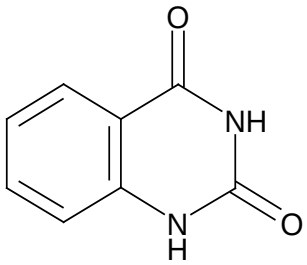
Figure 8: ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of 1*H*-quinazoline-2,4-dione (**91**)

A search with the mentioned NMR data in the Dictionary of Natural Products [116] resulted in **91** as a possible structure. This was confirmed by comparison of the NMR data with the literature. Compound **91** was thus assigned as 1*H*-quinazoline-2,4-dione.

1*H*-Quinazoline-2,4-dione (**91**) was isolated before from plants [113] and in our group also from a microorganism. [114] It has been reported that quinazolinones show anti-asthmatic, antirheumatic, antihypertonic, anti-anaphylactic, tranquilizing, neuro-stimulating, and benzodiazepine binding activity. [115-116] The quinazolindione moie-

ty, in particular, is widely found in natural purine bases, alkaloids and many biologically active compounds. [117] For example, 3-substituted quinazolinones, such as SGB-1534 and ketanserin have been found to have antihypertensive activities mediated via α-adrenoceptor and serotonic receptor antagonism, [118,119] respectively. 6,7-Dimethoxy-1*H*-quinazoline-2,4-dione is a key intermediate for the production of the medical drugs prazosin (Minipress), [120] bunazosin (detantol), [137] and doxazosin (cardenalin). [137] 7-Chloro-1*H*-quinazoline- 2,4-diones is a key intermediate for the production of drugs such as FK366 [121-122] and KF31327. [123]

The quinazolinedione ring system can generally be prepared by the reaction of anthranilic acid with urea, [124,125] anthranilamide with phosgene, [126] and anthranilic acid with potassium cyanate [127] or chlorosulfonyl isocyanate. [128]



91

4.1.2 Barakacin

Purification of fraction II using PTLC followed by Sephadex LH-20 afforded 25 mg of barakacin (**92**), a yellow solid. It showed an intense green fluorescence on TLC under UV (366 nm). The reaction of **92** with anisaldehyde/sulphuric acid gave an orange colour and with Ehrlich's reagent, the spot turned to red-violet (Figure 9) indicating an indole derivative.

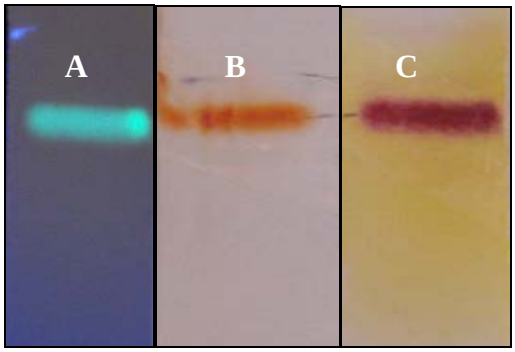


Figure 9: TLC of barakacin (**92**): **A:** under UV at 366 nm, **B:** after spraying with anisaldehyde/H_2SO_4 (orange), **C:** after spraying with Ehrlich's reagent (red-violet)

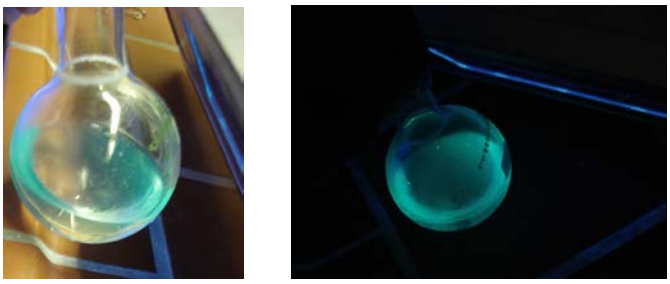


Figure 10: Barakacin (**92**) in $CDCl_3$ under UV at 366 nm

The ^{1}H NMR spectrum of compound **92** showed the pattern of three disubstituted aromatic systems. The first pattern showed two *ortho*-coupled 2H doublets at δ 7.50 (J = 7.9 Hz) and 7.34 (J = 8.1 Hz), and two 2H triplets of doublets at δ 7.20 (J = 7.1, 1.1 Hz) and 7.07 (J = 7.1, 1.0 Hz) indicating the presence of two 1,2-disubstituted benzene rings. Another 2H signal at δ 6.82 (2H, d, J = 1.7 Hz) and an H/D exchangeable broad signal at δ 7.94 (2H) together with the positive Ehrlich's reaction indicated a 3-substituted indole ring. Two *ortho*-coupled 1H doublets of doublets at δ 7.62 (J = 7.8, 1.5 Hz) and 7.02 (J = 8.3, 1.1 Hz), and two triplets of doublets at δ 7.29 (J = 7.3, 1.6 Hz) and 6.90 (J = 7.9, 1.1 Hz) indicated a third 1,2 -disubstituted benzene ring. Finally, the ^{1}H NMR spectrum displayed two narrow doublets at δ 6.89 (J = 0.7 Hz) and 6.11 (J = 0.8 Hz) along with a broad OH signal at δ 12.43.

Table 2: Physico-chemical properties of barakacin (**92**)

	Barakacin (**92**)
Appearance	Yellow solid
R_f	0.90 (CH_2Cl_2/2% MeOH); 0.61 (CH_2Cl_2)
Colour Reaction	Orange with Anisaldehyde/H_2SO_4 spraying reagent. Red-Violet with Ehrlich's reagent
Solubility	Soluble in $CHCl_3$, DMSO, MeOH, EtOH, and EtOAc. Insoluble in hexane, benzene and H_2O
Molecular formula	$C_{26}H_{19}N_3OS$
(+)-ESI-MS: *m/z* (%)	864.8 ($[2M+Na]^+$, 100), 444.1 ($[M+Na]^+$, 10), 422.0 ($[M+H]^+$, 15)
(-)-ESI-MS: *m/z* (%)	840.9 ($[2M-H]^-$, 92), 420.2 ($[M-H]^-$, 100)
(+)-HRESI-MS:	
Found	422.1321370 $[M+H]^+$
Calcd.	422.13216 $[M+H]^+$, for $C_{26}H_{20}N_3OS$
UV/VIS (0.20 mg/10 ml MeOH): λ_{max} (log ε)	(MeOH): 205 (4.43), 221 (4,77), 282 (4,27), 289 (4.25), 326 (4.09); (MeOH/HCl): 209 (4.66), 220 (4.76), 282 (4.22), 289 (4.23), 343 (4.03) (MeOH/NaOH): 205 (4.41), 223 (4.76), 283 (4.21), 361 (3.92) nm.

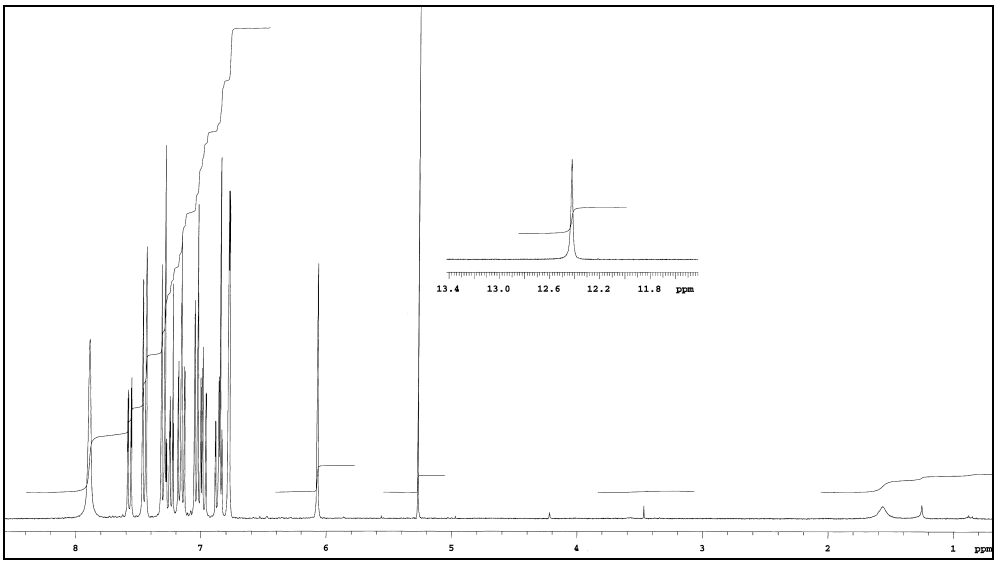


Figure 11: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of barakacin (**92**)

The ^{1}H and ^{13}C NMR spectra confirmed the presence of two indole moieties in a symmetrical orientation.

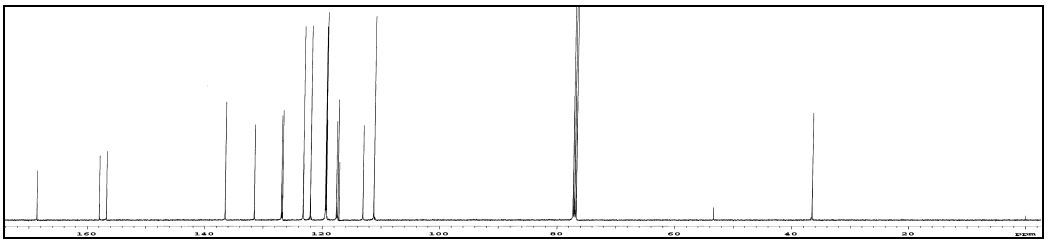


Figure 12: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of barakacin (**92**)

The HMBC spectrum of **92** indicated only 18 carbon signals of which seven were due to quaternary carbon atoms, and eleven methine carbons. All carbon signals were localised in the sp^2 region, except for the methine signal at δ_C 36.6 (δ_H 6.11). The quaternary carbon atom at δ_C 168.7 could be due to a carbonyl or indicate an sp^2 carbon localised between two heteroatoms as in a thiazole moiety (-N = C_q-S-). This agreed with the empirical formula and the strong green fluorescence, which resembled that of aeruginoic acid, [129-130] dihydroaeruginoic acid [149,131] and the recently isolated karamomycins A-C. [132]

The molecular weight was determined by ESI-MS: Three *quasi*-molecular ion peaks in positive and two in negative ESI-MS mode, respectively, confirmed the molecular weight of **92** as 421 Dalton. (+)-HRESI-MS of compound **92** delivered the molecular formula $C_{26}H_{19}N_3OS$. The ^1H-^1H COSY spectrum showed the expected correlations; the indole rings were further confirmed by HMBC cross signals (Figure 13). Correlations from H-2" (δ 6.82) to C-3", C-3"a, C-7"a, and C-6' along with the correlation from H-6' (δ 6.11) to C-2", C-3" and C-3"a supported the assignments of the indole moiety (fragment A, Figure 13).

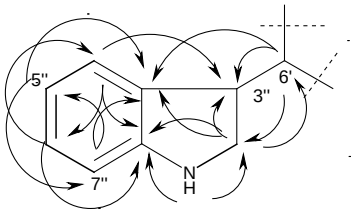


Figure 13: Fragment A

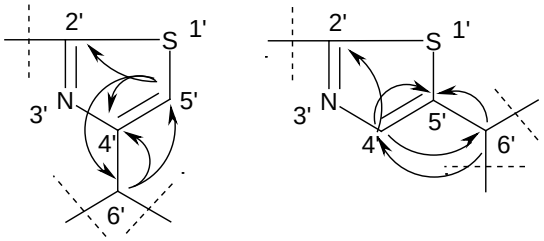


Figure 14: Fragments B1 and B2

Table 3: ^{13}C NMR (125 MHz) and ^{1}H NMR (300 MHz) data of barakacin (**92**) and pulicatin (**93**) in $CDCl_3$ [a)]. The indole part of **92** was compared with vibrindole A (3,3'-bis-indolylmethane); δ values, *J* in [Hz].

Position	Barakacin (92)		93
	δ_H (*J* in Hz)	δ_C	δ_C
1	-	156.7	156.6
1-OH	12.43 (s)	-	-
2	-	117.2	115.4
3	7.62 (dd, 7.8, 1.5)	126.9	126.8
4	6.90 (td, 7.9, 1.1)	119.3	119.2
5	7.29 (td, 7.3, 1.6)	131.5	131.2
6	7.02 (dd, 8.3, 1.1)	117.6	117.5
2'	-	168.7	166.3
4'	-	157.9	149.5
5'	6.89 (d, 0.7)	113.1	128.6
6'	6.11 (d, 0.8)	36.6	58.5/28.0[b]
(NH)	7.94 (2H, s)	-	-
2", 2"'	6.82 (2H, d, 1.7)	123.2	121.1
3", 3"'	-	117.4	121.8
3a", 3a"'	-	126.7	127.0
4", 4"'	7.50 (2H, d, 7.9)	119.5	119.7
5", 5"'	7.07 (2H, td, 7.1, 1.0)	119.4	119.0
6", 6"'	7.20 (2H, td, 7.1, 1.1)	122.0	121.7
7", 7"'	7.34 (2H, d, 8.1)	111.2	111.0
7a", 7a"'	-	136.5	136.6

[a)] referenced on $CDCl_3$ with δ_H = 7.27 and δ_C = 77.00; [b)] 58.5: C-6' of **93**; 28.0: CH_2 and values below are of vibrindole A

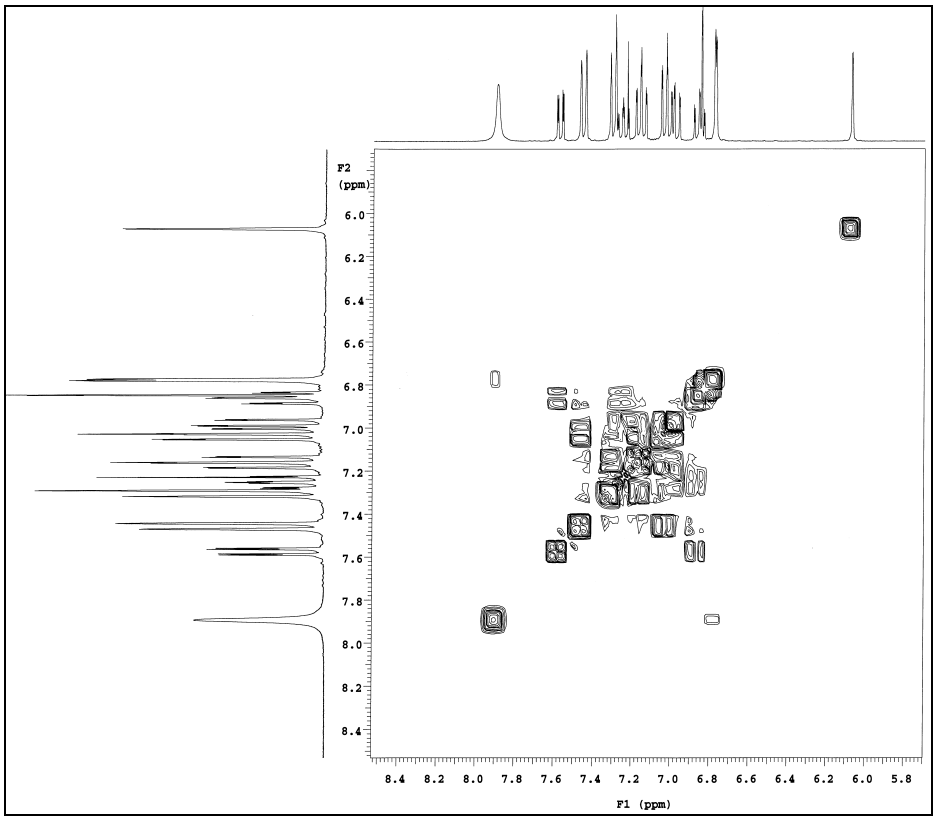


Figure 15: ^{1}H-^{1}H COSY spectrum ($CDCl_3$, 600 MHz) of barakacin (**92**)

The HMBC correlations of methines H-6 (δ7.02) to C-2 and C-4, and H-3 (δ7.62) to C-5, C-2' and the oxy-quaternary carbon C-1 indicated the presence of a 2-substituted phenol (δ_{OH} 12.43); H-3 showed an additional correlation with C-2' (Fragment C, Figure 16).

The proton doublet of H-6' at δ6.11 (J = 0.8 Hz) showed further couplings with C-4' and C-5' and the H-5' signal at δ6.89 (d, J = 0.7 Hz) correlated also with C-2', C-4' and C-6', giving two substructures B1 or B2 (Figure 14), which overlapped with atoms 6' and 2' in fragments A and C, respectively (Figure 13 and Figure 16).

These correlations revealed that the fragment A and C were connected *via* a thiazole ring B1 or B2 (Figure 14).

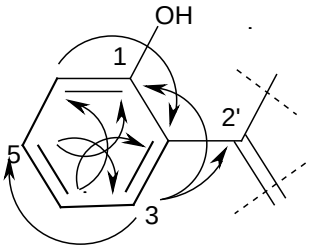


Figure 16: Fragment C

A distinction between the 2,4- and 2,5-disubstituted thiazoles B1 and B2 (Figure 14) was not possible on the basis of 2D correlations. However, comparison with pulicatin C (2) [133] indicated a close similarity for the phenylthiazole part, and the indole signals matched those of vibrindole A (3,3'-bis-indolylmethane [134] perfectly (see Table 3) As the shift of thiazol carbons are scarcely influenced by sp^3 C-residues, the above spectral information and the molecular formula finally established the structure of barakacin as 2-{4-[bis-(1*H*-indol-3-yl)-methyl]-thiazol-2-yl}-phenol (**92**, Figure 19). It is the first natural thiazolyl-indole alkaloid. [135]

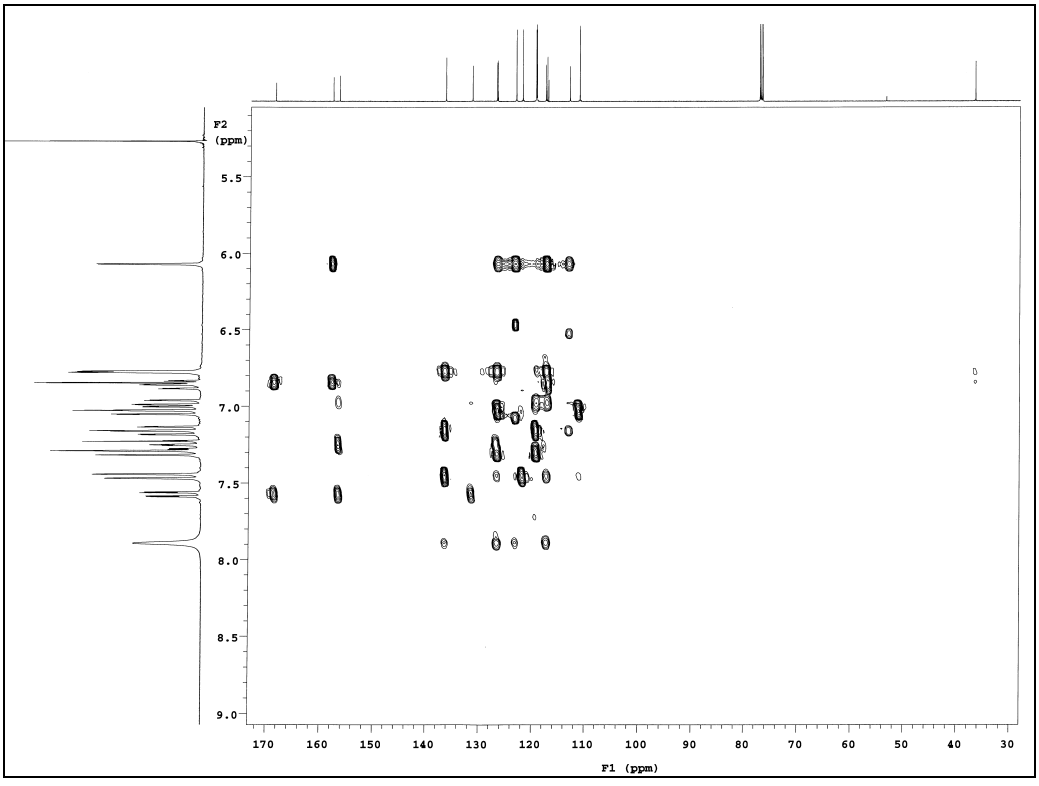
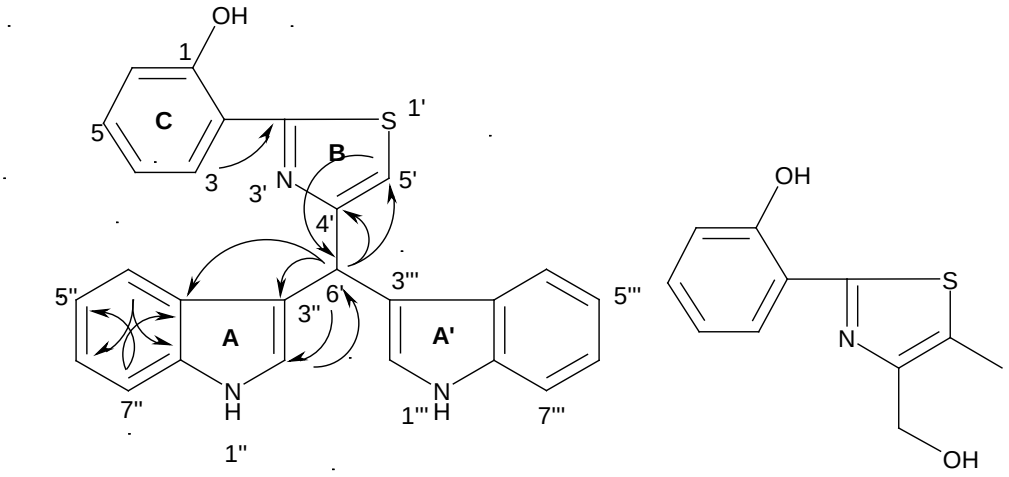


Figure 17: HMBC spectrum ($CDCl_3$, 600 MHz) of barakacin (**92**)



92 **93**

Figure 18: HMBC correlations (→) connecting fragments A~C of barakacin (**92**) and structure of pulicatin C (**93**).

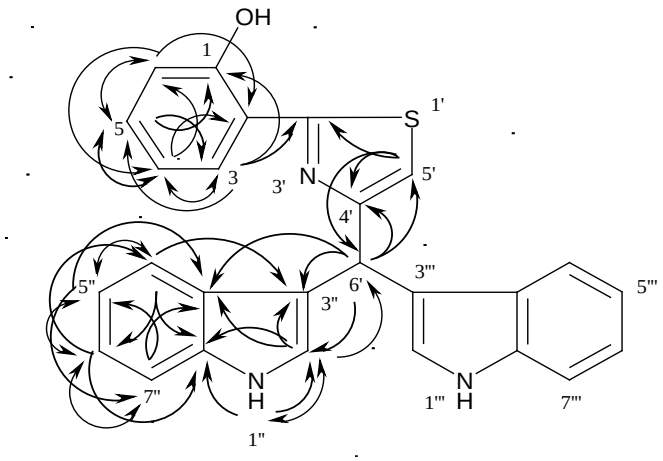
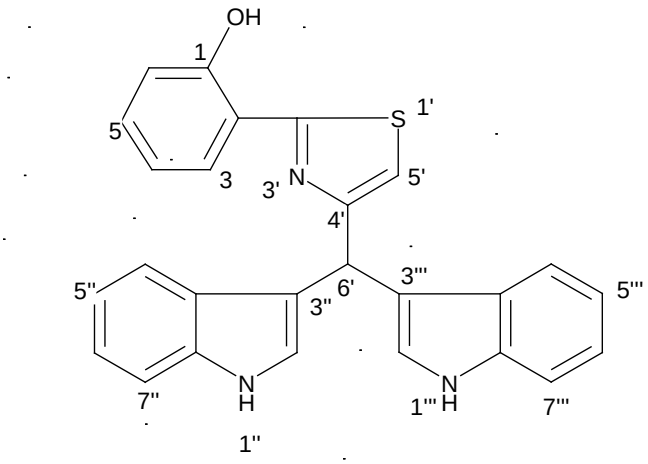


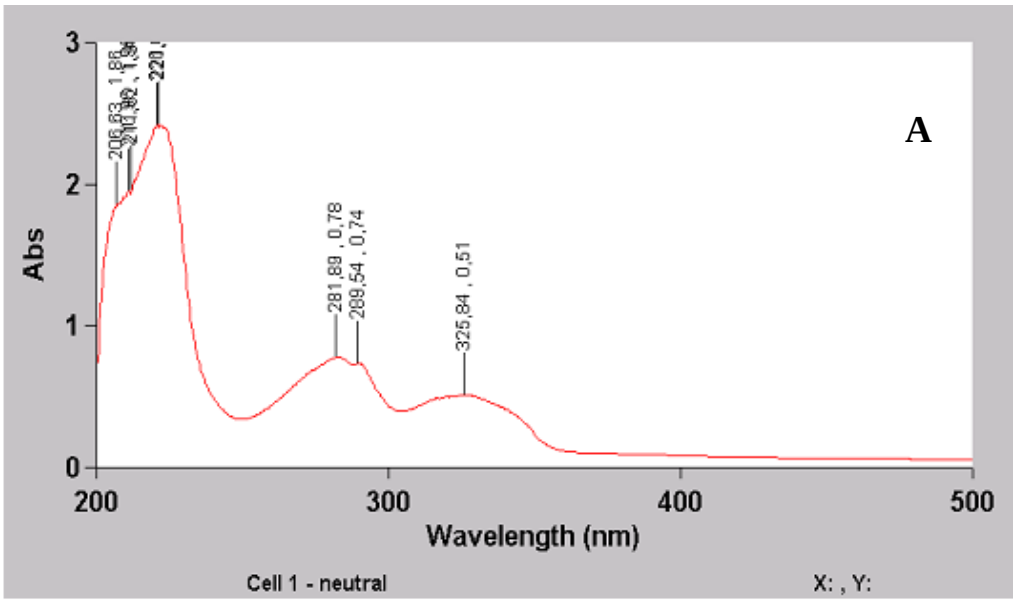
Figure 19: (↔) ^{1}H-^{1}H COSY and (→) HMBC correlations of barakacin (**92**)

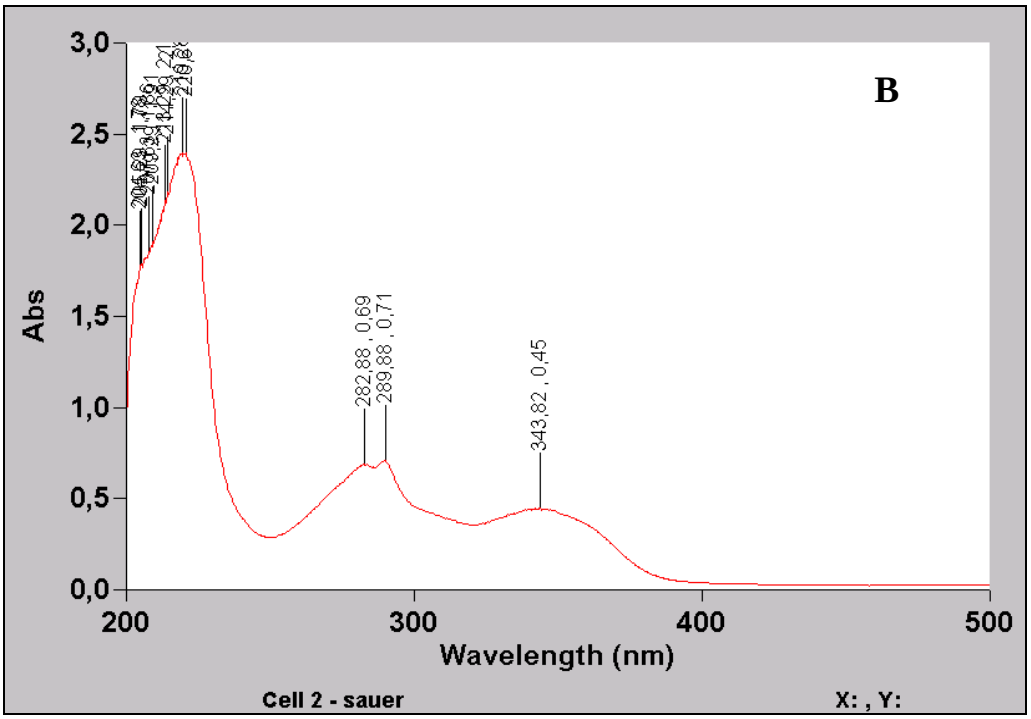
From the above spectral information, the molecular formula, and subsequently the double bond equivalence as well as the precise connectivities of fragments A-C, the structural formula of barakacin was finally established as 2-{4-[bis-(1*H*-indol-3-yl)-methyl]-thiazol-2-yl}-phenol (**92**). The structure was confirmed on the basis of the MS and NMR interpretation and by comparison with related spectra, as well as literature data. By a search in AntiBase, [115] the Dictionary of Natural products (DNP) [116] and the Chemical Abstract (CA) [117], no results were revealed, pointing to the novelty of **92** as the first thiazolyl-indol alkaloid isolated from nature, which was named as barakacin (**92**).



92

The UV spectra (MeOH) of **92** displayed four strong bands at λ_{max} = 221, 282, 290 and 326 nm in neutral solution. Under basic methanol conditions, the latter band showed a bathochromic shift to λ_{max} = 361 nm (Figure 20).





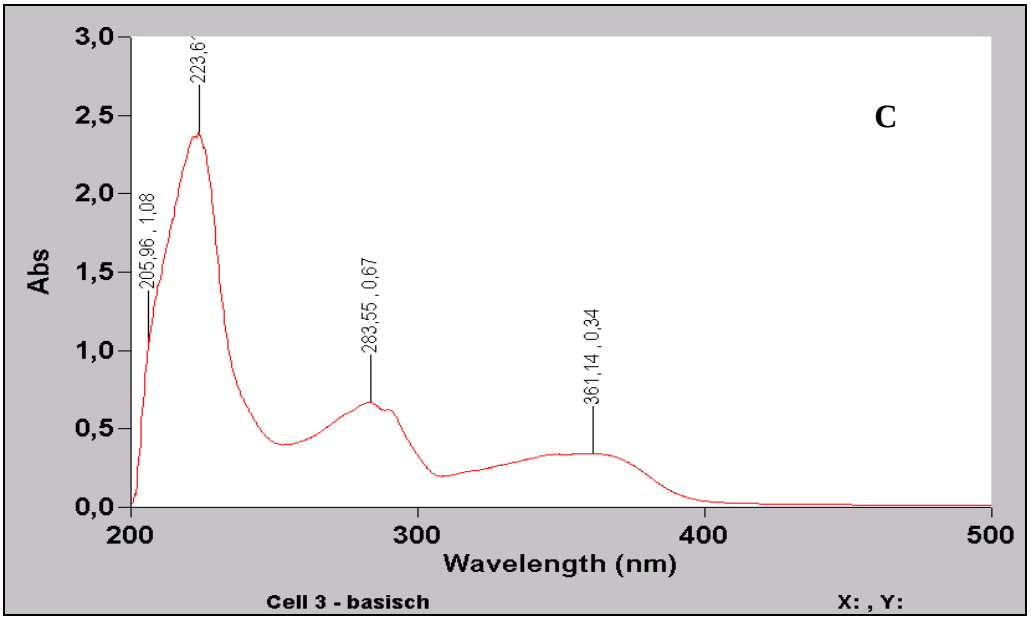


Figure 20: UV/vis spectra of barakacin (**92**); Neutral (MeOH), Acidic (MeOH + HCl), Basic (MeOH + NaOH)

The antibacterial and antifungal activities of barakacin (**92**) were qualitatively assessed using the agar diffusion method. Although a pronounced antibacterial activity of many indole-alkaloids against Gram-positive bacteria have been reported, barakacin (**92**) was inactive at 40 µg/paper disc against the tested organisms. The

strong activity of the crude extract of *P. aeruginosa* against the tested strains was due to phenazine-1-carboxylic acid (**43**).

Barakacin (**92**) showed, however, a weak and unselective cytotoxic activity against a range of human cancer cell lines LXFA 629L and LXFL 529L (lung), MAXF 401NL (breast), MEXF 462NL (melanoma), RXF 944L (kidney) and UXF 1138 (uterus) with a mean IC_{50} value of 2.8 μg/ml (mean IC_{70} = 5.4 μg/ml).

4.1.3 Rhamnolipid A (Glycolipid A):

Compound **94** was isolated from fraction IV as a polar oily compound, which turned to dark green after spaying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of **94** showed signals at δ 5.40, 4.80, 4.25, 3.80, 3.70, 3.40, 2.38, 2.50 and at δ 1.25. The signals at δ 0.82, 1.20-1.62 and δ 1.25 are characteristic of long chain aliphatic fatty acids.

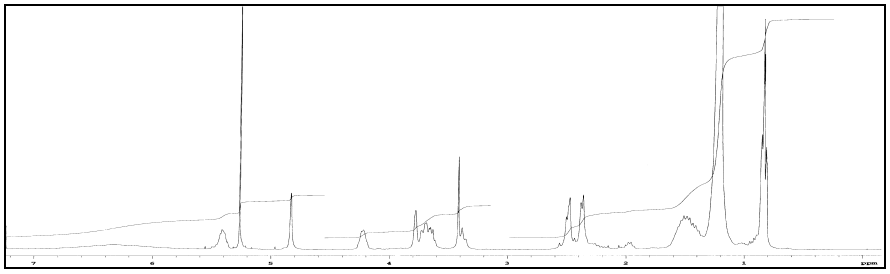
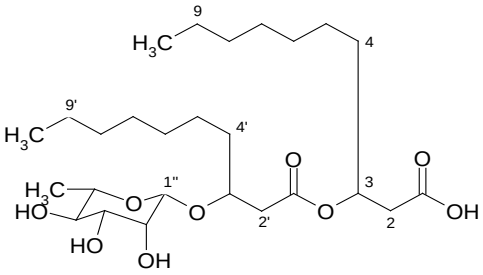


Figure 21: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of rhamnolipid A (**94**)

The ^{13}C NMR spectrum of **94** showed in the aliphatic region lipid signals at δ 22.5-34.5 for two chains of CH_2, a CH_3 signal at δ 14.1, and three CH signals between 71.2 and 73.8. The signals at δ 17.6, 67.7, 70.3, 71.2, 73.8, 95.1 are an indication of a sugar moiety. In the aromatic region, it displayed an ester and a carboxylic signal at δ 171.5 and δ 174.2.



94

Table 4: ^{1}H and ^{13}C NMR spectral data of rhamnolipid A (**94**)

Position	δ_H	δ_C	*C type*
C-1	2.50	71.3	CH
C-2	5.40	39.8	CH_2
C-3	–	171.5	=C-O-
C-4	4.25	71.5	CH
C-5	2.38	1.20	CH_2
C6-C9	1.20-1.62	22.5-34.5	CH_2
C-10	0.82	14.1	CH_3
COOH	–	174.2	COOH
C-1′-5′	1.20- 1.62	22.5-34.5	CH_2
C-6′	0.82	17.0	CH_3
C-1′′	4.81	95.1	CH
C-2′′	3.80	71.2	CH_2
C-3′′	3.40	67.7	CH_2
C-4′′	3.34	73.8	CH_2
C-5′′	3.70	70.3	CH_2
C-6′′	1.20	17.6	CH_3

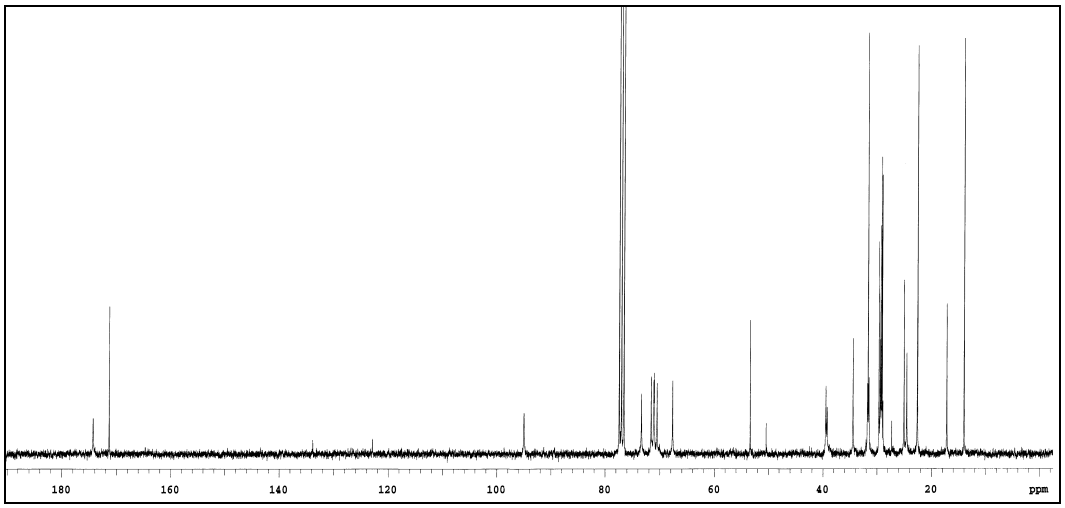
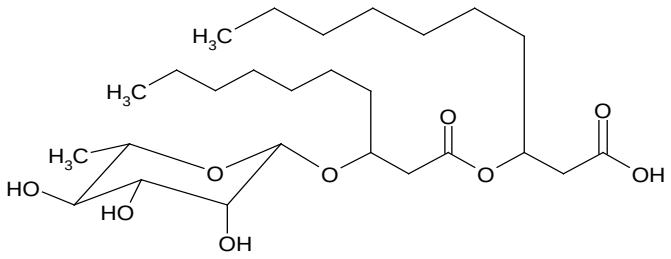


Figure 22: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of rhamnolipid A (**94**)

A search with the mentioned NMR data in the Dictionary of Natural Products [116] and according to the results given by mass spectrometry, resulted in **94** as the most plausible structure. Compound **94** was thus assigned as rhamnolipid A (**94**)



94

Cytotoxicity and proliferation assay of rhamnolipid A (**94**)

Antitumor activity of compound **94** was tested in a monolayer cytotoxicity and proliferation assay using human tumor cell lines as described previously. [385]

Briefly, the number of viable cells after 4 days of incubation with a test compound was determined using propidium iodide as a read-out. Antitumor activity including the induction of apoptosis and the inhibition of cell proliferation was recorded as a reduction of the viable cell number relative to control wells and expressed as T/C (test/control) value. The requirement of antitumor activity was a T/C value of <30%. Tumor selectivity was defined as a 3-fold lower individual IC_{70} value of a cell line

compared to the mean IC_{70} value over the 37-cell line panel. All compounds were tested in triplicate in a panel of 37 human tumor cell lines at five different concentrations ranging from 0.001 µg/ml up to 10 µg/ml. Twenty-four out of the thirty seven test cell lines had been established from patient-derived tumor xenografts growing in nude mice as described by Roth *et al.* [136]

The origin of the donor xenografts was described by Fiebig *et al.* 1992. [137] The remaining 12 cell lines were kindly provided by the US National Cancer Institute or purchased from the American Type Culture Collection (Rockville, MD, USA).[156]

Table 5: *In vitro* antitumor activity of rhamnolipid A (**94**) against tumor cell lines in a monolayer proliferation assay.

Tumor type	**Tumor cell line N°**	**Test/Control (%) at drug concentration (30 µg/ml)**
Colorectal	CFX HT29	6+++
Gastric	GXF 251L	14++
Lung A Adeno	LXF 529 NL	10 ++
Lung A Adeno	LXF 629 L	5 +++
Breast	MAXF	7 +++
Melanoma Xenograft	MEXF 462 NL	7 +++
Ovarien Cancer Xenograft	OVXF 899L	2 +++
Pancreas	PAXF	10 ++
Prostate	PRXF	14 ++
Pleuramesothelioma	PXF 1752L	15 ++
Renal	RXF 486L	25 ++
Uterus body	UXF 1138L	4 +

- (T/C = 50) + (30< = T/C < 50) ++ (10 < = T/C < 30) +++ (T/C < 10)

Table 6: *In vitro* antitumor activity of rhamnolipid A (**94**) against tumor cell lines in a monolayer proliferation assay.

Tumor type	Tumor cell line N°	IC_{50} µg/ml	IC_{70} µg/ml	IC_{90} µg/ml
Colorectal	CFX HT29	2.950	5.138	8.949
Gastric	GXF 251L	3.898	6.579	11.103
Lung A Adeno	LXF 529 NL	3.207	5.663	10.000
Lung A Adeno	LXF 629 L	3.080	5.198	8.773
Breast	MAXF	3.487	5.692	9.291
Melanoma Xenograft	MEXF 462 NL	2.900	5.158	9.172
Ovarien Cancer Xeno-graft	OVXF 899L	3.007	4.961	8.185
Pancreas	PAXF	2.682	5.179	10.000
Prostate	PRXF	3.814	6.515	11.130
Pleuramesothelioma	PXF 1752L	3.874	6.660	11.450
Renal	RXF 486L	4.780	8.627	15.570
Uterus body	UXF 1138L	3.467	5.495	8.709
	Mean n = 12	3.386	5.831	10.041

4.1.4 Phenazine-1-carboxylic acid

Compound **43** was obtained as a yellow solid from fraction III by further PTLC. Compound **43** showed UV absorbance under 254 nm and turned to yellow after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed signals for seven aromatic protons: two doublets of doublets at δ 9.00 (1H) and δ 8.58 (1H) and two multiplets between δ 8.40-8.20 (2H) and δ 8.10-7.98 (3H). From the intensities and coupling patterns, two aromatic 1,2-disubstituted and 1,2,3-trisubstituted rings could be derived. The down field shift pointed to their fusion with heteroatoms. The (+)-ESI mass spectrum of compound **43** fixed its molecular weight as 224 Dalton. The EI mass spectrum exhibited in addition to the molecular ion a base peak at *m/z* 180 resulting from the loss of a carboxy group, and the mass *m/z* 180 is indicative for the phenazine skeleton. A search in AntiBase [115] led to phenazine-1-carboxylic acid (**43**). The structure was further confirmed by comparing the data with authentic spectra and the literature. [138]

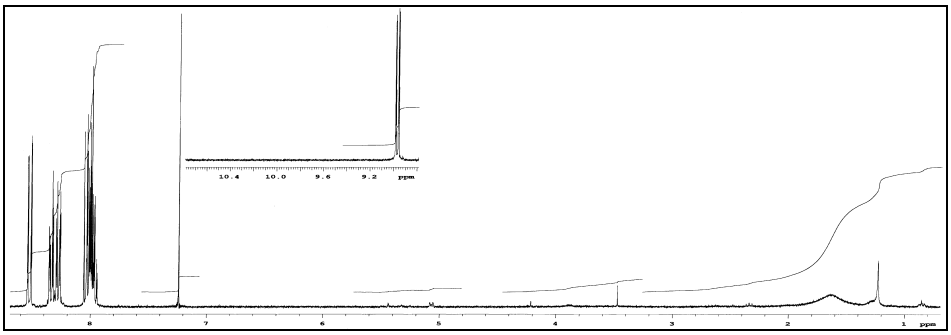
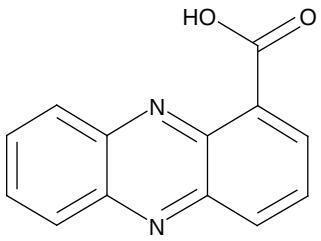


Figure 23: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of phenazine-1-carboxylic acid (**43**)

Phenazine-1-carboxylic acid (**43**), also known as tubermycin B was isolated previously from microorganisms *Pseudomonas*, [] *Streptomyces cinnamonensis*, [139] *Streptomyces misakiensis* [38] and *Actinomadura dassonvillei*. [140] It exhibits a weak activity against Gram-positive bacteria, and a moderate activity against both *Mycobacterium tuberculosis* BCG and *Mycobacterium tuberculosis* H37Rv (streptomycin resistant). [141] Other phenazines have been found to inhibit DNA-dependent RNA synthesis, and an intercalation mechanism has been proposed for their antibiotic action. [147,148148]



43

4.1.5 3-(Hydroxyacetyl)-indole

TLC of fraction III exhibited compound **95** as a middle polar UV absorbing band, which turned to orange when exposed to anisaldehyde/sulphuric acid. The compound was obtained as colourless solid.

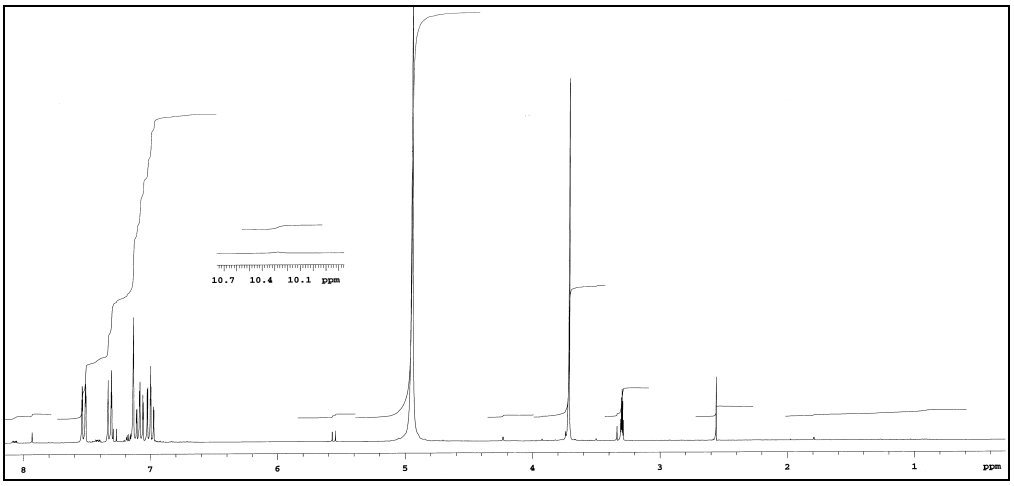
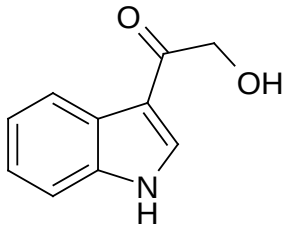


Figure 24: ^{1}H NMR spectrum (300 MHz, CD_3OD) of 3-hydroxyacetyl-indole (**95**)

The ^{1}H NMR spectrum showed a broad singlet of an acidic proton at δ 10.29, three signals with intensity of 5H protons at δ 7.58 (d), 7.38 (m), 7.08 (m), 7.00 (m, 2H). This resembled an indole skeleton substituted at 3-position. Additionally, in the aliphatic region, a 2H singlet at δ 3.74 of an oxygenated methylene group attached to an sp^2 carbon was observed.



95

A search in AntiBase [115] pointed to 3-hydroxyacetyl-indole (**95**), and the latter was further confirmed by direct comparison with an authentic spectrum and the literature. [142,143] Compound **95** was isolated previously from the fungus *Lactarius deliciosus*, [144] marine red algae [145] and sponges. [146] It was also isolated from myxobacteria *Archangium gephyra* [162] in addition to several other indole derivatives, which could be responsible for the antifungal properties of the extract. 3-Hydroxyacetyl-indole (**95**) was isolated as tryptophane metabolite from a staurosporine producer, *Streptomyces staurosporeus*. [147]

4.1.6 Indole-3-carbaldehyde

From fraction III, compound **96** was obtained as colourless solid by Sephadex LH-20 (CH_2Cl_2/ 40% MeOH), giving an UV absorbing spot on TLC which was stained to orange by anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum showed a doublet at δ 8.05 with a small coupling constant (J = 3.4 Hz) suggesting a *meta* coupled proton. Moreover, four aromatic protons at δ 7.95 (s, 1H), 7.41 (d, 1H), and 7.17 (m, 2H), indicated a 1,2-disubstituted aromatic ring. The ^{1}H NMR data pointed to an indole moiety substituted at 3-position. A search in AntiBase [115] confirmed the compound as indolyl-3-carbaldehyde (**96**), which was finally confirmed by direct comparison with authentic spectra.

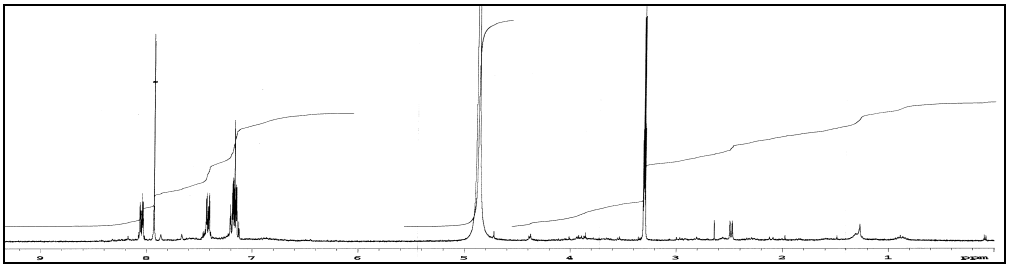
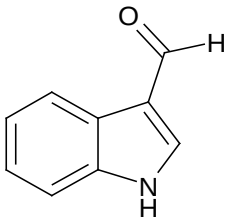


Figure 25: ^{1}H NMR spectrum (300 MHz, CD_3OD) of indole-3-carbaldehyde (**96**)



96

4.1.7 *Cyclo*(Phe,Pro)

Compound **97** was isolated as colourless solid from fraction IV by Sephadex LH-20 chromatography. It showed UV absorbance and turned to violet by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed a multiplet of a phenyl group at δ 7.30, a broad singlet at δ 6.08 of an acidic proton (NH), and two signals at δ 4.15 and δ 3.98 for two methines. The spectrum revealed multiplets at δ 3.62 (m, 1H, Ha-

10) and 3.58-3.45 (9-CH_2), and the ABX system of a methylene group attached to a heteroatom [2.80 (dd, 1 H, Hb-10) and 2.39 (m)]. In addition, multiplets of two further methylene groups at δ 2.10-1.85 were exhibited.

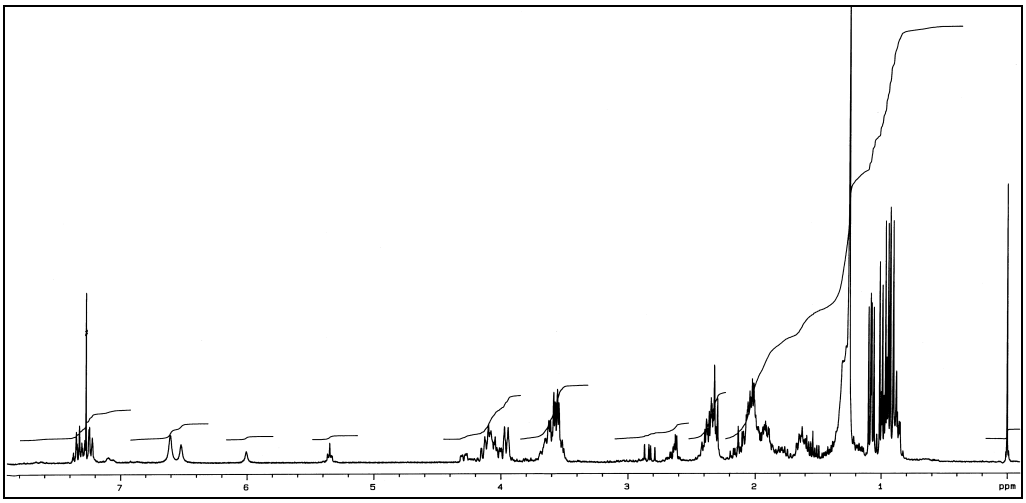
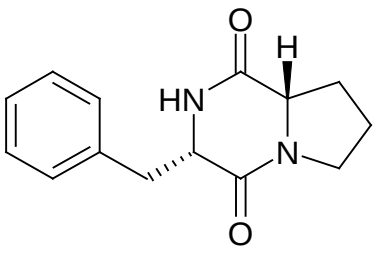
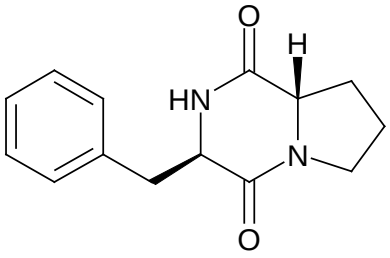


Figure 26: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of *cis-cyclo*(Phe,Pro) (**97**)

The ESI mass spectrum displayed a molecular ion peak at *m/z* 267 $[M+Na]^+$. A search in AntiBase [115] afforded two isomers, namely *cis-cyclo*(Phe,Pro) (**97**) and *trans-cyclo*(Phe,Pro) (**98**). Comparing the ^{1}H NMR and carbon values of CH-3 and CH_2-10 in both isomers with literature data [148,149] pointed to *cis-cyclo*(Phe,Pro) (**97**).



97



98

The cyclic dipeptides *cyclo*(Phe,Pro) have previously been isolated from *Rosellinia necatrix*. [150] *Cyclo*(Phe,Pro) was also isolated from *Streptomyces* sp. AMLK-335 and exhibited antimicrobial activity against four pathogenic microorganisms, such as *Bacillus subtilis* IAM 1069, *Micrococcus luteus* JCM 1464, *Staphylococcus aureus* TK 784, and *Saccharomyces cerevisiae* IFO 1008. [151]

4.1.8 Triethylamine

Compound **99** was isolated as oily material. It showed no UV absorbance at 254 nm, and was colourless after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum showed in the aliphatic region two signals: a methylene quartet at δ 3.22 due to the attachment to a heteroatom and a triplet at δ 1.15.

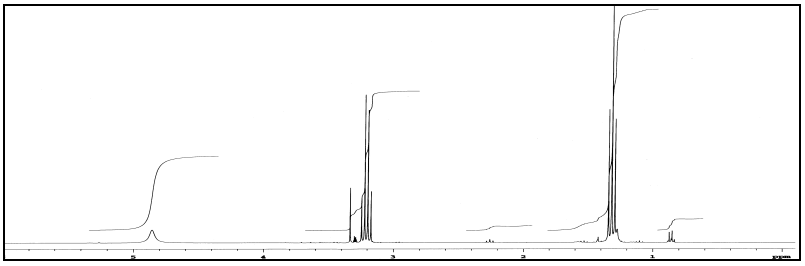


Figure 27: ^{1}H NMR spectrum (300 MHz, CD_3OD) of triethylamine (**99**)

A search in AntiBase [115] with these data gave triethylamine (**99**). It was further confirmed by the literature data. [152] As triethylamine is a highly volatile liquid, it was obviously isolated as an inorganic salt (as further carbon signals were missing).

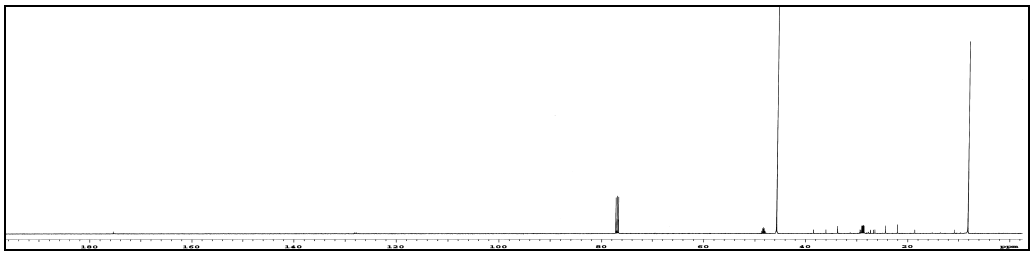
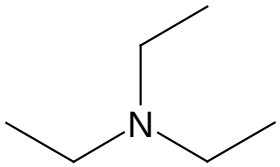


Figure 28: ^{13}C NMR spectrum (125 MHz, CD_3OD) of triethylamine (**99**)

Table 7: ^{13}C and ^{1}H NMR data (125, 300 MHz) of triethylamine (**99**) in comparison with literature data. [153]

	Experimental			**Literature** [172]	
Position	**δ_C**	**C type**	**δ_H (mult.)**	**δ_C**	**δ_H (mult.)**
1	8.1	CH_3 (t)	1.15 (t)	11.09	1.05 CH_3 (t)
2	45.9	CH_2 (q)	3.2 (q)	46.96	2.58 CH_2 (q)



99

Triethylamine (**99**) is commonly used in organic synthesis due to its basic properties and because it is liquid at room temperature. [154] Triethylamine (**99**) is often used in the preparation of esters and amides from acyl chlorides. [155] It is an organic acceptor in synthesis and a salt former in precipitation and purification operations. [156] Triethylamine (**99**) is mainly used in the production of quaternary ammonium compounds for textile auxiliaries and quaternary ammonium salts of dyes. [157] It is a stabilizer for chlorinated hydrocarbons and vinyl compounds, synthesis of semisynthetic penicillins and cephalosporins and solubilizers for herbicicides in combination with 2,4-dichlorophenoxyacetic acid. [158] It is useful as an intermediate for manufacturing medicines, pesticides and other chemicals. [177]

Triethylamine (**99**) is also used as an ingredient in sealing paint, in the manufacture of some paper and board adhesives, in reactions, as a stabilizer for the chlorinated solvents perchloroethylene and trichloroethylene, as an anti-livering agent for urea and melanine-based enamels, in the recovery of gelled paint vehicules, as an accelator and activator for rubber, as a corrosion inhibitor, as a propellant, [159] as a wetting, penetrating, and waterproofing agent of quaternary ammonium compounds, as an emulsifying agent for dyes, for the production of octadecyloxymethyltriethylammonium chloride (textile treatment agent), as an ingredient of photographic development accelerator, [175] and for many other applications.

Triethylamine (**99**) is the active ingredient in FlyNap, a product for anaesthetising *Drosophila melanogaster*. [160] Compound **99** is used in mosquito and vector control labs to anaesthetise mosquitoes. [179] It is used to prepare buffer solutions; also, the bicarbonate salt of triethylamine (often abbreviated TEAB, triethylammonium bicarbonate) is useful in reverse phase chromatography, often in a gradient to purify nucleotides and other biomolecules.

The toxicity of triethylamine (**99**) in experimentally and occupationally exposed individuals has been evaluated. Severe visual disturbances and changes in electrical activity in the cerebral cortex have been detected in human volunteers exposed to triethylamine by inhalation. Occupationally exposed workers have reported visual disturbances associated with exposure to triethylamine. Several case reports also indicate that exposure to triethylamine causes ocular and respiratory abnormalities.

4.2 Ruminal bacterium *Citrobacter freundii* ZIG

The ruminal bacterium *Citrobacter freundii* was selected due to its biological activity against human pathogenic bacteria such as *Staphylococcus aureus* and *Klebsiella pneumoniae* and the phytopathogenic fungi *Fusarium culmorum*, *Fusarium graminearum* and *Phoma tracheiphila.*

Several compounds were isolated and identified as oleic acid (**100**), myristic acid (**101**), palmitoleic acid (**102**), palmitic acid (**103**), 9,10-methanohexadecanoic acid (**104**), isoxanthohumol (**105**), *cyclo*(Pro,Leu) (**107**), *cyclo*(Tyr,Pro) (**109**), phenol (**111**) and adenine (**112**).

The bacterium formed separated colonies on LB medium after incubation for 24 hours at 37 °C. Pieces of well-grown agar plates were used to inoculate 120 of 1L Erlenmeyer flasks, each containing 250 ml of LB medium. The fermentation was carried out at 180 rpm on a rotary shaker for 3 days at 37 °C. The brown culture broth was harvested and filtered by a filter press, to separate the mycelium, which was extracted with ethyl acetate, while the culture filtrate was passed through Amberlite XAD-16. The column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluate was concentrated under reduced pressure and finally the aqueous residue extracted with ethyl acetate. On the other hand, the Celite phase was extracted with ethyl acetate (3 times) and acetone (2 times); the organic phases were then dried. Both crude extracts were combined based on TLC yielding a greenish-brown crude extract. The TLC showed several UV absorbing bands and with anisaldehyde/sulphuric acid some compounds gave pink colour.

Chromatography of the crude extract (3.25 g) on a silica gel column using a CH_2Cl_2-MeOH gradient resulted in three fractions I-III by TLC monitoring. They were further chromatographed in different ways to isolate the constituents in pure form.

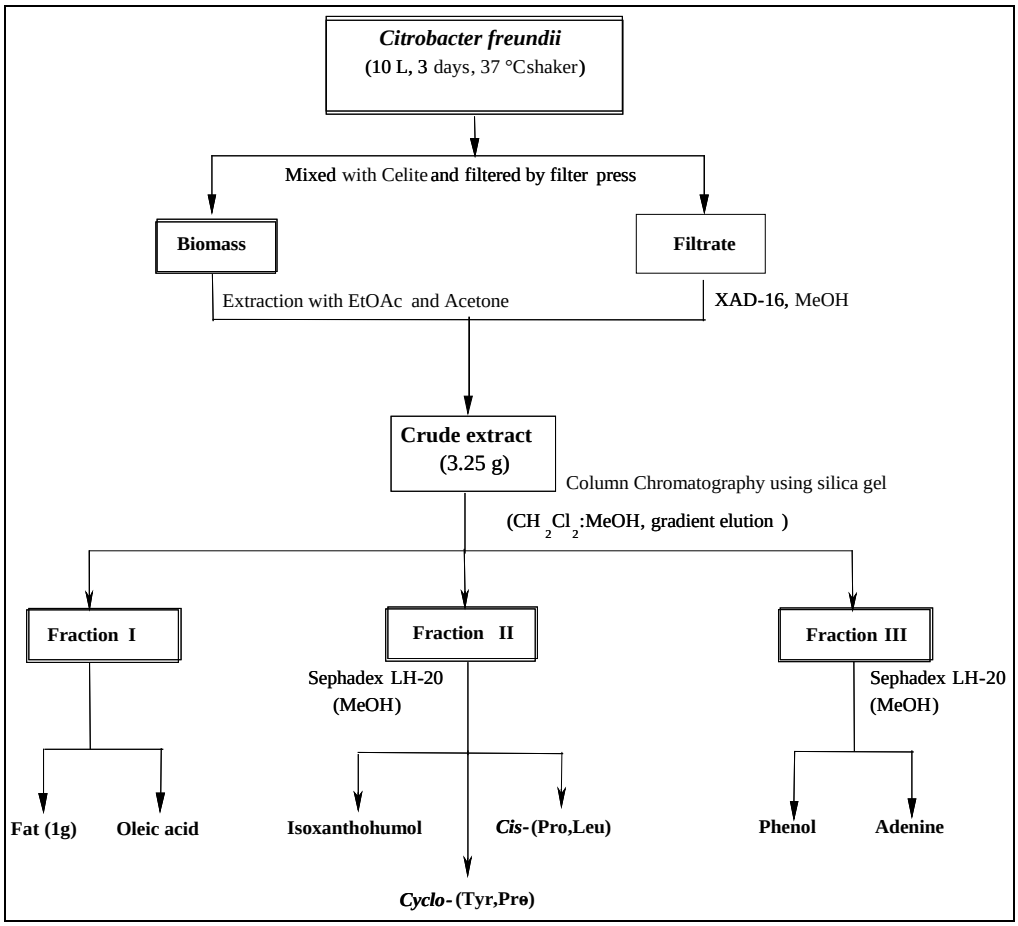
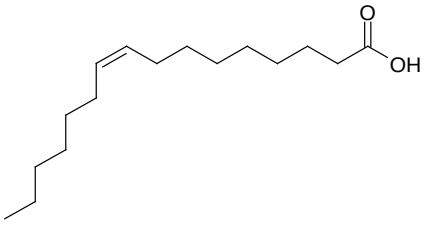


Figure 29: Work-up scheme of the ruminal *Citrobacter freundii*

4.2.1 Oleic acid

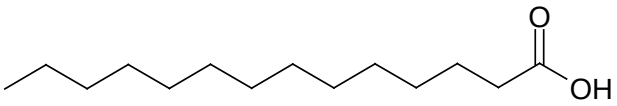
Compound **100** was isolated from fraction I as colourless, non-UV absorbing oil, which turned to blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum exhibited an exchangeable proton signal at δ 11.48, a multiplet at δ 5.36 of two olefinic protons (H-9, 10), three methylene signals connected to sp^2 carbons at δ 2.34 (t, CH_2-2), and 2.00 (m, CH_2-9, 10), as well as seven methylenes at δ 1.28. Additionally, one methyl triplet was observed at δ 0.85. By comparison of these data with reference mass spectra, this compound was identified as oleic acid (**100**).



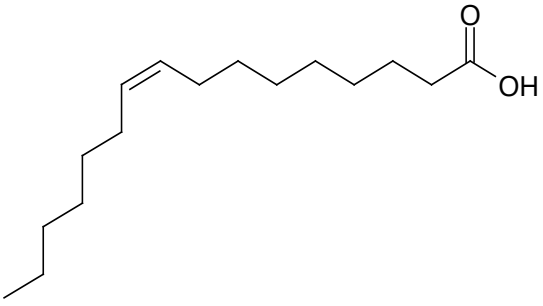
100

Oleic acid (**100**) is the main monounsaturated fatty acid of olive oil. It suppresses Her-2/neu over-expression, which synergistically interacts with anti-Her.2/neu immunotherapy by promoting apoptotic cell death of breast cancer cells with Her-2/neu oncogene amplification. [161] In addition, it was reported that oleic acid (**100**) is a potent inhibitor of fatty acid and cholesterol synthesis in C_6 glioma cells. Oleic acid (**100**) showed anticancer activity against breast cancer cells. [180] Furthermore, it was reported that oleic acid inhibits lipogenesis in C6 glioma cells. [162]

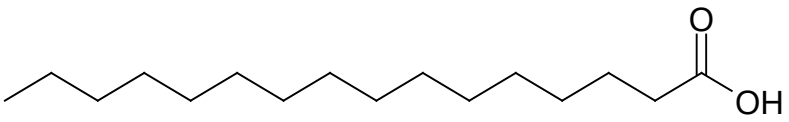
In addition to oleic acid (**100**), other oily compounds were obtained from the same fraction by GC-MS measurement and were characterized by gas chromatographic comparison with NIST spectra as one saturated fatty acid (myristic acid, **101**) and three unsaturated acids (palmitoleic acid, **102**), palmitic acid (**103**), and 9,10-methanohexadecanoic acid (**104**).



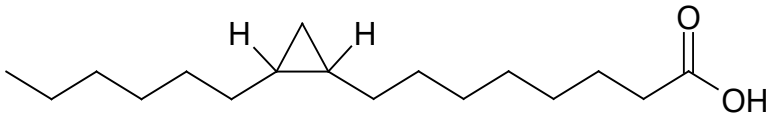
101



102



103



104

4.2.2 Isoxanthohumol

Compound **105** was isolated as colourless solid from fraction II; it exhibited a UV absorbing band, which showed no colour with anisaldehyde/sulphuric acid and an UV fluorescence pale yellow band at 366 nm. The ^{1}H NMR spectrum of **105** displayed two doublets each with intensity of 2H at δ 7.30 and 6.80 as an indication of a 1,4-disubstituted aromatic ring. In addition, a downfield 1H singlet at δ 6.04 could be due to an aromatic proton between two electron-donating OH-groups. Furthermore, an oxymethine proton gave a dd at δ 5.22, which could be adjacent to an additional methylene group present as ABX between δ 2.95-2.60. A 1H triplet at δ 5.17 of an olefinic proton linked to a methylene group was observed as dd at δ 3.20. Furthermore a 3H singlet at δ 3.78 of a methoxy group was observed. Finally, it showed two methyl singlets at δ 1.61 and 1.57, which could be attached to an olefinic carbon. The partial structures derived thereof are given below (Figure 31).

The search in AntiBase [115] with these data and comparison with reported literature values [163] gave a hit for isoxanthohumol (**105**). Comparison of the ^{1}H NMR spectrum with authentic spectra from our collection confirmed this assignment.

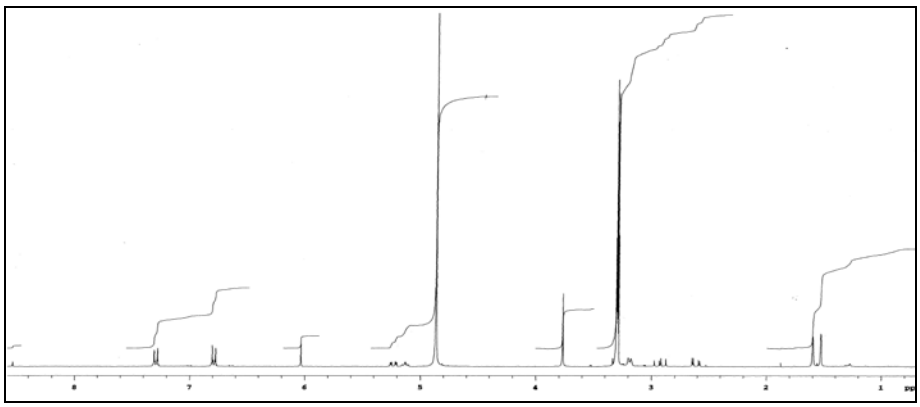


Figure 30: ^{1}H NMR spectrum (300 MHz, CD_3OD) of isoxanthohumol (**105**)

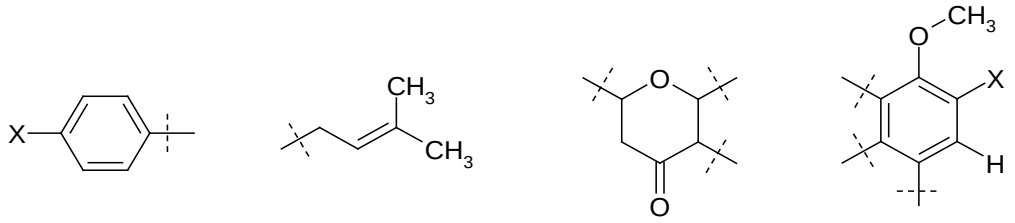
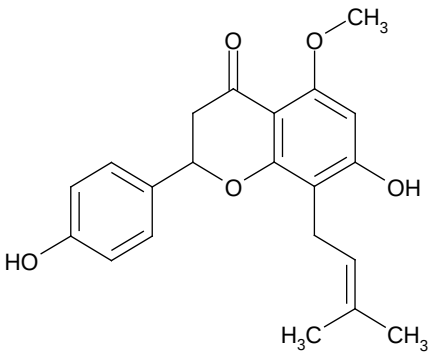
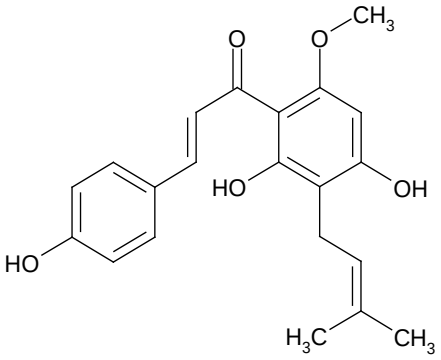


Figure 31: Partial structures of isoxanthohumol (**105**)

Isoxanthohumol (**105**) is an isomer of the prenylated flavonoid xanthohumol (**106**), which is the most important prenylated chalcone of hop (*Humulus lupulus*). [164] The two isomers were both isolated from the widely cultivated plant *Humulus lupulus L.* (Cannabianacae), and from the roots of *Sophora flavescens.* [165] Isoxanthohumol (**105**) was first isolated (and named humulol) and later identified as a flavanone by Verzele et *al.* in 1957. [166]



105



106

Xanthohumol (**106**) is converted to isoxanthohumol by thermal treatment and increased pH value during the brewing process. [167] Xanthohumol (**106**) has been shown to have antiproliferative and cytotoxic effects in human cancer cell lines. [168] It has also been displayed to inhibit diacylglycerol acetyltransferase (DGAT). [169]

Isoxanthohumol (**105**) has received much attention as a proestrogen, [170] and antiviral agent, [171] as antioxidant, [172] and in recent years as a cancer chemopreventive agent. [173,174]

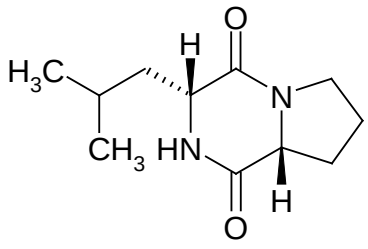
4.2.3 *Cis-C*yclo(Pro,Leu)

Compound **107** was isolated as colourless solid from fraction II on Sephadex LH-20. It showed a UV absorbance and turned to violet by anisaldehyde/sulphuric acid.

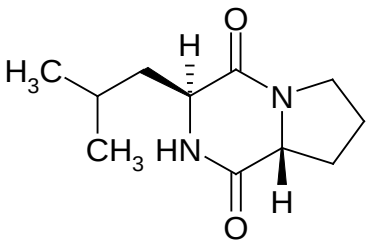
The ^{1}H NMR spectrum showed a broad 1H singlet of an amide NH at δ 5.98, two methine protons at δ 4.13 (t), and 4.02 (dd). Furthermore, a 2H multiplet between 3.64-3.53 (9-H2), a 1H multiplet at δ 2.45-2.28 (10-Ha), and a multiplet between δ 2.24-1.64 of 5H were observed. In addition, a 1H multiplet at δ 1.61-1.45 (7-Hb), and two 3H doublets of two equivalent methyl groups were found at δ 1.0 and 0.96, delivering an isopropyl system.

The ^{1}H NMR spectrum was compared with authentic spectra from our collection. A search in AntiBase [115] resulted in two possible isomers; *cis-cyclo*(Leu,Pro) (**107**) and *trans-cyclo*(Leu,Pro) (**108**). The comparison of the spectral data with reported literature pointed to *cis-cyclo*(Leu,Pro) (**107**). [175]

Cis-cyclo(Leu,Pro) (**107**) is a cyclic dipeptide, produced e.g. by *Rosellinia necatrix*. [169] It has a potent antifungal activity against a wide range of fungal pathogens, including eyespot, scab, powdery mildews, leaf spot disease, club root, dallar spot, and grey mould. [176] *Cis-cyclo*(Leu,Pro) (**107**) isolated from *Streptomyces* KH-614 showed antimicrobial activity against *Candida albicans* IAM 4905, *Mucor ramannianus* IAM6218, *Rhizoctonia solani* IFO 6218, *Aspergilus fumigatus* ATCC 42202, *Glomerella cingulata* IFO 9767, IFO5994, *Trichophton mentagrophytes* ATCC 18749, and *Trichophyton rubrum* ATCC 44766. [195] *Cis-cyclo*(Leu,Pro) (**107**) showed also antifungal activity against *Pyricularia oryzae*, which causes blast disease of rice. [195]



107



108

4.2.4 *Cyclo*(Tyr,Pro)

Compound **109** was isolated as colourless solid from fraction II on Sephadex LH-20. It showed a UV absorbance and turned to violet after spraying with anisaldehyde/sulphuric acid. It showed also a colour staining to blue by chlorine/anisidine reaction, pointing to an additional peptide moiety.

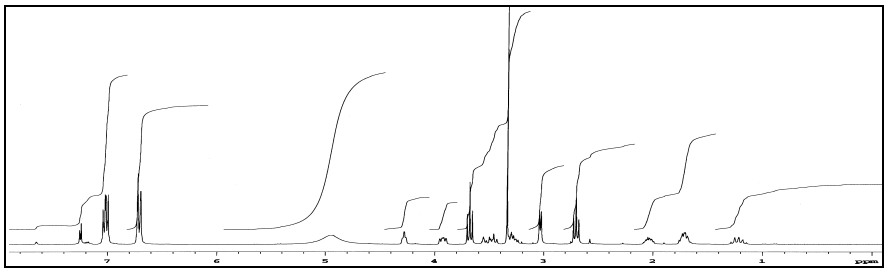
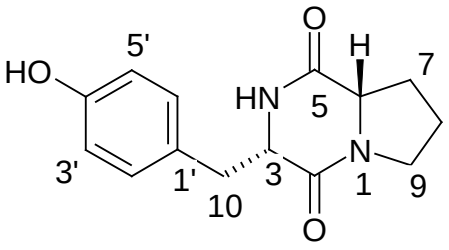


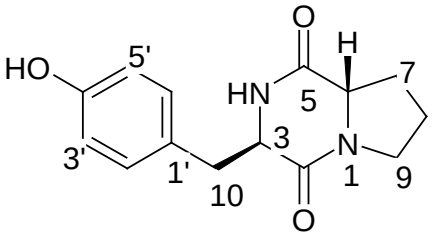
Figure 32: ^{1}H NMR spectrum (300 MHz, CD_3OD) of *cis-cyclo*(Tyr,Pro) (**109**)

The ^{1}H NMR spectrum showed a 1,4-disubstituted aromatic ring due to the presence of AA',BB' signals at δ 7.01 and 6.68. It displayed a broad singlet at 4.97 of an acidic proton (NH), as well as two methines attached to electron withdrawing substituents at δ 4.28 and 3.98. The spectrum revealed doublet signals at δ 3.67 and 2.70 for the ABX system of a methylene group, and a multiplet at δ 3.68-3.30 (9-CH_2) for a methylene group attached to a heteroatom. In addition, multiplets of two further methylene groups at δ 1.2, 1.70 and 2.30 were exhibited.

A search in AntiBase [115] led to the structural isomers *cis-cyclo*(Tyr,Pro) (**109**) and *trans-cyclo*(Tyr,Pro) (**110**). Comparing the chemical shifts of H-3 and CH_2-10 in both isomers (**109**), and (**110**) with the discussed data, confirmed the compound as *cis*-form (**109**).



109



110

The cyclic dipeptides *cyclo*(Tyr,Pro) **109** and **110** have previously been isolated from *Altemaria alternata*. [177] The diketopiperazines are characterised by the presence of two chiral centres at positions 3 and 6 to afford four isomers. They have antimicrobial activity. [178-179]

4.2.5 Phenol

Compound **111** was isolated as colourless solid with the typical smell of phenol. The ^{1}H NMR spectrum showed overlapping signals at δ 6.72 (d) and 6.78 (t) and one triplet at δ 7.18. The ^{13}C NMR spectrum showed two CH signals with double intensity at δ 116.2 and δ 130.4 and a CH at δ 120.5, also a quaternary carbon at δ 158.2.

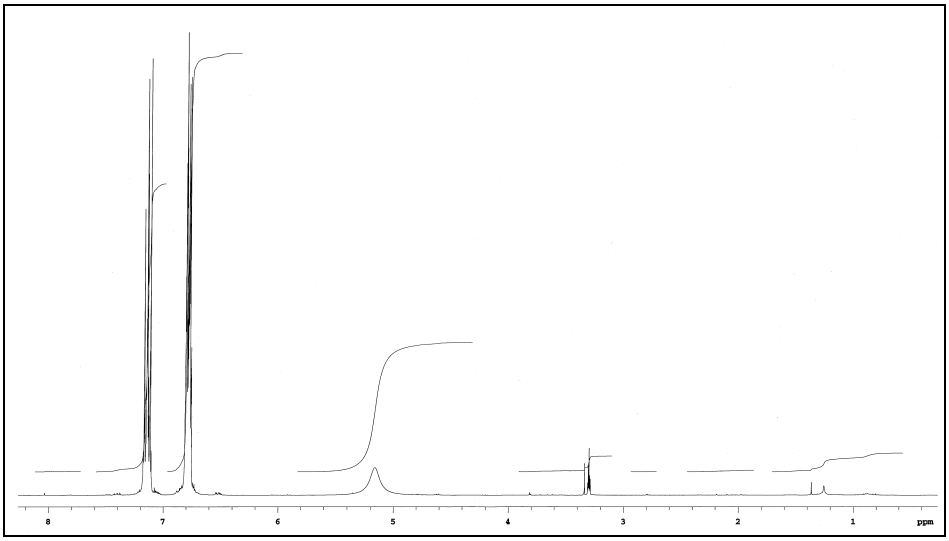


Figure 33: ^{1}H NMR spectrum (300 MHz, CD_3OD) of phenol (**111**)

Table 8: ^{13}C and ^{1}H NMR data of phenol (**111**)

Position	δ_C	**C type**	δ_H **(mult.)**
1	158.2	C_q	–
3,5	130.4	CH	6.78 (t)
4	120.5	CH	6.72 (t)
2,6	116.2	CH	7.18 (d)
-OH	–	–	5.18 (s, br, 1H)

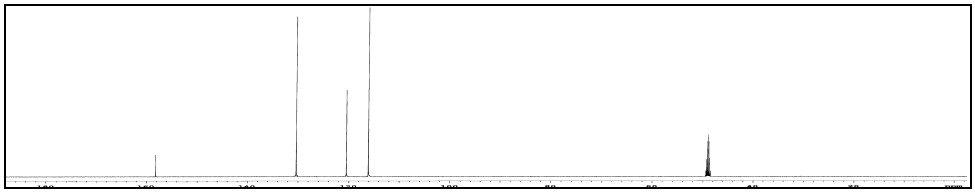
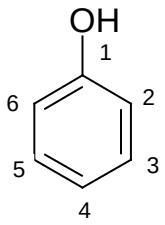


Figure 34: ^{13}C NMR spectrum (125 MHz, CD_3OD) of phenol (**111**)

A search in AntiBase [115] with the NMR data led to phenol (**111**). The typical smell and comparison of this compound with a phenol sample from our lab collection confirmed its identity.

Phenol (**111**) is a versatile precursor for a large collection of drugs, most notably aspirin but also many herbicides and pharmaceuticals. Phenol (**111**) was widely used as an antiseptic, especially as carbolic soap, from the early 1900s through the 1970s and in wounds heavily contaminated with antibiotic-resistant organisms. [180] Phenol (**111**) is also used in the preparation of cosmetics including sunscreen, [181] hair dyes, and skin lightening preparations. Phenol (**111**) and its vapours are corrosive to the eyes, the skin, and the respiratory tract. [182] Repeated or prolonged skin contact with phenol may cause dermatitis, or even second and third-degree burns due to phenol's caustic and defatting properties. [183] Inhalation of phenol vapour may cause lung oedema. [205] The substance may cause harmful effects on the central nervous system and heart, resulting in dysrhythmia, seizures, and coma. [184]



111

4.2.6 Adenine

On TLC, a polar band UV absorbing at 254 nm was detected in fraction III, which stained to blue by spraying with anisaldehyde/sulphuric acid. It was isolated and purified using PTLC followed by Sephadex LH-20 chromatography to afford a colour-

less solid. The ^{1}H NMR spectrum in DMSO-d_6 showed two highly downfield shifted singlets each with intensity of 1H at δ 8.1 and 8.05 and one broad H/D-exchangeable singlet at 7.05 of 2H. A search in AntiBase [115] with the spectral data and a comparison with authentic spectra from our collection pointed to adenine (**112**).

Adenine (**112**) named also angustmycin B is widespread throughout in animal and plant tissues. It was isolated first from the *Actinomycete* sp. 6 A-704 by Yüntsen et *al*. [185] as an antibiotic with *in vitro* activity against *Mycobacterium tuberculosis* 607 and *Mycobacteria phlei*. Additionally, it showed antiviral activity and pharmaceutical useful properties to extend the storage time of whole blood preparates. [186] Adenine (**112**) is a purine component of DNA, RNA, of coenzymes and of biosynthetic intermediates.

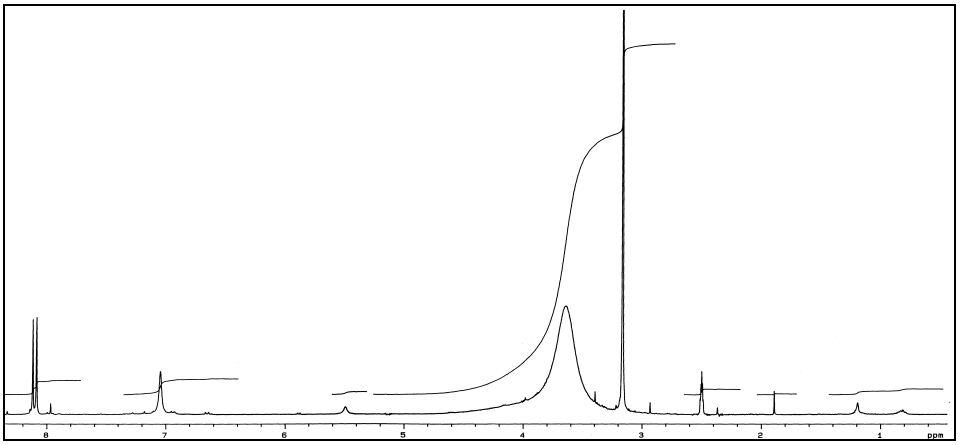
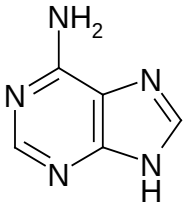


Figure 35: ^{1}H-NMR-spectrum (DMSO-d_6, 300 MHz) of adenine (**112**)



112

4.3 Ruminal bacterium *Gemella morbillorum* ZIK

The ruminal bacterium ZIK was cultivated on LB medium for 24 hours at 37 °C. Pieces of well grown agar plates were used to inoculate 20 of 1L Erlenmeyer flasks, each containing 250 ml of LB medium.

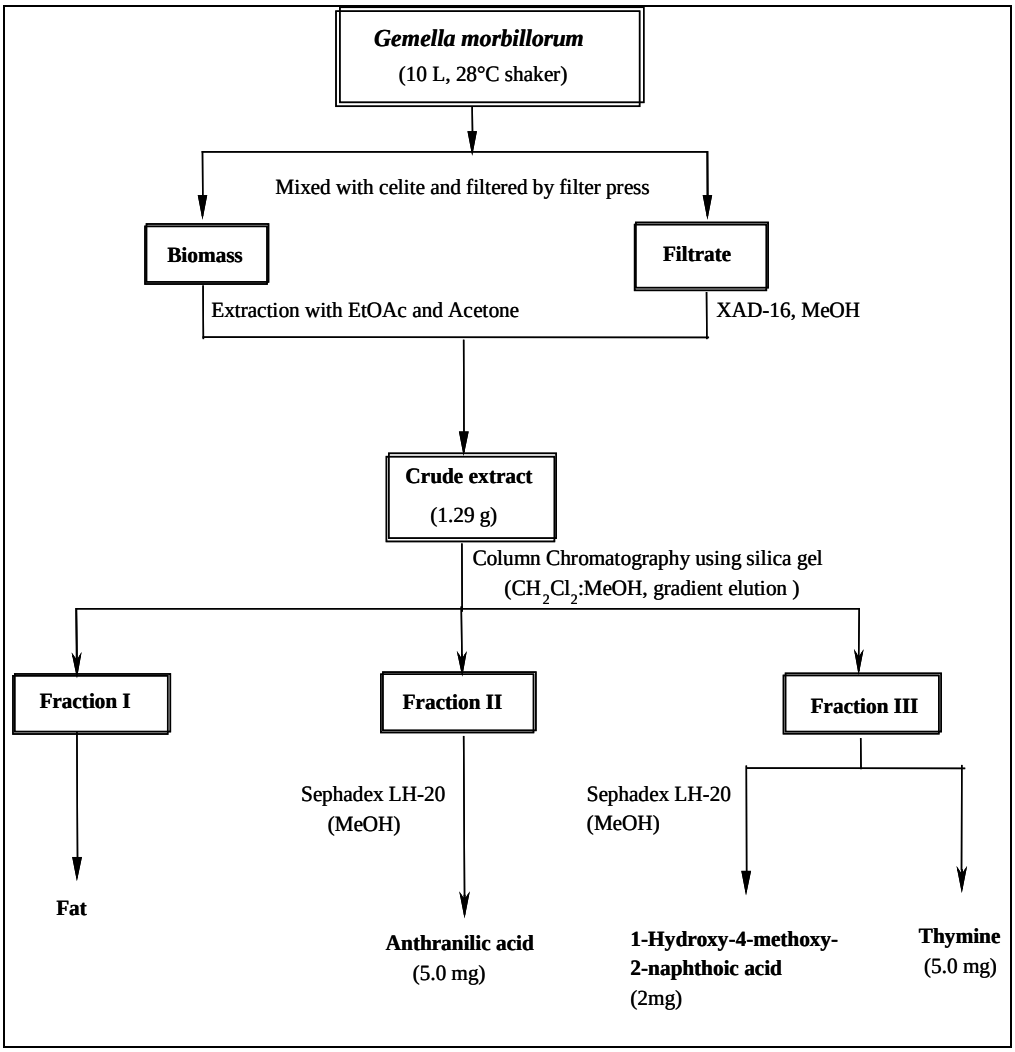


Figure 36: Work-up scheme of the ruminal *Gemella morbillorum*

The fermentation was carried out at 180 rpm on a rotary shaker for 3 days at 37 °C. The culture broth was harvested and filtered to separate the biomass, which was extracted with ethyl acetate (3 times) and acetone (2 times), respectively. The culture filtrate was then passed through Amberlite XAD-16. The column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluates were concentrated under reduced pressure and finally extraction of the residue was done with

ethyl acetate. The organic phases were dried. Both crude extracts were mixed prior to TLC yielding 1.29 g of a crude extract. Chromatography of the crude extract on a silica gel column using a CH_2Cl_2-MeOH gradient with successively increasing polarity resulted in three fractions I-III according to monitoring by TLC.

4.3.1 Anthranilic acid

Compound **113** was isolated as pale yellow solid from fraction II using Sephadex LH-20 MeOH. It showed a blue spot under UV 254 nm and an intensive blue UV fluorescence at 366 nm and turned to yellow after spray with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum revealed four protons in the aromatic region, two doublets at δ 6.65 and δ 7.95 and two triplets of doublets at δ 6.68 and δ 7.37 forming a 1,2-disubstituted aromatic system. Based on NMR and MS data and a search in Anti-Base, [115] compound **113** was elucidated as anthranilic acid.

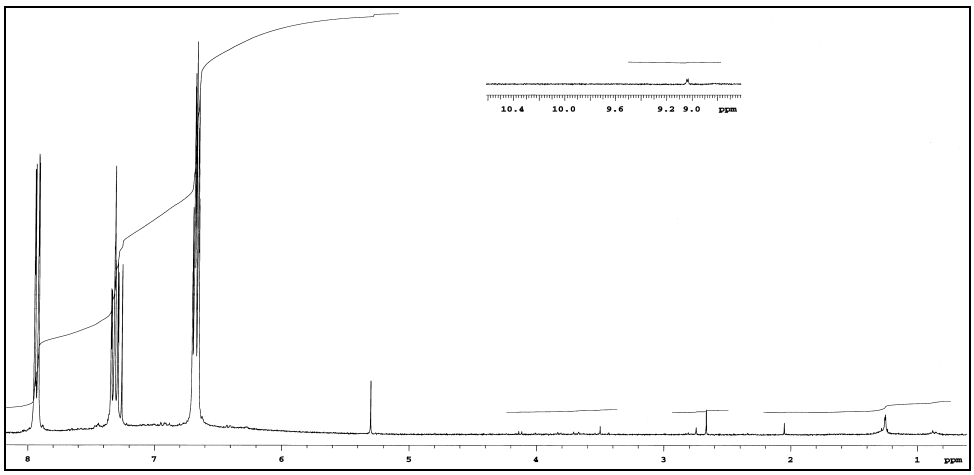
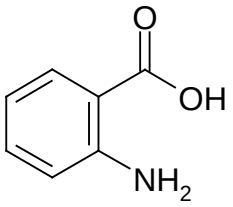
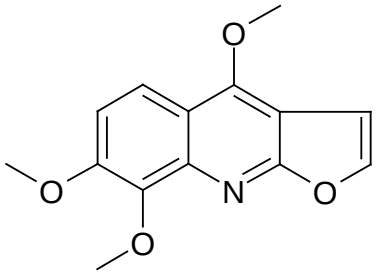


Figure 37: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of anthranilic acid (**113**)

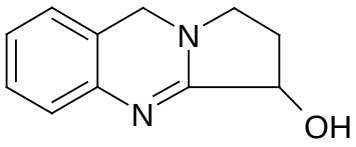
Anthranilic acid (**113**) is widely abundant in bacteria and fungi. It is a biosynthetic key intermediate for several alkaloids having the quinoline, acridine, quinazoline skeletons. [187,188] Examples are skimmiamine (**114** [210,189] or peganine (**115**) which are frequently present in the *Rutaceae* family and of the phenazines, which formally can be divided into two molecules of anthranilic acid. [190]



113



114



115

4.3.2 1-Hydroxy-4-methoxy-2-naphthoic acid

Compound **116** was isolated from fraction III as colourless solid. On TLC, it gave an intense blue UV fluorescence; the zone turned light brown with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of compound **116** showed a typical pattern of four *ortho*-coupled protons (two doublets at δ 8.20 and δ 8.05 and two overlapping triplets at δ 7.43) indicating a 1,2-disubstitued aromatic system. A 1H singlet (7.35) indicated a further penta-substituted aromatic ring. Finally, the singlet of an sp^2-bound methoxy group was located at δ 3.85.

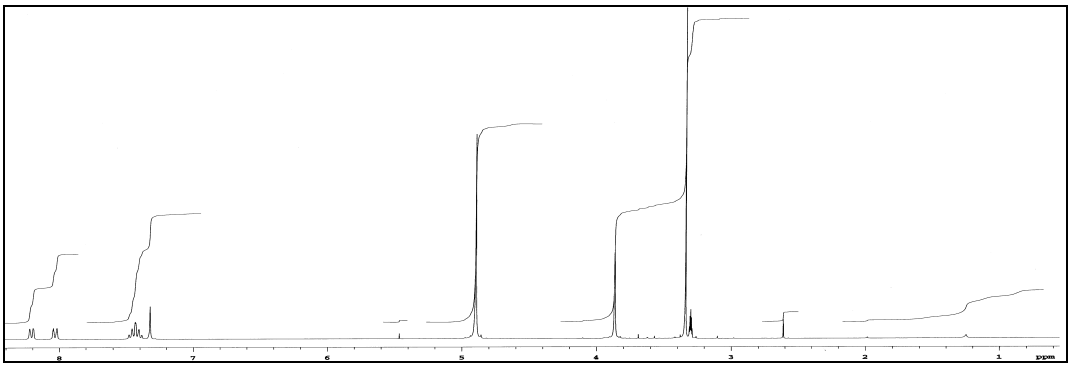


Figure 38: ^{1}H NMR spectrum (300 MHz, CD_3OD) of 1-hydroxy-4-methoxy-2-naphthoic acid (**116**)

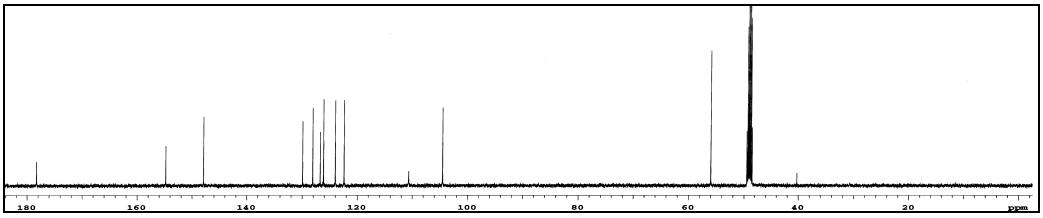
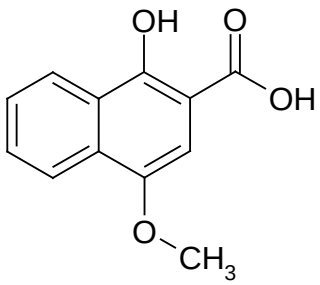


Figure 39: ^{13}C NMR spectrum (125 MHz, CD_3OD) of 1-hydroxy-4-methoxy-2-naphthoic acid (**116**)

The molecular weight of compound **116** was determined as 218.0 Dalton by EI-MS. HREI-MS deduced the molecular formula $C_{12}H_{10}O_4$.



116

Based on the spectroscopic data and a search in AntiBase, [115] this compound was determined as 1-hydroxy-4-methoxy-2-naphthoic acid (**116**) and confirmed by comparison with authentic spectra from our collection and with the literature. [191]

Table 9: ^{1}H and ^{13}C NMR data of 1-hydroxy-4-methoxy-2-naphthoic acid (**116**) in comparison with literature values [214] in CD_3OD

Position	δ_H	δ_H Literature [214]	δ_C	δ_C Literature [214]
1	–	–	155.1	155.3
2	–	–	110.2	125.8*
3	7.35 (s)	7.19 (s)	104.3	101.8
4	–	–	148.0	147.6
4a	–	–	130.0	129.8
5	8.05	8.18 (1H, d)	122.2	121.8
6	7.35	7.62 (1H,ddd)	128.0	128.4
7	7.43	7.56 (1H, ddd)	126.4	126.1
8	8.20	8.33 (1H, d)	124.0	123.4
8a	–	–	126.0	125.8
9	–	–	178.2	173.9
10	3.85	3.96 (3H, s)	56.0	55.1

*Wrong value reported in literature

4.4 Ruminal bacterium *Enterobacter amnigenus* ZIH

The bacterium ZIH was cultivated in small scale. The first 5 l culture of *Enterobacter amnigenus* was grown in 1 l Erlenmeyer flasks filled with 300 ml of LB-medium (peptone 8 g, yeast extract 5 g, NaCl 5 g per liter distilled water, adjusted to pH 7.8) on a linear shaker with 95 rpm at 37 °C. After three days of fermentation, the culture broth was used to inoculate a Braun Biostat U fermenter, filled with 20 l of LB medium. The following conditions were maintained: stirring at 200 rpm, 37 °C, pH 6.5 ± 1.5, aeration 1.5 m^3/h. The culture broth was harvested after 5 days.

The biomass was filtered off by means of a pressure filter and extracted with ethyl acetate and acetone. On basis of their similar chromatograms, both extracts were combined, yielding 3.3 g of crude extract, which was chromatographed by silica gel column chromatography (CC; CH_2Cl_2/MeOH, stepwise gradient) under TLC monitoring to afford four fractions I-IV.

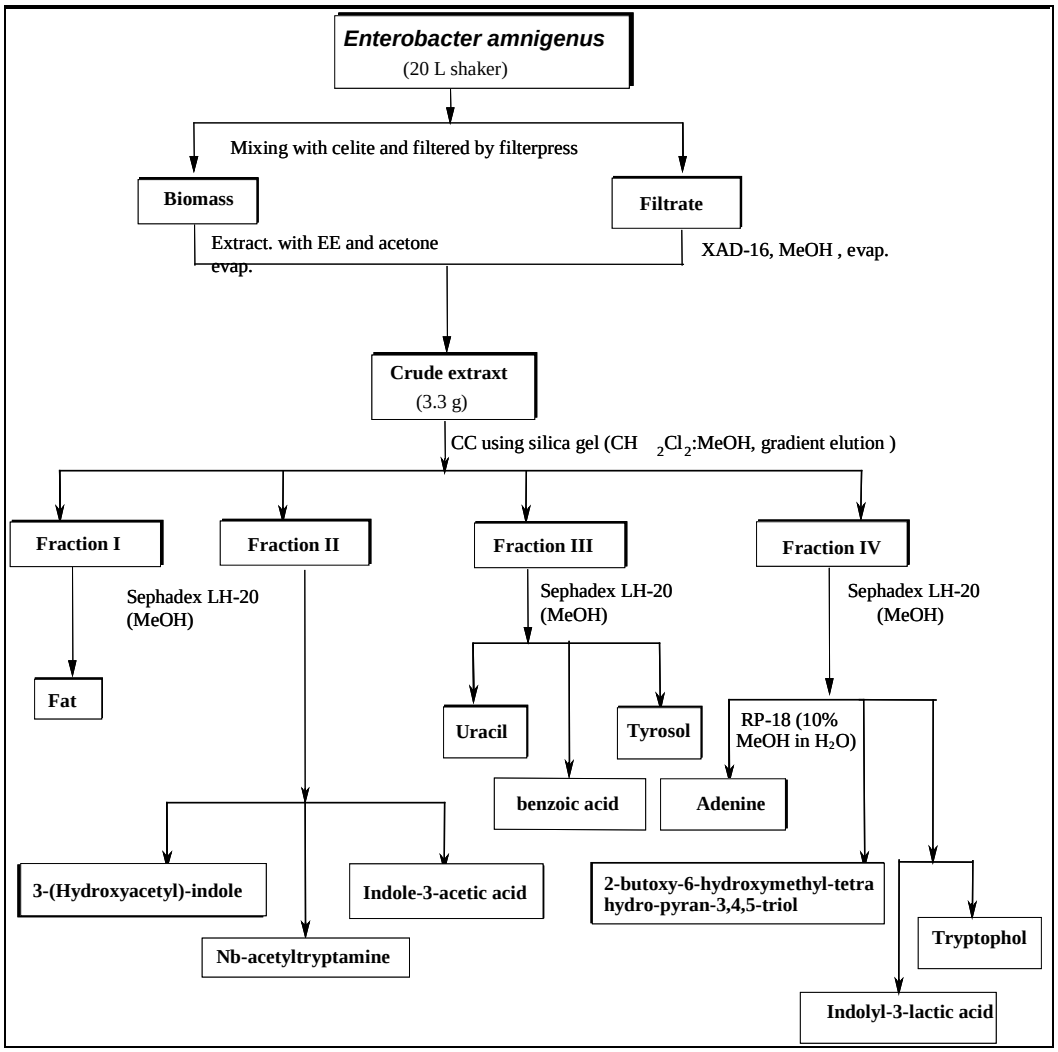


Figure 40: Work-up scheme for the ruminal *Enterobacter amnigenus*

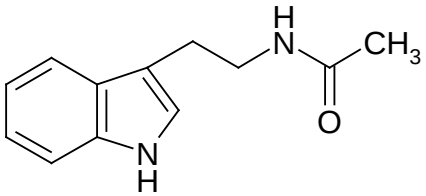
4.4.1 $N_{ß}$-Acetyltryptamine

Compound **117** was isolated as colourless solid from fraction II; it showed a UV absorbing band, which turned to orange/violet by anisaldehyde/sulphuric acid and pink by Ehrlich's reagent, as indication of an indole derivative.

The ^{1}H NMR spectrum displayed a broad NH singlet at δ 8.30, five signals of aromatic protons as well as three signals in the aliphatic region. In the aromatic region, it showed two doublets each with intensity of 1H at δ 7.60 and 7.38, two overlapped triplets at δ 7.26-7.08 forming an 1,2-disubstituted benzene ring, as well as a doublet at δ 7.03 of a proton with a *m*-coupling or next to an amino group. In the aliphatic region, a broad singlet at δ 5.70 of an NH moiety was observed, a quartet at δ 3.60 for methylene protons, which showed a triplet after H/D exchange, confirming that it

is adjacent to an amino group. Furthermore, a triplet at δ 2.98 of methylene protons indicated an ethandiyl group, while a singlet of a methyl group typical for an acetyl group was observed at δ 1.93. A search in AntiBase [115] led to N_{β}-acetyltryptamine (**117**), which was further confirmed by comparison with an authentic spectrum and the literature. [192]

$N_{ß}$-Acetyltryptamine (**117**) is known as secondary metabolite from different species of plants [193] e.g. leaves of *Prosopis nigra* (*Liguminosae*), [116] as well as from bacteria e.g myxobacterium *Archangium gephyra* strain Ar T205; it is known for its antifungal properties. [162]



117

4.4.2 Tyrosol

Fraction III yielded on Sephadex-LH 20 compound **118**, which was isolated as colourless oil. It was absorbing at 254 nm, and turned to a red colour when sprayed with anisaldehyde/sulphuric acid.

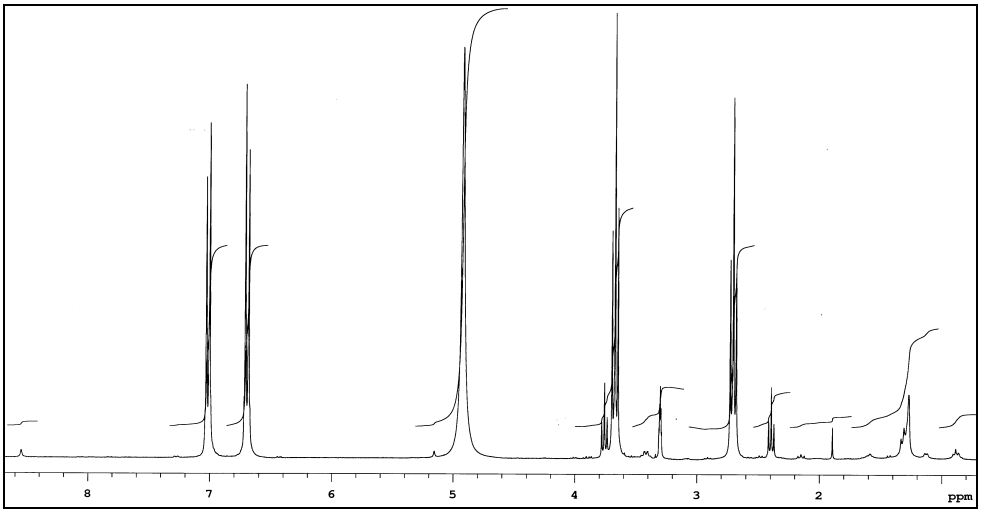
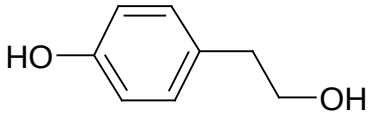


Figure 41: ^{1}H NMR spectrum (300 MHz, CD_3OD) of tyrosol (**118**)

The ^{1}H NMR spectrum of compound **118** displayed a pair of doublets at δ 7.09 and 6.74 integrating for two protons each and corresponding to a A_2B_2 system with the same coupling constant, characteristic of a 1,4-disubstituted aromatic ring. The up-field shift of these signals is the evidence of electron donating groups on the aromatic ring. At δ3.65, a triplet appeared integrating for two protons and corresponding to the two geminal protons of a hydroxymethylen group present in the molecule. At δ2.72, another triplet appeared which integrated for two protons that corresponded to a methylene linked to the aromatic ring.



118

A search in AntiBase [115] using the above spectroscopic data and comparison of the ^{1}H NMR spectroscopic data with those in the literature [194-194] resulted in tyrosol (**118**), which has been further confirmed by comparing with authentic spectra as well as literature data. [195,196]

Tyrosol (**118**) is a compound of pharmacological interest showing antioxidating activity. It has been used in atherosclerosis treatment, protecting low-density lipoproteins (LDL) from oxidation, which played an important role in the initiation and progress of cardiovascular disease. [197-198] Tyrosol (**118**) is present in a variety of natural sources. The principal source in the human diet is olive oil. In olive oil, tyrosol forms esters with fatty acids. [199] As an antioxidant, tyrosol can protect cells against injury due to oxidation. [200] Tyrosol (**118**) was also reported to be antibiotically weakly active against *Saccharomyces cerevisiae* (>100), *Nematospora corlyi* (> 100) as well as to be moderately phytotoxic and antifungal. [201] Compound **118** is widespread in fungi e.g. *Candida* sp., [226] *Ceratocystis fimbriata*, *Cochliobolus lunata*, *G*. fuikuroi, *Pyricularia* oryzae [202] as well as several *Ceratocystis* sp. isolated from leaves of *Ligustrum ovalifolium* and the bark of *Fraxinus excelsior*, or *Gibberella fuikuroi* and peanuts. [203]

4.4.3 Phenol

From the same fraction III compound **111** was isolated as colourless solid and gave pink colour after spray by anisaldehyde /sulphuric acid. It is UV absorbing at 254 nm. Compound **111** was isolated before from the ruminal ZIG and described in paragraph 4.2.5 in CD_3OD.

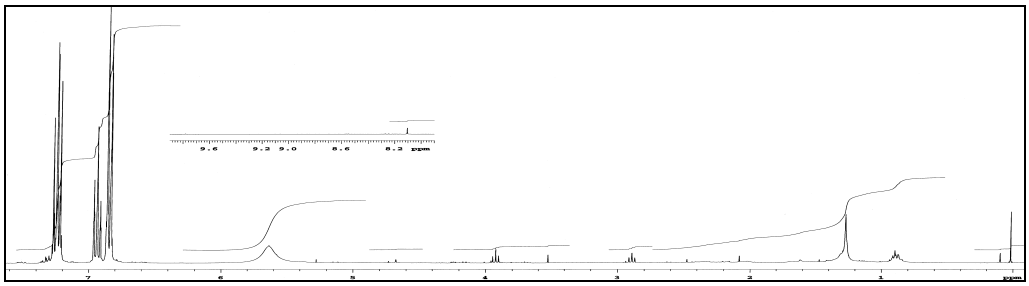


Figure 42: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of phenol (**111**)

The ^{1}H NMR spectrum of **111**, in $CDCl_3$, displayed three resonances in the aromatic region at δ 7.28 (2H, d, J = 8.5 Hz, H-2, 6), at δ 6.87 (1H, t, J = 8.5 Hz, H-4), at δ 6.82 (2H, t, J = 8.5 Hz, H-3, 5). It also displayed an exchangeable proton for an OH group at δ 5.62 (1H, br, s). On the basis of the above data as well as by comparison with the literature, it was deduced that compound **111** was phenol.

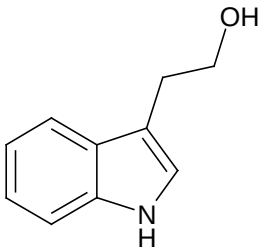
4.4.4 Tryptophol

Compound **119** was isolated as brown solid from fraction IV, by Sephadex LH-20 (MeOH). It showed a UV absorbing band at 254 nm, and was stained to violet by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **119** showed a 1H signal for an exchangeable proton at δ = 8.05 (s, br, 1 H, NH). In the aromatic region, it showed also a 1,2-disubstituted aromatic ring due to the presence of four 1H signals at δ 7.63 (d, 1 H, 4-H), 7.39 (d, 1 H, 7-H), 7.21 (dd, 1 H, 5-H), 7.19 (dd, 1 H, 6-H), and one singlet at 7.10 (s, 1 H, 2-H); the OH signal was not invisible.

In the aliphatic region, it showed two 2H triplets at δ 3.93 (t, ^3J = 6.6 Hz, 2 H, 9-CH_2) and 3.05 (t, ^3J = 6.6 Hz, 2 H, 8-CH_2): According to the shifts, a 1,2-disubstituted ethylene group could be attached at one side to an sp^2 carbon and at the other side to a hetero atom (O or N).

EI-MS showed a molecular ion peak at *m/z* 161 followed by a base peak at *m/z* 130 due to the indolyl-3-methylene ion. A search in AntiBase [115] led to the identification of compound **119** as tryptophol, which was further confirmed by comparison of the sample with the data of authentic NMR spectra from our collection and in the literature.

Tryptophol (**119**) is a very common metabolite of plants and fungi. [204] Tryptophol was firstly obtained by Ehrlich from fermentation of amino acids by yeast. [205] It was also isolated and easily identified from this strain. It is reported to inhibit the growth of Gram-positive bacteria and *Candida albicans*. [206]



119

4.4.5 Brevinic acid

Compound **120** was isolated from fraction III as a purple pigment. It showed UV absorbance under 254 nm. The ^{1}H NMR spectrum showed in the aromatic region signals at δ 7.61, 7.64, 7.91 and 7.94. In the aliphatic region it displayed signals at δ 2.10, 2.50, 3.09, 3.68 and 5.22.

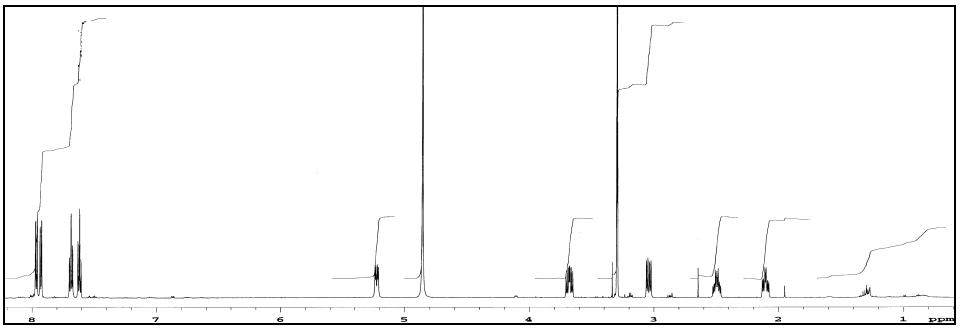


Figure 43: ^{1}H NMR spectrum (300 MHz, CD_3OD) of brevinic acid (**120**)

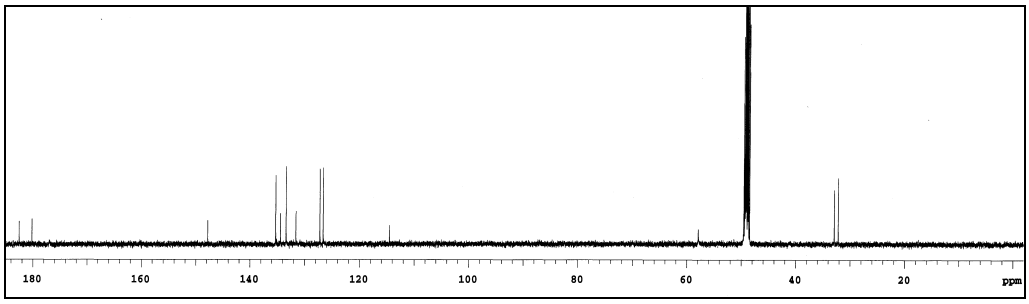
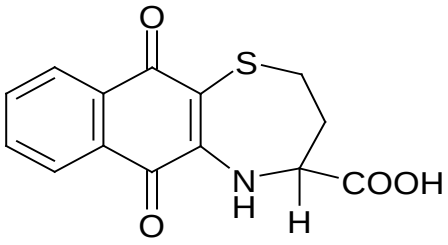


Figure 44: ^{13}C NMR spectrum (125 MHz, CD_3OD) of brevinic acid (**120**)

Table 10: ^{13}C and ^{1}H NMR data of brevinic acid (**120**)

Position	δ_C	**C type**	δ_H **(mult.)**
1	126.6	CH	δ 7.94 (d, 1H)
2	135.6	CH	7.61 (t, 1H)
3	133.4	CH	7.64 (t, 1H)
4	128.2	CH	7.91 (d, 1H)
4a	131.6	C_q	–
5	182.5	C_q	–
5a	114.5	C_q	–
6	–	–	–
7a	32.0	CH_2	3.09 (dd, J = 16 Hz, 6 Hz, 1H)
7b	32.0	CH_2	3.68 (dq, J = 16 Hz, 6 Hz, 1H)
8a	32.5	CH_2	2.10 (m, 1H)
8b	32.5	CH_2	2.50 (m, 1H)
9	58.0	CH	5.22 (dd, 1H)
10	–	–	–
10 a	147.9	C_q	–
11	180.2	C_q	–
11 a	134.2	C_q	–



120

A search in AntiBase [115] using the above spectroscopic data and comparison of the NMR spectroscopic data with those reported in the literature resulted in brevinic acid (**120**).

4.4.6 Indole-3-lactic acid

Compound **121** was isolated as colourless oil from fraction IV. It exhibited UV absorption at 254 nm and turned red by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **121** revealed five aromatic protons, which were indicative for a 3-substituted indole moiety. The spectrum showed two doublets of doublets at δ 7.65 and 7.37 and two triplets of doublets at δ 7.05 and 6.95, respectively, of a 1,2-disubstituted benzene ring. In addition there was a singlet at δ 7.11. In the aliphatic region, the signal of an oxygenated methine group at δ 4.21 and signals of diastereotopic methylene protons at δ 2.90 and 2.95 were visible, which indicated the neighbourhood of a stereogenic centre and possibly of an sp^2-carbon or a hetero atom.

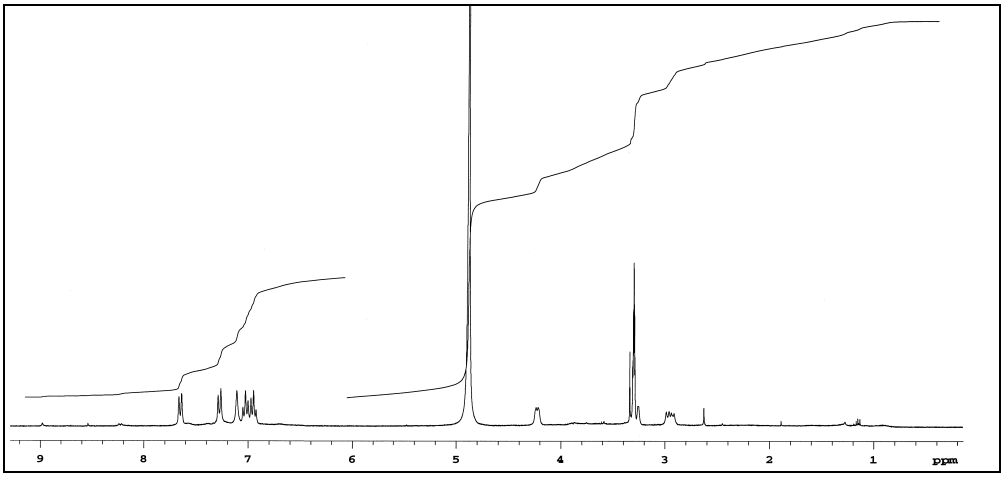
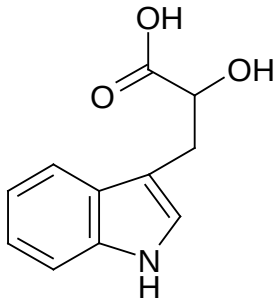


Figure 45: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of indole-3-lactic acid (**121**)

The ESI mass spectrum gave a *pseudo*molecular ion peak at *m/z* 228 $[M+Na]^+$, which fixed the molecular weight to 205 Dalton. Based on the above spectroscopic data, the isolated compound was identified as indole-3-lactic acid (**121**), which was further confirmed by comparing the data with the authentic spectra.

Compound **121** was frequently found in bacteria, fungi and yeasts and exhibited activity against *Candida albicans*. It is a naturally occurring indole derivative, preferably detected in soil bacteria and fungi and only in low amounts in plants. [207] It is a weak auxin analogue. [233] Indole-3-1actic acid is formed from tryptophane via tryptamine. [208]



121

4.4.7 Butyl glycoside

Compound **122** was isolated from fraction IV as colourless polar compound after purification by using Sephadex LH-20 and RP-18 chromatography columns. It was not UV absorbing and turned to green after spraying with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum displayed at δ 4.75 (d, J = 1.5 Hz) a doublet of an anomeric proton (which suggested the presence of a sugar moiety), a multiplet of four oxygenated methines between δ 3.76-3.50 and two oxygenated methylene groups at δ 3.40, 3.72 and 3.68, 3.80. In addition, it showed two multiplets of two methylenes at δ 1.40 and 1.60 and one methyl group at 0.93.

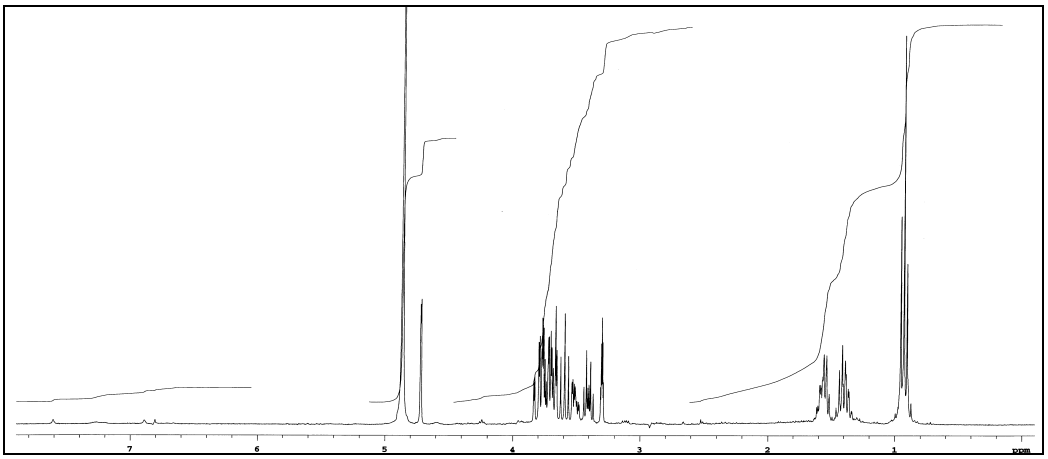


Figure 46: ^{1}H NMR spectrum (300 MHz, CD_3OD) of butyl glycoside (**122**)

The ^{13}C NMR spectrum revealed ten carbon atoms, seven of them were oxygenated. The resonance at δ_C 101.5 was again characteristic for the acetal carbon of a sugar skeleton. The other oxygenated carbon signals were observed at δ_C 74.6 (CH-2'), 72.7 (CH-3'), 72.3 (CH-4'), 68.6 (CH-5'), 68.3 (CH_2-1), and 62.9 (CH_2-6'), respectively. Additionally, three signals at δ_C 32.7, 20.5 and 14.2 were assigned as two methylenes and one methyl of an aliphatic chain.

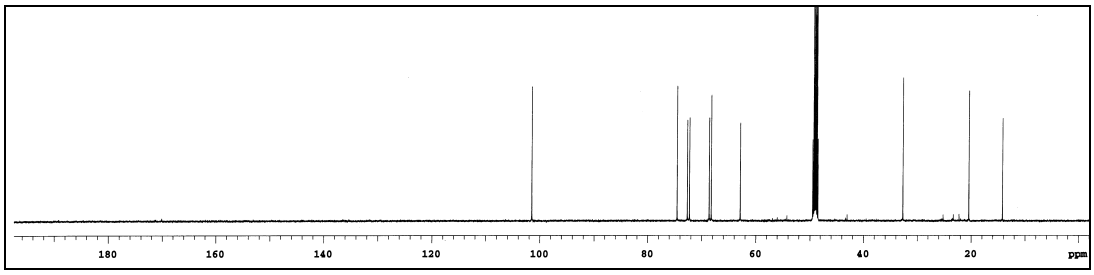
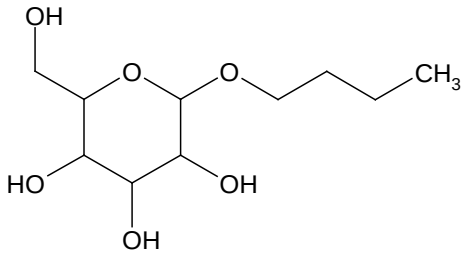


Figure 47: ^{13}C NMR spectrum (125 MHz, CD_3OD) of butyl glycoside (**122**)



122

Table 11: ^{1}H and ^{13}C NMR data, HMBC and COSY correlations of butyl glycoside (**122**) in CD_3OD

Position	δ_C	C	δ_H (mult.; *J* in Hz)	COSY	HMBC
1	68.3	CH_2	3.40	2	1'
		CH_2	3.72	2	1'
2	32.7	CH_2	1.60 (m, 2H)	3	–
3	20.5	CH_2	1.40 (m, 2H)	2, 4	1, 2, 4
4	14.2	CH_3	0.91 (t, ^{3}J = 7.3 Hz, 3H)	3	2, 3
1'	101.5	CH	4.75 (d, ^{3}J = 1.5 Hz, 1H)	2'	1, 2', 3'
2'	74.6	CH	3.50 (m)	3'	–
3'	72.7	CH	3.76 (m)	4'	–
4'	72.3	CH	3.76 (m)	5'	–
5'	68.6	CH	3.60 (m)	6'	3', 4'
6'	62.9	CH_2	3.68 (m)	5'	5'

The oxymethylene group at δ 3.40/3.72 (δ_C 68.3) coupled with the anomeric carbon and with the n-propyl residue by COSY and HMBC, respectively. Compound **122** was correspondingly elucidated as a butyl hexopyranoside **122**, in agreement with the (+)-ESI MS data (*m/z* = 259 for $[M+Na]^+$). A search in AntiBase [115] and the Chemical Abstract for compound **122** resulted in no hits. The compound is obviously new; the configuration remains, however, open at present, but can probably solved best by comparison with a synthetic sample.

4.4.8 Other metabolites

From the same strain, uracil (**126**) and adenine (**124**) were isolated. Compound **126** is a pyrimidine base of RNA; it base pairs with adenine and replaces thymine during DNA transcription. Methylation of uracil produces thymine.

Uracil (**126**) helps in the body to carry out the synthesis of many enzymes necessary for cell function through bonding with ribose and phosphate. [209] Uracil (**126**) serves as coenzyme for reactions in the human body and in plants. [209]

Compound **126** is also involved in the biosynthesis of polysaccharides and the transportation of sugars containing aldehyde functions. It can also increase the risk for cancer in cases in which the body is extremely deficient in folate. [210] The deficiency in folate leads to increased ratio of (dUMP)/ (dTMP) and uracil misincorporation into DNA and eventually low production of DNA.[236] Uracil (**126**) can be used to determine microbial contamination of tomatoes. The presence of uracil is an indication of lactic acid bacteria contamination in the fruit. [211]

From this bacterium *Enterobacter amnigenus* ZIH, 3-hydroxyacetyl-indole (**95**) was also isolated described previously in 4.1.5), together with two diketopeparazines, *cyclo*(Phe,Pro) (**97**) and *cyclo*(Pro,Leu) (**107**), which were already described previously in paragraphs 4.1.7 and 4.2.3, respectively.

4.5 Ruminal *Serratia rubidae* ZIE

The strain *Serratia rubidae* ZIE was grown in LB medium at pH 7 in 80 of 1 L Erlenmeyer flasks each containing 250 ml. The culture was cultivated on a shaker at 37 °C.

Figure 48: Culture of *Serratia rubidae*

After 3 days, the pink culture was harvested and was filtered with the aid of the filter press (Figure 4). The water phase was given on an Amberlite XAD-16 column and eluted with methanol.

Figure 49: Water phase adsorbed on XAD-16 column

The mycelium was extracted with ethyl acetate and acetone. The two extracts were brought to dryness under vacuum and finally combined, as the TLC of both extracts were same, resulting 6.2 g of dark pink crude extract.

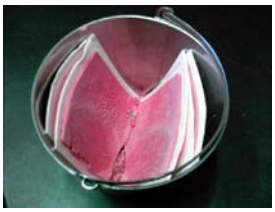
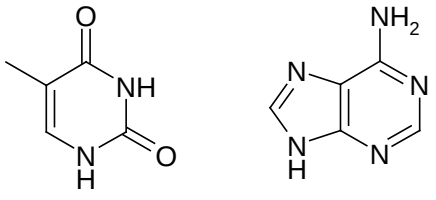
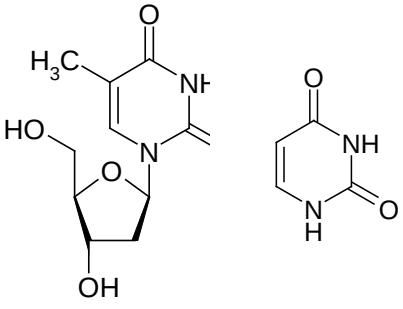


Figure 50: Mycelium obtained after filtration by filter press

From this ruminal bacterium *Serratia rubidae*, eight compounds were isolated, some of which had been previously described in this thesis; they were identified as thymine (**123**)**,** adenine (**124**)**,** thymidine (**125**), uracil (**126**), prodigiosin (**127**), indole-3-acetic acid (**128**), 1-acetyl-β-carboline (**129**), actinomycin D (**131**) and 1-hydroxy-4-methoxy-2-naphthoic acid (**134**). However, from HPLC-MS of the crude extract, a compound with a molecular weight of *m/z* 1049 was observed. Preliminary ESI-MS/MS studies revealed that the compound was potentially a new cyclopeptide structurally related to surfactin C.



123 **124**



125 **126**

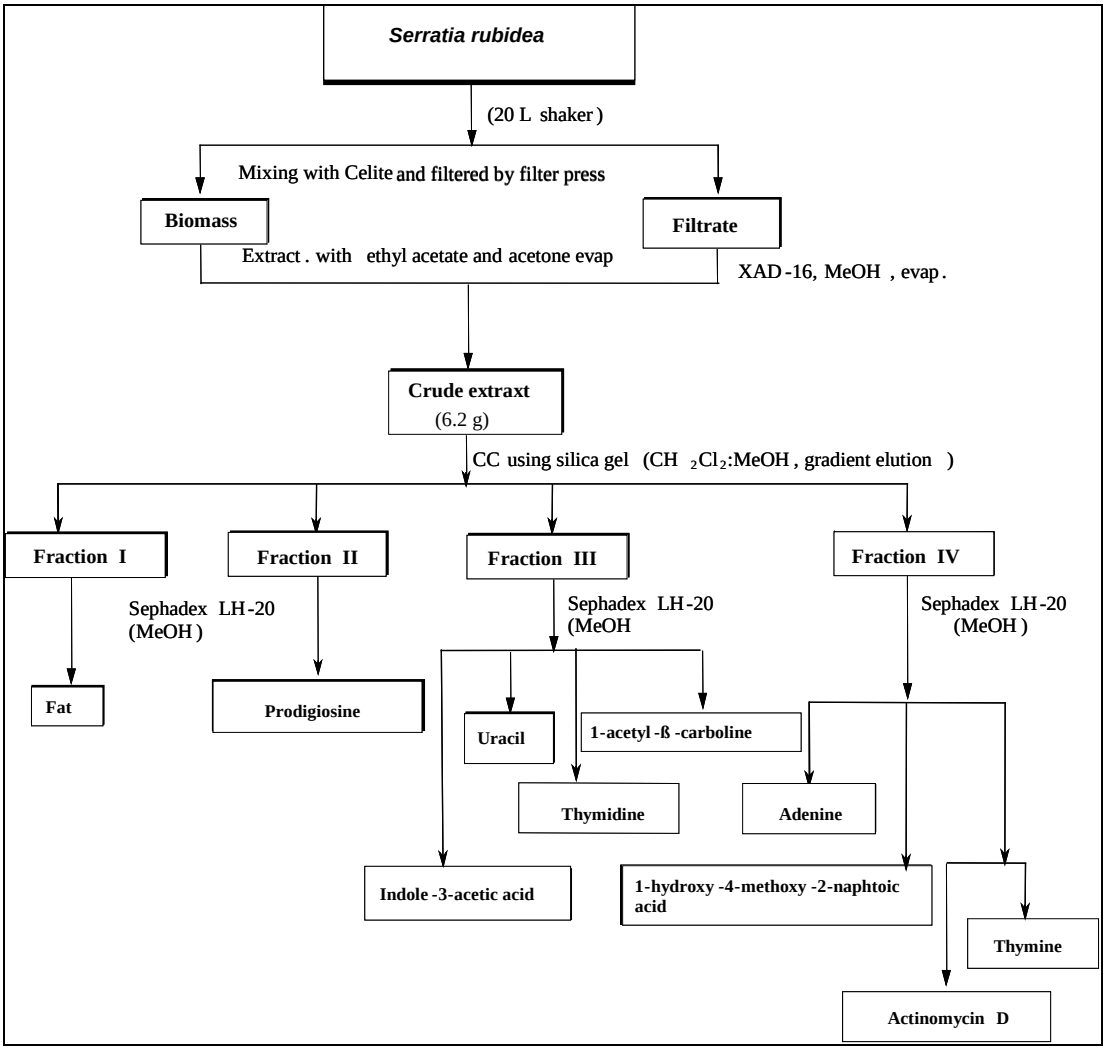


Figure 51: Work-up scheme of the ruminal *Serratia rubidae* ZIE

4.5.1 Surfactin derivative

The crude extract of this strain was submitted to HPLC-ESI-MS, where the presence of peptide homologues with quasimolecular ions at *m/z* values of 1058.8, 1072,9 and 1086.9 were detected.

A compound with molecular weight *m/z* 1049 was observed and further investigated by HR-MS to obtain the molecular formula $C_{54}H_{95}N_7O_{13,}$ and then submitted to HPLC-ESI-MS/MS (Figure 52).

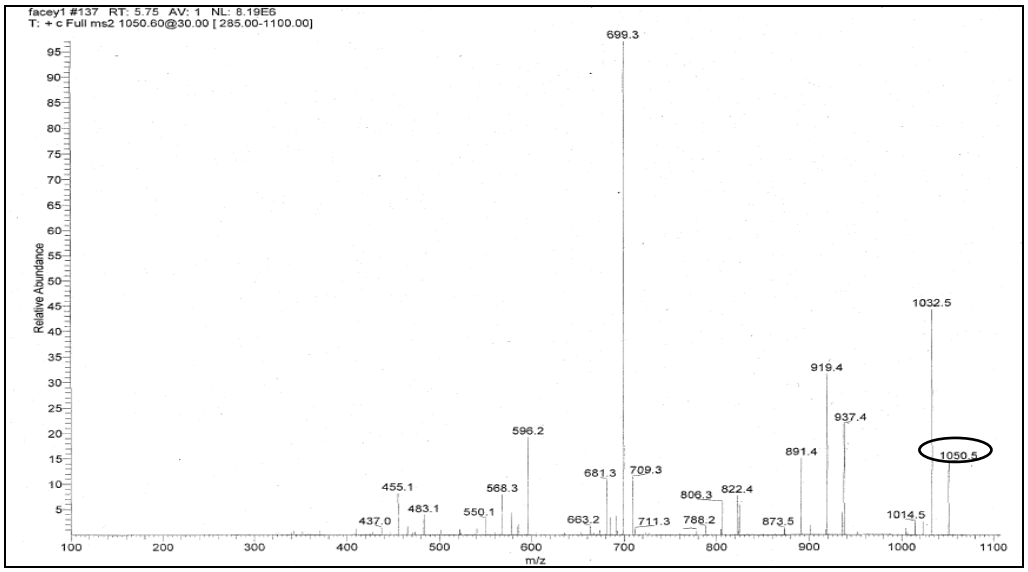


Figure 52: Tandem Mass Spectrometry. HPLC-ESI-MS/MS of the selected peak *m/z* 1050 $[M+H]^+$

ESI-MS/MS is particularly useful in the elucidation of peptides as it induces amide-bond cleavage and hence the amino acid sequence of peptides can be obtained.

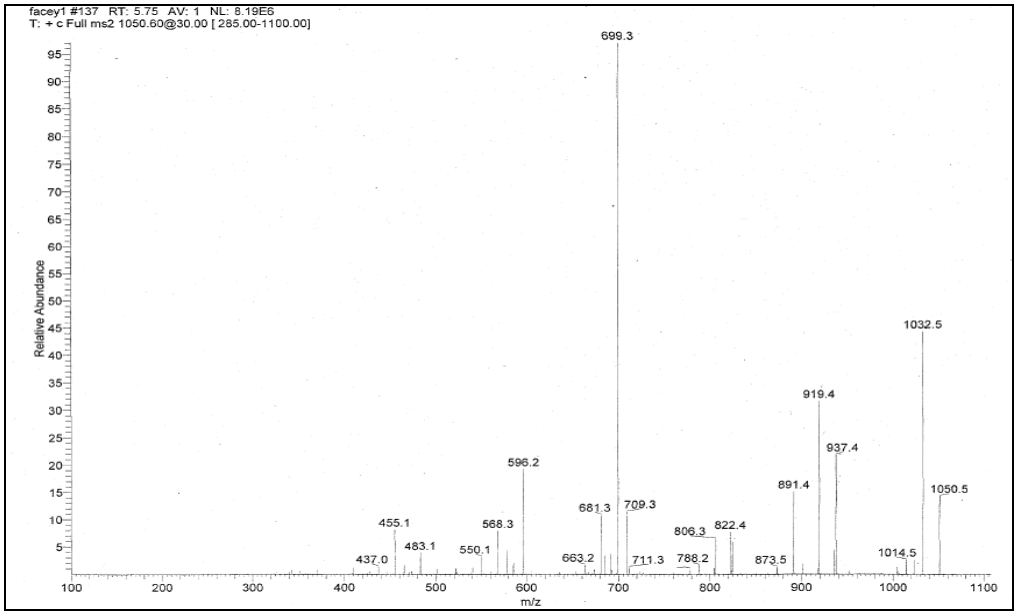


Figure 53: Partial amino acid sequence [Leu-Leu-Asp-Leu-Leu] of the peptide with the molecular weight *m/z* 1049

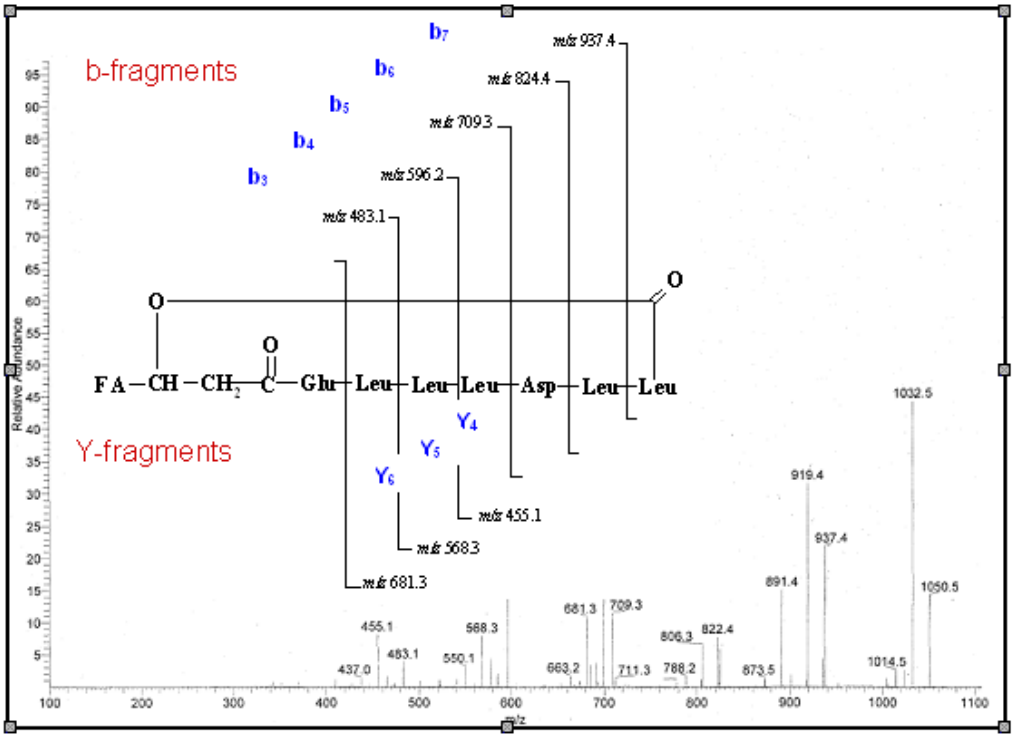


Figure 54: Tandem Mass Spectrometry

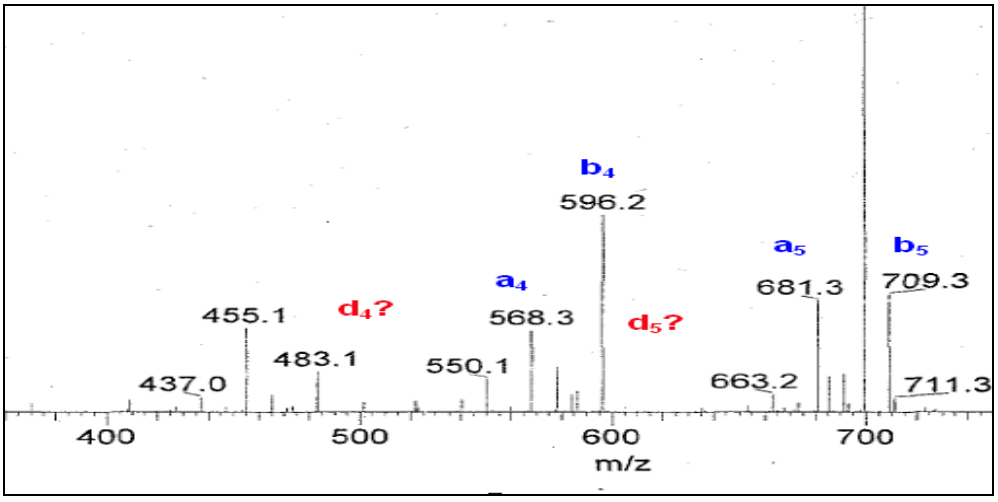


Figure 55: Tandem Mass Spectrometry

Preliminary ESI-MS/MS studies revealed that the compound was a potentially new cyclopeptide structurally related to surfactin C, Figure 54. Further mass spectrometry measurements are needed to confirm the sequence of this peptide.

A number of surfactins have been isolated from *Streptomyces* sp. as well as *Bacillus* spp. Surfactins obtained from *Bacillus subtilis* [212] are most efficient biosurfactants, and exhibit potent antifungal, and antitumor activities against Ehrlich ascites carcinoma cells. They are known to inhibit fibrin clot formation, as well as cyclic adenosine 3',5'-monophosphate phosphodiesterase. [213,214]

4.5.2 Prodigiosin

Fraction II exhibited gradient pink spots on TLC, and was subjected to Sephadex LH-20 MeOH. The resulting sub-fraction was further purified by PTLC (5% MeOH/DCM) and then again by Sephadex LH-20 MeOH.

Figure 56: Fraction II from the crude extract of the ruminal *Serratia rubidae* eluted on Sephadex LH-20 (MeOH)

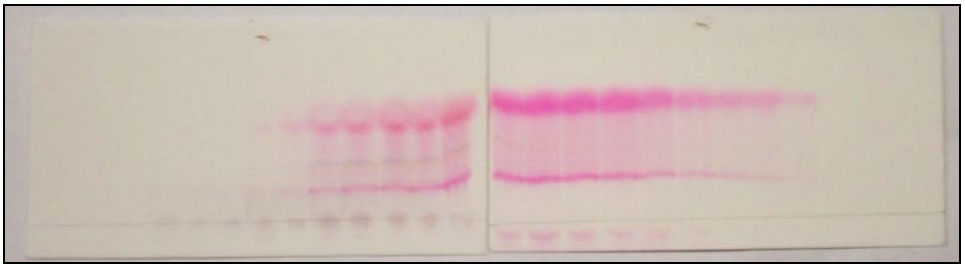
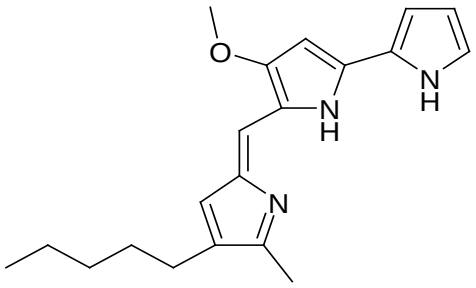


Figure 57: TLC showing prodigosines, which turned to yellow with NaOH

The pink spots on TLC turned to yellow after moistening with sodium hydroxide, as indication of prodigosins; they turned to blue after spraying with anisaldehyde/sulphuric acid. The blue compound (see Figure 56) could not be isolated.

Prodigosines were reported to have antibacterial, [215] antifungal, [241] antiprotozoal, antimalarial, [216] immunosuppressive, [217] and anticancer properties. [218]

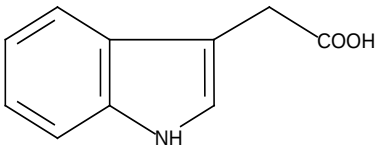


127

4.5.3 Indole-3-acetic acid

Working up of fraction III on Sephadex LH-20 led to compound **128** as a colourless solid. It showed a UV absorbing band, which turned to orange and pink with anisaldehyde/sulphuric acid and Ehrlich's reagent, respectively. The ^{1}H NMR spectrum of compound **128** showed in the aromatic region two 1H doublets at δ 7.53, δ 7.37, two 1H triplets at δ 7.09 and 7.01 for 1,2-disubstituted benzene ring, as well as a singlet at 7.17; this signal pattern was indicative for an indole group. In the aliphatic region a methylene singlet was found at δ 3.94. By searching in AntiBase, [115] compound **128** was identified as indole-3-acetic acid. [219]

Indole-3-acetic acid (**128**) is widespread in human urine, fungi, corn, and can be categorised as an auxin (phytohormon). [220] It is one of the important hormones responsible for the acceleration of growth of higher plants (plant growth regulator). It plays an important role in a number of plant activities, including fruit development, abscission, and root initiation. [221]



128

4.5.4 1-Acetyl-β-carboline

Compound **129** was isolated as pale yellow solid from fraction III. It formed a strongly UV absorbing band with blue fluorescence that gave a yellowish-green colour with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **129** showed a broad NH or OH signal at δ 10.31, two doublets at δ 8.55 and δ 8.17 with the coupling constant of 4.9 Hz, which is smaller than the *ortho* coupling and larger than a *meta* coupling of a benzene system; thus, a heteroaromatic ring was supposed. The doublet at δ 8.16 (1H), two doublets of doublets at δ 7.61 (2H), and a multiplet at δ 7.34 (1H) revealed a 1,2-disubstituted benzene ring. In addition, the spectrum exhibited one singlet at δ 2.90 of an acetyl group. A search in AntiBase [115] resulted in 1-acetyl-β-carbolin (**129**), which was further confirmed by comparison with the authentic spectra as well as literature data. [222]

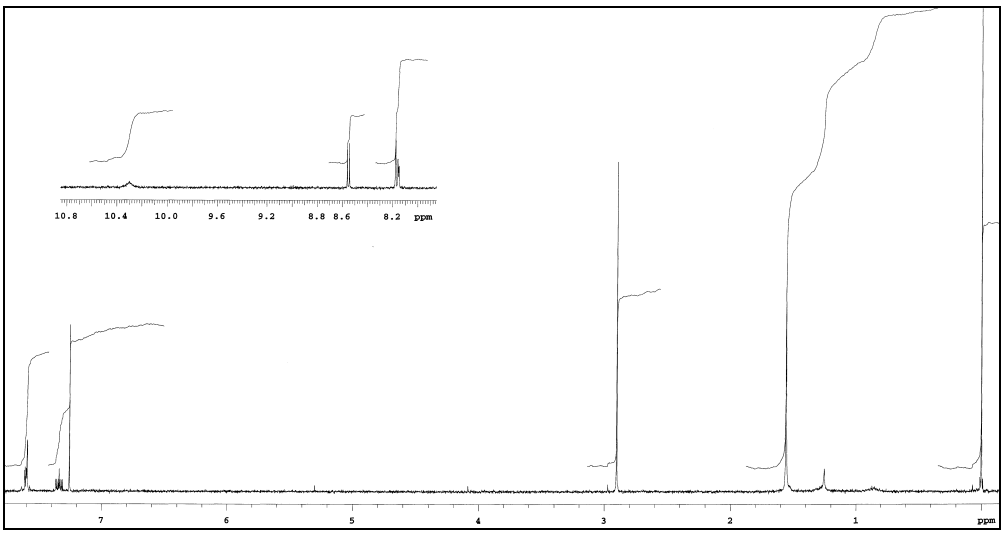
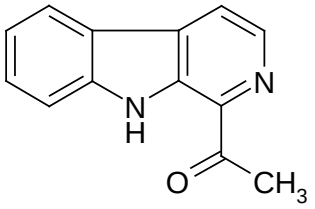


Figure 58: ^{1}H NMR-spectrum ($CDCl_3$, 300 MHz) of 1-acetyl-β-carboline (**129**)



129

β-Carboline alkaloids are metabolites from plants, [223,224] bacteria and fungi, many of them exhibit phamacological activities [225] e.g are enzyme inhibitors (monoamineoxidase, *c*AMP-phosphodiesterase) or antiviral agents [226] and of special interest because of their strong affinity to the benzodiazepine receptor. β-Carbolines are also used as herbicides and fungicidal agents. [227]

4.5.5 4-Hydroxy-5-methyl-furan-3-one

Compound **130** was oily and showed a UV absorbing band at 254 nm, which turned to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **130** showed in the aliphatic region two singlets at δ 2.32 (s, 3H) and at δ 4.60 (s, 2H).

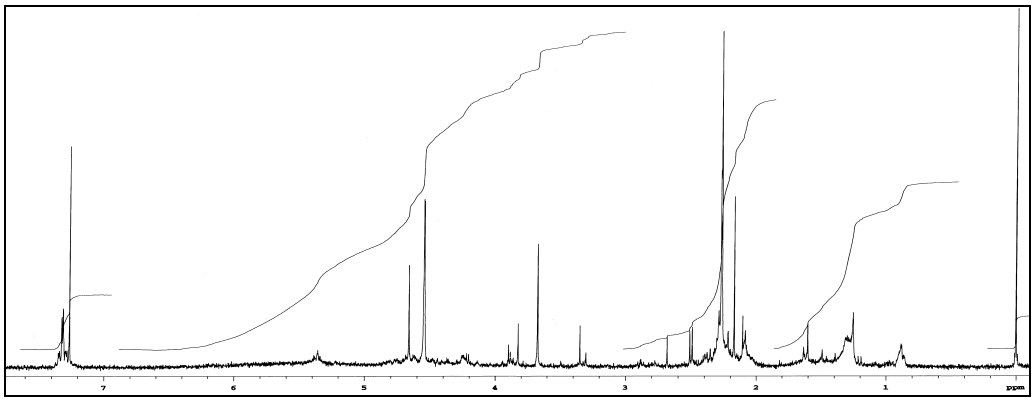
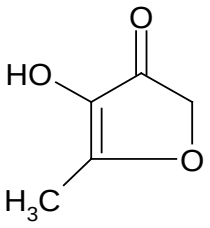


Figure 59: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of 4-hydroxy-5-methyl-furan-3[2*H*]-one (**130**)

4-Hydroxy-5-methyl-furan-3[2H]-one (**130**) was extracted before from *Pinaceae* species (plants) and has also been isolated in parallel to the present thesis in our group;[228] it has strong anti-oxidant properties and a skin-whitening activity. It is not irritating and could be applied in cosmetics. [229]

The antioxidant activity of compound **130** was more interesting than that of ascorbic acid and its derivatives which are unstable in an aqueous medium and liable to oxida-

tions, and cause irritations to the skin. Kojic acid also has drawbacks as its skin-whitening activity is insufficient and it is unstable. Compound **130** has the ability to inhibit melanine formation and it is stable for long period of time. Compound **130** has also been used as flavouring agent for food. [255]



130

4.5.6 Actinomycin D

Compound **131** was isolated from fraction IV by Sephadex LH-20 in methanol as reddish-orange coloured solid. It was UV absorbing and showed no colour change with NaOH, but turned to red with concentrated sulphuric acid.

The ^{1}H NMR spectrum showed four NH doublets between δ 8.1 and 7.2, indicative for a peptide. The spectrum showed also two *ortho*-coupled protons at δ 7.39 (d, ^{3}J = 7.9 Hz, 1H, H-7), and 7.67 (d, ^{3}J = 7.9 Hz, 1H, H-8) of a 1,2,3,4-tetrasubstituted aromatic ring, and two 3H singlets at δ 2.20 and 2.43 for methyl groups attached to an aromatic system. This is characteristic for the phenoxazinone chromophore (Figure 60) in actinomycins.

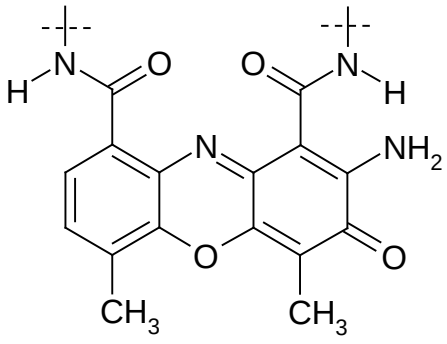


Figure 60: Phenoxazinone chromophore

The spectrum also exhibited eight proton signals of oxygenated or α-amino acid protons between δ 6.0 and 4.5. Further signals were observed between 4.03-3.40 for many methylene and methine groups. Six 3H singlets were observed between δ 2.93-2.23, four of them for N-methyl groups, and two for methyl groups attached to an aromatic ring. Additionally, the spectrum showed five multiplets between 0.99-0.82.

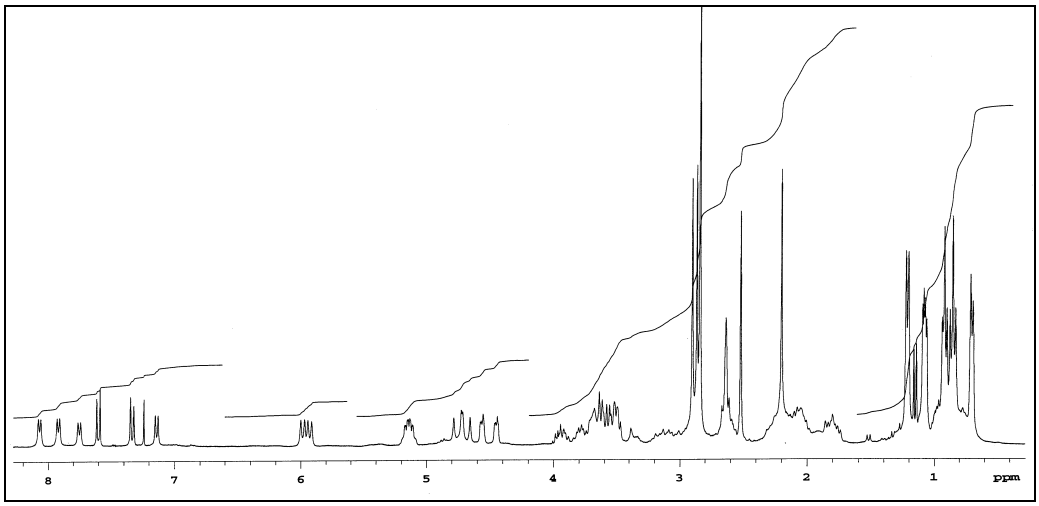


Figure 61: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of actinomycin D (**131**)

The ^{13}C NMR spectrum exhibited 11 signals between δ 100.1-148.0 and one signal at δ 178.5, which are attributed to the twelve ring-carbon atoms of the phenoxazinone system. Twelve sp^2 carbonyls were observed around δ 166.0-174.0 and are characteristic to those of amide and lactone-carbonyl groups of a peptide. The α-carbon atoms of the amino acids were shown in the region δ 46.0-60.0. In addition, two signals of oxygenated methine carbons were at δ 71.0 and δ 75.0.

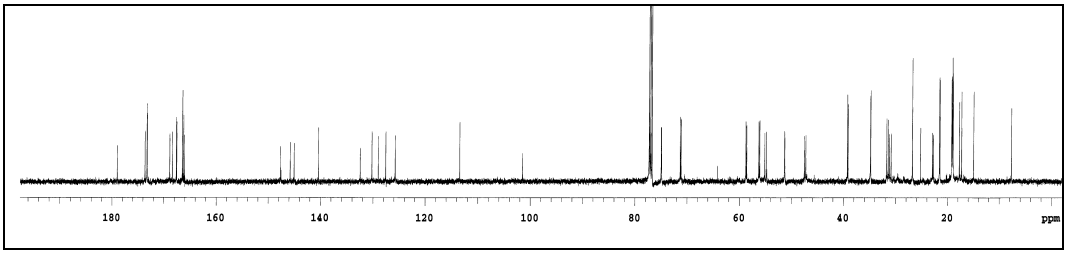
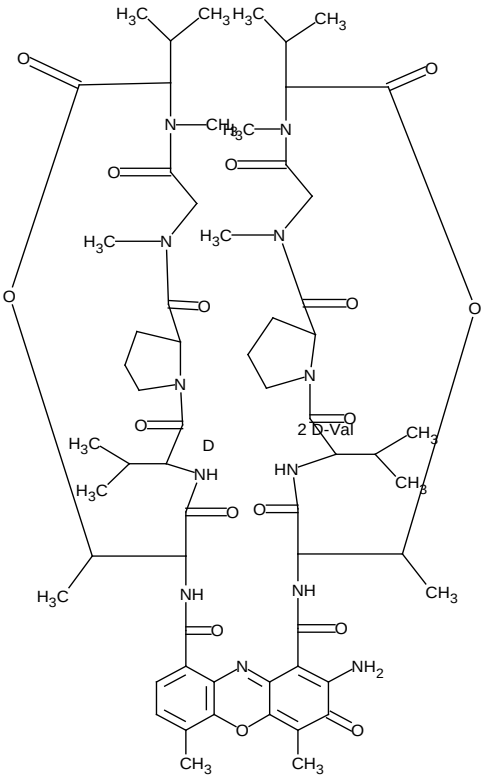


Figure 62: ^{13}C NMR spectrum (300 MHz, $CDCl_3$) of actinomycin D (**131**)



131

A search in AntiBase [115] resulted in actinomycin D (**131**). Compound **131** was further confirmed by comparing the spectra with authentic ones as well as the literature. [161,230] The actinomycins form a family of chromopeptide antitumor antibiotics isolated from various *Streptomyces* strains. [231-232] They have a phenoxazinone chromophore in common and are varying only in the amino acid content of their two depsipentapeptide moieties. [233]

Actinomycin complexes termed A, B, C, D, I, X, Z, as well as other actinomycin analogues have often been reviewed. [234] Actinomycin C3 and D (**131**) have found clinical application as anticancer drug, particularly in the therapy of Wilm's tumor [235] and soft tissue sarcoma in children. [236] Actinomycin D has been proposed as a therapeutic agent for AIDS, because it is a potent inhibitor of HIV-1 minus-strand transfer. [237]

4.6 Ruminal Klebsiella pneumoniae ZIC

The ruminal bacterium ZIC was cultivated on LB medium for 24 hours at 37 °C. Pieces of well grown agar were used to inoculate 20 of 1L Erlenmeyer flasks, each

containing 250 ml of LB medium. The fermentation was carried out at 180 rpm on a rotary shaker for 3 days at 37 °C. The culture broth was harvested and filtered to separate the biomass, which was extracted with ethyl acetate (3 times) and acetone (2 times), respectively. The culture filtrate was then passed through Amberlite XAD-16. The column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluates were concentrated under reduced pressure and finally extraction of the residue was done with ethyl acetate. The organic phases were dried. Both crude extracts were mixed according to TLC yielding 1.41 g of a crude extract. Chromatography of the crude extract on a silica gel column using a CH_2Cl_2-MeOH gradient with successively increasing polarity resulted in three fractions I-III, according to the TLC monitoring.

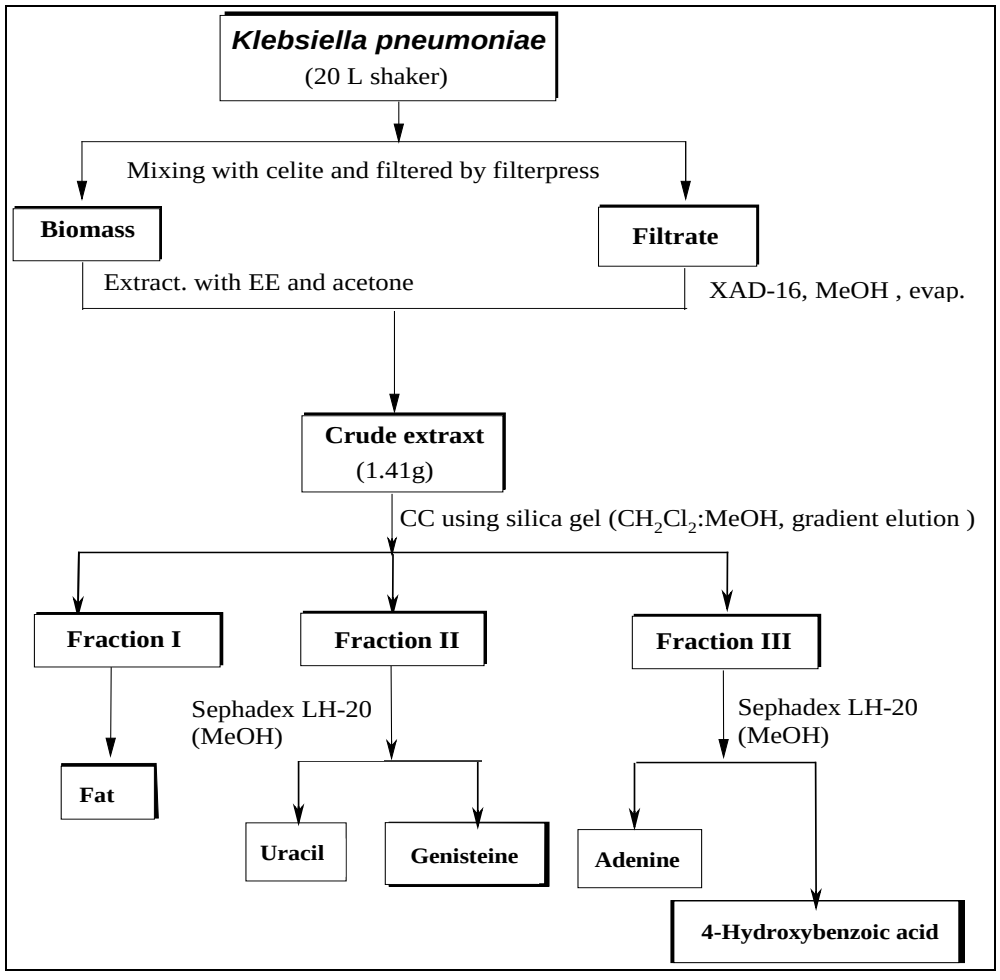


Figure 63: Work-up scheme of the ruminal *Klebsiella pneumoniae*

Four compounds were isolated from the ruminal *Klebsiella pneumoniae*; all of them were known and were identified as 4',5,7-trihydroxyisoflavone (genisteine, **133**), uracil (**126**), 4-hydroxybenzoic acid (**134**), and adenine (**124**).

4.6.1 4',5,7-Trihydroxyisoflavone (genisteine)

Compound **133** was isolated from fraction II as white powder, which showed UV absorption at 254 nm. The molecular formula $C_{15}H_{10}O_5$ was deduced by HRESIMS. The aromatic region of the ^{1}H NMR spectrum of compound **133** showed signals at δ 8.03 (d), 6.38 (dd), 6.19 (d) and an A_2B_2 pattern at δ 7.38 (d) and 6.82 (d).

The above data indicated that compound **133** could be an isoflavone. A search in AntiBase [115] by using the above spectroscopic data resulted in genisteine (**133**).

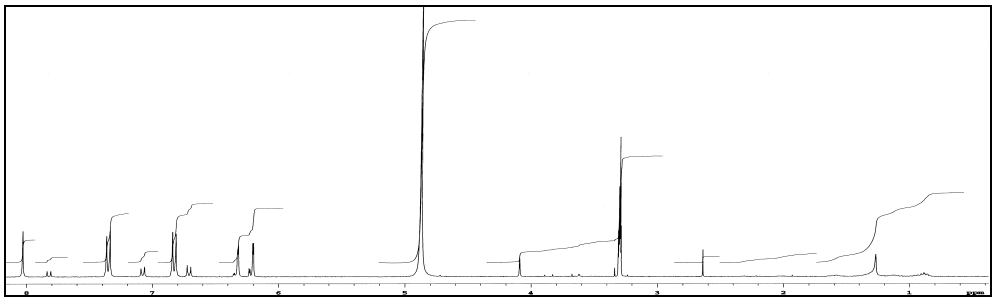


Figure 64: ^{1}H NMR spectrum (300 MHz, CD_3OD) of 4',5,7-trihydroxyisoflavone (**133**)

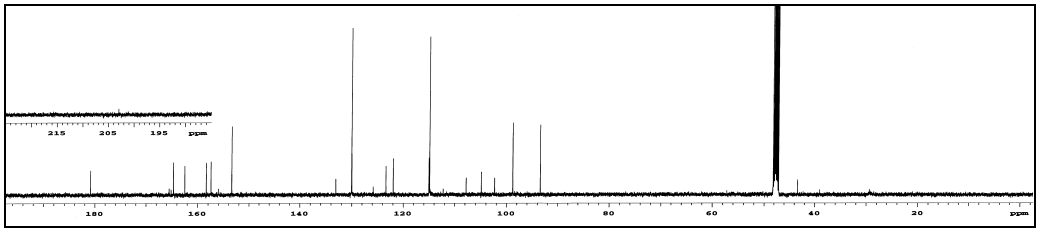
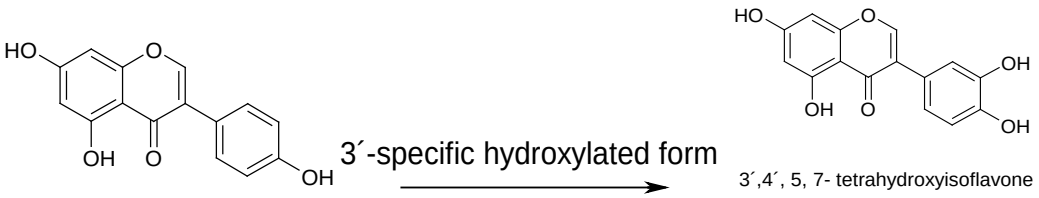


Figure 65: ^{13}C NMR spectrum (125 MHz, CD_3OD) of 4′,5,7-trihydroxyisoflavone (**133**)

4',5,7-Trihydroxyisoflavone (genisteine) (**133**) is a phenolic phytoestrogen found in numerous plants, soybeans and fava beans. [238,239] Isoflavones like compound **133** have been reported to act as antioxidants, free radical scavengers, metal chelators, and antibacterial agents. [265-240] In addition, isoflavones are known to have medicinal and chemopreventive activities on human health. [241-242]

The hydrogenated product 3′,4′,5,7-trihydroxyisoflavone (**132**) [243] of genisteine (**133**) has potent antioxidant properties that contribute to their cholesterol-lowering effects, cardiovascular protection, antitumor effects, and anticarcinogenic properties.[244-245] Furthermore, compounds with the *ortho*-dihydroxy group are known to exhibit anti-inflammatory and anti-allergic activities, [277] and express anticarcinogenic proprieties due to the inhibition of protein tyrosine kinases [246] by acting as potent tyrosinase inhibitors [247] and active inhibitors of lipoxygenases. [248] Hydroxylated isoflavones are invaluable compounds for lowering the incidence of cancer-related diseases. [277,249]



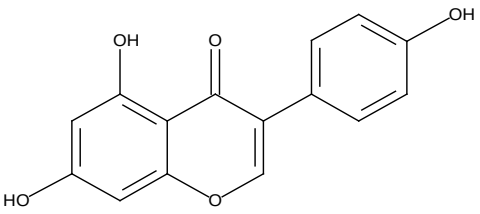
133 **132**

Figure 66: 3´-specific hydrogenation of genisteine (**133**)

Genistein (**133**) itself is a specific inhibitor of protein tyrosine kinase, has been shown to inhibit UVB induced skin carcinogenesis in hairless mice. [250] It inhibits the growth and proliferation of testicular cells and is a potential diagnostic and therapeutic tool in testicular pathophysiological research. [251]

As genisteine is a common plant product, **133** and derivatives were isolated from fermentations in media containing plant-derived nutrients such as soybean meal or cotton seed meal likely originate from the medium components and not from microbial biosynthetic origin. [252]

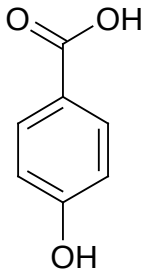
Genistein was found to be a strong topoisomerase inhibitor, similarly to some chemotherapeutic anticancer drugs like etoposide and doxorubicin. [253, 254]
In high doses it was found to be strongly toxic to normal cells. [255] This effect may be responsible for both the anticarcinogenic and carcinogenic potential of the substance. [256,257]



133

4.6.2 4-Hydroxybenzoic acid

Compound **134** was also obtained from fraction II as white crystalline solid and showed UV absorption at 254 nm. The ^{1}H NMR spectrum of compound **134** showed only two doublets as A_2X_2 pattern at δ 7.78 (2H, d, J = 8.5 Hz, H-3,5) and 6.45 (2H, d, J = 8.5 Hz, H-2,4) and a carboxylic proton at δ 11.42 (1H, br s). A search in Anti-Base [115] resulted in 4-hydroxybenzoic acid (**134**), which was further confirmed by comparison with the authentic spectra as well as literature data.



134

4-Hydroxybenzoic acid (**134**) is widespread in many plants, as well as in microorganisms as the free acid or in form of derivatives. It was firstly isolated from fruits of *Catalpa bignonioides*. [116]

4.7 Ruminal bacterium *Pseudomonas aeruginosa* ZIL

The ruminal bacterium *Pseudomonas aeruginosa* 210 was isolated in Tunisia from the rumen of a cow. After three days of fermentation, the culture broth was used to inoculate a Braun Biostat U fermenter, filled with 20 l of LB medium. The culture broth was harvested after 5 days. The biomass was filtered off by means of a pressure filter and extracted with ethyl acetate and acetone, while the filtrate was passed through an Amberlite XAD-16 column. After concentration, the aqueous residue was

extracted with ethyl acetate. The extracts from the mycelium and the water phase were similar on TLC and were combined, yielding 3.54 g of a greenish-brown resin. Silica gel column chromatography (CC; CH_2Cl_2/MeOH, stepwise gradient) under TLC monitoring afforded four fractions I-IV. Fraction II obtained from CH_2Cl_2: CH_3OH 9.9:0.1 showed a single spot on TLC, which on final purification using silica gel (CH_2Cl_2/ 5 % MeOH) and Sephadex LH-20 (CH_3OH) CC resulted in 1-phenazinol (20 mg) and phenazine-1-carboxamide (**42**) (25 mg). Fraction III obtained with 0.2 % MeOH was again subjected to CC on silica gel and finally purified by Sephadex LH-20 (CH_3OH) to provide 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) (100 mg). Fraction IV obtained with 0.3 % MeOH afforded 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**) (15 mg) after Sephadex LH-20 (CH_3OH) and PTLC (CH_2Cl_2: CH_3OH, 9.3:0.7). A mixture of rhamnolipid A (**94**) and B (**140**) (50 mg) was obtained from fraction V (0.5 % MeOH) by using Sephadex LH-20 (CH_3OH).

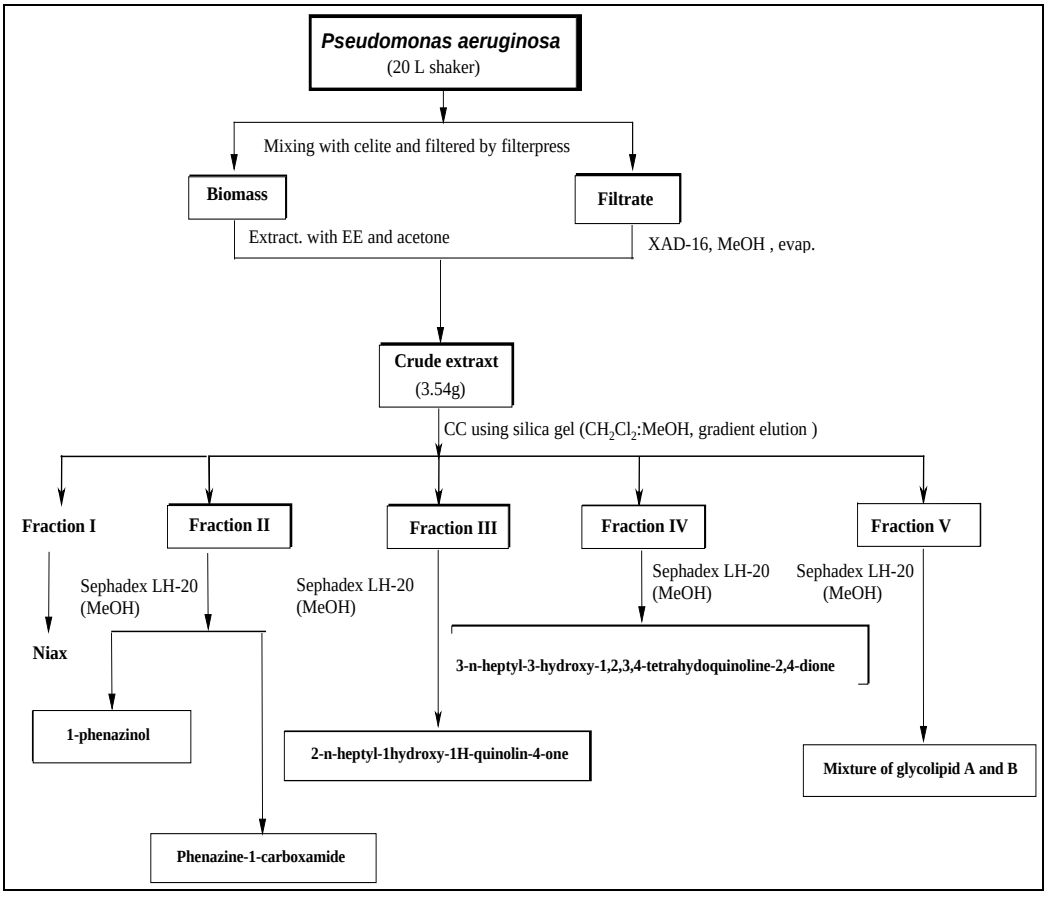


Figure 67: Work up scheme for the ruminal *Pseudomonas aeruginosa*

4.7.1 2-*n*-Heptyl-1-hydroxy-1*H*-quinolin-4-one

Compound **135** was isolated as a colourless crystalline solid. The UV spectrum showed absorption bands at 355, 254 and 215 nm for aromatic and α,β-unsaturated systems. The molecular formula $C_{16}H_{21}NO_2$ was deduced through HRESIMS of the *pseudo*molecular ion peak at *m/z* 260.16450 $[M+H]^+$. The aromatic region of the ^1H NMR spectrum showed signals of an *ortho*-disubstituted benzene (Table 12), two 1H doublets at δ 8.23, 8.05 and two 1H triplets at δ 7.55 and 7.33, J = 7.8 Hz). An additional 1H singlet appeared at δ 6.22, and signals between δ 2.60 and 0.81 indicated an *n*-heptane chain. At δ 11.9, the singlet of a D_2O replaceable proton was visible. A search in AntiBase [115] with these data pointed to the quinolone antibiotic 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**), a tautomer of KF-8940 (**136**). [258] The 2D NMR data confirmed the identity of **135** (Table 12).

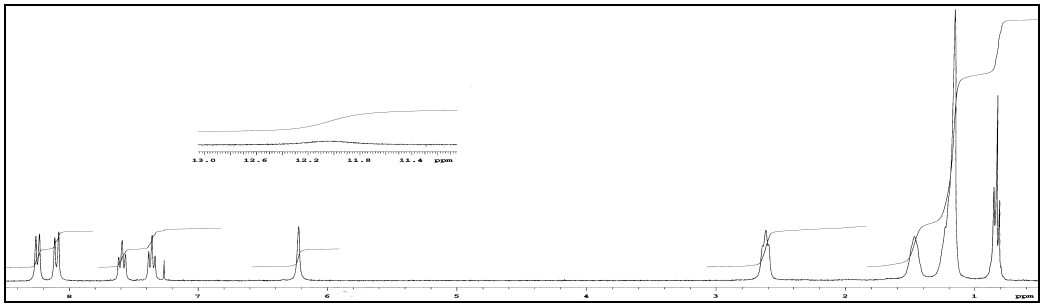


Figure 68: ^1H NMR spectrum (300 MHz, $CDCl_3$) of 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**)

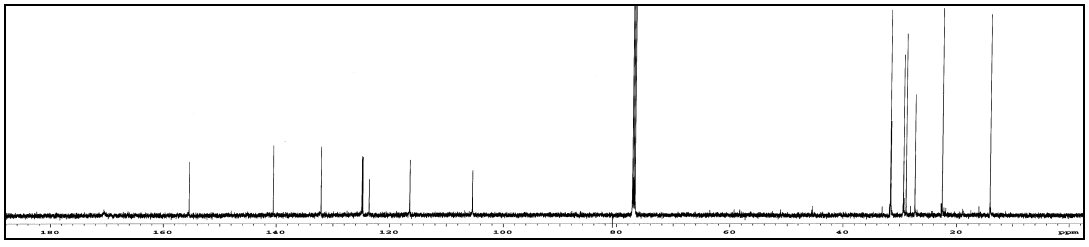
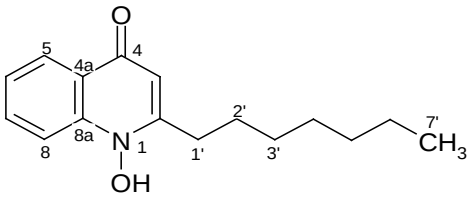
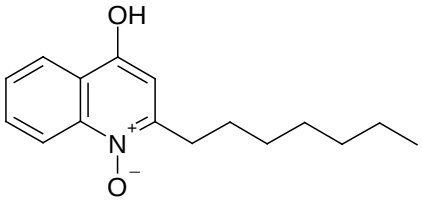


Figure 69: ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**)



135



136

Table 12: ^{1}H and ^{13}C NMR data, HMBC and COSY correlations of 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) in ($CDCl_3$)

	Experimental				**Literature[302]**	
Position	δ_C [a]	δ_H [b] (*J* in Hz)	**HMBC[c] (H→C)**	**COSY[c] (H→H)**	δ_H [b] (*J* in Hz)	δ_C [a]
2	155.5	–	–	–	–	155.6
3	105.4	6.23, s	2, 4, 4a, 1'	–	6.39	105.5
4	170.5	–	–	–	–	170.4
4a	123.6	–	–	–	–	123.7
5	124.7	8.23, d (7.8)	4, 7, 8a	H-6	8.27	132.1
6	124.9	7.33, t (7.8)	8, 4a	H-5,7	7.58	124.9
7	132.1	7.55, t (7.8)	5, 8a	H-6,8	7.36	124.8
8	116.5	8.05, d (7.8)	6, 4a, 8a	H-7	8.07	116.5
8a	140.5	–	–	–	–	140.6
1'	31.6	2.67, t (7.6)	2, 3, 3'	H_2-2'	2.78	31.7
2'	29.4	1.50, m	–	H_2-1',3'	1.6	27.5
3'	27.4	1.23, br s	–	–	1.2	29.5
4'	28.9	1.23, br s	–	–	1.2	29.0
5'	31.7	1.23, br s	–	–	1.2	31.7
6'	22.6	1.23, br s	–	–	1.2	22.6
7'	14.0	0.80, t (6.3)	5', 6'	H_2-6'	0.84	14.0

[a] ^{1}H-NMR spectrum at 300 MHz; [b] ^{13}C-NMR spectrum at 150 MHz; [c] HMBC and COSY spectra at 300 MHz

Compound **135** was tested *in vitro* for anticancer activity in a panel of 37 human tumor cell lines derived from solid human tumors comprising bladder, central nervous

system, colon, gastric, head and neck, lung, mammary, ovarian, pancreatic, prostate and renal cancers, as well as cell lines established from human melanoma, pleuramesothelioma and the uterus body. Compound **135** exhibited a mean IC_{50} value of 28.852 µg/ml, a mean IC_{70} value of 29.442 µg/ml and a mean IC_{90} value of 31.842 µg/ml (Table 14).

Table 13: *In vitro* antitumor activity of 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) against tumor cell lines in a monolayer proliferation assay.

Tumor type	**Tumor cell line N°**	**Test/Control (%) at Drug Concentration (30 µg/ml)**
Lung A Adeno	LXF 529 NL	13 ++
Lung A Adeno	LXF 629 L	9 +++
Melanoma Xenograft	MEXF 462 NL	44 +
Uterus body	UXF 1138L	40+

- (T/C = 50) + (30< = T/C < 50) ++ (10 < = T/C < 30) +++ (T/C < 10)

Table 14: *In vitro* antitumor activity of 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) against tumor cell lines in a monolayer proliferation assay.

Tumor type	**Tumor cell line N°**	**IC_{50} µG/ml**	**IC_{70} µG/ml**	**IC_{90} µG/ml**
Lung A Adeno	LXF 529 NL	4.896	13.044	34.749
Lung A Adeno	LXF 629 L	9.619	16.753	29.179
Melanoma Xenograft	MEXF 462 NL	21.418	65.854	>30.000
Uterus body	UXF 1138L	20.030	44.932	>30.000
	Mean n = 12	28.852	29.442	31.842

Compound **135** was tested for its effects on the motility of the zoospores of the grapevine downy mildew pathogen, *P. viticola*. Compound **135** inhibited motility of zoospores and subsequent lysis of the cells in a dose- and time-dependent manner (Table 15). In presence of **135**, zoospores moved very slowly in their axis, instead of displaying straight swimming in a helical fashion. This phenomenon continued for several minutes depending on the dose of the compound **135** and then the zoospores lysed. This is the first report on motility inhibitory and lytic activities of a natural product isolated from a ruminant bacterium, *Pseudomonas aeruginosa*.

Table 15: Motility inhibitory and lytic activities (% ± s.e) of compound **135** isolated from the ruminal bacterium *Pseudomonas aeruginosa* against the zoospores of grapevine downy mildew pathogen *Plasmopara viticola*

		15 min		**30 min**		**45 min**		**60 min**	
	Dose (µg ml^{-1})	**Motility inhibition**	**Lysis**	**Motility inhibition**	**Lysis**	**Motility inhibition**	**Lysis**	**Motility inhibition**	**Lysis**
Compound 135	5	32±4	7±1	51±5	37±3	75±7	62±4	78±9	68±7
	10	77±5	42±7	88±6	72±5	90±8	82±7	97±4	86±5
	25	100±0	100±0	nt	nt	nt	nt	nt	nt
	50	100±0	100±0	nt	nt	nt	nt	nt	nt

Data presented here are average value ± s.e. of at least three applications in each dose of test compound

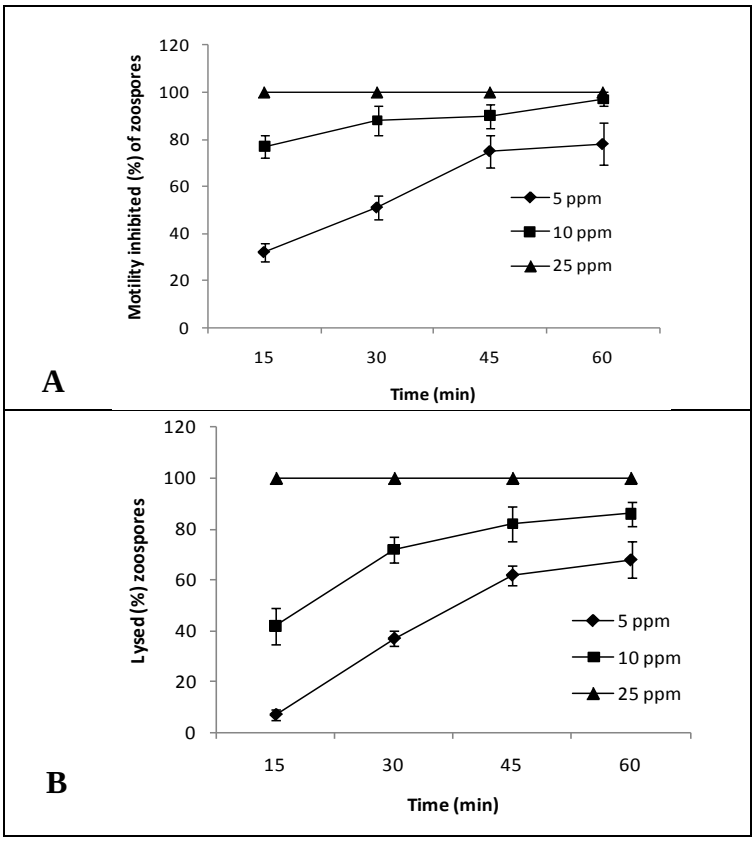


Figure 70: Motility inhibitory (**A**) and lytic (**B**) activities of 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) against zoospores of grapevine downy mildew pathogen, *Plasmopara viticola*.

Peronosporomycetes are distinct from fungi and are phylogenetic relatives of brown algea and diatoms. [259] They cause many destructive diseases in plants, animals, fishes and humans. *Plasmopara viticola* is a serious pathogen of grapevine world-wide. Many fungicides are ineffective against this phytopathogen, and hence, bioactive compounds with new mode of action are needed to combat this economically important pest. Under favourable environmental conditions, the fungus *P. viticola* infects grapevine leaves by means of characteristic biflagellated motile zoospores released from airbone sporangia coming from other infected plants. The zoospores aggregate to stomata of the grapevine leaf by swimming through water films and then rapidly encyst to become round cystospores by shedding their flagella. [384, 260]

The cytospores then rapidly germinate to form germ tubes and penetrate host tissue through the stomata. Interruption of any of these asexual stages eliminates the potential for pathogenesis. [261]

The success of any zoosporic pathogen can be attributed in part to the speed of asexual differentiation to generate bi-flagelled motile zoospores and their ability to find hosts through chemotaxis. [262] Therefore, compounds that can interfere with normal swimming behaviour and early development of *P. viticola* are supposed to be important as lead compounds in the management of this phytopathogen.

2-*n*-Heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) was isolated before by Hays *et al.* as antibiotic from *Pseudomonas aeruginosa*.[263] Lightbown and Jackson revealed that 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) was an antagonist of dihydrostreptomycin and an inhibitor of electron transport through the cytochrome b~c_1 segment of the respiratory chain.[264,265] Kitamura *et al.* proved that 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**) at high concentrations inhibited 12-lipoxygenase and cyclooxygenase.[290]

4.7.2 3-*n*-Heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione

Compound **137** was also isolated as a colourless crystalline solid. The aromatic region of the ^{1}H NMR spectrum showed signals of an *ortho*-disubstituted benzene (Table 16), namely two 1H doublets at δ 7.81, 7.05 and two 1H triplets at δ 7.58 and 7.18. Additional signals between δ 2.60 and 0.81 indicated an *n*-heptane chain. The ESI mass spectrum revealed the *pseudo*molecular ion peak at *m/z* 275 $[M+Na]^+$.

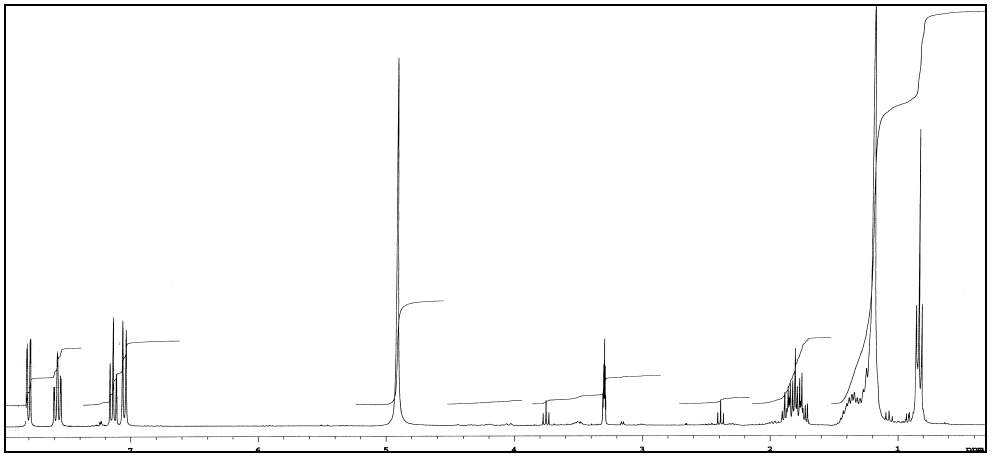


Figure 71: ^{1}H NMR spectrum (300 MHz, CD_3OD) of 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**)

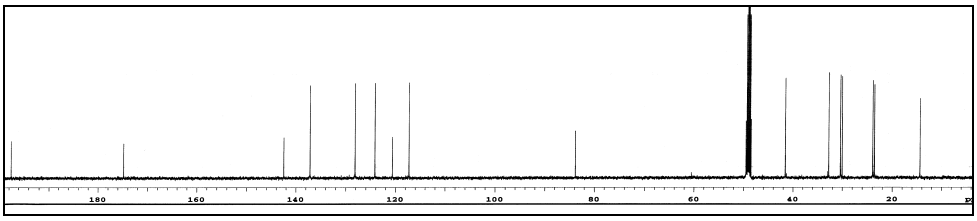
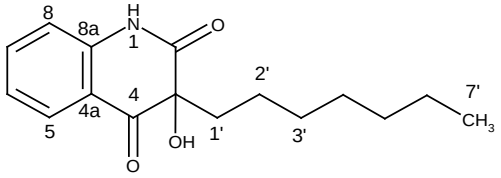


Figure 72: ^{13}C NMR spectrum (125 MHz, CD_3OD) of 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**)

A search in AntiBase [115] by using the above spectroscopic data as well as the molecular weight suggested that the isolated compound was 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**) having the molecular formula $C_{16}H_{21}NO_3$. This was further confirmed by comparing with literature data. [290] 3-*n*-Heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**) was isolated before by Neuenhaux *et al.* as a metabolite of *Pseudomonas aeruginosa*. [266]

Compounds **135** and **137** were described as 5-lipoxygenase inhibitors. [290] Compound **135** was a potent and selective inhibitor of the 5-lipoxygenase of rat basophilic leukemia cells in a dose-dependent manner: the half maximal inhibitory concentration (IC_{50}) was 1.5 x 10^{-7} M according to Kitamura *et al.* [290]

Quinolones are forming a rather new class of clinically versatile antibiotics, which are predominantly useful in the treatment of a broad spectrum of gram-negative pathogens. [267] For many of them, an inhibition of the bacterial enzyme gyrase has been described. [268] Their medicinal application originates from the treatment of urinary infections, [269] but recently also an extremely strong activity against *Helicobacter pylori* has been described. [270] Also some naturally occurring derivatives are known, but their number is still low. Most of them are bearing an acetate-derived or isoprenoid side chain at C-2 or C-3.



137

Table 16: ^{1}H and ^{13}C NMR data, HMBC and COSY correlations of 3-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (**137**) in CD_3OD

	Experimental in CD_3OD		**Literature[290] in $CDCl_3$**	
Position	**δ_C[a]**	**δ_H[b] (*J* in Hz)**	**δ_C[a]**	**δ_H[b] (*J* in Hz)**
1-NH	–	–	–	9.30
2	197.6	–	155.6	–
3	83.8	–	108.2	3.83
4	174.8	–	178.9	–
4a	120.6	–	124.9	–
5	128.1	7.81, d	131.7	7.91
6	124.1	7.18, t	125.2	7.18
7	137.2	7.58, t	123.6	7.59
8	117.3	7.05, d	118.7	7.07
8a	142.5	–	140.8	–
1'	41.6	2.8, m	34.4	1.8
2'	32.7	1.21, m	29.2	1.2
3'	30.4	1.21, br s	29.2	1.2
4'	30.0	1.21, br s	29.0	1.2
5'	23.9	1.21, br s	31.6	1.2
6'	23.6	1.21, br s	22.6	1.2
7'	14.4	0.80, t	14.0	0.83

4.7.3 Polypropylenglycol

Compound **138** was obtained as colourless oil; it displayed a non-UV active zone and gave white zone on a pink background by spraying with anisaldehyde/sulphuric acid reagent and heating. The ^{1}H NMR spectrum of compound **138** showed only two peaks in the aliphatic region, one methyl doublet at δ 1.10 for CH_3 groups and a 3H multiplet at δ 3.48 of overlapping oxygenated methine and oxymethylene groups. After a search in AntiBase [115] using the mentioned spectroscopic data, the isolated compound was assigned as polypropylenglycol (**138**).

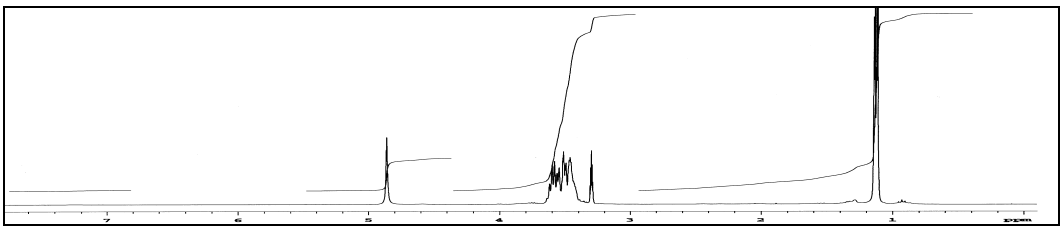
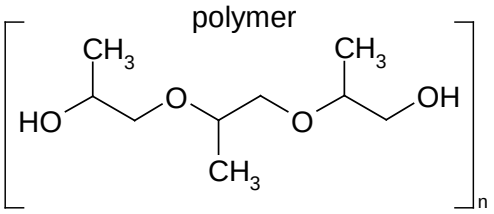


Figure 73: ^{1}H NMR spectrum (300 MHz, CD_3OD) of polypropylenglycol (**138**)

Polypropylenglycol (**138**) is a polymer of $CH(CH_3)$-CH_2OH units and often used as antifoaming agent in fermenter cultures. We realized here and with other strains that it is also a natural product.



138

4.7.4 1-Phenazinol

Compound **139** was obtained as green needles from fraction IV by further applying PTLC. The ^{1}H NMR spectrum showed in the aromatic region seven aromatic protons: two doublets of doublets at δ 8.22 (1 H) and 8.18 (1 H) and two multiplets at δ 7.82-7.84 (2 H), two others doublets at δ 7.22 and δ 7.81 and a triplet at δ 7.76. Hence, an aromatic 1,2-disubstituted and a 1,2,3-trisubstituted ring could be derived. The displayed downfield shift pointed to a connection with heteroatoms. A search in AntiBase [115] led to 1-phenazinol. The compound was further confirmed by comparing the data with authentic spectra.

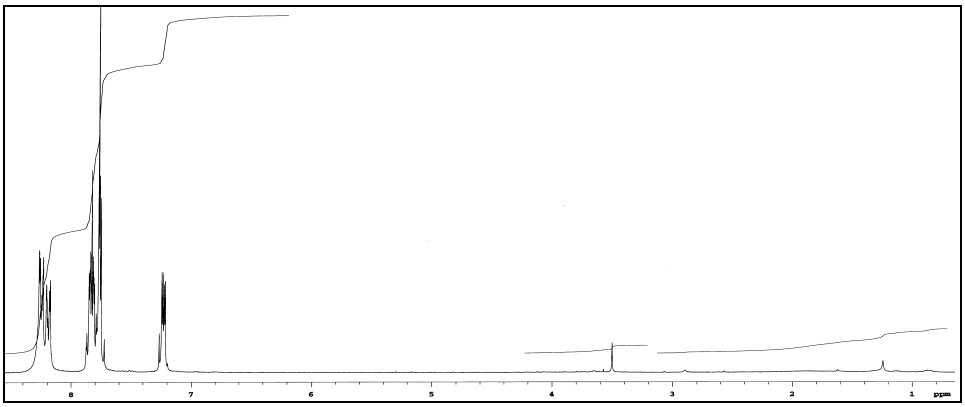


Figure 74: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of 1-phenazinol (**139**)

The ^{13}C NMR spectrum displayed in total twelve carbon signals in the aromatic region between δ 108.8 and δ 151.6, five of quaternary atoms and seven of methine groups).

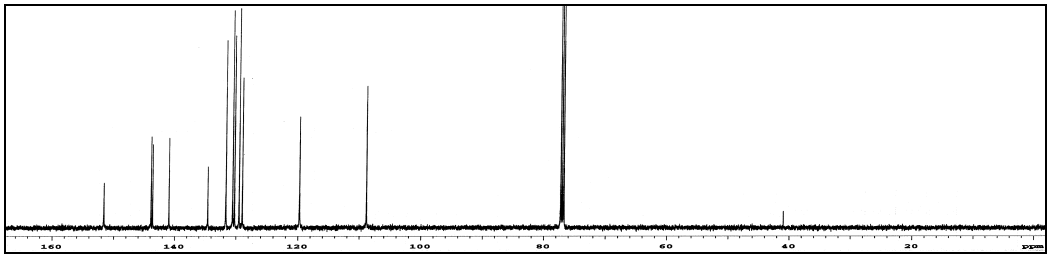
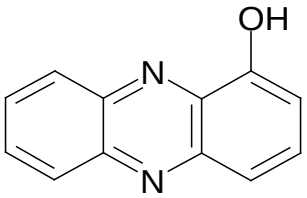


Figure 75: ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of 1-phenazinol (**139**)



139

1-Phenazinol (**139**) is a brightly green pigment that is one of the characteristic secondary metabolites of the bacterial genus *Pseudomonas*; it is believed to function as antagonistic agent in microbial competitiveness. [271]

Table 17: ^{1}H and ^{13}C NMR data, HMBC and COSY correlations of 1-phenazinol (**139**) in $CDCl_3$

	1-Phenazinol (139)			
Position	**δ_H[a] (*J* in Hz)**	**δ_C[b]**	**HMBC[c] (H→C)**	**COSY[c] (H↔H)**
1	–	151.6	–	–
2	7.22 (d)	108.8	1, 4, 4a, 10a,	7.81
3	7.76 (t)	131.8	1, 4a	–
4	7.81 (d)	119.9	–	7.22
4a	–	143.8	–	–
5a	–	144.1	–	–
6	8.18 (dd)	129.1	5a	7.82
7	7.82 (m)	130.4	6	8.22
8	7.84 (m)	130.7	–	8.22
9	8.22 (dd)	129.8	7, 8, 9a	7.82, 7.84
9a	–	141.1	–	–
10a	–	134.6	–	–

[a]: 300 MHz, [b]: 125 MHz, [c]: 600 MHz

4.7.5 Phenazine-1-carboxamide

Compound **42** was isolated as pale yellow-greenish solid. The ^{1}H NMR spectrum showed seven aromatic proton signals, three were adjacent in a first spin system at δ 9.02 (dd 1H, H-2), 7.97 (dd, 1H, H-3), 8.42 (dd, 1H, H-4), and four in a second di-substituted benzene ring at δ 8.28 (m, 1H, H-6), 7.93 (dd, 1H, H-7), 7.89 (dd, 1H, H-8) and 8.23 (m, 1H, H-9); in addition two exchangeable protons were observed at δ 10.74 (brs, 1H, 1-NH) and at δ 6.38 (brs, 1H, 1-NH). Signals in the aliphatic region were absent. The ESI mass spectrum delivered a *pseudo*molecular ion peak at *m/z* 246 for $[M+Na]^+$, which gave a molecular weight of 223 Dalton. The HRESI mass spectrum established the molecular formula $C_{13}H_9N_3O$. By a search in AntiBase [115]

and comparison of the spectroscopic data with authentic spectra and literature data, the isolated compound was assigned as phenazine-1-carboxamide (**42**).

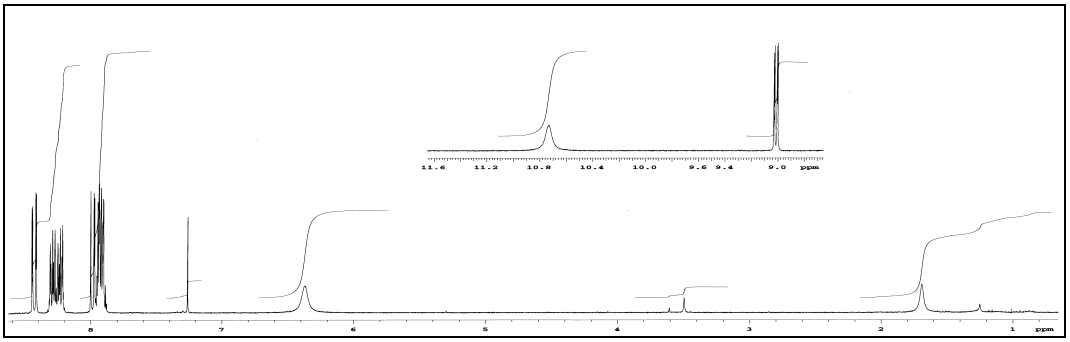
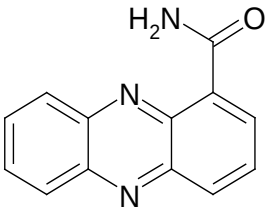


Figure 76: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of phenazine-1-carboxamide (**42**)



42

4.7.6 Rhamnolipid A and rhamnolipid B

Compounds **94** and **140** were tentatively characterized according to the chromatographic and spectroscopic data as a mixture of two lipids, giving on TLC two polar spots which turned to intense green after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum (Figure 77) showed signals at δ 4.83, 3.80, 3.40, 3.65-3.75, and at δ 1.25. The signals at δ 0.88, 1.15-1.62 and δ 1.25 are characteristic of a long aliphatic chain. Comparing the spectrum of the mixture **94**/**140** with the spectrum of rhamnolipid A (**94**, sometimes also named as glycolipid A, Figure 21) suggested that both compounds were chemically related.

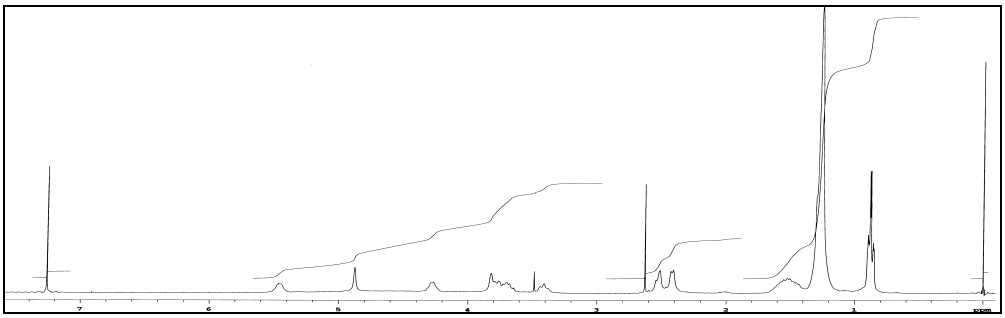
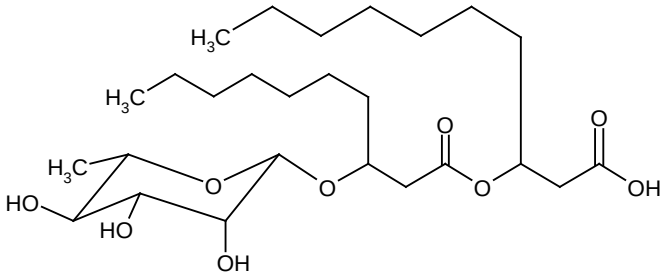
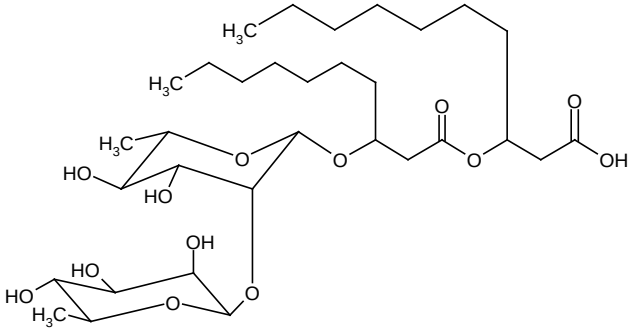


Figure 77: ^{1}H-NMR-spectrum ($CDCl_3$, 300 MHz) of rhamnolipid A (**94**) and rhamnolipid B (**140**)



94



140

According to the results of mass spectrometry, two pseudomolecular ions were detected at *m/z* = 503.4 $[M-H]^-$ and 649.6 $[M-H]^-$. According to these data, a search in AntiBase identified compounds **94** and **140** as rhamnolipids A and B.

Due to their detergent properties, glycolipids can be used in drug delivery, clean up of oil spills, and enhanced oil recovery. Among these biosurfactants, the sugar parts are variable and may consist of rhamnose, trehalose, mannose and sophorose as hydrophilic moieties. In addition to surface activity, recently microbial glycolipids were shown to have biological activities to induce cell differentiation, [272,273] have antimi-

crobial activities for plant disease control [274,275] and even some immunological activities. [276] Rhamnolipid B had bactericidal activity against *Mycobacterium tuberculosis*. [277]

Rhamnolipids could be utilized as biodegradable surfactants and antimicrobial agents [307] for their amphiphilic nature and also as a source of rhamnose, which is used as a fine chemical for the synthesis of a widely used flavour, 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (Furaneol). [278,279]

These substances are of commercial interest because of their biodegradability and low toxicity. Applications can be found in food, pharmaceutical, cosmetics and especially chemical industries, basically in any industry or processes where surface activity properties in multiphase systems are encountered. [280]

The production of rhamnolipids could be improved by media optimisation and use of waste materials. Recently, biorenewable waste was used as a carbon source for production of lipopeptides and glycolipids. [281, 282]

5 Terrestrial bacteria

5.1 Terrestrial *Bacillus* sp. ZIR

The terrestrial *Bacillus* sp. ZIR isolated from the rhizosphere of a palm tree in Tunisia was inoculated from well grown plates into 40 1L Erlenmeyer flasks, each containing 250 ml of LB medium. The fermentation was carried out at 95 rpm on a linear shaker for 3 days at 28 °C. It formed a brown culture broth, which was filtered over Celite and adsorbed on Amberlite XAD-16, while the mycelium was extracted with ethyl acetate and acetone. The crude extract (4.50 g) was subjected to silica gel column chromatography and then to size exclusion chromatography. The oily fractions I-III were subjected to GC-MS analysis. One major compound was isolated from fraction IV.

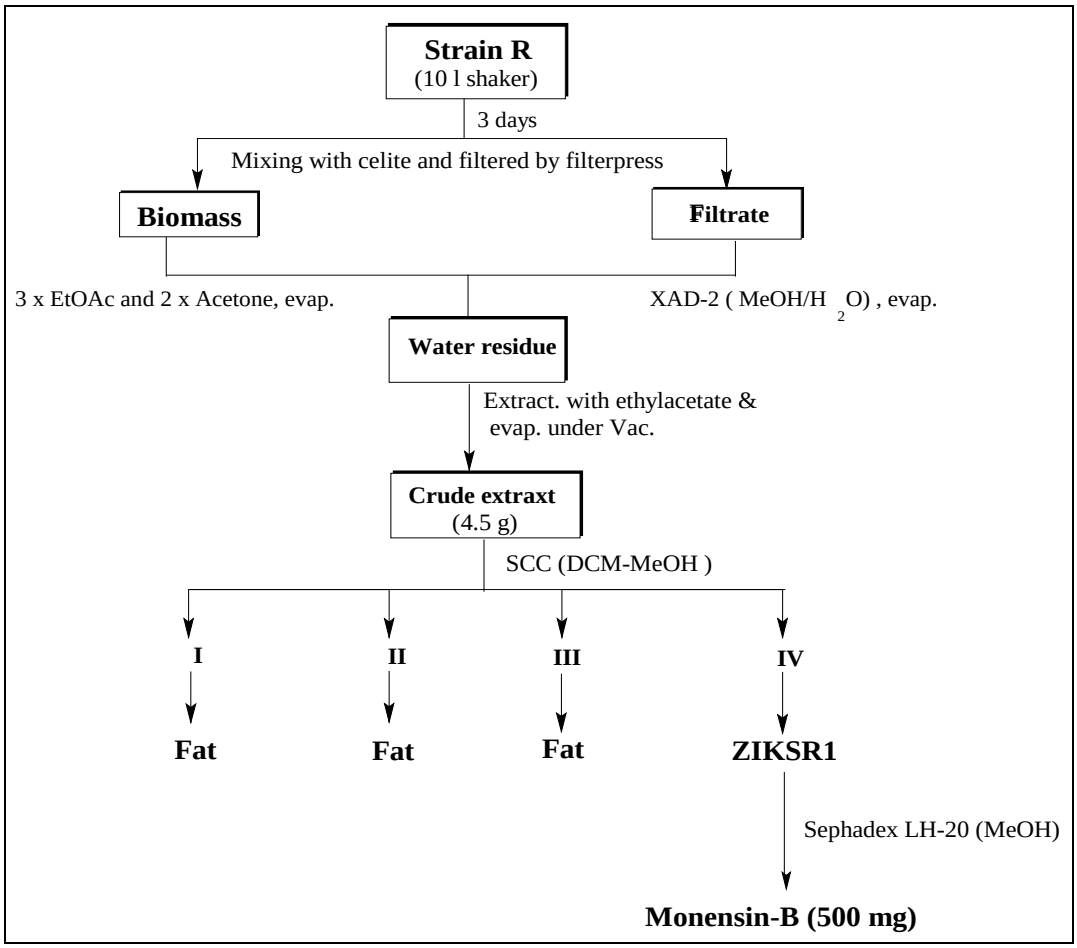


Figure 78: Work-up scheme of the *Bacillus* strain ZIR

5.1.1 Monensin B

TLC of fraction IV exhibited a colourless and non-UV absorbing spot, which turned dark brown after spraying with anisaldehyde/sulphuric acid and heating. Purification of this fraction using Sephadex LH-20 with methanol resulted in monensin B (**141**) as brownish solid. The ESI mass spectrum of **141** exhibited *pseudo*molecular ion peaks at *m/z* 679 $[M + Na]^+$ in the positive mode. HRSIMS analysis suggested a molecular formula of $C_{35}H_{60}O_{11}$.

The ^1H NMR spectrum of **141** in $CDCl_3$ exhibited signals for seven oxymethine protons, one oxymethylene at δ 3.92 (H-26) as well as a methoxy group at δ 3.37 (H-35). In addition overlapped signals for six methine and seven methylenes between 1.3-2.5 were observed. Finally the ^1H NMR spectrum showed signals for eight methyl groups in the range of δ 1.45-0.83.

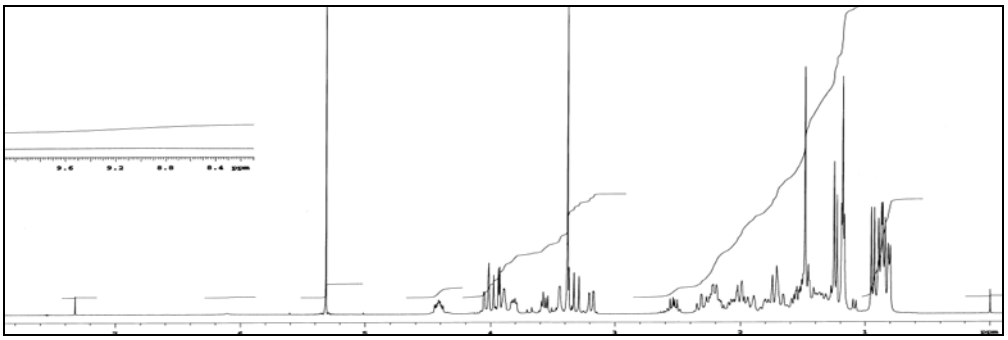


Figure 79: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of monensin B (**141**)

The ^{13}C NMR spectrum of **141** in $CDCl_3$ afforded evidence of one carboxylic acid CO at δ 181.2 (C-1), thirteen signals of oxygenated sp^3 carbons including four quaternary carbons at δ 106.4 (C-9), 88.1 (C-25), 86.2 (C_q-12), and 83.8 (C_q-16), seven methines in the range of δ 85.5-70.1, one methylene at δ 64.2 (C-26) as well as one methoxy group at δ 58.0 (C-35). There were further 28 signals between δ 54 and 10. Searching in AntiBase [115] and Chemical Abstracts using the previous NMR and mass data resulted in monensin B (**141**). The structure was further confirmed by comparison with authentic spectra as well as literature data.

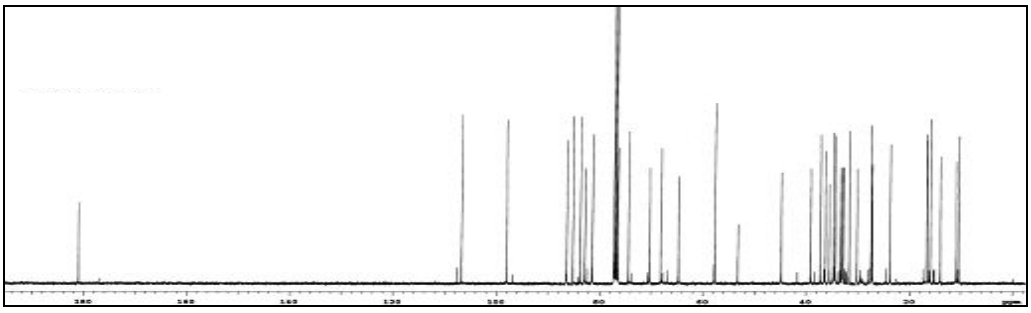


Figure 80: ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of monensin B (**141**)

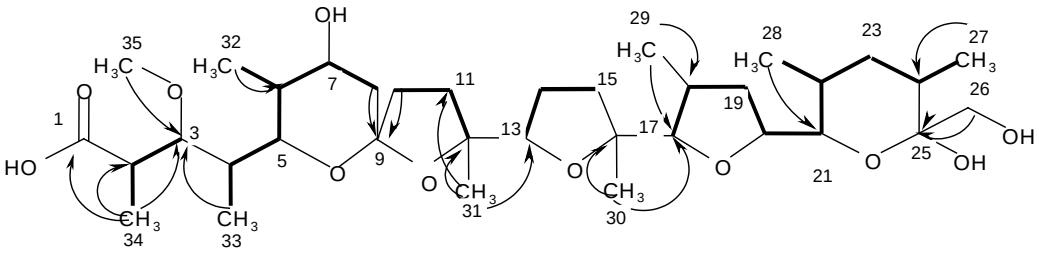
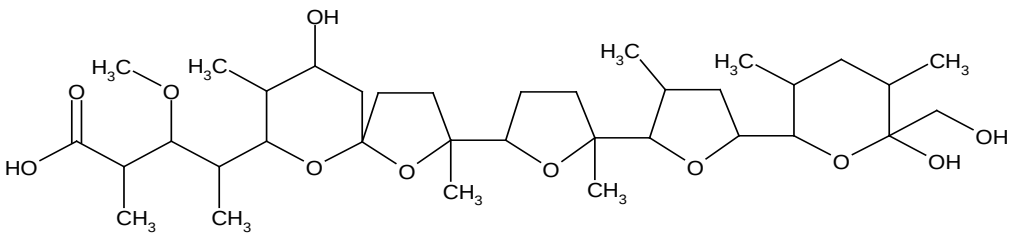


Figure 81: ^{1}H-^{1}H COSY (▬) and HMBC (⟶) correlation of monensin B (**141**)

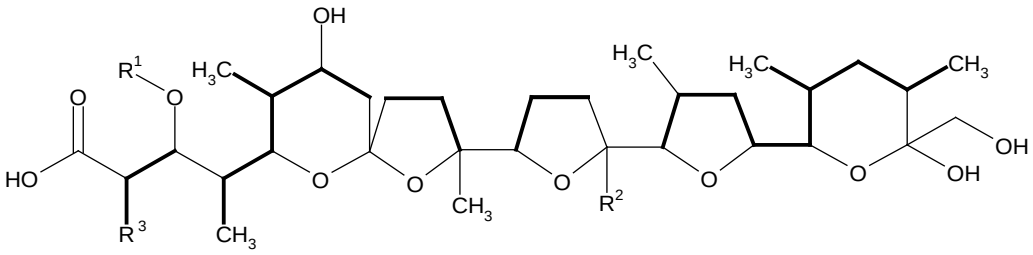
Table 18: ^{1}H NMR (300 MHz) and ^{13}C NMR data (125 MHz) of monensin B (**141**) in $CDCl_3$

monensin B (**141**)					
Position	**^{1}H (Int., mult., *J* [Hz])**	**^{13}C**	**Position**	**^{1}H (Int., mult., *J* [Hz])**	**^{13}C**
1	–	181.2	19	2.32, 1.61 (2H, m)	34.0
2	2.45 (1H, dd, 6.7, 10.2)	45.9	20	3.12 (1H, m, 9.6, 3.9)	77.8
3	3.12 (1H, dd, 10.2, 1.3)	83.9	21	3.84 (1H, m)	76.3
4	2.06 (1H, m)	38.6	22	1.44 (1H, m)	32.9
5	4.00 (1H, dd, 11.3, 2.1)	69.1	23	1.39 (2H, m)	36.9
6	2.00 (1H, m)	36.2	24	1.56 (1H, m)	37.4
7	3.88 (1H, m)	71.6	25	–	88.1
8	1.94, 1.61 (2H, m)	34.5	26	3.92 (1H, m)	64.2
9	–	106.4	27	0.85 (3H, d, 3.9)	16.5
10	1.96	40.2	28	0.83 (3H, d, 3.2)	17.0
11	1.96	34.0	29	0.91 (3H, d, 6.9)	14.5
12	–	86.2	30	1.19 (3H, s)	24.1
13	3.65 (1H, dd, 15.5, 5.2)	82.7	31	1.45 (3H, s)	28.2
14	1.88, 1.52 (2H, m)	28.3	32	0.94 (3H, d, 7.2)	11.0
15	2.32, 1.52 (2H, m)	31.2	33	1.10 (3H, d, 6.9)	11.5
16	–	83.8	34	1.17 (3H, d, 6.7)	16.8
17	3.92 (1H, d, 3.5)	88.0	35	3.37 (3H, s)	58.0
18	2.27 (2H, m)	36.0			



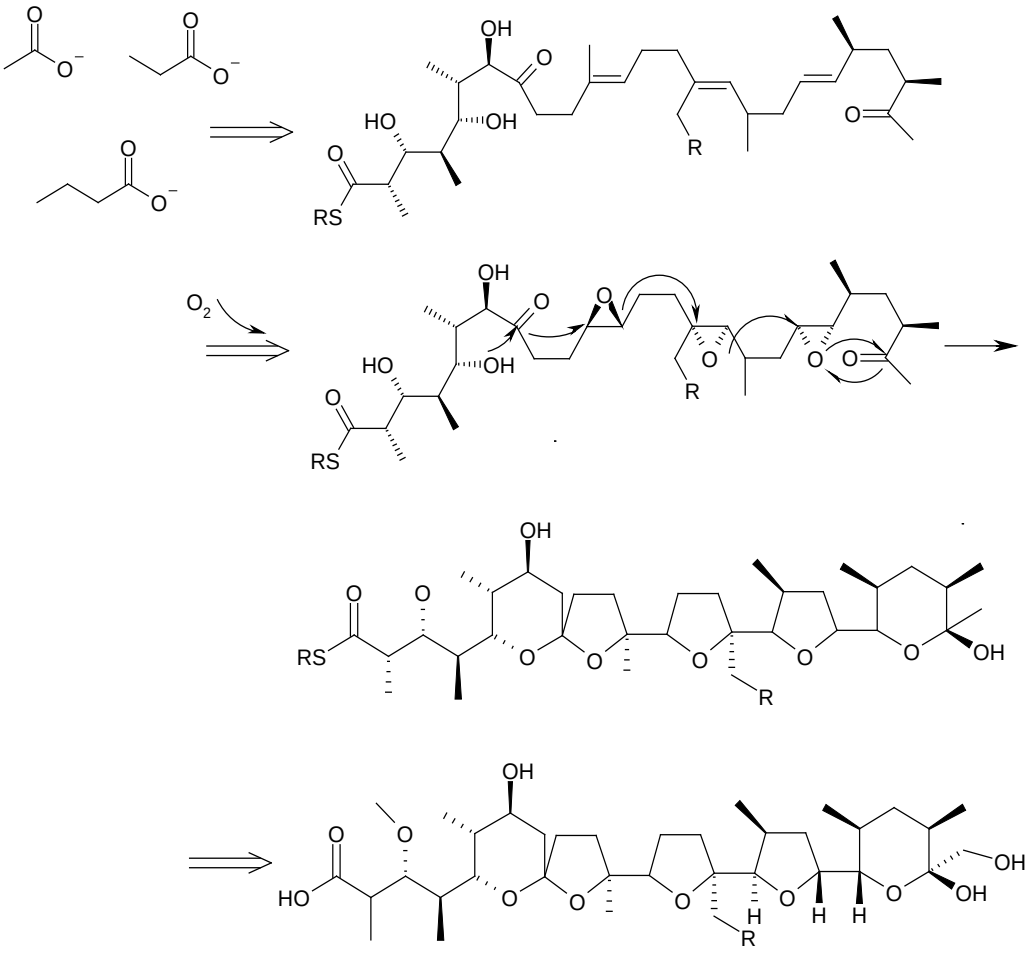
141

Monensins are important polyether ionophore antibiotics, isolated from *Streptomyces cinnamonensis*. For example, monensin (**142**) was broadly used as an anticoccidial agent for poultry and to improve the efficiency of feed used in ruminant animals.



	R^1	**R^2**	**R^3**
Monensin B (**141**)	CH_3	CH_3	CH_3
Monensin A (**15**)	CH_3	CH_3CH_2	CH_3
Monensin (**142**)	CH_3	H	CH_3

Monensin A (**15**) and B (**141**) also displayed activities against *Bacillus subtilis*, [283] which agrees with the result from the biological pre-screening. The biosynthetic pathway of monensin (**142**) has attracted a great deal of interest [284,285] and it has been proposed that the cyclic ether groups in monensin might proceed via a cascade of cyclisation steps on a triepoxy-intermediate, which was supported by isotope labelling experiments. [286-287]



	R
monensin A (**15**)	CH_3
monensin B (**141**)	H

Figure 82: Biosynthetic pathway for monensin A (**15**) and B (**141**)

5.2 Terrestrial *Pseudomonas* sp. ZIPS

The terrestrial *Pseudomonas aeruginosa* ZIPS was cultivated on LB medium for 24 hours at 28 °C. Pieces of well grown agar plates were used to inoculate 20 of 1L Erlenmeyer flasks, each containing 250 ml of LB medium. The fermentation was carried out on a rotary shaker for 3 days at 28 °C. The culture broth was harvested and filtered to separate the biomass, which was extracted with ethyl acetate and acetone, respectively. The culture filtrate was then passed through Amberlite XAD-16. The column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluates were concentrated under reduced pressure and finally extraction of the

residue was done with ethyl acetate. The organic phases were dried. Both crude extracts were mixed yielding 3.5 g of a crude extract. Chromatography of the crude extract on silica gel column using CH_2Cl_2-MeOH gradient with successively increasing polarity resulted in four fractions I-IV followed by monitoring with TLC.

Four known compounds were isolated and were identified as phenazine-1-carboxylic acid (**43**), linoleic acid (**89**), anthranilic acid (**113**), and *cis-cyclo*(Tyr,Pro) (**109**).

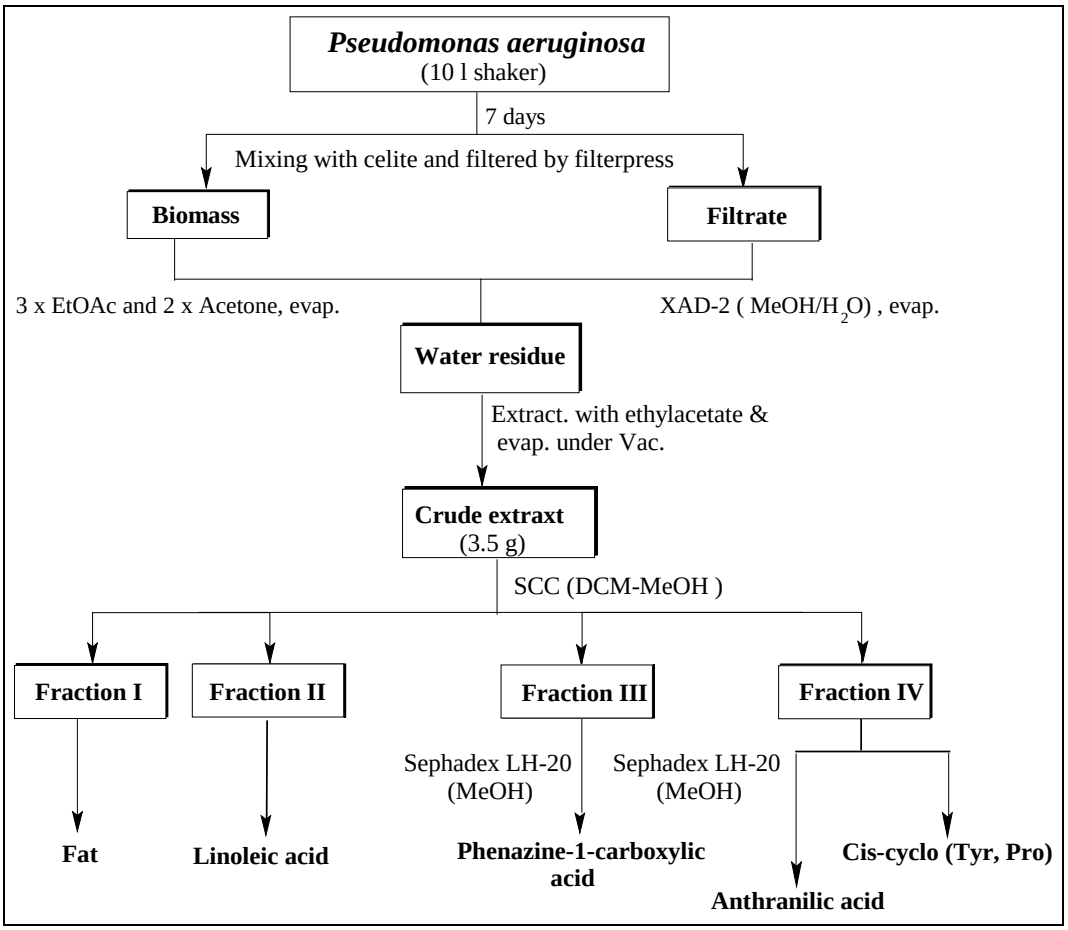


Figure 83: Work-up scheme of *Pseudomonas* sp. ZIPS

5.2.1 Linoleic acid

Compound **89** was obtained as low polar colourless UV absorbing oil, which stained to blue by anisaldehyde/sulphuric acid. This colour reaction was a hint for a terpene, steroid or unsaturated fatty acid. The ^{1}H NMR spectrum exhibited a broad 1H singlet at δ 11.2 of a free carboxylic acid group. In addition, a 4H multiplet was observed

between δ 5.41-5.25 (m, 4 H, 9,10,12,13-CH), which could be assigned to two olefinic double bonds. Furthermore, a 2H triplet of methylene group adjacent most likely to a sp^2 carbon was displayed at δ 2.78 (t, ^3J = 6.0 Hz, 2 H, 11-CH_2). Furthermore, a triplet of two magnetically equivalent methylene groups likely adjacent to sp^2 carbons was displayed at δ 2.38 (t, ^3J = 7.2 Hz, 2 H, 2-CH_2). Additionally, two multiplets were observed at δ 2.08 (m, 4 H, 8,14-CH_2) and 1.63 (m, 4 H, 3-CH_2), each of 4H as of two methylene groups; the first of them could be linked to a sp^2 carbon. Moreover, a multiplet of 10 protons was observed in the range of δ 1.40-1.20, pointing to a side chain of 5 methylene groups. At the end, a 3H triplet was observed at δ 0.85 (m, 3 H, 18-CH_3). This agrees with linoleic acid. The structure was further confirmed by comparison with authentic spectra as well as literature data.

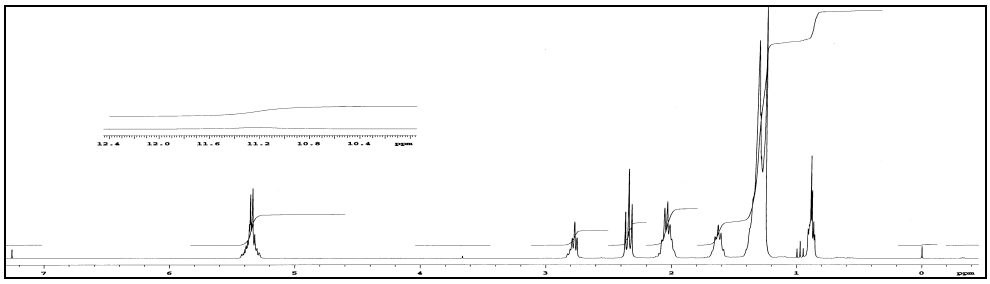
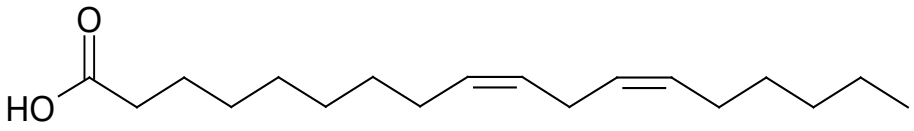


Figure 84: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of linoleic acid (**89**)

Linoleic acid (**89**) is known as a constituent of most vegetable and animal fats and is used biosynthetically as essential fatty acid for the production of prostaglandin, and was frequently isolated from marine brown algae. [288] Compound **89** was isolated also recently from a *Micrococcus* species using GC/MS methods. [289]



89

5.3 Terrestrial *Streptomyces* sp. 195

The terrestrial *Streptomyces* sp. 195 was cultivated on M_2 agar medium at 28 °C for five days. This culture was used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2 medium (25 L). After incubation at 28 °C on a linear shaker for

seven days, the dark brown culture broth was filtered under vacuum. The biomass was extracted with ethyl acetate followed by acetone. The filtrate was subjected to an XAD-16 column; the resin was washed with distilled water and eluted with methanol. The extracts were concentrated under vacuum to obtain a brown oily crude extract (1.75 g), which showed on TLC a non-absorbing band giving a blue-violet colour with anisaldehyde/sulphuric acid. The crude extract was subjected to silica gel column chromatography (CC) eluting with CH_2Cl_2, followed by stepwise addition of CH_3OH to yield two fractions I and II. Fraction I contained fat and was not further investigated. Fraction II was subjected to Sephadex LH-20 (MeOH) followed by reverse phase RP-18 chromatography to afford (1R,2S,4S)-2-(1-hydroxy-6-methylheptyl)-4-hydroxymethyl-butanolide (**143**).

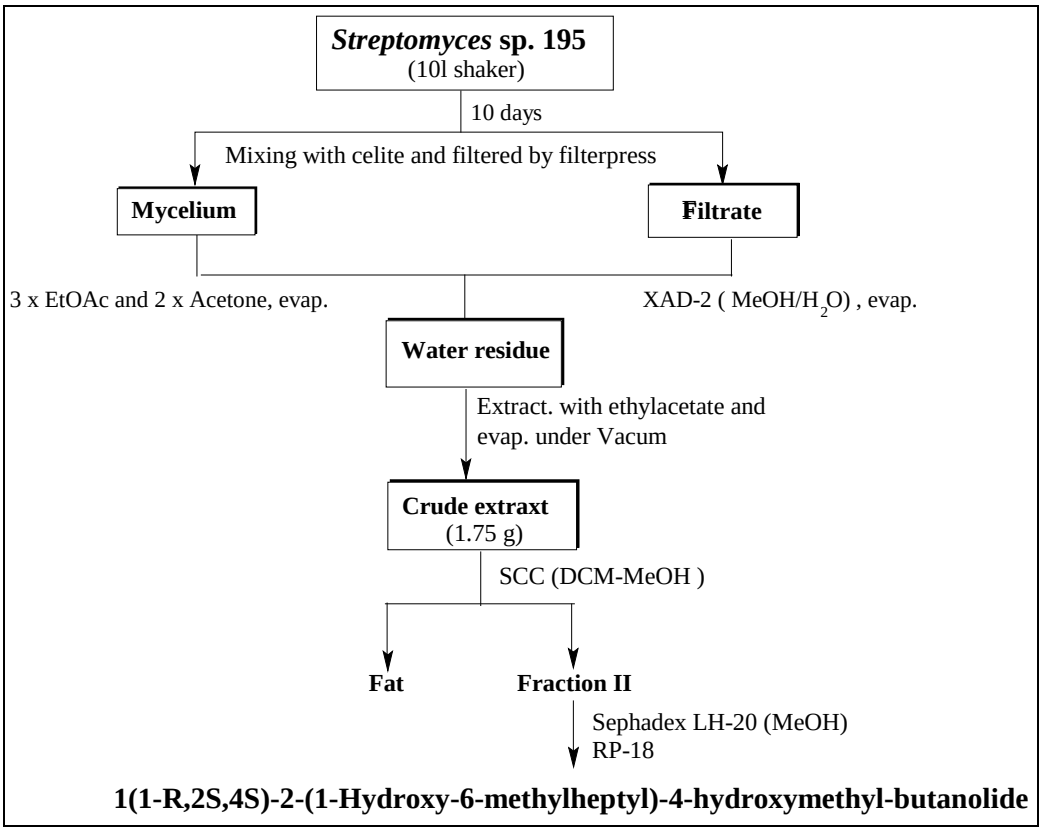


Figure 85: Work-up scheme of bacterial *Streptomyces* sp. 195

5.3.1 5-Hydroxymethyl-3-(1-hydroxy-6-methyl-heptyl)-dihydrofuran-2-one

Compound **143** was isolated as UV inactive pale yellow oil, which turned blue-violet after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **143** displayed methylene protons at δ 4.20 and 4.60 as ABX system; their downfield shift

was explained by their attachment to oxygen. By HREIMS, a molecular formula of $C_{13}H_{24}O_4$ was determined, which agreed with the ^{1}H and ^{13}C NMR data.

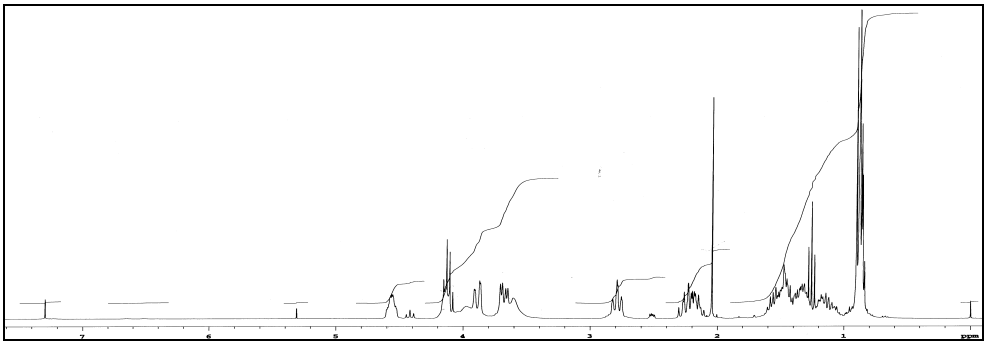


Figure 86: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of 5-hydroxymethyl-3-(1-hydroxy-6-methyl-heptyl)-dihydrofuran-2-one (**143**)

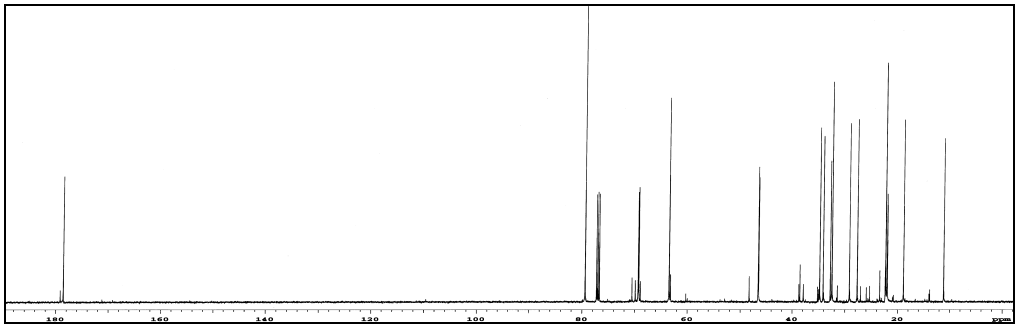
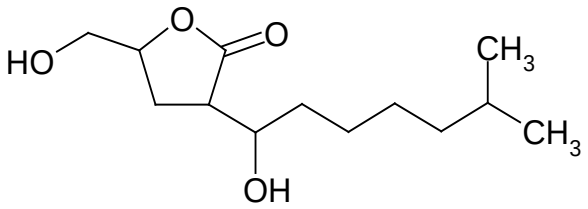


Figure 87: ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of 5-hydroxymethyl-3-(1-hydroxy-6-methyl-heptyl)-dihydrofuran-2-one (**143**)



143

Table 19: ^{1}H NMR and ^{13}C NMR data of 5-hydroxymethyl-3-(1-hydroxy-6-methyl-heptyl)-dihydrofuran-2-one (**143**) in $CDCl_3$

Position	δ_C	δ_H (mult.; *J* in Hz)
1	179.9	–
2	46.3	2.78 (ddd, *J* = 2.6 Hz, *J*' = 8Hz, *J*" = 10.5 Hz, lH)
3	23.0	2.05 (ddd, *J* = 4Hz, *J*' = 10 Hz, *J*" = 12.8 Hz, 1H)
		2.35 (ddd, *J* = J' = 8 Hz, *J*" = 12.8 Hz, lH, H_α-3)
4	80.0	4.60 (m, 1H)
5	64.4	3.55 (dd, *J* = 7Hz, *J*' = 2.5Hz, lH)
1´	69.3	4.08 (m, 1H, br)
2´	34.8	1.05- 1.55 aliphatic side chain
3´	32.5	1.05- 1.55 aliphatic side chain
4´	27.7	1.05- 1.55 aliphatic side chain
5´	22.4	3.83 (dd, 1H, *J* = 7 Hz, *J*' = 1.5 Hz, H-5')
6´	29.2	(lH)
Me-7´	22.5	0.81 (d, *J* = 7.2 Hz, 3H)
Me-8´	22.5	0.81 (d, *J* = 7.2 Hz, 3H)

5-Hydroxymethyl-3-(1-hydroxy-6-methyl-heptyl)-dihydrofuran-2-one (**143**) is similar to the previously described virginiae butanolides, but possesses a different substitution pattern in the γ-lactone ring. Total synthesis and absolute configuration of this compound were established and determined by Pathirana *et al.* [290] The examination of the isolated compound **143** exhibited high cytotoxic activity against brine shrimps (*Artemia salina*).

5.4 Terrestrial *Streptomyces* sp. Ank 315

The strain *Streptomyces* sp. Ank 315 formed grey mycelial colonies on M_2 agar after 10 days at 28 °C.

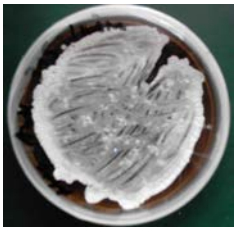


Figure 88: Culture of *Streptomyces* sp. Ank 315 on agar plate

The strain *Streptomyces* sp. Ank 315 was selected due to its biological activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, the fungus *Mucor miehei* (Tü284) and the yeast *Candida albicans.*

The *Streptomyces* sp. Ank 315 was cultivated on 25 L and 60 L scale using 1L Erlenmeyer flasks containing 250 mL of M_2 medium at 28 °C for 8 days on a linear shaker (250 rpm).

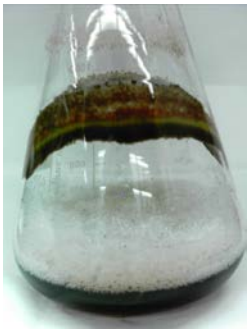


Figure 89: Culture of *Streptomyces* sp. Ank 315 in M_2 liquid medium

The culture broth was mixed with Celite and filtered with a filter press. The filtrate was passed through an Amberlite XAD-16 column, the resin was washed water and eluted with methanol. The methanol phase was concentrated and the aqueous residue was extracted with ethyl acetate. The mycelium was extracted sequentially with ethyl acetate and then acetone. The extracts showed similar compositions on TLC and were combined. They were further chromatographed in different ways to get their constituents in pure form.

Six new chromophenazines A-F (**144-152**) [9-methyl-5-(3'-methyl-but-2'-enyl)-5*H*-benzo[a]phenazine-7-one (**144**), 9-methyl-5-(3'-methyl-but-2'-enyl)-7-oxo-5,7-dihydro-benzo[a]phenazine-1-carboxylic acid (**145**), 5-(3'-methyl-but-2'-enyl)-7-oxo-5,7-dihydro-phenazine-1-carboxamide (**146**), 3-benzoyl-5-(3'-methylbut-2'-enyl)-5,10-dihydro-phenazine-1-carboxylic acid (**147**), 3,7-dibenzoyl-5-(3'-methyl-but-2'-enyl)-5,10-dihydro-phenazine-1-carboxylic acid (**151**), and 3,7-dibenzoyl-5-(3'-methyl-but-2'-enyl)-5,10-dihydro-phenazine-1-carboxamide (**152**)], together with phenazine-1-carboxylic acid (**43**), anthranilic acid (**113**), tryptophol (**119**) and 1-phenazinol (**139**) were isolated from *Streptomyces* sp. Ank 315.

Compounds **144** and **145** were obtained from the 25 L cultivation, whereas compounds **146, 147, 151** and **152** were isolated from the 60 L cultivation. Details of the isolation are shown in Figure 90 and Figure 91.

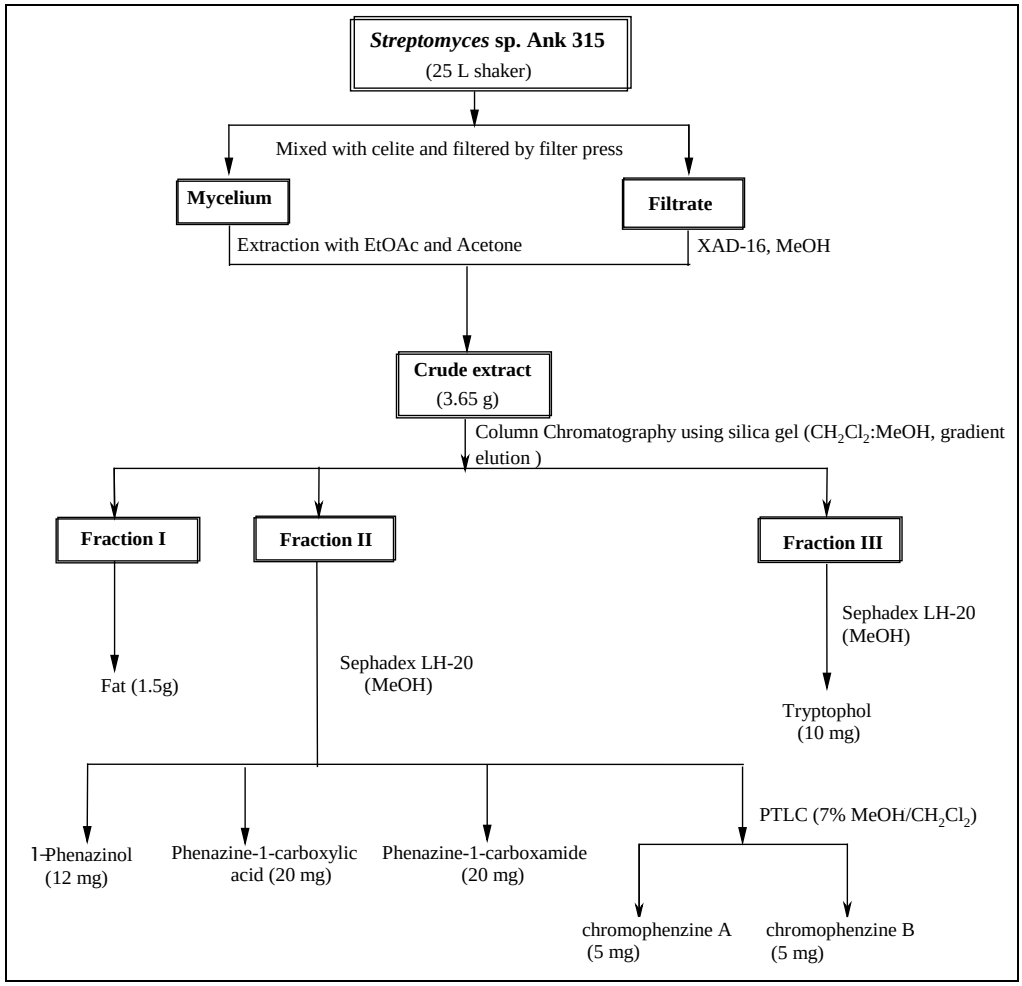


Figure 90: Isolation of chromophenazine A (**144**) and chromophenazine B (**145**)

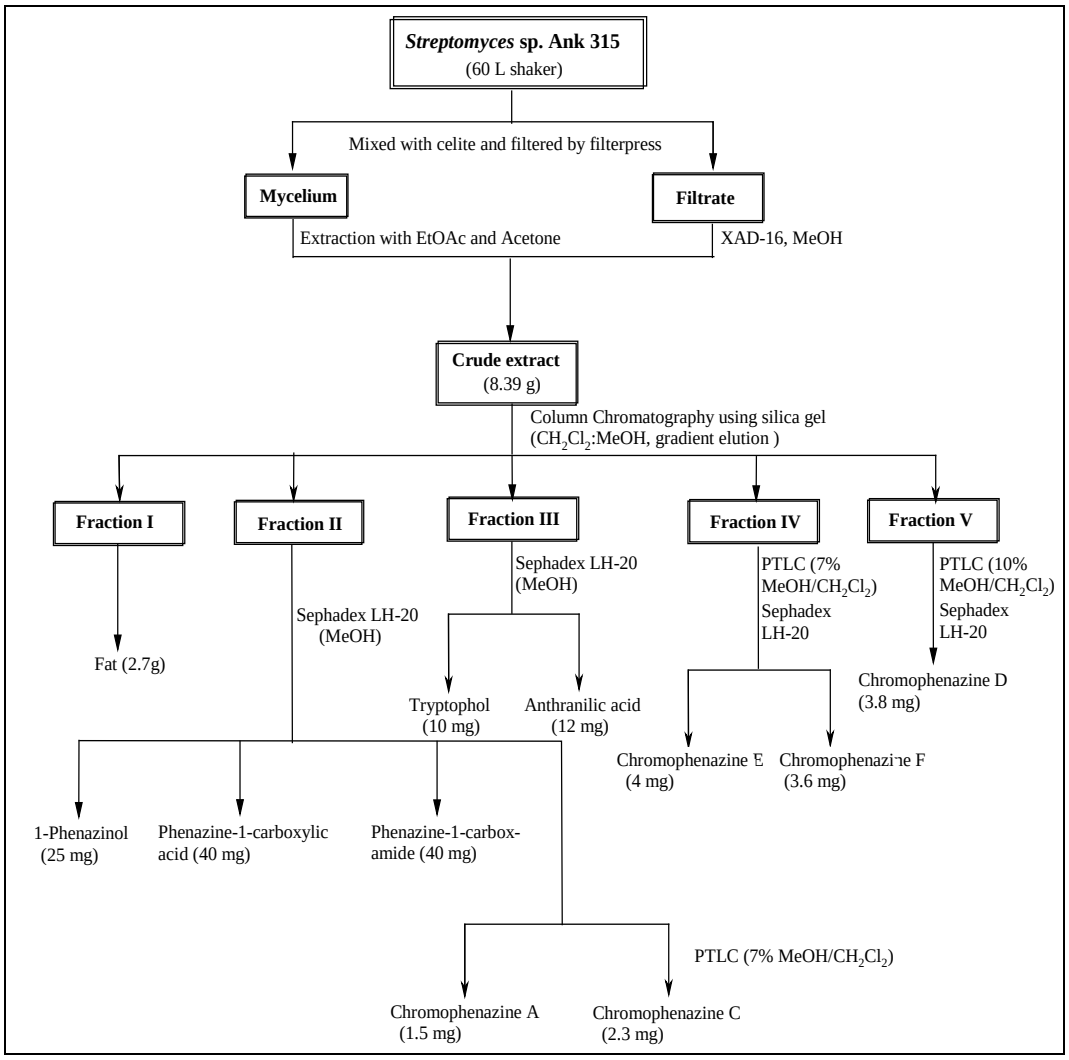


Figure 91: Isolation of chromophenazines C (**146**) - F (**152**)

5.4.1 Chromophenazine A

Fraction II was subjected to Sephadex LH-20 in methanol, where the sub-fraction three showed by PTLC monitoring an intense orange fluorescence under UV at 366 nm. The coloured zone became colourless on TLC after spraying with anisaldehyde/sulphuric acid.

Compound **144** was isolated as an orange powder with long-wavelength absorptions at λ_{max} 463 sh, 490, and 519 sh nm. It showed an intense orange fluorescence on TLC under UV at 366 nm. The molecular mass was determined by ESIMS in the positive

and negative mode, respectively, and the molecular formula $C_{22}H_{20}N_2O$ resulted by HRESIMS of the $[M+H]^+$ signal at *m/z* 329.

The ^1H NMR spectrum of **144** (Table 20) displayed eight aromatic protons of disubstituted and trisubstituted aromatic systems: The first pattern showed partially overlapping *ortho*-coupled 1H doublets at δ 7.96 (dd, J = 7.9, 1.5 Hz) and 7.32 (d, J = 7.9 Hz) and triplets at δ 7.36 and 7.56, indicating a 1,2-disubstituted benzene ring. The second pattern indicated with 1H doublets at δ 8.74 (J = 8.2 Hz), 8.14 (J = 1.7 Hz) and a dd signal at δ 7.54 (J = 8.2, 1.7 Hz) a 1,3,4-trisubstituted benzene ring. An additional 1H singlet was observed at δ 6.12, and a 3H singlet at δ 2.52 indicated the presence of an *ar*-Me group.

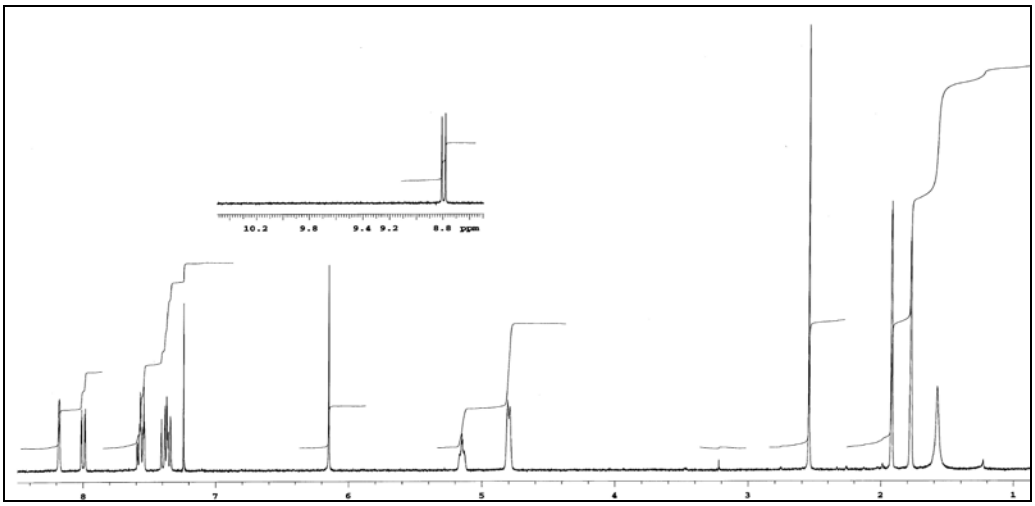


Figure 92: ^1H-NMR-spectrum (300 MHz, $CDCl_3$) of chromophenazine A (**144**)

The ^{13}C NMR and HSQC spectra of **144** (Table 20) disclosed in total 22 carbon signals for three methyls, one methylene, nine sp^2 methine and nine quaternary sp^2 carbon atoms. The downfield signal at δ 181.9 was due to an α,β-unsaturated ketone. The signals at δ 138.2 (C_q-3'), 116.5 (C-2'; δ_H 5.14, m), 45.7 (C-1'; δ_H 4.76, d, J = 5.4 Hz, CH_2), 25.6 (δ_H 1.77, Me), and 18.7 (δ_H 1.91, Me) pointed to the presence of a prenyl group, which was obviously connected to nitrogen, as the shift of the methylene group indicated. [291,292] Respectively, there were HMBC cross signals from the 1'-CH_2 group to C-4a and C-5a, indicating an N-prenylated diphenylamine derivative.

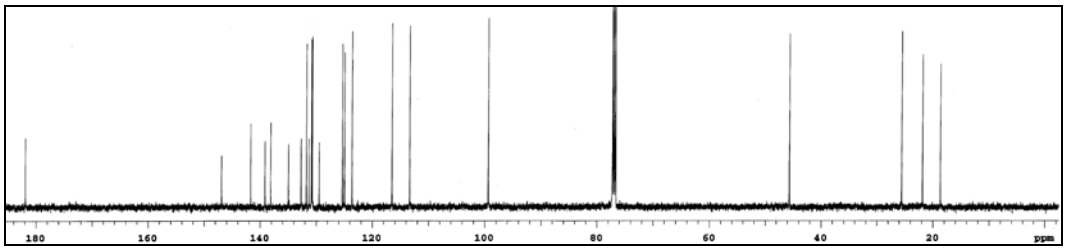


Figure 93: ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of chromophenazine A (**144**)

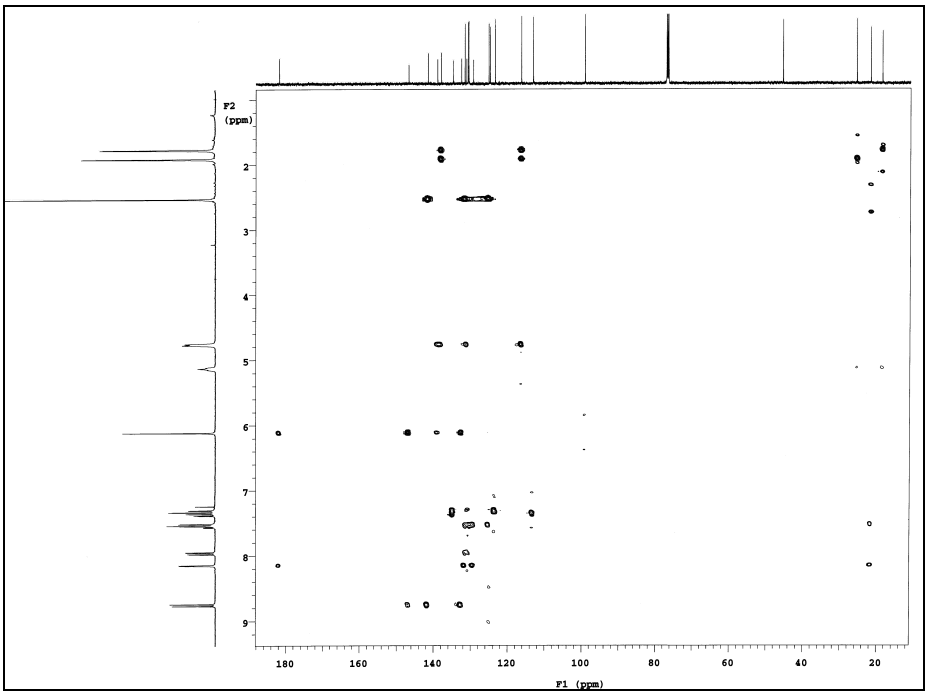


Figure 94: HMBC spectrum (300 MHz, $CDCl_3$) of chromophenazine A (**144**)

The position of the prenyl unit was further confirmed by 1D NOE experiments, in which the irradiation into the methylene signal (δ 4.80) showed an enhancement of the H-4 (δ 7.32) and H-6 (δ 6.14) signals.

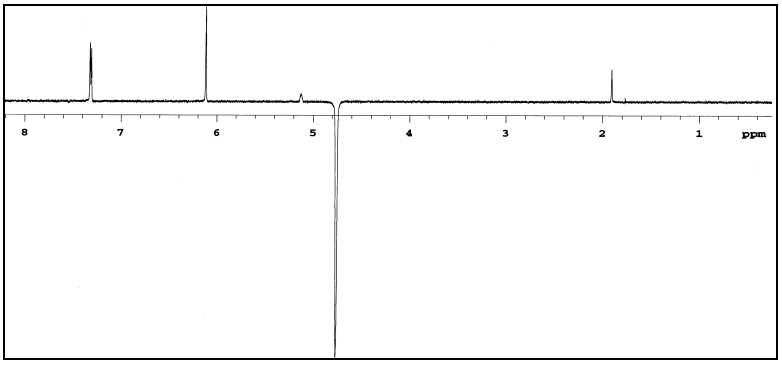


Figure 95: 1D NOE spectrum (125 MHz, $CDCl_3$) of chromophenazine A (**144**)

To explain the colour, a ring closure forming a phenoxazinone, a 10*H*-acridin-3-one or a 10*H*-phenazin-2-one must be assumed. While phenoxazinones (the chromophore of actinomycins) are already excluded by the elemental composition and 10*H*-acridin-3-ones have never been isolated from nature, the UV data of **144** resembled strongly those of endophenazine B (**38**).[30] The 2D NMR data confirmed indeed a phenazin-2-one skeleton: According to the COSY data and the signal multiplicity, ring A was *ortho*-disubstituted. The connection of the remaining atoms followed mainly from HMBC cross signals of H-6 with C-5a, 7, 7a and 11b. The *meta*-coupling proton H-8 correlates with the carbonyl C-7. All other correlations were in full agreement with structure **A** (Table 20). Compound **144** was subsequently elucidated as 9-methyl-5-(3'-methyl-but-2'-enyl)-5*H*-benzo[a]phenazin-7-one (name according to IUPAC rules), for which we suggest the name chromophenazine A (**144**).

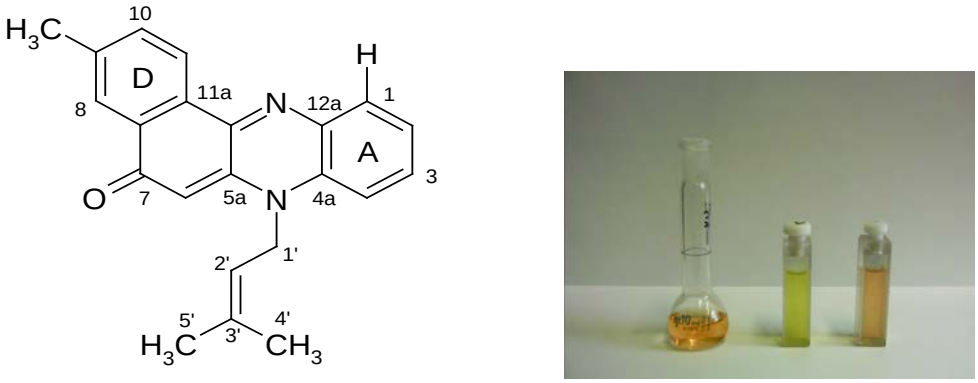


Figure 96: Structure of chromophenazine A (**144**) and solutions in neutral, acidic, and basic methanol (from left to right)

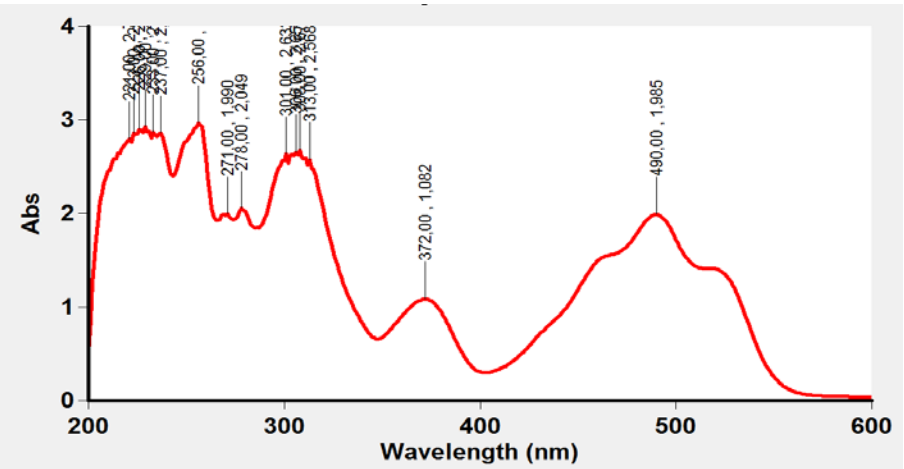


Figure 97: UV spectrum of chromophenazine A (**144**) in CD_3OD

Table 20: ^{1}H and ^{13}C NMR data HMBC and COSY correlations of chromophenazines A (**144**) in $CDCl_3$

Position	Chromophenazine A (**144**)			
	δ_H[a] (*J* in Hz)	δ_C[b]	HMBC[c] (H→C)	COSY[c] (H↔H)
1	7.96 (dd, 7.9, 1.5)	130.8	3, 4a	H2
2	7.36 (t, 7.9)	123.6	4,12a	H1/H3
3	7.56 (t, 7.9)	130.9	4a	H2/H4
4	7.32 (d, 7.9)	113.4	2,3,12a	H3
4a	–	131.3	–	–
5a	–	139.2	–	–
6	6.12 s	99.4	5a,7, 7a,11b	–
7	–	181.9	–	–
7a	–	132.7	–	–
8	8.14 (d, 1.7)	125.4	10,11a, 9-Me	H9-Me
9	–	141.7	–	–
9-Me	2.52 s	21.9	8, 9,10	H8
10	7.54 (dd, 8.2, 1.7)	131.8	8, 11a, 9-Me	H11
11	8.74 (d, 8.2)	125.0	7a, 9,11b	H10
11a	–	129.5	–	–
11b	–	146.9	–	–
12a	–	135.0	–	–
1'	4.76 (d, 5.4)	45.7	4a,5a,2'	H2'/H4'/H5'
2'	5.14 m	116.5	4',5'	H1'
3'	–	138.2	–	–
4'	1.77 s	25.6	2',3',5'	H1'/H5'
5'	1.91 s	18.7	2',3',4'	H1'/H4'

[a] ^{1}H NMR spectra were recorded at 300 MHz; [b] ^{13}C NMR spectra were recorded at 125 MHz; [c] HMBC and COSY spectra were recorded at 600 MHz

5.4.2 Chromophenazine B

A second minor component **145** was isolated as orange powder as well, with intense orange fluorescence on TLC under UV (366 nm). The UV and NMR data were very

similar to those of **144**, but ring A was now 1,2,3-trisubstituted instead of 1,2-disubstituted, as two clearly separated doublets and a triplet in the aromatic range indicated (Table 21). The molecular formula $C_{23}H_{20}N_2O_3$ (determined by HRESIMS of the *pseudo*-molecular ion peak at *m/z* 373.15459 ($[M+H]^+$) differs by CO_2 from **144**, and the proton data pointed to a carboxylic group at position 1 or 4 in ring A. ^{13}C data could not be measured due to the small available amount; there was an NOE signal between CH_2-1' and H-6, confirming the prenyl residue again at N-5; the expected correlation with H-4 was, however, not visible. Our assignment of chromophenazine B as 9-methyl-5-(3'-methyl-but-2'-enyl)-7-oxo-5,7-dihydrobenzo[a]phenazine-1-carboxylic acid (**145**) is therefore based solely on the similarity of the shifts of H-2 – H-4 with the respective protons in **146**.

For the biosynthesis of **144** and **145**, a 5,9-diprenylated phenazine precursor related to **38** or to the aglycone of aestivophoenin C [293] can be assumed, whose cyclization would give rise to ring D in **144** and **145**, respectively.

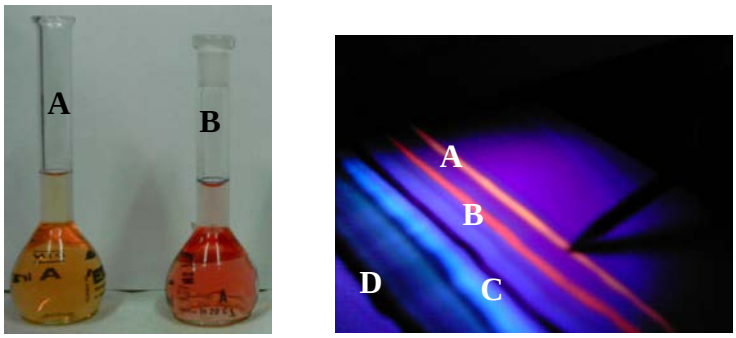


Figure 98: **A:** Chromophenazine A (**144**), **B:** Chromophenazine B (**145**) (left) in MeOH and (right) under UV at 366 nm; C: anthranilic acid. D: starting line.

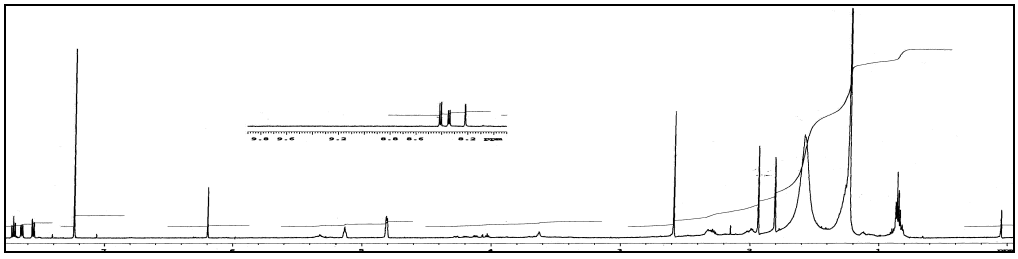
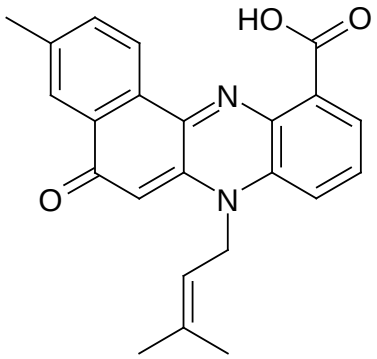


Figure 99: ^1H NMR spectrum (600 MHz, $CDCl_3$) of chromophenazine B (**145**)



145

Table 21: ^{1}H NMR data of chromophenazines B (**145**) in $CDCl_3$

Position	chromophenazine B (145)	Position	chromophenazine B (145)
	δ_H[a] (*J* in Hz)		δ_H[a] (*J* in Hz)
1	–	9-Me	2.58 s
2	8.34 (d, 8.1)	10	7.56 (d, 8.4)
3	7.71 (t, 8.2)	11	8.41 (d, 8.2)
4	7.64 (d, 8.3)	11a	–
4a	–	11b	–
5a	–	12a	–
6	6.19 s	1'	4.80 (d, 5.4)
7	–	2'	5.13 (t, 5.5)
7a	–	3'	–
8	8.22 s	4'	1.80 s
9	–	5'	1.93 s

[a] ^{1}H NMR spectrum was recorded at 300 MHz

5.4.3 Chromophenazine C

The violet chromophenazine C (**146**) showed absorptions at 224, 281, 361 and 531 nm; the molecular formula $C_{18}H_{17}N_3O_2$ was established by HRESIMS of the *pseudo*molecular ion peak $[M+H]^+$ at *m/z* 308. The ^{1}H NMR spectrum of **146** (Table 22) displayed signals of a 1,2,3-trisubstituted benzene ring with similar shifts as for **145**. In addition, a doublet at δ 7.58 (1H, d, J = 7.6 Hz), a doublet of a doublet at δ 7.12

(1H, J = 7.6, 1.2 Hz) and a narrow doublet at δ 6.16 (1H, d, J = 1.2 Hz) pointed to a 1,2,4-trisubstituted benzene ring. The presence of an *N*-prenyl group was again indicated by signals at δ 5.10 (1H, m), 4.81 (2H, d, J = 4.8 Hz), 1.96 (3H, s), and 1.78 (3H, s).

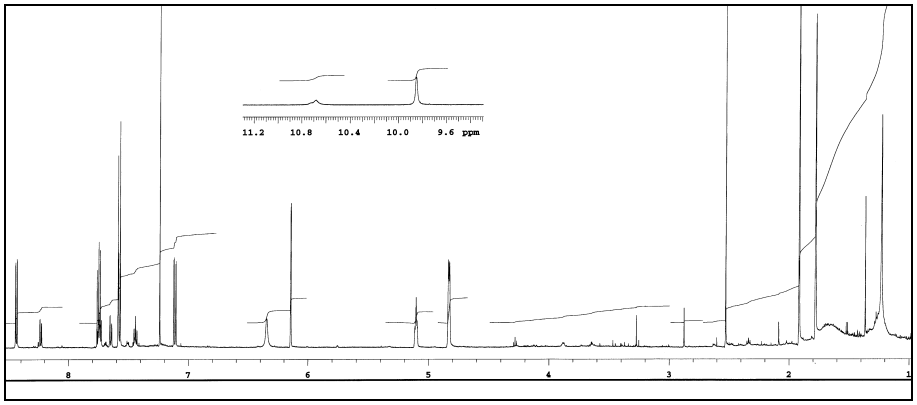


Figure 100 ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of chromophenazine C (**146**)

The ^{13}C NMR/HSQC data of **146** (**Table 22**) disclosed 18 carbon signals for two methyls, one methylene, seven methines and eight quaternary carbon atoms. The downfield signals at δ 183.9 and 166.3 indicated the presence of a conjugated ketone and an amide or acid group. In comparison with **144** and **145**, the annulated benzene ring D was missing in **146**. The position of the prenyl group was the same as in **144** and **145**, as CH_2-1' (δ 4.81) showed $^{3}J_{C,H}$ correlations with C-4a (δ 131.8) and C-5a (δ 137.1). This was again supported by an NOE enhancement of H-4 (δ 7.58) and H-6 (δ 6.16) after irradiation of CH_2-1' (δ 4.81) and CH-2'.

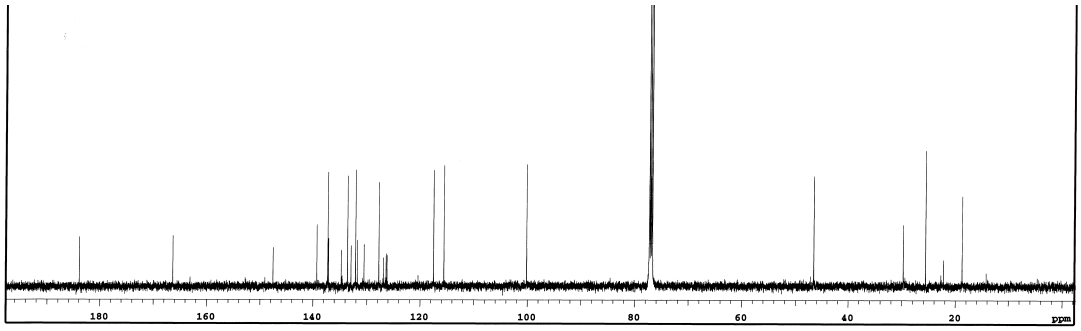
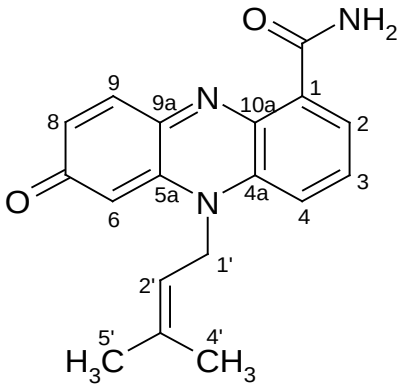


Figure 101: ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of chromophenazine C (**146**)



146

The amide carbonyl (δ 166.3) showed an HMBC correlation with H-2 (δ 8.42) thus confirming the position at C-1. The acidic amide proton at δ 6.46 showed a COSY cross signal with the second amide proton at 9.86 and additionally a weak HMBC correlation with C-1. On the basis of these data, chromophenazine C must be 5-(3'-methyl-but-2'-enyl)-7-oxo-5,7-dihydrophenazine-1-carboxamide (**146**).

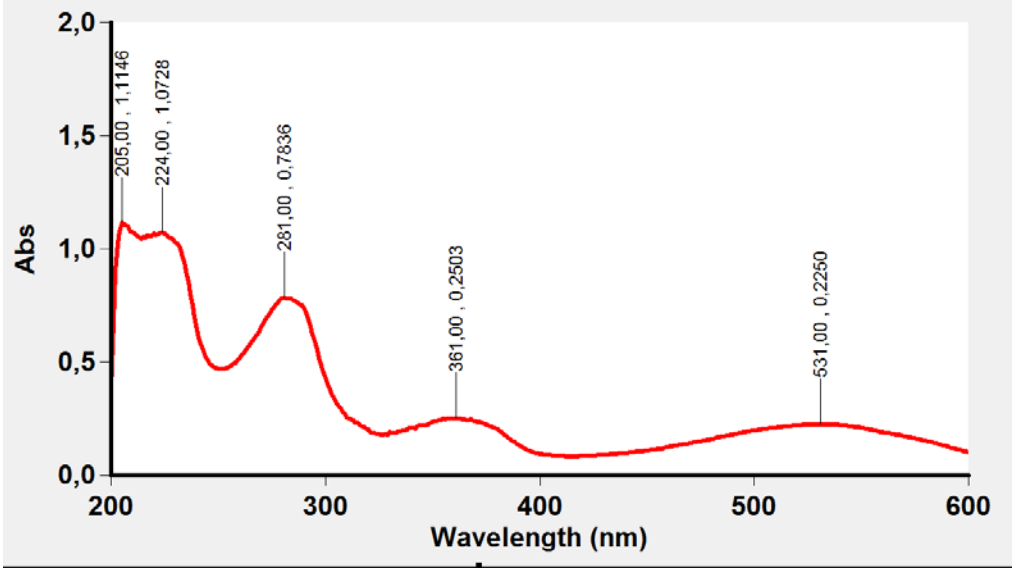


Figure 102: UV/Vis spectrum of chromophenazine C (**146**)

As chromophenazine C (**146**) and endophenazine B (**38**) are having the same chromophore, the similarity of their absorption spectra (λ_{max} = 531 for **146** and 545 nm sh for **38**, respectively) is expectable; the colour of the chromophore has also been confirmed by synthetic products. [294] DFT calculations [295] explained also why **144** and **145** absorbed at shorter wavelength than **146**, as their HOMO/LUMO energy difference is substantially larger.

Table 22: ^{1}H and ^{13}C NMR data HMBC and COSY correlations of chromophenazine C (**146**) in $CDCl_3$

	Chromophenazine C (**146**)			
Position	δ_H[a] (*J* in Hz)	δ_C[b]	HMBC[c] (H→C)	COSY[c] (H↔H)
1	–	130.5	–	–
2	8.42 (d, 7.6)	127.8	4, 4a,10a, $CONH_2$	H3/H4
3	7.75 (t,7.6)	132.1	1,2,4,4a,10a	H2/H4
4	7.58 (d,7.6)	117.5	1,2,10a	H2/H3/H1'
4a	–	131.8	–	–
5a	–	137.1	–	–
6	6.16 (d, 1.2)	100.2	5a,7,9a	H1'/H8
7	–	183.9	–	–
8	7.12 (dd, 7.6, 1.2)	137.2	6,9a	H6/H9
9	7.58 (d, 7.6)	133.6	5a,7	H8
9a	–	147.5	–	–
10	–	–	–	–
10a	–	132.9	–	–
1'	4.81 (d, 4.8)	46.5	2',3',4a,5a	H2'/H4/H6/H4'/H5'
2'	5.10 (m)	115.5	4',5'	H1'/H4'/H5'
3'	–	139.3	–	–
4'	1.96 (s)	18.8	2', 3', 5'	H1'/H2'
5'	1.78 (s)	25.6	2', 3', 4'	H1'/H2'
1"	–	–	–	–
2",6"	–	–	–	–
3",5"	–	–	–	–
4"	–	–	–	–
C = O	–	–	–	–
COR^1	–	166.3	–	–
NH_2	9.86, 6.35 (2s)	–	–	–

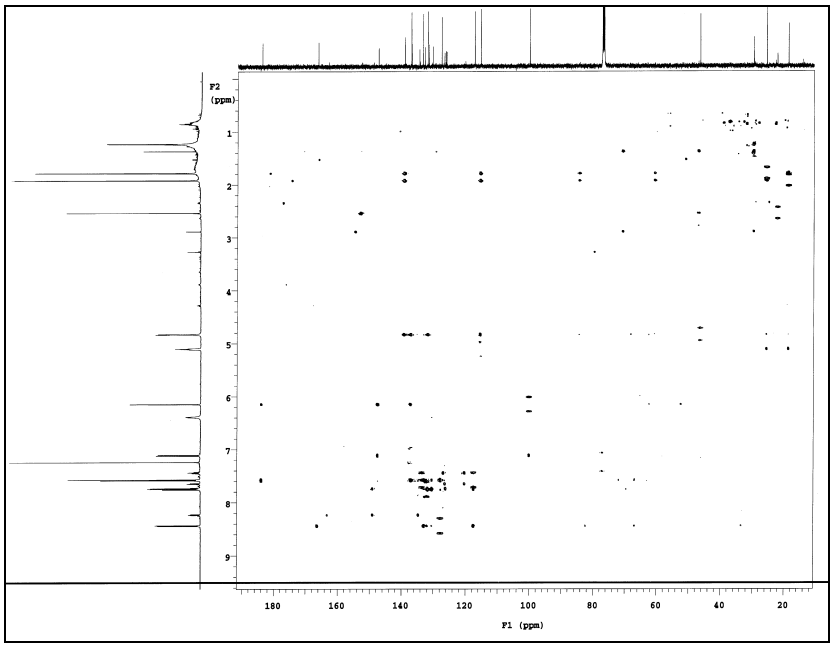


Figure 103: HMBC spectrum (600 MHz, $CDCl_3$) of chromophenazine C (**146**)

The 10*H*-phenazin-2-ones are keto-tautomers of 2-hydroxyphenazines. corresponding to *semi*-empirical calculations, [328] the phenolic hydroxyphenazine tautomers are more stable than the keto forms, and so it is understandable that amongst the about 30 natural 2/3-hydroxyphenazines, only one was claimed to be the keto tautomer: in the violet endophenazine B (**38**), [296] N-5 is alkylated as in **144-145**, so that a rearrangement to the more stable phenol is blocked. A single N-5 monosubstituted 3-phenazinol has been described, but in the dark red 1,8-phenazinediol-10-oxide,[297] the long-wavelength absorption at 540 nm is perhaps better explained by the tautomeric 9,10-dihydroxy-10*H*-phenazin-2-one form.

5.4.4 Chromophenazine D

Chromophenazine D (**147**) was isolated as a dark red solid with absorption bands at 389 and 491 nm in the visible range. The broad signal at 3446 cm^{-1} in the IR spectrum gave a hint for the presence of a carboxylic group, which was confirmed by a CO signal at δ 169.0. The molecular formula $C_{25}H_{22}N_2O_3$ was established by HRESIMS of the *pseudo*-molecular ion peak $[M-H]^-$ at *m/z* 397. The NMR spectra of **147** (Table 23) indicated again the presence of a prenyl group as in **144-146**, and an additional benzoyl residue (Figure 104).

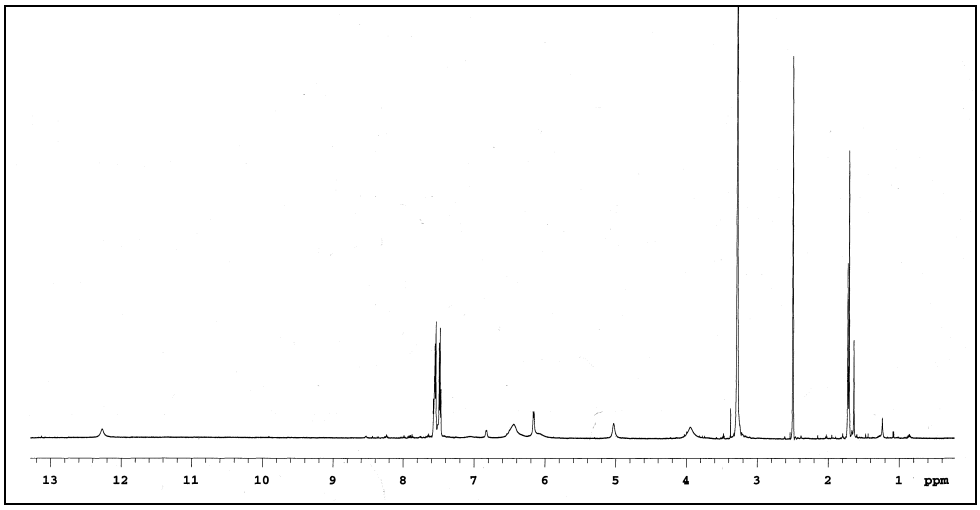


Figure 104: ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of chromophenazine D (**147**)

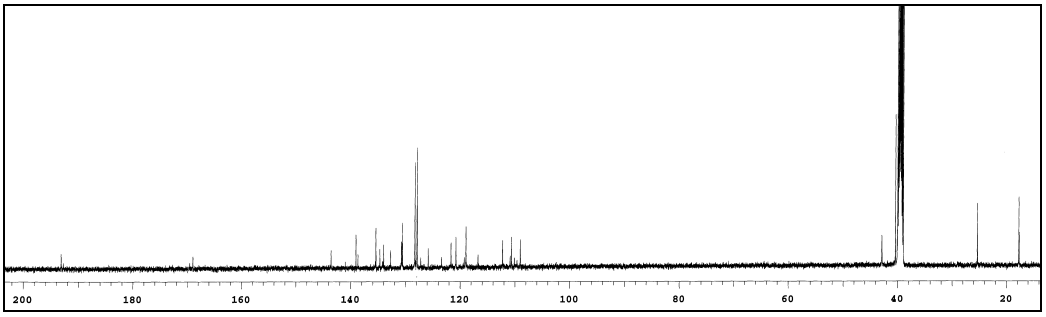


Figure 105: ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of chromophenazine D (**147**)

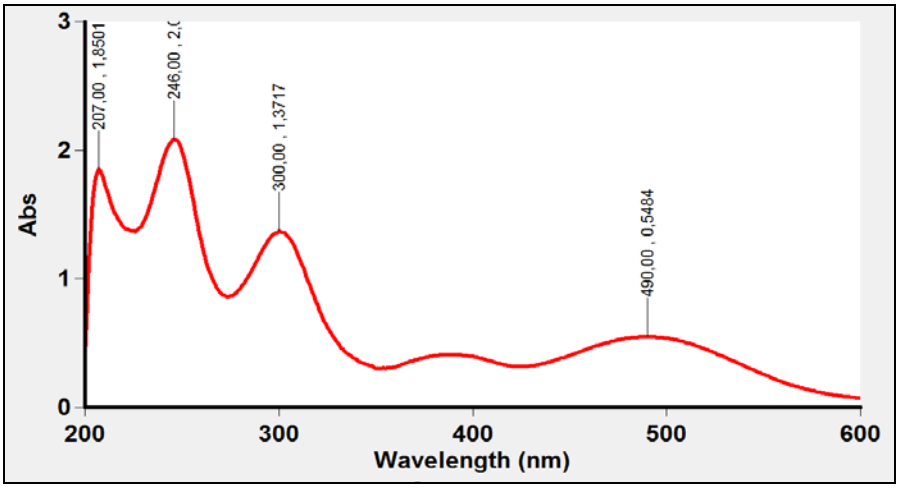


Figure 106: UV/Vis spectrum of chromophenazine D (**147**) in CD_3OD

A search in AntiBase [115] with an *N*-prenyldihydrophenazine substructure, a benzoyl fragment and a carboxy group resulted in aestivophoenins A (**148**), [298] B (**149**) and benthophoenin (**150**), respectively. The position of the carboxylic acid, benzoyl and prenyl moieties were confirmed to be the same as in **148** by 2D NMR spectra, especially ^{1}H,^{1}H COSY and HMBC correlations (Table 23). In the HMBC spectra, H-2 (δ 7.51) showed a ^{3}J correlation with the acid carbonyl (δ 169.0) confirming its position at C-1, and the attachment of the benzoyl group at C-3 was obvious from the ^{3}J correlation of H-6 (δ 6.16) and H-8 (δ 6.43) with the benzoyl carbonyl (δ 193.1). According to these data, chromophenazine D (**147**) was confirmed as 3-benzoyl-5-(3'-methylbut-2'-enyl)-5,10-dihydrophenazine-1-carboxylic acid, an isomer of the so far undescribed aglycone of aestivophoenin A (**148**).

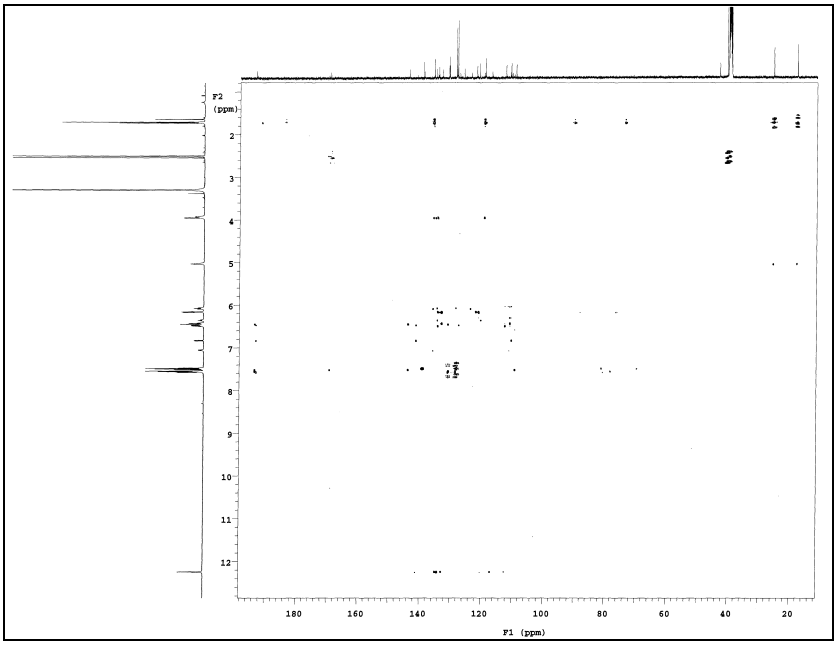
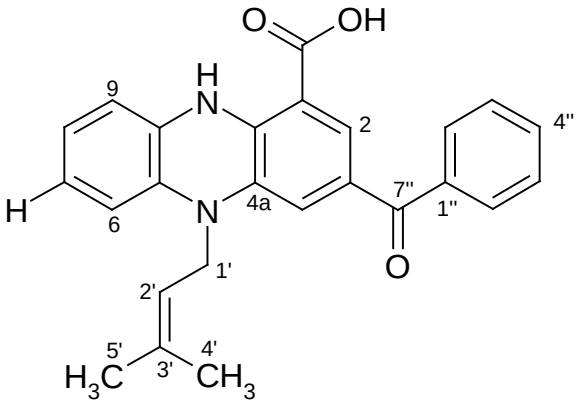
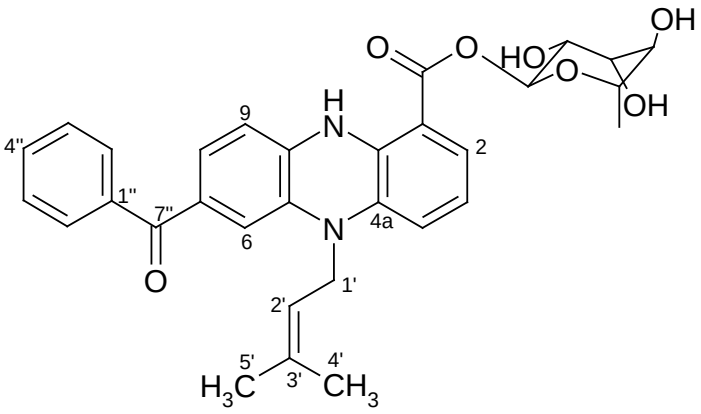


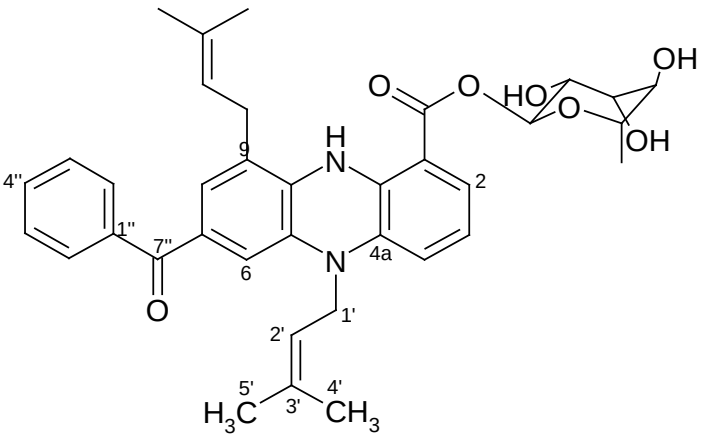
Figure 107: HMBC spectrum (600 MHz, DMSO-d_6) of chromophenazine D (**147**)


O
OH
H
N
9
2
4''
H
6
N
4a
7''
1''
1'
O
2'
5'
4'
3'
H3C
CH3

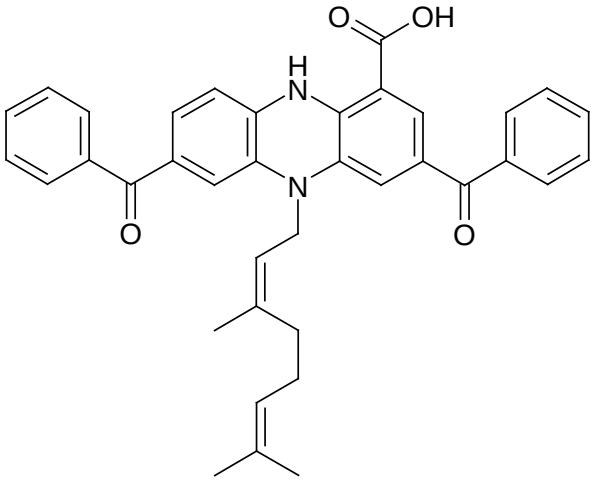
147


OH
O
O
HO
O
OH
H
N
9
2
4''
1''
7''
6
N
4a
O
1'
2'
5'
4'
3'
H3C
CH3

148


OH
O
O
HO
O
OH
H
N
9
2
4''
1''
7''
6
N
4a
O
1'
2'
5'
4'
3'
H3C
CH3

149



150

Table 23: ^{1}H, ^{13}C NMR, HMBC and COSY correlations of chromophenazine D (**147**) in DMSO-d_6

Position	Chromophenazine D (147)			
	δ_H[d] (*J* in Hz)	δ_C[b]	HMBC[c] (H→C)	COSY[c] (H↔H)
1	–	116.7	–	–
2	7.51 (d, 1.8)	130.7	4,10a,CO_2H,C = O	H4
3	–	135.5	–	–
4	6.45 (s br)	109.0	2,10a	H2
4a	–	134.7	–	–
5a	–	134.0	–	–
6	6.16 (d br, 7.7)	110.6	7,8,5a,9a	H7/H8
7	6.49 (t, 7.7)	121.7	5a,9	H6
8	6.43 (t, 7.4)	120.8	6,9a	H6/H9
9	6.15 (d, 7.4)	112.3	7,8,5a,9a	H8
9a	–	132.7	–	–
10	12.24 (s br)	–	1,4a,5a,9,9a,10a	–
10a	–	143.6	–	–
1'	3.95 (d, 4.3)	42.9	4a,5a,2',3'	H2'/H4'/H5'
2'	5.03 (m)	119.0	4',5'	H1'/H4'/H5'
3'	–	135.4	–	–
4'	1.70 (s)	17.8	2',3', 5'	H1'/H2'
5'	1.72 (s)	25.3	2',3', 4'	H1'/H2'
1"	–	139.0	–	–
2",6"	7.55 (m)	128.2	2, 4", C = O	H3",5"/ H4"
3",5"	7.48 (t, 7.0)	127.9	1"	H2", 6"/ H4"
4"	7.56 (m)	130.5	2",6", C = O	H3",5"/ H2", 6"
C = O	–	193.1	–	–
COR[1]	–	169.0	–	–
NH_2	–	–	–	–

[a] ^{1}H NMR spectra recorded at 600 MHz; [b] ^{13}C NMR spectra were recorded at 125 MHz; [c] HMBC and COSY spectra were recorded at 500 MHz; [d] recorded at 300 MHz

5.4.5 Chromophenazine E

For the pink chromophenazine E (**151**), the molecular formula $C_{32}H_{26}N_2O_4$ was deduced by means of HRESIMS of the *pseudo*-molecular ion peak $[M-H]^-$ at *m/z* 501. The ^{1}H NMR spectrum of **151** (Figure 108) showed signals for a 1,2,4-trisubstituted and *meta*-coupled proton signals of a tetrasubstituted benzene ring (Table 24). In addition, signals for two benzoyl groups at δ 7.61-7.55 (10H, signals overlapping; 2 CO at δ 193.4, 193.0) and a carboxy group (δ_{CO} 168.2) were observed (Figure 109). An *N*-prenyl residue was again found, and a singlet at δ 13.1 indicated NH-10 of a further dihydrophenazine. A substructure search [115] resulted in benthophoenin, [325] which had, however, at N-5 a C_{10}-prenyl residue instead of a C_5-prenyl group.

Figure 108: ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of chromophenazine E (**151**)

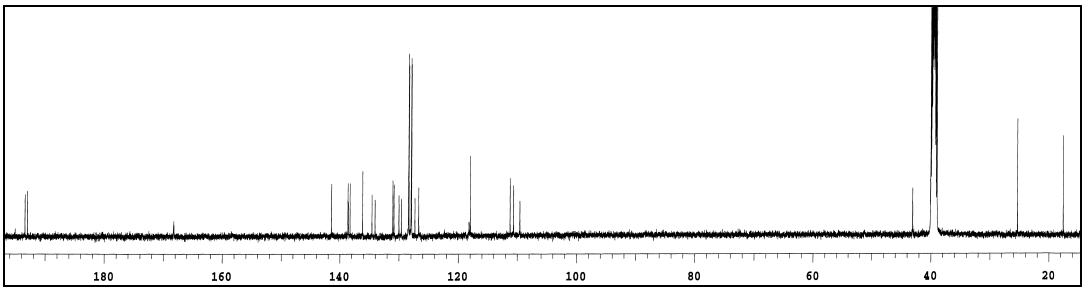


Figure 109: ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of chromophenazine E (**151**)

Irradiation into the allyl methylene group C-1' caused an enhancement of the signals of H-4 and H-6, so that N-5 and not N-10 must be prenylated as in the previous chromophenazines (Figure 110).

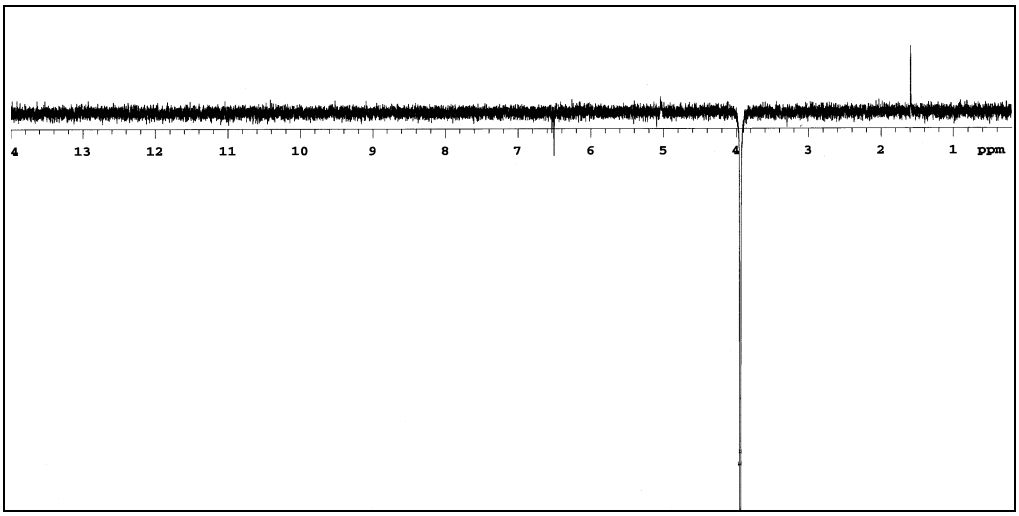


Figure 110: NOE spectrum (600 MHz, DMSO-d_6) of chromophenazine E (**151**)

The attachment of the carboxy group at C-1 and a benzoyl group at C-3 was confirmed by HMBC spectra, in which H-2 (δ 7.51) showed ^3J correlations with the acid carbonyl (δ 168.2) and the benzoyl carbonyl (δ 193.4). The position of the second benzoyl residue at C-7 was derived from ^3J correlations of H-6 (δ 6.53) and H-8 (δ 6.87) with the carbonyl at δ 193.0 (Figure 112).

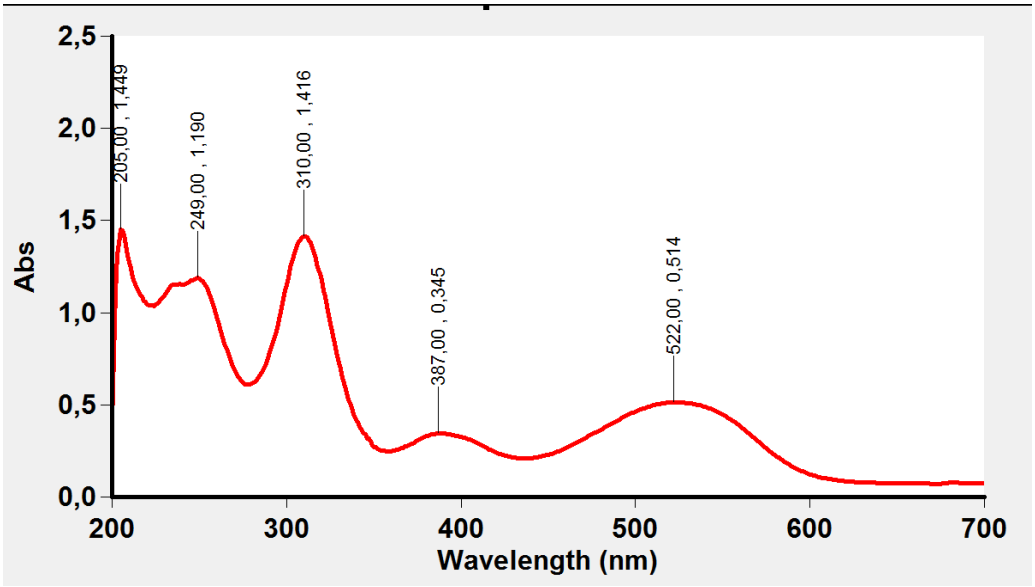


Figure 111: UV/vis spectrum of chromophenazine E (**151**) in CD_3OD

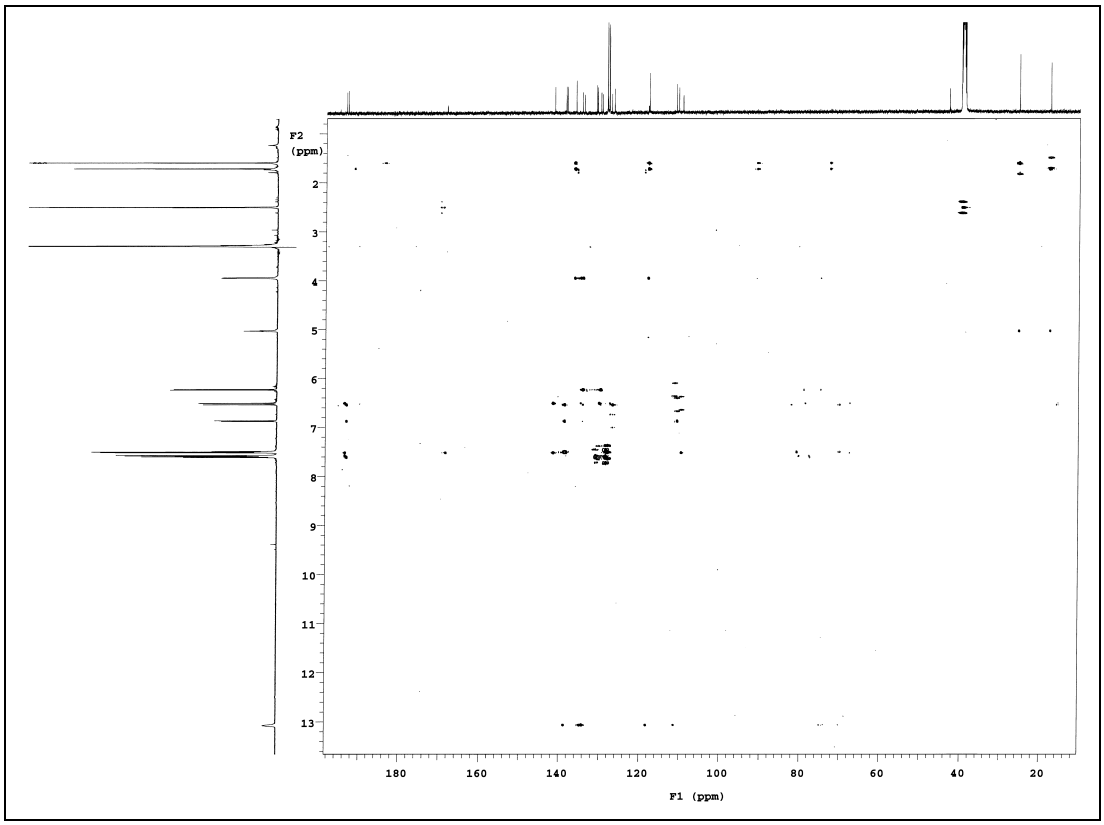
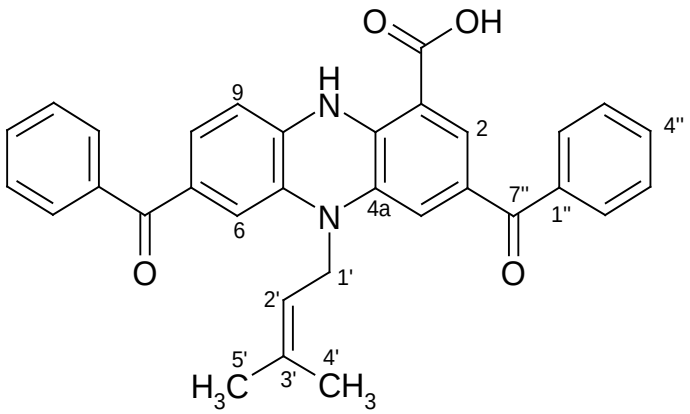


Figure 112: HMBC spectrum (600 MHz, DMSO-d_6) of chromophenazine E (**151**)

Hereby the structure of chromophenazine E was established as 3,7-dibenzoyl-5-(3-methyl-but-2-enyl)-5,10-dihydrophenazine-1-carboxylic acid (**151**).



151

Table 24: ^{1}H and ^{13}C NMR data, HMBC and COSY correlations of chromophenazine E (**151**) in DMSO-d_6

	Chromophenazine E (151)			
Position	**δ_H[a] (*J* in Hz)**	**δ_C[b]**	**HMBC[c] (H→C)**	**COSY[c] (H↔H)**
1	–	118.2	–	
2	7.51 (d, 1.2)	130.0	4,9a,10a, 7"	H4
3	–	130.9	–	–
4	6.50 (d, 1.8)	109.6	2,4a,10a, 7"	H2
4a	–	134.6	–	–
5a	–	134.1	–	–
6	6.53 (d, 1.1)	110.6	8,5a,9a,7"'	H8
7	–	129.6	–	–
8	6.87 (dd, 7.9,1.8)	126.7	6,9a,7"'	H6/H9
9	6.23 (d, 7.9)	111.2	5a,7	H8
9a	–	138.6	–	–
10	13.12 (s)	–	1,4a,5a,9,9a	–
10a	–	141.5		–
1'	3.94 (d, 5.4)	43.0	4a, 5a,2',3'	H2'/H4'/H5'
2'	5.03 (th, 5.9, 1.4)	117.9	4', 5'	H1'/H4'/H5'
3'	–	136.2	–	–
4'	1.59 (s)	17.6	2', 3', 5'	H1'/H2'/H5'
5'	1.71 (s)	25.4	2', 3', 4'	H1'/H2'/H5'
1"		138.7[d]	–	–
2",6"	7.60 (m)	128.5[d]	7"	H2",6"/H3",5"
3",5"	7.50 (m)	128.1[d]	1"	H3",5"/H2",4",6"
4"	7.60 (m)	131.1[d]	2",6",7"	H4"/H3",5"
7"	–	193.4[d]	–	–
1"'	–	138.3[d]	–	–
2"',6"'	7.60 (m)	128.4[d]	4"',7"'	H2"',6"'/H3"',5"'
3"',5"'	7.50 (m)	128.0[d]		H3"',5"'/H2"',4"',6"'
4"'	7.60 (m)	130.9[d]	2"',6"', 7"'	H4"'/H3"',5"'
7"'	–	193.0	–	–
1-*C*OR[1]	–	168.2	–	–

[a] ^{1}H NMR spectra were recorded at 300 MHz; [b] ^{13}C NMR spectra were recorded at 125 MHz; [c] HMBC and COSY spectra were recorded at 500 MHz; [d] the values of the benzoyl shifts cannot be distinguished and are tentatively assigned according to calculations using ACD

5.4.6 Chromophenazine F

Chromophenazine F (**152**) was isolated as red powder with a absorption band at 511 nm at longest wavelength. The molecular formula $C_{32}H_{27}N_3O_3$ was determined by HRESIMS of the *pseudo*-molecular ion peak $[M-H]^-$ at *m/z* 500. The proton spectrum of **152** (Figure 113) was nearly identical with that of **151**, and the 2D data led to the same carbon skeleton (Figure 114).

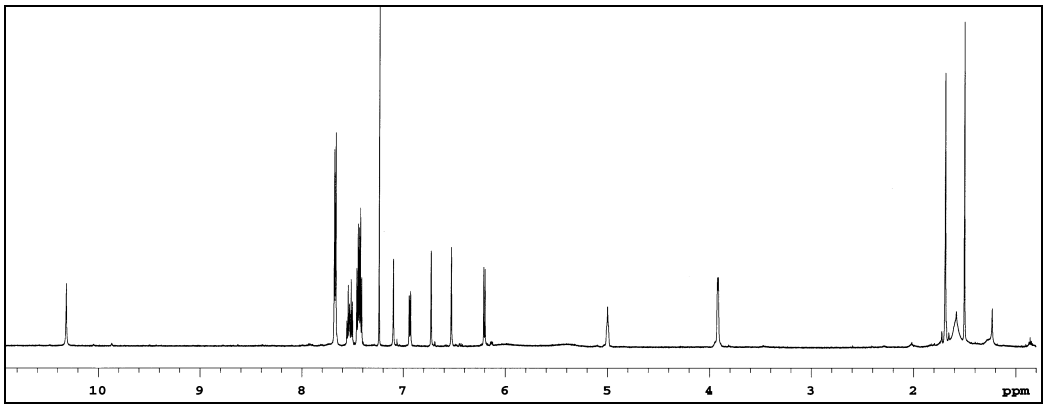


Figure 113: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of chromophenazine F (**152**)

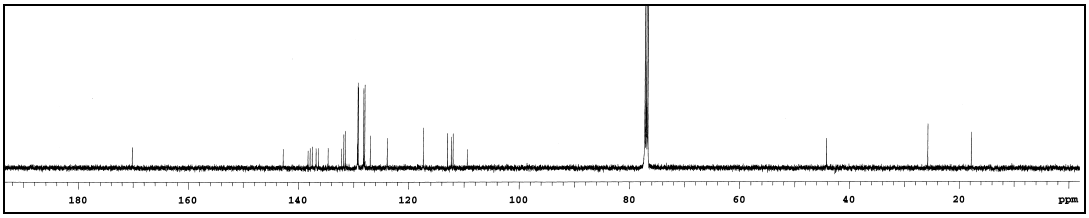


Figure 114: ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of chromophenazine F (**152**)

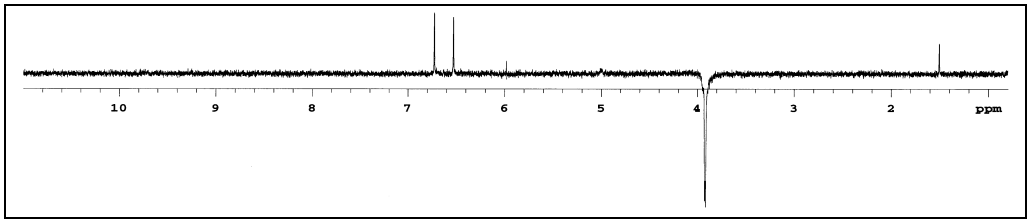


Figure 115: 1D-NOESY spectrum (600 MHz, $CDCl_3$) of chromophenazine F (**152**)

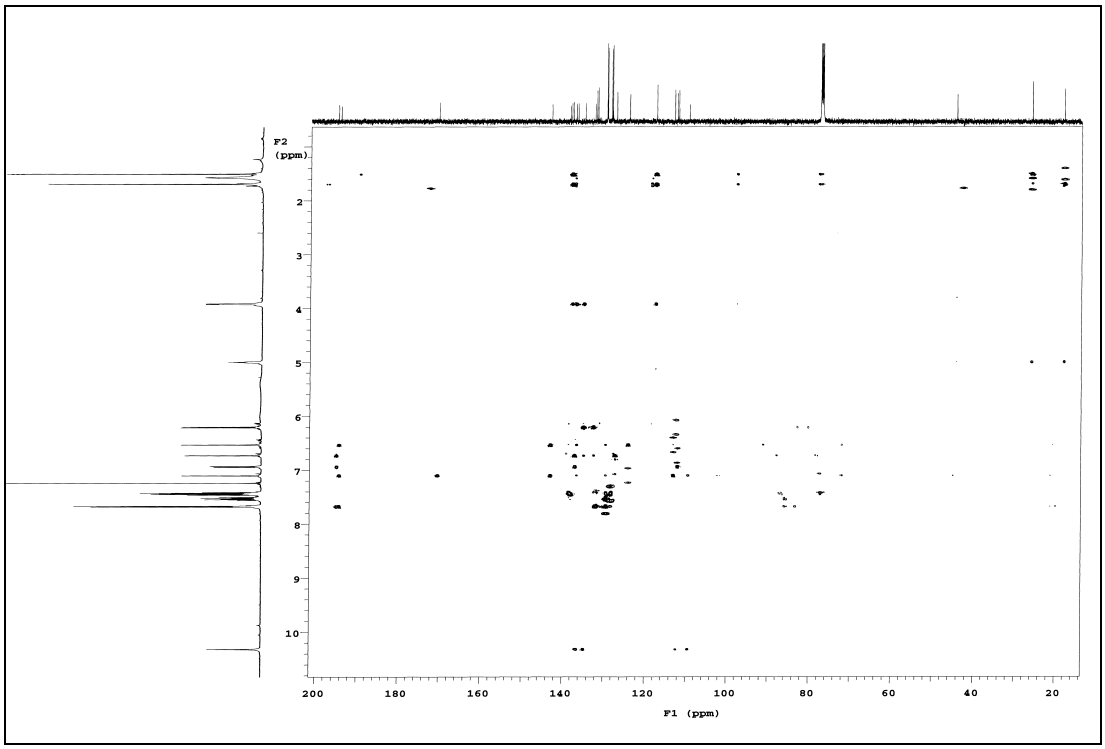
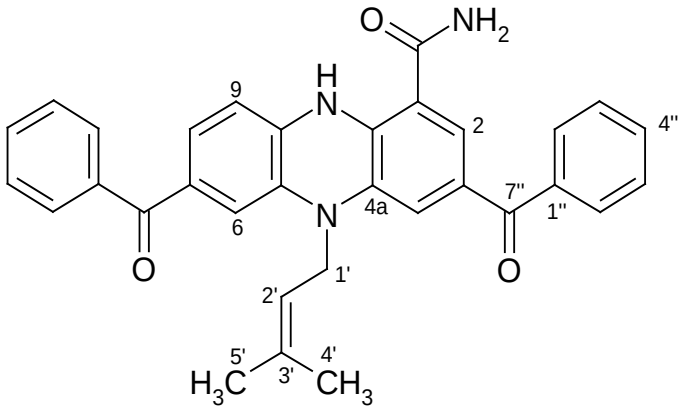


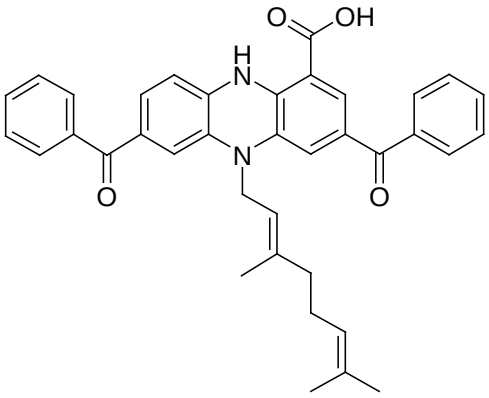
Figure 116: HMBC spectrum (600 MHz, $CDCl_3$) of chromophenazine F (**152**)

The positions of the substituents were derived from 2D NMR data as for **151** and found to be the same. With respect to the empirical formula, the signal at δ 170.1 was, however, assigned to an amide group instead of an acid. These data identified chromophenazine F (**152**) as 3,7-dibenzoyl-5-(3-methyl-but-2-enyl)-5,10-dihydro-phenazine-1-carboxamide.

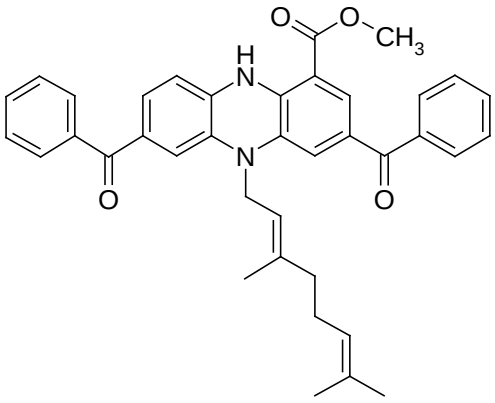


152

Chromophenazines E (**151**) and F (**152**) are closely related with benthophoenin (**150**) isolated from *Streptomyces prunicolor* and to the methyl ester **153** thereof. [324] The ^{1}H and ^{13}C data resemble those of the benthophoenins, as expected (Table 25).



150



153

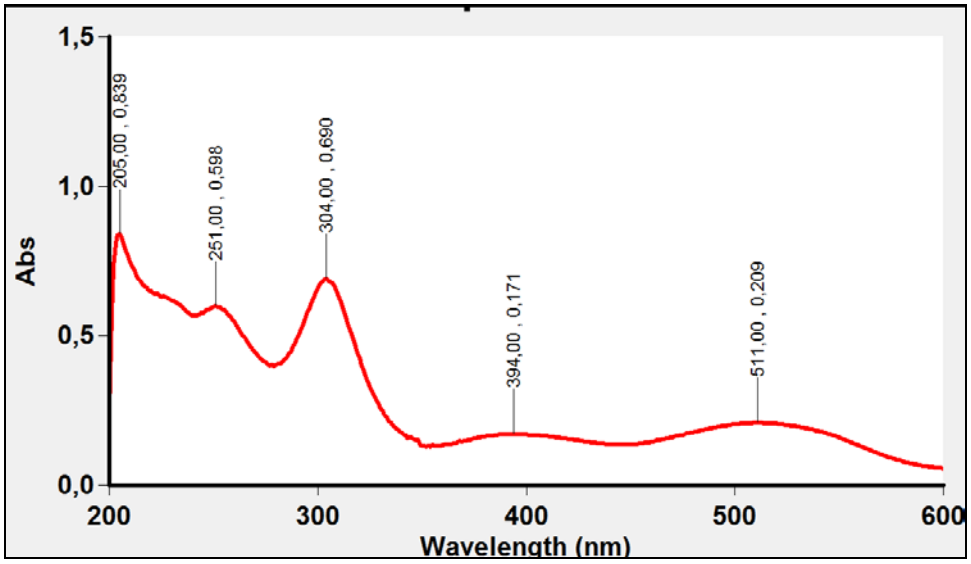


Figure 117: UV/Vis spectrum of chromophenazine F (**152**)

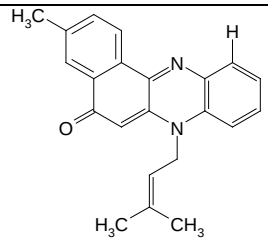
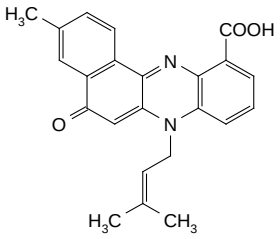
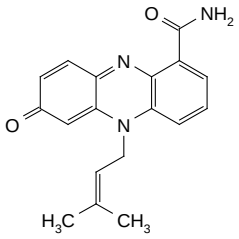
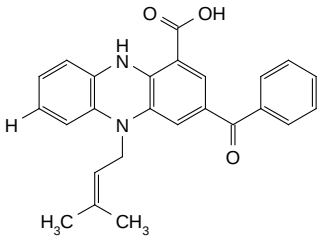
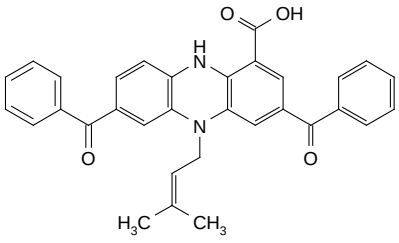
Table 25: ^{1}H and ^{13}C NMR data HMBC and COSY correlations of chromophenazine F (**152**) in $CDCl_3$

Position	Chromophenazine F (**152**)			
	δ_H[a] (*J* in Hz)	δ_C[b]	HMBC[c] (H→C)	COSY[c] (H↔H)
1	–	109.4	–	–
2	7.10 (d, 1.4)	123.9	1,3,4,4a,10a, 7'''	H4/H9
3	–	129.3	–	–
4	6.52 (s br)	113.0	2,3,4a,10a,7''	H2/H10
4a	–	136.4	–	–
5a	–	134.7	–	–
6	6.72 (d, 1.2)	112.0	7,8,5a,9a,7''	H8/H10
7	–	132.2	–	–
8	6.93 (dd, 7.9,1.4)	127.0	6,9a,7'''	H6/H9
9	6.20 (d, 7.9)	112.3	5a,7	H2/H8
9a	–	136.8	–	
10	10.3 (s)	–	1,4a,5a,9	H4/H6
10a	–	142.8	–	–
1'	3.91 (d, 6.3)	44.2	4a, 5a, 2', 3'	H1'/H4'/H5'
2'	4.99 (m)	117.4	4', 5'	H2'/H4'/H5'
3'	–	137.5	–	–
4'	1.50 (s)	17.8	2', 3', 5'	H1'/H2'/H5'
5'	1.68 (s)	25.8	2', 3', 4'	H1'/H2'/H4'
1''	–	137.9[d]	–	–
2'',6''	7.67 (d, 7.8)	129.3[d]	1'',4'',7''	H2'',6''/H3'',5''
3'',5''	7.45 (t, 7.8)	128.0[d]	1''	H3'',5''/H2'',4'',6''
4''	7.51 (tt, 7.5, 1.2)	131.6[d]	2'',6''	H4''/H3'', 5''
7''	–	194.1[d]	–	–
1'''	–	138.3[d]	–	–
2''',6'''	7.67 (d, 7.8)	129.2[d]	4''',7'''	H2''',6'''/H3''',5'''
3''',5'''	7.42 (t, 7.8)	128.2[d]	1'''	H3''',5'''/H2''',4''',6'''
4'''	7.54 (tt, 7.5, 1.3)	131.8[d]	2''',6'''	H4'''/H3''',5'''
7'''	–	194.7	–	–
1-*C*OR[1]	–	170.2	–	–

[a] ^{1}H NMR spectra were recorded at 300 MHz; [b] ^{13}C NMR spectra were recorded at 125 MHz; [c] HMBC and COSY spectra were recorded at 500 MHz; [d] the values of the benzoyl shifts cannot be distinguished and are tentatively assigned according to calculations using ACD

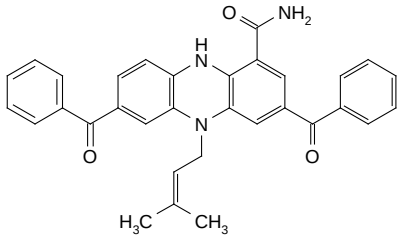
Table 26: ^{1}H, ^{13}C NMR HMBC and COSY correlations of chromophenazine E (**151**) in DMSO-d_6 and chromophenazine F (**152**) in $CDCl_3$, in comparison with related compounds; coupling constants *J* in [Hz].

Atom no.	Chromophenazine E (151)		Chromophenazine F (152)		Benthophoenin (150)		Benthophoenin methyl ester (153)	
	δ_H[a] (*J* in Hz)	δ_C[b]	δ_H[c] (*J* in Hz)	δ_C[b]	δ_H[a] (*J* in Hz)	δ_C[b]	δ_H[a] (*J* in Hz)	δ_C[b]
1	–	118.2	–	109.4	–	124.9	–	107.5
2	7.51 (d, 1.2)	130.9	7.10 (d, 1.4)	123.9	7.73	131.8	7.53	127.5
3	–	130.0	–	129.4	–	136.8	–	129.5
4	6.50 (d, 1.8)	110.6	6.52 (s br)	113.0	Np*	112.7	6.67	113.2
4a	–	134.6	–	136.4	–	132.8	–	136.1
5a	–	134.1	–	134.7	–	132.8	–	134.8
6	6.53 (d, 1.1)	109.6	6.72 (d, 1.2)	112.0	6.69	113.0	6.76	112.4
7	–	129.6	–	132.2	–	135.9	–	132.7
8	6.87 (dd, 7.9,1.8)	126.7	6.93 (dd, 7.9,1.4)	127.0	6.99	128.3	6.98	126.6
9	6.23 (d, 7.9)	111.2	6.20 (d, 7.9)	112.3	6.32	112.9	6.25	112.4
9a	–	138.6	–	136.8	–	139.9	–	136.4
10	13.12 (s)	–	10.3 (s)	–	–	–	9.80	–
10a	–	141.5	–	142.8	–	143.8	–	143.8
1'	3.94 (d, 5.4)	43.0	3.91 (d, 6.3)	44.2	Np*	44.9	–	44.2
2'	5.03 (td, 5.9, 1.4)	117.19	4.99 (m)	117.4	5.06	119.5	5.02	117.1
3'	–	136.9	–	137.5	–	141.7	–	141.4
4'	1.59 (s)	17.6	1.50 (s)	17.8	2.08	16.5	2.08	16.3
5'	1.71 (s)	25.4	1.68 (s)	25.8	1.68	40.5	1.62	39.5
6'	–	–	–	–	2.08	27.2	2.01	26.4
7'	–	–	–	–	5.06	124.9	5.02	123.7
8'	–	–	–	–	–	132.5	–	131.7
9'	–	–	–	–	1.61	17.8	1.61	17.7
10'	–	–	–	–	1.59	25.8	1.56	25.6
1"	–	138.7[d]	–	137.9[d]	–	139.9	–	138.4
2", 6"	7.60[d]	128.5[d]	7.67 (d, 7.8)[d]	129.3[d]	7.66	130.3	7.69	129.4
3", 5"	7.50[d]	128.1[d]	7.45 (t, 7.8)[d]	128.0[d]	7.50	129.3	7.43	128.1
4"	7.60[d]	131.1[d]	7.51 (tt, 7.5,1.2)[d]	131.5[d]	7.59	132.6	7.53	131.7
7"	–	193.4[d]	–	194.1[d]	–	197.1	–	194.8
1"'	–	138.3[d]	–	138.3[d]	–	139.9	–	138.1
2"',6"'	7.60[d]	128.4[d]	7.67 (d, 7.8)[d]	129.2[d]	7.66	130.3	7.69	129.4
3"', 5"'	7.50[d]	128.0[d]	7.42 (t, 7.8)[d]	128.2[d]	7.50	129.3	7.45	128.1
4"'	7.60[d]	130.9[d]	7.54 (tt, 7.5,1.3)[d]	131.8[d]	7.59	132.6	7.55	131.7
7"'	–	193.0	–	194.7	–	197.1	–	194.2
1-COR[1]	–	168.2	–	170.2	–	173.8	–	168.2
1-CO_2Me	–	–	–	–	–	–	3.81	52.1

Compound	Structure
Chromophenazine A (**144**)	
Chromophenazine B (**145**)	
Chromophenazine C (**146**)	
Chromophenazine D (**147**)	
Chromophenazine E (**151**)	

Chromophenazine F

(**152**)



5.4.7 Chromophenazines: Unusual ESI-MS and CID-MS/MS Fragmentations

Surprisingly, in the positive ion mode electrospray mass spectra of chromophenazine D, E, and F intensive signals were observed at *m/z* 398 (chromophenazine D, **147**), *m/z* 502 (chromophenazine E, **151**) and *m/z* 501 (chromophenazine F, **152**) corresponding to the odd-electron $M^{+\bullet}$ ions. The identity of these species was confirmed by HR-ESIMS (Table 27). Typically, under electrospray condition even-electron *quasi*-molecular ions were formed by protonation or as adducts with ions from the solution phase (e.g. Na^+, K^+, NH_4^+). However, in (+)-ESI, oxidation of the analyte molecules can occur leading to the $M^{+\bullet}$ ions described above. Additionally, $[M+H]^+$ ions were detected with low intensity for chromophenazine E (**151**) and F (**152**), but not for chromophenazine D (**147**). However, the formation of $[M]^{+\bullet}$ species was not observed for chromophenazine A (orange, **144**), B (orange, **145**), and C (violet, **146**).

Obviously, the red coloured chromophenazines D (**147**), E (**151**), and F (**152**) with at least one benzoyl group in conjugation to the phenazine moiety are able to stabilize a radical within the molecule in the gas phase and so they can be oxidized very easily under electrospray conditions (positive ion mode) forming radical cations preferably.

Furthermore, the isoprene subunit can be cleaved very rapidly by tandem mass spectrometry using collision-induced dissociation. For chromophenazines D (**147**), E (**151**), and F (**152**) a loss of $\Delta m/z$ 69 was observed corresponding to the cleavage of an odd-electron isoprenyl radical $[C_5H_9^{\bullet}]$ from the radical molecular ion peak $[M^{+\bullet}]$ (chromophenazine D (**147**), *m/z* 398 to *m/z* 329, chromophenazine E (**151**), *m/z* 502 to *m/z* 433, chromophenazine E (**151**), *m/z* 501 to *m/z* 432) forming even-electron fragment ions (Table 28). In contrast, for chromophenazines A (**144**) and C (**146**), loss of a neutral even-electron isoprene unit (*m/z* 68, C_5H_8) was observed. On the other hand, the even-electron negative ions of chromophenazines D (**147**), E (**151**),

and F (**152**) formed by deprotonation in the (-)-ESI mode were also able to cleave off an odd-electron isoprenyl radical [$C_5H_9^{\bullet}$] forming negatively charged odd-electron fragment ions [chromophenazine D (**147**), *m/z* 397 to *m/z* 328, chromophenazine E (**151**), *m/z* 501 to *m/z* 432, chromophenazine F (**151**), *m/z* 500 to *m/z* 431].

Table 27: Ionic species of chromophenazines D (**147**), E (**151**), and F (**152**) formed by (+)-ESI-MS.

Compounds	Proposed species	Elemental composition	Exact mass measured	Exact mass calculated	Relative intensity
Chromophenazine A (144)	$[M]^{+\bullet}$	$C_{22}H_{20}N_2O$	n.d.	328.1570	-
	$[M+H]^+$	$C_{25}H_{23}N_2O$	329.1650	329.1648	high
	$[M-H]^+$	$C_{22}H_{19}N_2O$	n.d.	327.1492	-
Chromophenazine C (146)	$[M]^{+\bullet}$	$C_{18}H_{17}N_3O_2$	n.d.	307.1315	-
	$[M+H]^+$	$C_{18}H_{18}N_3O_2$	308.1396	308.1394	high
	$[M-H]^+$	$C_{18}H_{16}N_3O_2$	n.d.	306.1237	-
Chromophenazine D (147)	$[M]^{+\bullet}$	$C_{25}H_{22}N_2O_3$	398.1624	398.1625	high
	$[M+H]^+$	$C_{25}H_{23}N_2O_3$	n.d.	399.1703	-
	$[M-H]^+$	$C_{25}H_{21}N_2O_3$	397.1546	397.1546	low
Chromophenazine E (151)	$[M]^{+\bullet}$	$C_{32}H_{26}N_2O_4$	502.1888	502.1887	high
	$[M+H]^+$	$C_{32}H_{27}N_2O_4$	503.1984*	503.1966	low
	$[M-H]^+$	$C_{32}H_{25}N_2O_4$	501.1810	501.1809	low
Chromophenazine F (152)	$[M]^{+\bullet}$	$C_{32}H_{27}N_3O_3$	501.2049	501.2047	high
	$[M+H]^+$	$C_{32}H_{28}N6_3O_3$	502.2140*	502.2125	low
	$[M-H]^+$	$C_{32}H_{26}N_3O_3$	500.1972	500.1969	low

* peaks not completely resolved; n.d. = not detected

Table 28: MS/MS fragmentation of selected ions from chromophenazines D (**147**), E (**151**), and F (**152**).

Compounds	Selected Ions	Fragmentation	Exact mass measured	Exact mass calculated
Chromophenazine A (**144**)	$[M+H]^+$, 329.2	$[M-C_4H_8]^+$	273.1024	273.1022
		$[M-C_5H_8]^+$	261.1024	261.1022
		$[M-C_4H_8-CO]^+$	245.1074	245.1073
		$[M-C_5H_8-CO]^+$	233.1075	233.1073
Chromophenazine C (**146**)	$[M+H]^+$, 308.1	$[M-C_5H_8]^+$	240.0768	240.0768
Chromophenazine D (**147**)	$[M]^{+\bullet}$, 398.2	$[M-C_5H_9]^+$	329.0925	329.0921
		$[M-C_5H_9-H_2O]^+$	311.0819	311.0815
		$[M-C_5H_9-H_2O-CO]^+$	283.0869	283.0866
			255.0918	255.0917
		$[M-C_5H_9-H_2O-CO-CO]^+$	328.0845*	328.0853
	$[M-H]^-$, 397.2	$[M-H-C_5H_9]^{-\bullet}$		
Chromophenazine E (**151**)	$[M]^{+\bullet}$, 502.2	$[M-C_5H_9]^+$	433.1190	433.1183
		$[M-C_5H_9-H_2O]^+$	415.1082	415.077
		$[M-C_5H_9-H_2O-CO]^+$	387.1132	381.1128
			359.1182	359.1179
	$[M-H]^-$, 501.2	$[M-C_5H_9-H_2O-2\,CO]^+$	432.1122	432.1116
		$[M-H-C_5H_9]^{-\bullet}$		
Chromophenazine F (**152**)	$[M]^{+\bullet}$, 501.2	$[M-C_5H_9]^+$	432.1347	432.1343
		$[M-C_5H_9-NH_3]^+$	415.1081	415.1077
		$[M-C_5H_9-NH_3-CO]^+$	387.1129	381.1128
			431.1290	431.1275
	$[M-H]^-$, 500.2	$[M-H-C_5H_9]^{-\bullet}$		

5.5 Terrestrial *Streptomyces* Ank 223

The strain *Streptomyces* sp. Ank 223 formed grey mycelial colonies after incubation on M_2 agar medium for 10 days at 28 °C. It was selected due to its biological activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, the fungus *Mucor miehei* (Tü284) and the yeast *Candida albicans.*

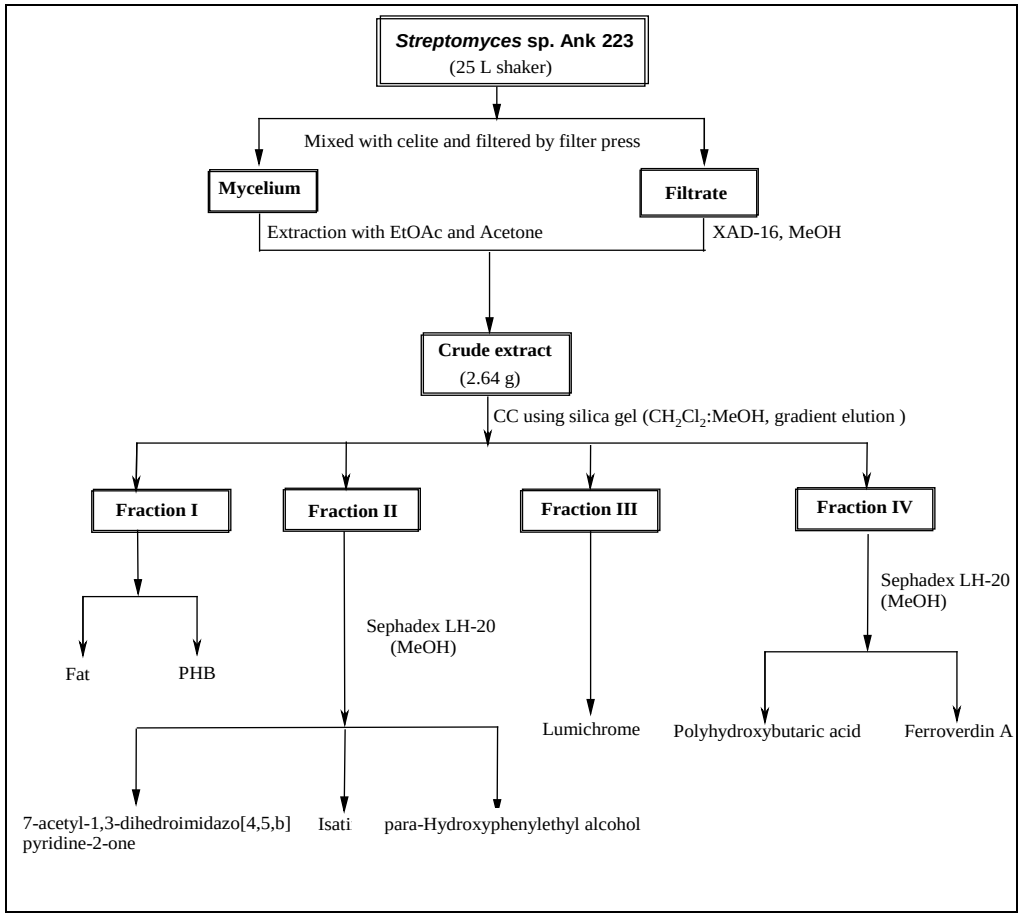


Figure 118: Work-up scheme of *Streptomyces* sp. Ank 223

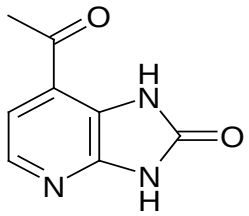
The *Streptomyces* sp. Ank 223 was cultivated on 25 L scale using 1L Erlenmeyer flasks containing 250 mL of M_2 medium at 28 °C for 8 days on a linear shaker (250 rpm). The culture broth was mixed with Celite and filtered with a filter press. The filtrate was passed through an Amberlite XAD-16 column, the resin was washed with water, and eluted with methanol. The methanol phase was concentrated and the aqueous residue was extracted with ethyl acetate. The mycelium was extracted sequentially with ethyl acetate and then acetone. The extracts showed similar composi-

tions on TLC and were combined. They were further chromatographed in different ways to get their constituents in pure form.

Five compounds were isolated from *Streptomyces* sp. Ank 223, all of them were known and were identified as 7-acetyl-1,3-dihydroimidazo[4,5,b]pyridine-2-one (**154**), isatin (**155**), polyhydroxybutyric acid (**156**), lumichrome (**157**), ferroverdin A (**158**).

5.5.1 7-Acetyl-1,3-dihydroimidazo[4,5,b]pyridine-2-one

Compound **154** was obtained as white solid. The ^{1}H NMR spectrum of **154** showed two doublets in the aromatic region at δ 7.15 and at δ 7.15 and a methyl singlet at δ 2.60. A search in AntiBase [115] using the above spectroscopic data identified **154** as 7-acetyl-1,3-dihydroimidazo[4,5,b]pyridine-2-one (**154**).



154

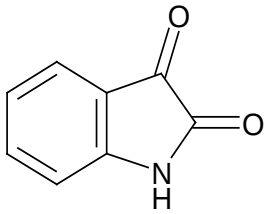
5.5.2 Isatin

Compound **155** was isolated as orange powder and showed UV absorption at 254 nm and no colour change after spray with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **155** showed two doublets at δ 7.58 and 6.85 and two triplets at δ 7.55 and 7.08, indicating a 1,2-disubstituted benzene ring. A search with the data above in AntiBase [115] and by comparison of the above spectroscopic data with literature identified the compound as isatin (**155**).

Isatin (**155**) is responsible for the protection of shrimp embryos of *Palaemon macrodactylus* from an infection by pathogenic fungus *Lagenidium callinectes*. [299] Only

six isatin derivatives are known from microorganisms [115], while bromo-isatin is the only one, which was reported from higher organisms (mollusks). [300]

Isatin (**155**) was reported as signalling substance between microorganisms for production of antibiotics. In addition, it is active as inhibitor of xanthine oxidase of milk [301] and monoamine oxidase. [302] Isatin (**155**) is known as fungal pigment from a mutant of *Schizophyllum commune*. [303] Synthetically, **155** was obtained for the first time during oxidative degradation of indigo by nitric or chromic acid.



155

5.5.3 Polyhydroxybutyric acid (PHB)

Compound **156** was isolated from fraction IV as white polymer and showed no UV absorption and no colour after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **156** showed a methyl doublet at δ 1.23, two multiplets at δ 2.40 and 2.60 and a sharp ABX signal of an oxygenated methine group at δ 5.23. By a search in AntiBase [115] using the above spectroscopic data, the compound was confirmed as polyhydroxybutyric acid (**156**).

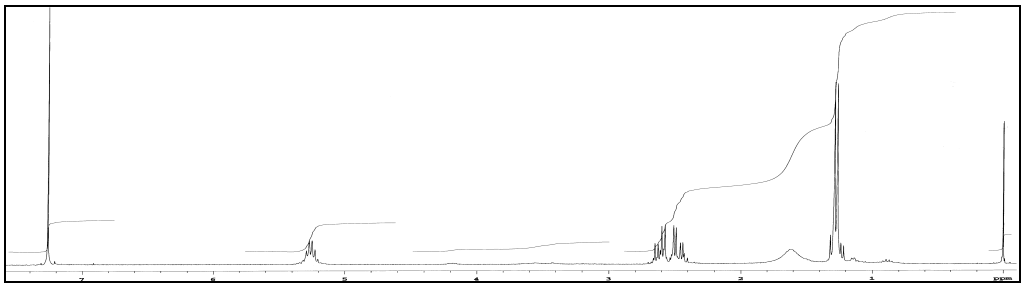
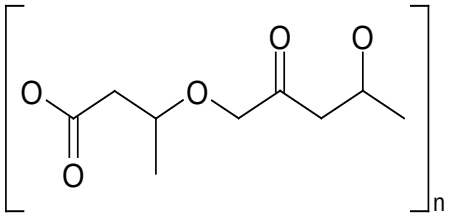


Figure 119: ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of PHB (**156**)



156

5.5.4 Lumichrome

Compound **157** was isolated from fraction III using Sephadex LH-20 (methanol) as yellow pale solid, which was UV absorbing at 254 nm and stained to yellow with anisaldehyde/sulphuric acid.

The ^{1}H NMR spectrum of lumichrome (**157**) exhibited four singlets, two 1H singlets in the aromatic region at δ 8.03 and 7.85 along with one broad 2H singlet of acidic groups at δ 13.9, and two in the aliphatic region at δ 2.32 and 2.25 attributed to two methyl groups possibly connected with an aromatic ring. A search in AntiBase [115] and a comparison with authentic spectra from our collection resulted in lumichrome (**157**).

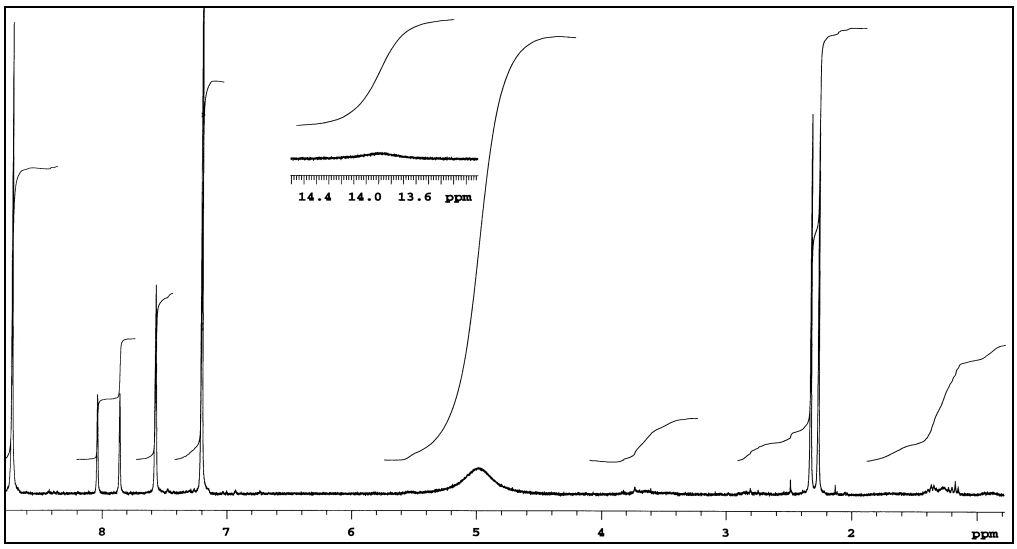
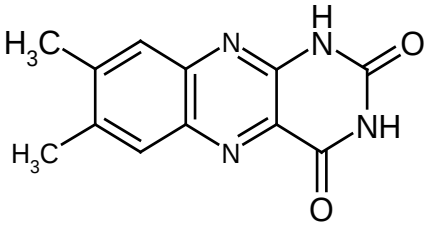


Figure 120: ^{1}H NMR spectrum (Pyridine-d_5, 300 MHz) of lumichrome (**157**)

Compound **157** was previously isolated from a Chinese marine sponge *Cinachyrella australiensis* by Liya et *al*. in 2004, [304] from the actinomycete *Micromonospora* sp.

strain Tü 6368 [305] and the fungus *Aspergillus oniki* 1784. [338] It was reported to act as a testosterone 5α-reductase inhibitor. [306]



157

5.5.5 Ferroverdin A

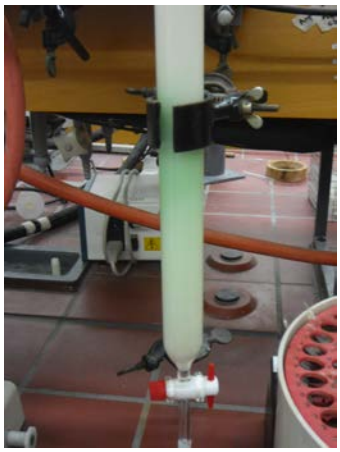


Figure 121: Separation of ferroverdin A (**158**) on Sephadex LH-20 in methanol.

Compound **158** was isolated from fraction IV as a green powder, whose colour is due to the iron content. The ^{1}H NMR spectrum displayed in the aromatic region five signals at δ 8.10, δ 7.74, δ 7.18, δ 7.53, δ 7.22. Further more three signals at δ 6.75, δ 5.26 and at δ 5.82 . The molecular formula of ferroverdin A was determined to be $C_{45}H_{30}N_3O_{12}Fe$ on the basis of HR-ESIMS measurement (*m/z*, found 861.1257, calcd. 861.1258 for $C_{45}H_{31}N_3O_{12}Fe$ $[M+H]^+$).

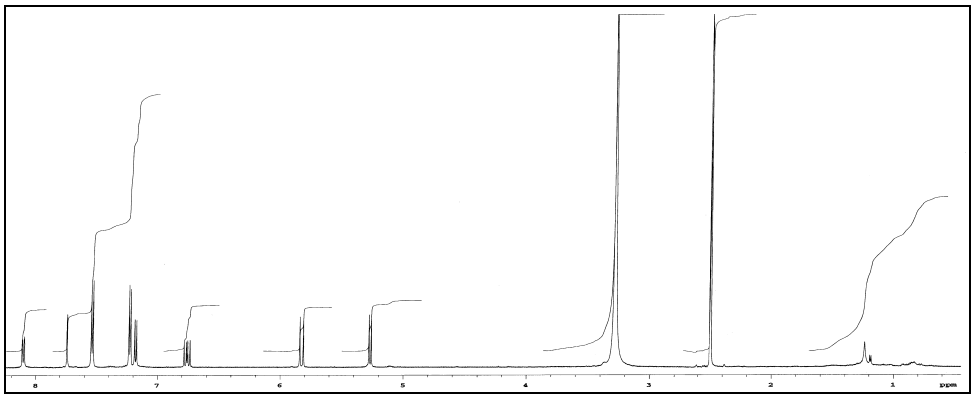


Figure 122: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ferroverdin A (**158**)

The ^{13}C NMR spectrum contained 15 carbon signals, which were classified into two sp^2 methylenes, seven sp^2 methines and six sp^2 quaternary carbons by analysis of the DEPT spectra. Some carbon signals overlapped.

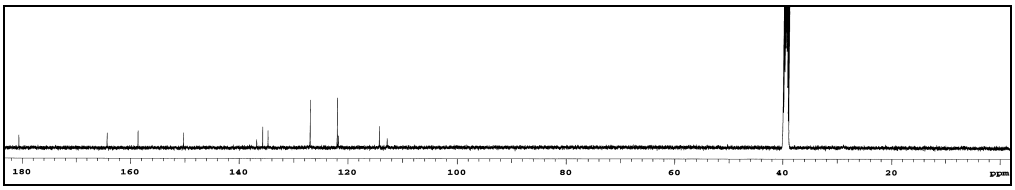


Figure 123: ^{13}C NMR spectrum (DMSO-d_6, 300 MHz) of ferroverdin A (**158**)

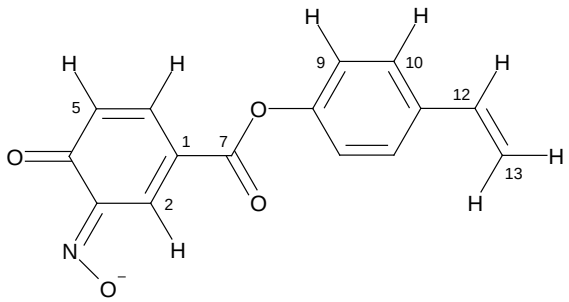
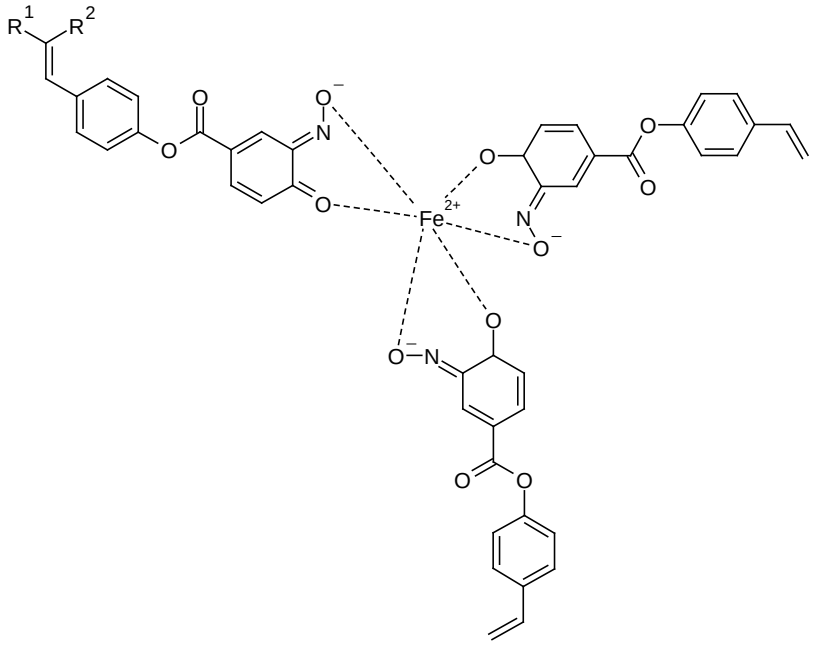


Figure 124: Monomer of ferroverdin A (158)

Table 29 ^{1}H and ^{13}C NMR data of ferroverdin A (**158**) in DMSO-d_6

Position	δ_H **(*J* in Hz)**	δ_C	δ_H **(*J* in Hz) Literature** [307]	δ_C **(*J* in Hz) Literature** [340]
C-1	–	114.4	–	116.9
C-2	7.74 (d, 2.2)	112.8	7.90	114.7
C-3	–	158.7	–	160.6
C-4	–	180.7	–	181.9
C-5	7.18 (d, 9.2)	121.8	7.20	122.7
C-6	8.10 (dd, 9.2, 2.0)	136.8	8.20	138.3
C-7	–	164.3	–	166.5
C-8	–	150.3	–	152.1
C-9	7.22 (d, 8.6)	122.0	7.10	123.0
C-10	7.53 (d, 8.6)	127.0	7.50	128.2
C-11	–	134.7	–	136.8
C-12	6.75 (dd, 11.1, 11.1)	135.7	6.70	137.3
C-13	5.26 (d, 11.1)	114.3	5.30	114.2
	5.82 (d, 17.7)	114.3	5.75	114.2

Ferroverdins are green siderophores produced in the mycelium of *Streptomyces* species. Ferroverdins consists of three *p*-vinylphenyl-3-nitroso-4-hydroxybenzoate ligands complexed with a ferrous ion. There are three ferroverdin derivatives: A (**158**), B (**159**), and C (**160**).



Ferroverdin	R_1	R_2
A (**158**)	H	H
B (**159**)	OH	H
C (**160**)	H	COOH

zzzz All ferroverdins showed a dose-dependent inhibitory activity against human cholesteryl ester transfer protein (CETP). The IC_{50} values were 21, 0.62 and 2.2 μm for ferroverdins A (**158**), B (**159**) and C (**160**), respectively, indicating that ferroverdin B is one of the most potent CETP inhibitors of microbial origin. [308]

5.6 *Streptomyces sp.* WO 668

For the pre-screening, the terrestrial *Streptomyce*s sp. WO 668 was cultivated in Erlenmeyer flasks in M_2^+ medium at 28 °C for five days. After work-up, the resulting crude extract showed on TLC, UV absorbing bands, which gave a pink colour with anisaldehyde/sulphuric acid.

A culture on agar was used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2^+ medium (25 L). After incubation at 28 °C on a linear shaker for seven days, the dark brown culture broth was filtered under vacuum. The biomass was extracted 3 times with ethyl acetate followed by two times with acetone. The filtrate was subjected to XAD-16; the resin has been washed with distilled water. The methanolic eluate was concentrated under vacuum to obtain a crude extract (2.70 g), which was subjected to silica gel column chromatography (CC) eluting with CH_2Cl_2, followed by stepwise addition of CH_3OH to yield twelve fractions FI to FXII.

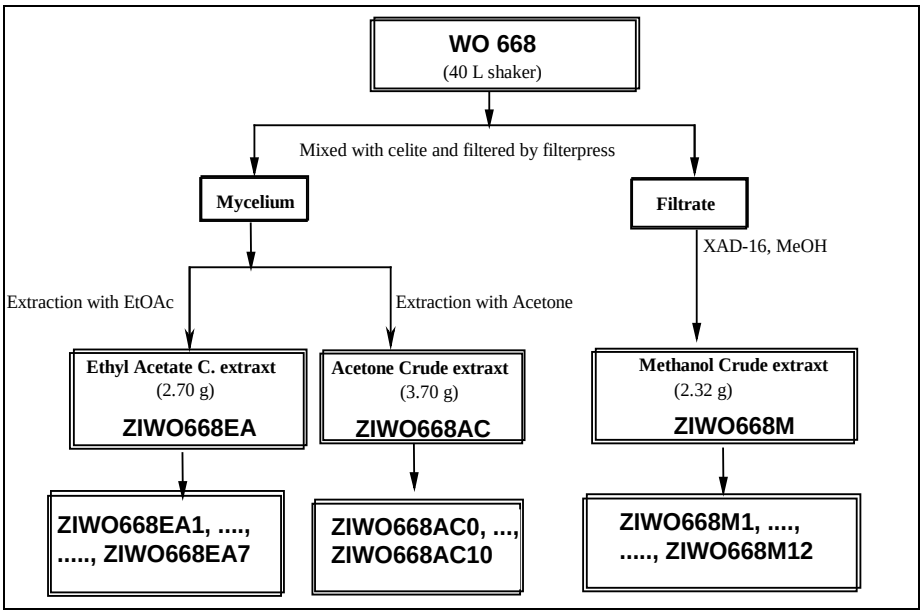


Figure 125: Work-up scheme of *Streptomyces* sp. WO668

The ethyl acetate extract was concentrated under vacuum to obtain a crude extract (2.70 g), which was subjected to silica gel column chromatography (CH_2Cl_2/MeOH) to yield seven fractions I to VII. The acetone extract was concentrated under vacuum to obtain a further crude extract (3.70 g), which was also subjected to silica gel column chromatography (CH_2Cl_2/MeOH) to yield eleven fractions.

All methanol extract fractions were subjected to Sephadex LH-20 (MeOH) to yield indole-3-carbaldehyde (**96**), tryptophol (**119**), indole-3-lactic acid (**121**), uracil (**126**), *p*-hydroxybenzoic acid (**134**), 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**161**), and 13-hydroxy-12-methyl-tetradecanoic acid (**163**).

The acetone crude extract was submitted to HPLC-ESI-MS and delivered peptide homologues.

5.6.1 Peptide homologues

The acetone crude extract of this strain was submitted to HPLC-ESI-MS. The presence of peptides homologues was then detected. It was noticed that the ionisation gave different *quasi*molecular ions for $[M+H]^+$ at *m/z* 882, 897, 911 and 925, indicating a series of homologues. The selected peaks were subjected to HR-MS to obtain a molecular formula $C_{41}H_{74}N_{10}O_{11}$, $C_{42}H_{76}N_{10}O_{11}$, $C_{43}H_{78}N_{10}O_{11}$, respectively.

The sample was then submitted to HPLC-ESI-MS/MS. Preliminary ESI-MS/MS studies revealed that the compounds were potentially structurally related to fusaricidin A, fusaricidin B, LI-F06a.

5.6.2 3-Hydroxy-4-(4-hydroxyphenyl)-butan-2-one

Compound **161** was isolated as colourless solid from fraction IV of the methanol extract. It showed middle polarity on TLC, was not UV absorbing and colourless after spraying with anisaldehyde/sulphuric acid. The molecular weight of **161** was deduced as 180 Dalton according to ESI MS spectra, and the corresponding molecular formula was established as $C_{10}H_{12}O_3$ on the bases of HRESIMS.

The ^{1}H NMR spectrum of compound **161** displayed two 2H doublets (J = 8.5 Hz) at δ 7.04 and 6.68, indicating a 1,4-disubstitued benzene ring. Additionally, a signal for one oxygenated methine was visible at δ 4.23 (ABX) together with a methylene 2H at 2.93 (dd) and 2.72 (m) given by a benzene-attached methylene group. A methyl singlet was finally shown at 2.18, representing most likely an acetyl group.

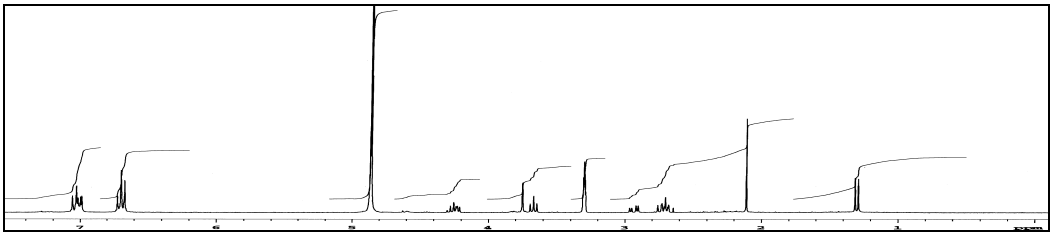


Figure 126: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 3-hydroxy-4-(4-hydroxyphenyl)-butan-2-one (**161**)

The ^{13}C NMR/HMQC spectra displayed ten carbon signals, which were classified into several categories: One acetyl carbonyl (213.0), six aromatic carbons, among them two quaternary (157.1 and 129.4) and two 2 CH methine signals (131.4 and 116.1), confirming the 1,4-disubstituted aromatic residue to bear an oxygenated sp^2 carbon (157.1), also an sp^3 oxymethine (79.5), a methylene (40.0) and methyl (26.3) carbon signals were recognized, as expected from the ^1H NMR spectrum.

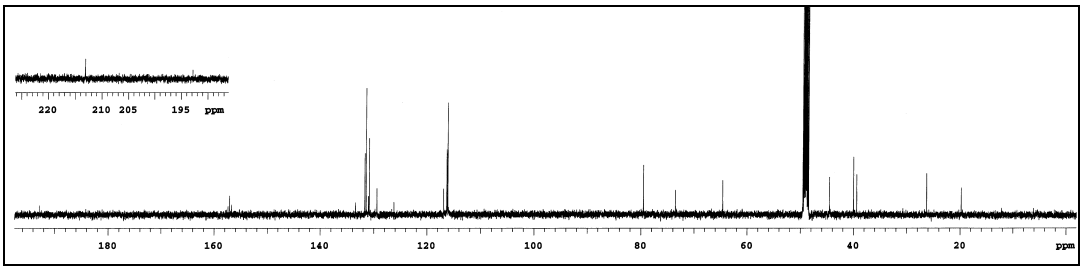


Figure 127: ^{13}C NMR spectrum (CD_3OD, 300 MHz) of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**161**)

Table 30: ^1H and ^{13}C NMR Data of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**161**) in CD_3OD

Position	**δ_H (J in Hz)**	**δ_C**
C-1	–	129.4
C-2	7.04 (d, 8.5)	131.4
C-3	6.68 (d, 8.5)	116.1
C-4	–	157.1
C-5	6.68 (d, 8.5)	116.1
C-6	7.04 (d, 8.5)	131.4
C-1'	2 .72 (m)	40.0
	2.93 (dd, 14.1, 4.7)	40.0
C-2'	–	79.5
C-3'	–	213.0
C-4'	2.18 (s)	26.3

In the $^{1}H,^{1}H$ COSY experiment, the proton signals of the oxymethine (δ_H 4.23) and the sp^2-bound methylene (δ_H 2.93 and 2.72) showed correlations, confirming their direct neighbourhood.

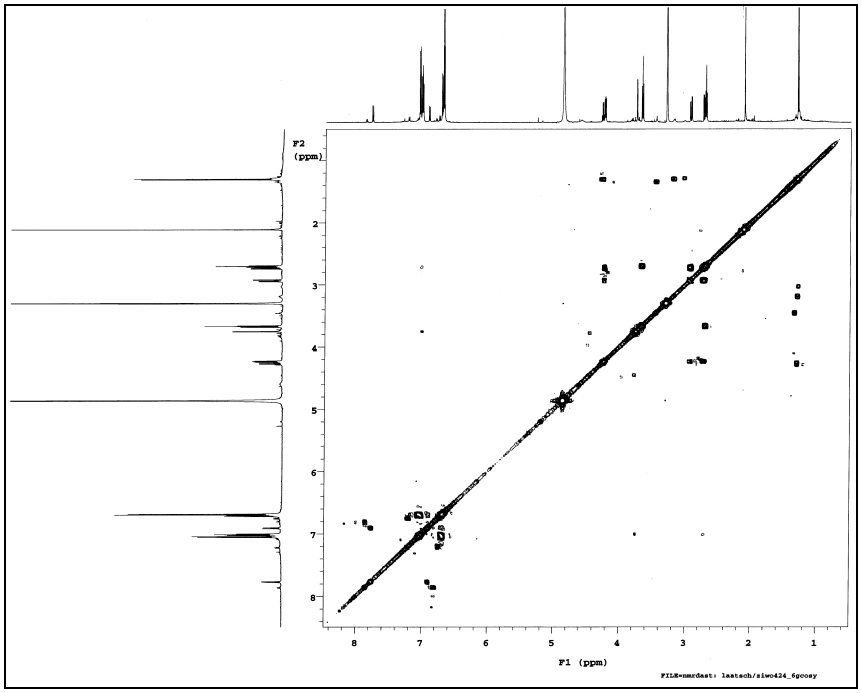


Figure 128: ^{1}H-^{1}H COSY spectrum (CD_3OD, 600 MHz) of 3-hydroxy-4-(4-hydroxyphenyl)-butan-2-one (**161**)

According to the HMBC experiment, the methylene group (CH_2-4) was directly connected to the aromatic quaternary carbon C-1' (129.4), as correlations with both C-1' (^{2}J) and CH-2'/6' (131.4, ^{3}J), and vice versa were visible. The 4-substituted phenol residue was confirmed by further HMBC correlations (Figure 129), resulting in 4-(2-hydroxy-ethyl)-phenol as partial structure. On the other hand, the methyl singlet (δ_H 2.18) showed a coupling with the carbonyl C-2 (δ_C 213.0) and the oxymethine carbon C-3 (δ_C 79.5), confirming the attachment of a terminal acetyl group to the oxymethine C-3 (δ_C 79.5). Furthermore, the methylene protons H-4a/b (δ_H 2.93, 2.72) and the oxymethine (H-3, δ_H 4.23) displayed diagnostic ^{3}J and ^{2}J correlations with the acetyl carbonyl (C-2, 213.0).

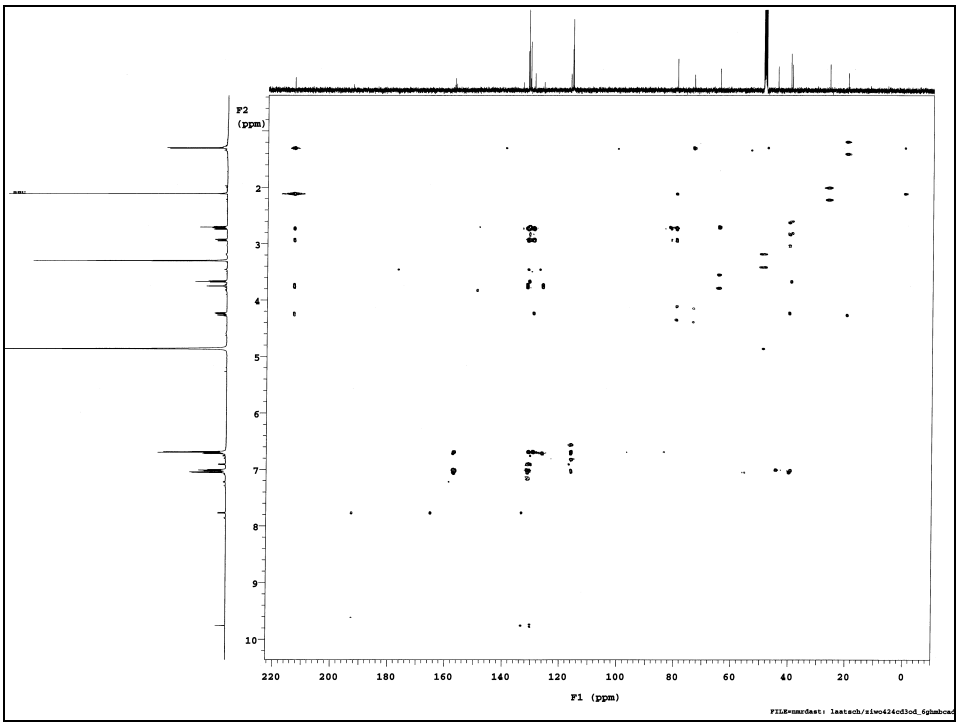


Figure 129: HMBC spectrum (CD_3OD, 600 MHz) of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**161**)

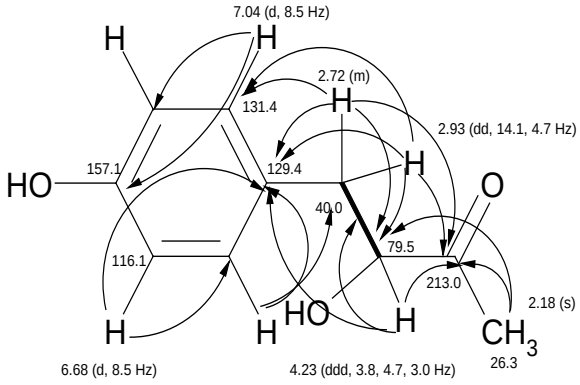
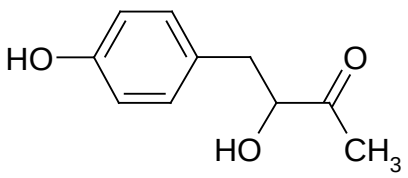


Figure 130: ^1H-^1H COSY and HMBC correlations of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**161**)



161

Accordingly, compound **161** was elucidated as 3-hydroxy-4-(4-hydroxyphenyl)-butan-2-one (**161**). Based on a search in AntiBase [115] and the Dictionary of Natural Products, compound **161** is reported here for first time as natural product. It was, however, already obtained by synthesis. [309]

5.6.3 13-Hydroxy-12-methyl-tetradecanoic acid

Compound **163** was isolated as oily substance. The ^{1}H NMR spectrum showed signals in the aliphatic region at δ 2.25 (t, H-2), 1.6 (q, H-3), 1.22-1.31 (aliphatic chain, H-5-12), 1.18 (m, H-13), (0.87, 13-CH_3), 3.6 (m, H-14) and 1.15 (m, 14-CH_3).

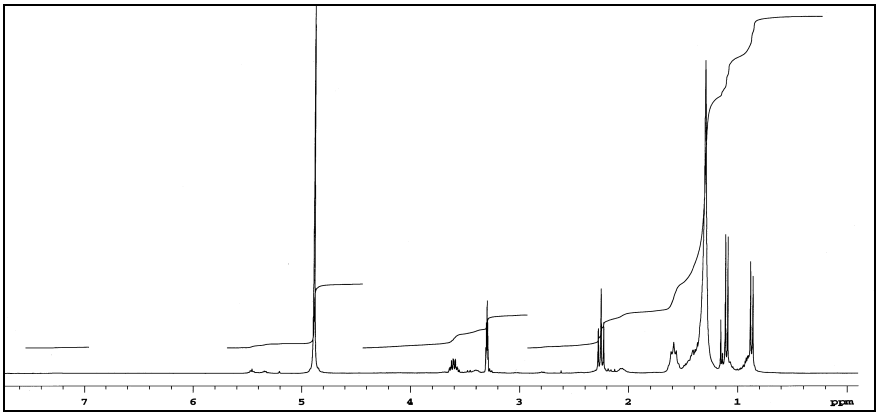


Figure 131: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 13-hydroxy-12-methyl-tetradecanoic acid (**163**)

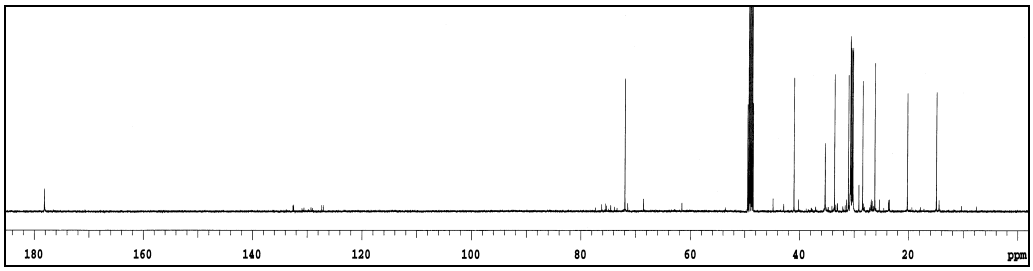


Figure 132: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 13-hydroxy-12-methyl-tetradecanoic acid (**163**)

Table 31: ^{1}H, ^{13}C and 2D NMR data of 13-hydroxy-12-methyl-tetradecanoic acid (**163**) in CD_3OD

Position	δ_H [a]	δ_C [b]	HMBC [c] (H→C)	COSY [c] (H↔H)
1	–	178.5	–	–
2	2.25 (t)	35.4	26.2	1.6
			30.2-30.8	
3	1.6 (q)	26.2	30.2-30.8	1.3, 2.25
			35.4	
4	1.3	28.5	–	1.6
5	1.22-1.31	30.2	–	–
6	1.22-1.31	30.3	–	–
7	1.22-1.31	30.4	–	–
8	1.22-1.31	30.6	–	–
9	1.22-1.31	30.7	–	–
10	1.22-1.31	30.8	–	–
11	1.22-1.31	33.7	–	–
12	1.18 (m)	41.0	30.2-30.8	–
12-CH_3	0.87	14.9	20.2, 33.7, 41.0, 71.0	1.3
13	3.60 (m)	71.9	–	1.1
14 (CH_3)	1.10	20.2	14.9, 41.0, 71.0	3.6

In the COSY spectrum, the methyl doublet at δ 1.10 (H_3-14) correlated with the oxymethine signal at 3.60 (C-13), indicating a CH(OH)-CH_3 fragment. Both methyl groups showed a strong HMBC correlation with the oxy-methine carbon C-13. As there was also an HMBC correlation from the second methyl signal at δ 0.87 with C-14, a CH_3-CH(OH)-CH(CH_3)- fragment was confirmed. According to the empirical formula $C_{15}H_{28}O_3$ (by ESI-HRMS), only the double bond equivalent of one carbonyl group was present; a ring was therefore excluded. The only way to connect the remaining atoms is to place group mentioned above at the end of *n*-undecanoic acid, resulting in 13-hydroxy-12-methyl-tetradecanoic acid (**163**). This agrees also with

the triplet at δ 2.25, which is in the expected region for an α-methylene group in a fatty acid.

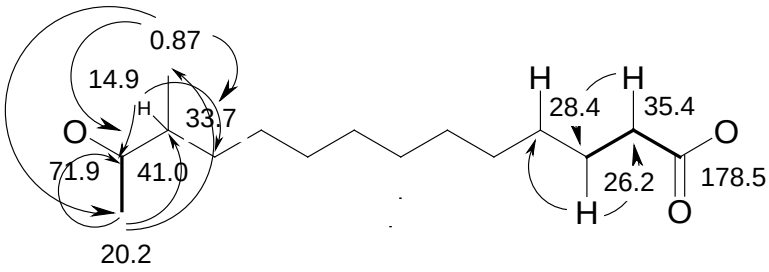
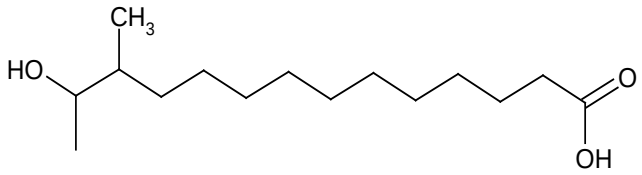


Figure 133: ^{1}H-^{1}H COSY (—) and HMBC (↔) correlations of 13-hydroxy-12-methyl-tetradecanoic acid (**163**)



163

Terminally hydroxylated *anteiso*-fatty acids are very rare in nature, and compound **163** had not been described before. The closest relation is found with 4,11-dihydroxy-10-methyldodec-2-en-1,4-olide, which has, however, a shorter chain and is additionally hydroxylated in position 4.

5.6.4 Aspernigrin A

Compound **165** was isolated as white crystals; it was UV absorbing under 254 nm. The ^{1}H NMR spectrum (Figure 134) showed the presence of a benzyl moiety deduced from the characteristic signals of a monosubstituted aromatic ring (δ 7.24-7.31, 5H) in conjunction with a signal at δ 3.95 integrating for two protons (H_2-7). Furthermore, two doublets at δ 6.34 (H-5) and 8.52 (H-2) were observed (Table 32).

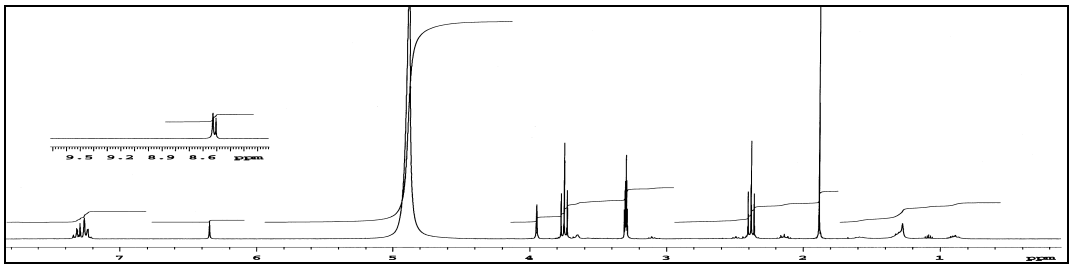


Figure 134: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of aspernigrin A (**165**)

Table 32: ^{1}H and ^{13}C NMR data of aspernigrin A (**165**) in CD_3OD

Position	δ_H	δ_C
1	–	–
2	8.52 s	145.7
3	–	117.7
4	–	180.6
5	6.34 s	119.3
6	–	155.7
7	3.95 s (2H)	40.7
8	–	138.1
9/13	7.31	129.8
10/12	7.28	130.1
11	7.24	128.1
14	–	170.5
$CONH_2$	–	–

The ^{13}C NMR spectrum displayed chemical shifts at 145.7 (C-2), 117.7 (C-3), 180.6 (C-4), (δ_C 119.3, C-5), δ 155.7 (C-6), 37.6 (C-7), 138.1 (C-8), (129.8, C-9/C-13), (130.1, C-10/C-12), (128.1, C-11). Hence, compound **165** was found to be aspernigrin A (**165**).

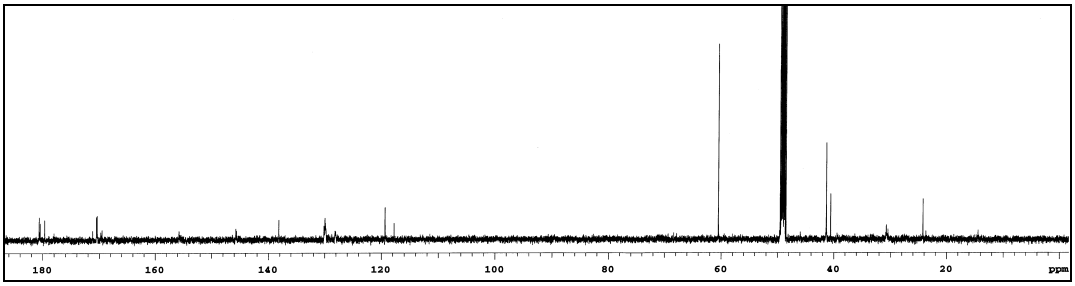
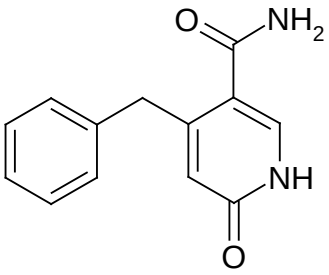
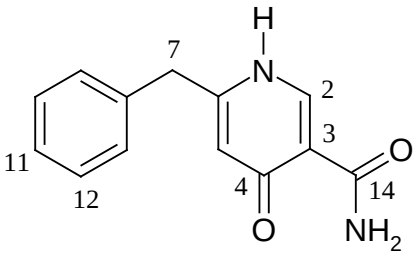


Figure 135: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of aspernigrin A (**165**)

The structure of aspernigrin A, previously elucidated to be 4-benzyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**164**), was revised as 6-benzyl-4-oxo-1,4-dihydro-pyridine-3-carboxamide (**165**) on the basis of additional NMR spectroscopic data and the X-ray crystallographic analysis. [310]



164



165

6 Marine bacteria

6.1 Marine *Streptomyces* sp. B 909-417

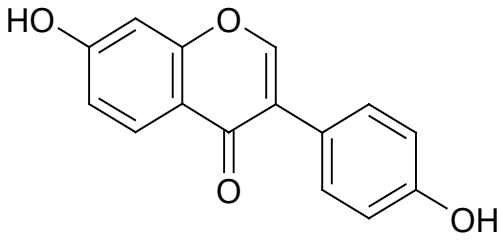
The marine *Streptomyces* sp. B 909-417 was selected due to its antibacterial and high cytotoxic activities on brine shrimps. The strain was cultivated on M_2^+ medium for 10 days at 28 °C. Pieces of well grown agar were used to inoculate 100 of 1L Erlenmeyer flasks, each containing 250 ml of LB medium. The fermentation was carried out at 180 rpm on a rotary shaker for 3 days at 28 °C. The culture broth was harvested and filtered to separate the biomass, which was extracted with ethyl acetate (3 times) and acetone (2 times), respectively. The culture filtrate was then passed through Amberlite XAD-16. The column was washed with 25 L demineralised water

and eluted with 15 L methanol. The eluates were concentrated under reduced pressure and final extraction of the residue was done with ethyl acetate. The organic phases were dried. Both crude extracts were mixed yielding a crude extract of 2.64 g. The chromatography of the latter on silica gel using a dichloromethane-methanol gradient with successively increasing polarity resulted in three fractions I-III according to monitoring by TLC.

Fraction I contained fat and was not further investigated. Two compounds were isolated from fraction II and were identified as daidzein (**166**) and 4-hydroxybenzoic acid (**134**).

6.1.1 Daidzein

Compound **166** was isolated from fraction II as white powder, which showed UV absorption at 254 nm. The molecular formula $C_{15}H_{10}O_4$ was deduced by HRESIMS of the *pseudo*-molecular ion peak at *m/z* 255 in the positive mode and 253 in the negative mode. The aromatic region of the ^{1}H NMR spectrum of **166** showed an ABX pattern at δ 7.85 (d), 6.73 (dd), 6.58 (d) and an A_2B_2 pattern at δ 7.26 (d) and 6.82 (d). It showed also a singlet at δ 8.56. The above data indicated that **166** could be an isoflavone. A search in AntiBase [115] by using the above spectroscopic data resulted in daidzein (**166**), which was further confirmed by comparison with reported literature data.



166

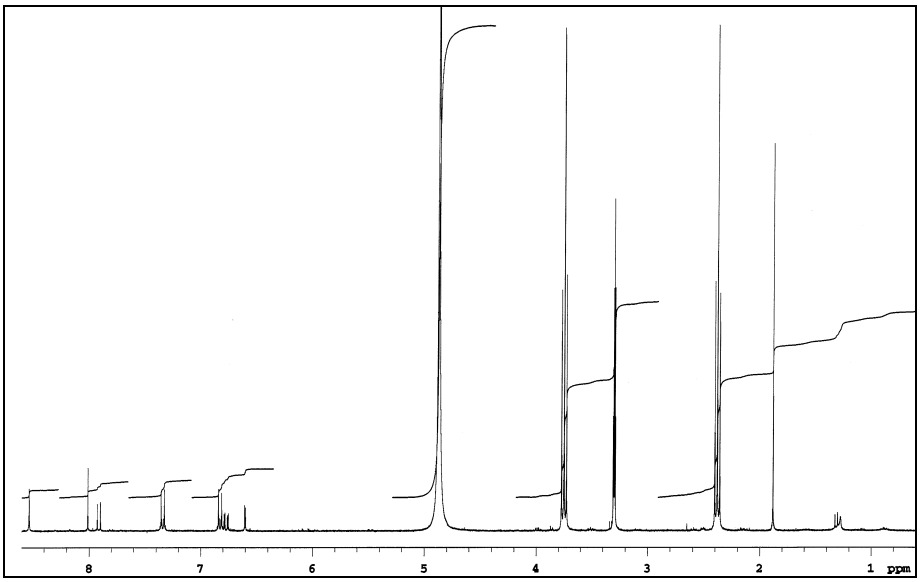
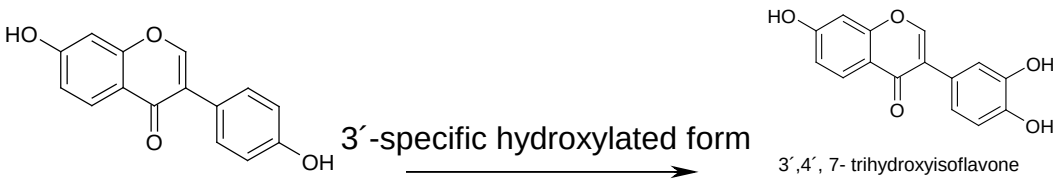


Figure 136: ^{1}H NMR spectrum (CD_3OD, 600 MHz) of daidzein (**166**)



166 **132**

Figure 137: Chemical structure of 3'-specific hydroxylated form [272]

Daidzein (4´,7-dihydroxyisoflavone, **166**) is found in relatively high concentrations in almost all soy foods made from soy protein [281,311] and is generally present in the form of various glycosidic conjugates. [312,313] These are efficiently hydrolyzed by intestinal glucosidases, and daidzein is released and either further metabolized or absorbed unchanged. [314] Daidzein (**166**) and genisteine (**133**) are isoflavones often isolated from fermentations in media containing plant-derived nutrients such as soybean meal or cotton seed meal. [284]

The important biological properties of isoflavones like **133** and **166** include their ability to inhibit tyrosine kinases [278,315] and growth factors that regulate cell growth and proliferation.[316] Isoflavones have been extensively investigated as potential dietary and pharmacological agents in a wide range of indications, including cardiovascular disease, cancers, osteoporosis, and conditions associated with acute ovarian oestrogen deficiency. [317,318]

Hydroxylated products of daidzein (**166**) have potent antioxidant properties that contribute to their cholesterol-lowering effects, cardiovascular protection, antitumor effects, and anticarcinogenic properties. [275,319]

6.2 Marine *Streptomyces* B 7936

The crude extract of the marine *Streptomyces* sp. B7936 exhibited high activity against *Staphylococcus aureus*, *Mucor miehei* (Tü284), *Streptomyces viridochromogenes* (Tü57), moderate activity against *Candida albicans*, *Staphylococcus aureus*, the algae *Chlorella vulgaris*, and *Chlorella sorokiniana*, and weak activity against *Scenedesmus subspicatus*.

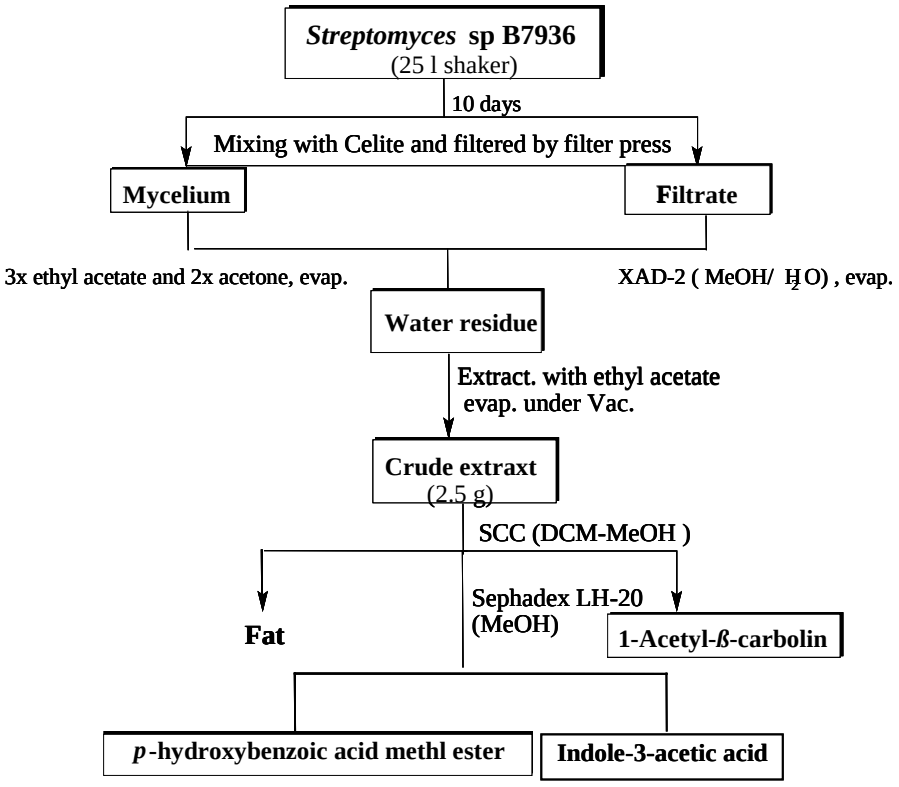


Figure 138: Work up scheme of *Streptomyces* sp. B 7936

Fermentation of the strain was carried out primarily in shaker flasks on a small scale on M_2^+ medium (50% artificial Sea water) for three days at 28 °C. The seed culture was used to inoculate a 25-liter jar fermenter. After 3 days, the culture was extracted with ethyl acetate. The reddish-brown crude extract was defatted and applied to silica

gel column chromatography, eluting by a dichloromethane-methanol gradient to give three fractions. Fraction I contained fat, but purification of II and III led to the isolation of indole-3-acetic acid (**128**), 1-acetyl-β-carbolin (**129**) and *p*-hydroxybenzoic acid methyl ester (**167**).

6.2.1 *p*-Hydroxybenzoic acid methyl ester

Compound **167** was isolated as a white crystalline solid from fraction II. It was UV absorbing and turned to pink after spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **167** showed two doublets at δ 7.95 (d, 2H, ^{3}J = 8.9 Hz) and δ 6.87 (d, 2H, ^{3}J = 8.9 Hz, AA'BB' system), in addition to one methoxy singlet at δ 3.87 (s, 3H, OCH_3). The search with the spectroscopic data provided two hits, namely *p*-hydroxybenzoic acid methyl ester (**167**) and *p*-methoxybenzoic acid (**168**). The compound was finally assigned by comparison with authentic spectra as *p*-hydroxybenzoic acid methyl ester (**167**).

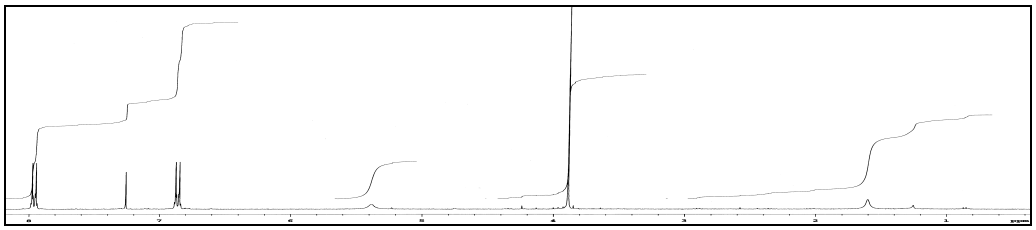
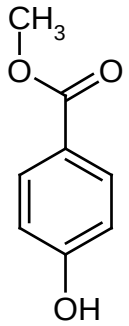
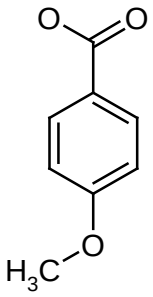


Figure 139: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of *p*-hydroxybenzoic acid methyl ester (**167**)



167



168

7 Hospital area bacterium

7.1 Hospital area bacterium *Bacillus pumilus* 1 ZIBP1

The terrestrial *Bacillus pumilus* ZIBP1 was cultivated on LB medium for 24 hours at 28 °C. Pieces of well grown agar were used to inoculate 40 of 1L Erlenmeyer flasks, each containing 250 ml of LB medium. The fermentation was carried out at 180 rpm on a linear shaker for 3 days at 28 °C. The culture broth was harvested and filtered to separate the biomass, which was extracted with ethyl acetate (3 times) and acetone (2 times), respectively. The culture filtrate was then passed through Amberlite XAD-16. The column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluates were concentrated under reduced pressure and finally extraction of the residue was done with ethyl acetate. The organic phases were dried. Both crude extracts were mixed yielding 2.5 g of a crude extract. Chromatography of the latter on silica gel column using dichloromethane-methanol gradient with successive increasing in polarity resulted in four fractions I-IV followed by monitoring with TLC.

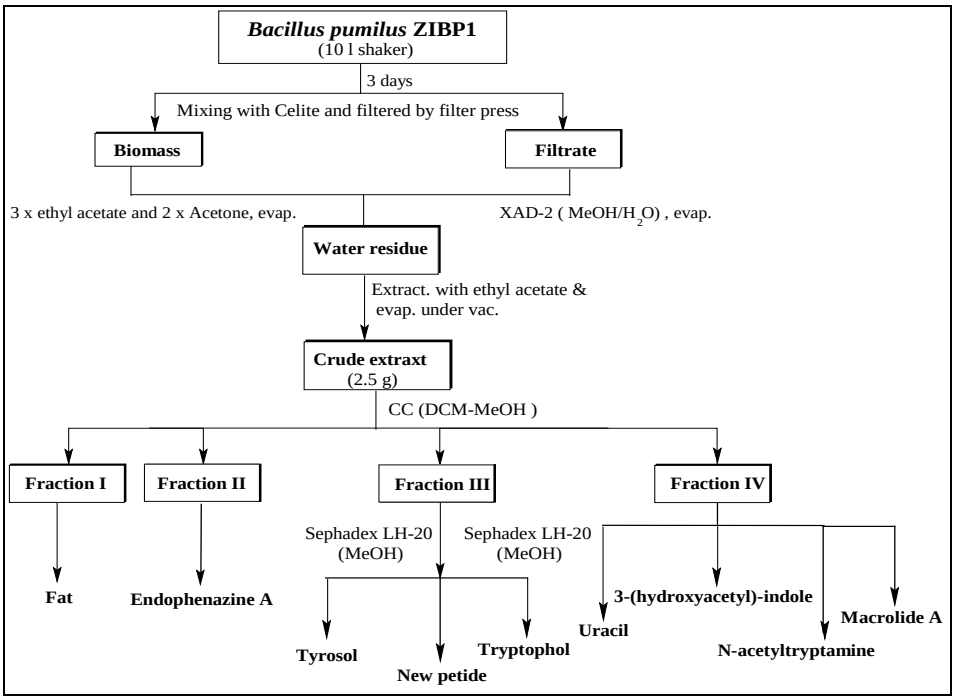


Figure 140: Work-up scheme of *Bacillus pumilus* ZIBP1

Six compounds were isolated from *B. pumilus* ZIBP1. All of them were known and were identified as endophenazine A (**37**)**,** 3-(hydroxyacetyl)-indole (**95**), N_{β}-

acetyltryptamine (**117**), tyrosol (**118**), tryptophol (**119**), uracil (**126**), and macrolactin A (**169**).

7.1.1 Endophenazine A

Compound (**37**) was isolated from fraction II as pale yellow compound with UV absorption at 254 nm. The ^{1}H NMR spectrum of **37** showed six aromatic protons at δ 8.95 (dd, 1H, J = 7.5, 1.5 Hz), 7.85 (dd, 1H, J = 8.5, 7.5 Hz), 8.50 (dd, 1H, J = 8.5, 1.5 Hz), 8.19 (dd, 1H, J = 8.5, 1.5Hz), 8.00 (dd, 1H, J = 8.5, 7.0 Hz), 7.80 (dd, 1H, J = 7.0, 1.5 Hz). In the aliphatic region proton signals were observed at δ 4.02 (d, 2H, J = 7.0Hz), 5.45 (t, 1H, J = 7.0Hz, H-2'), and 1.79 (s, 6H).

The ESI mass spectrum showed a *pseudo*-molecular ion peak at *m/z* 292 $[M+H]^{+}$. The high-resolution mass spectrum gave the molecular formula $C_{18}H_{17}N_2O_2$ for 293.1314. The previous data pointed to endophenazine A (**37**). It was further confirmed by the reported literature data.

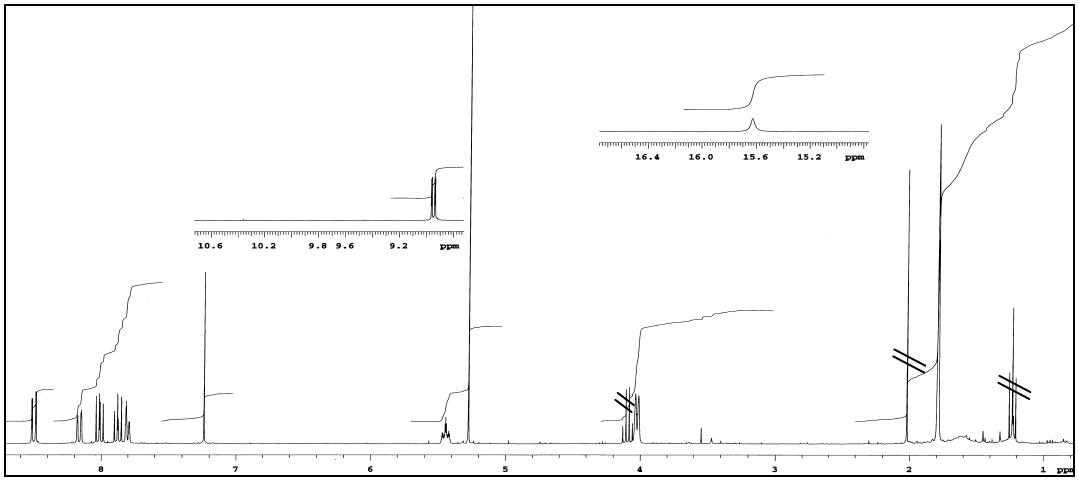


Figure 141: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of endophenazine A (**37**)

The ^{13}C NMR spectrum displayed 18 carbon atoms, 12 carbon atoms for the phenazine skeleton, one carbonyl of a carboxylic group at δ 166.0 (1-COOH) and 5 carbon atoms for a prenyl unit: one methylene at δ 29.8 (C-1'), a methine at 120.3 (C-2'), a quaternary carbon at 135.4 (C-3') and two methyls at 25.7 (C-4') and 18.0 (C-5').

Table 33: ^{1}H, ^{13}C NMR HMBC and COSY correlations of endophenazine A (**37**) in $CDCl_3$

Position	**δ_H (*J* in Hz)**	**δ_C**	**δ_H (*J* in Hz)**	**δ_C Literature [320]**
1	–	124.9	–	126.3
2	8.95 (dd, 1H, *J* = 7.5, 1.5 Hz)	137.0	8.92 (1H)	128.4
3	7.85 (dd, 1H, *J* = 8.5, 7.5Hz)	130.0	7.99 (1H)	132.6
4	8.50 (dd, 1H, *J* = 8.5, 1.5 Hz)	134.8	8.46 (1H)	118.1
4a	–	142.9	–	132.7
5a	–	144.4	–	138.7
6	8.19 (dd, 1H, *J* = 8.5, 1.5Hz)	128.0	8.13 (1H)	100.6
7	8.00 (dd, 1H, *J* = 8.5, 7.0Hz)	131.8	7.86 (1H)	183.9
8	7.80 (dd, 1H, *J* = 7.0, 1.5Hz)	131.5	7.78 (1H)	135.6
9	–	139.14	–	142.9
9a	–	139.04	–	147.7
10a	–	139.00	–	131.8
1'	4.02 (d, 2H, *J* = 7.0Hz)	29.8	4.04 (2H)	29.1
2'	5.45 (t, 1H, *J* = 7.0Hz, H-2')	120.3	5.43 (1H)	118.0
3'	–	135.4	–	137.0
4'	1.79 (s, 3H)	25.7	1.78	25.8
5'	1. 79 (s, 3H)	18.0	1.78	18.0
1-COOH	15.61 (br, 1H)	166.0	15.60 (br, 1H)	165.5

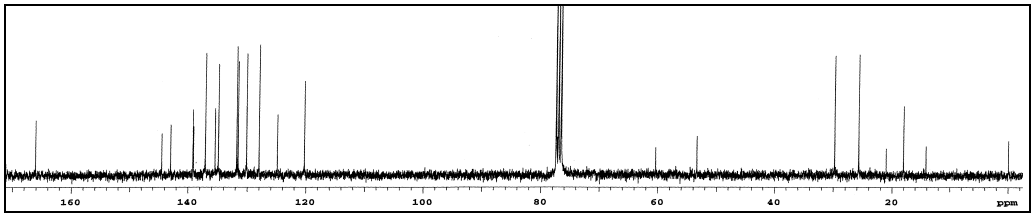
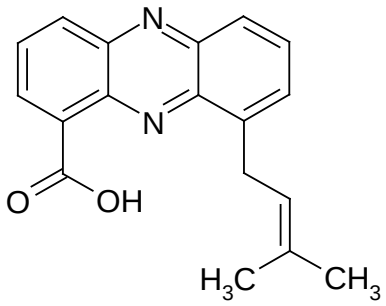


Figure 142: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of endophenazine A (**37**)



37

7.1.2 Macrolactin A

Compound **169** was isolated from fraction IV using Sephadex LH-20 (MeOH). It was isolated as yellow oil, which showed a UV absorbing band at 254 nm and turned to dark blue with anisaldehyde/sulphuric acid.

The ^1H NMR spectrum exhibited twelve olefinic protons between δ 5.40 and 7.20. Moreover, it also showed four oxymethine protons at δ 5.00, 4.28, 4,25 and 3.80. Additionally, six methylene groups in the upfield region between δ 0.97 and 2.55 and one methyl doublet at 1.22 were also observed. The search in AntiBase with these spectroscopic data resulted in macrolactin A (**169**), which was further confirmed by comparison with authentic spectra.

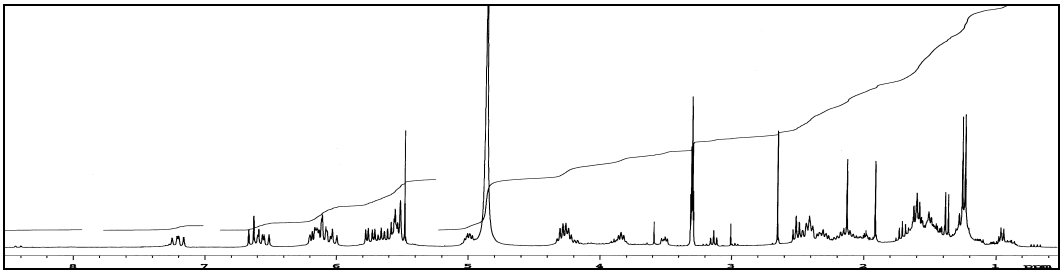
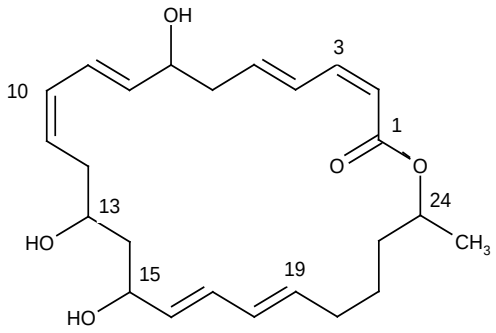


Figure 143: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of macrolactin A (**169**)

Macrolactin A (**169**) was first isolated from a deep-sea bacterium by Gustafson et *al.*, in 1989. [321] It was the first member of the macrolactin family and it was found to be active against selected reference microorganisms and clinical multiresistant gram-positive and gram-negative pathogens. [322,323] Macrolactin A (**169**) exhibits significant antiviral and anticancer properties, including inhibition of B16-F10 murine melanoma cells. [324] The lack of an adequate supply of macrolactin for therapeutic purposes has resulted in attempts to synthesize macrolactin A and analogues. [355,356]



169

8 Fungal-derived metabolites

8.1 *Aspergillus flavus*

The sub-culture of the fungus *Aspergillus flavus* was used to inoculate 4 P-flasks containing each 200 g of rice mixed with 300 ml of M_2 medium and incubated for 30 days at 20 °C. The mycelium was extracted three times with ethyl acetate, two times with acetone and two times with methanol. The organic phases were evaporated to dryness and combined to afford the crude extract (25.43 g). The extract was evaporated to dryness and separated by silica gel column chromatography to afford aurasperone A (**170**), aurasperone C (**171**), a mixture of lichexanthone and rubrofusarin B (**173**), and 5-hydroxymethylfuran-3-carboxylic acid (**174**).

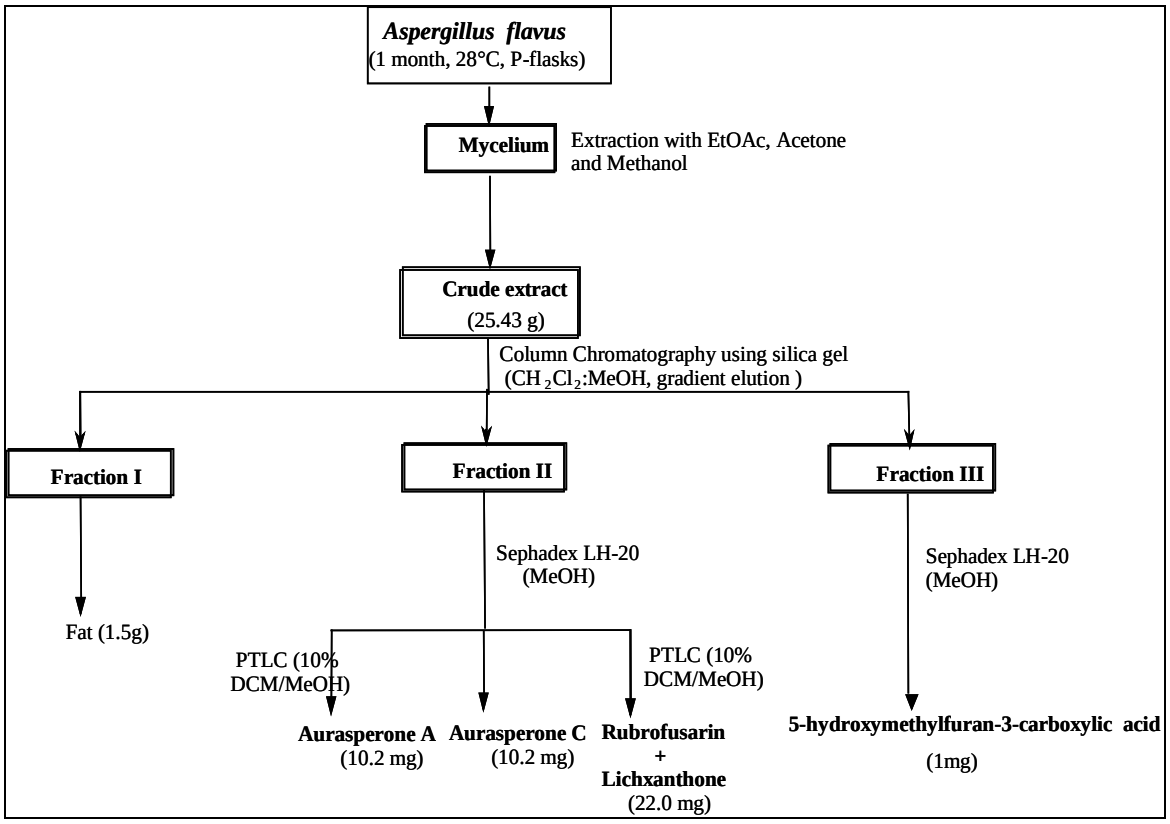


Figure 144: Work up scheme of *Aspergillus flavus*

8.1.1 Aurasperone A

Compound **170** was isolated as yellow pigment giving yellow fluorescence under UV at 366 nm. The search in AntiBase with of the spectroscopic data mentioned in Table 33 resulted in aurasperone A (**169**) that was further confirmed by comparison with reported literature data. [325]

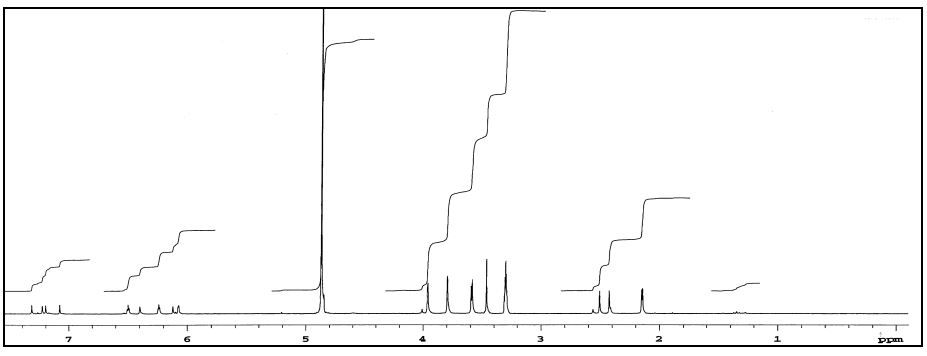
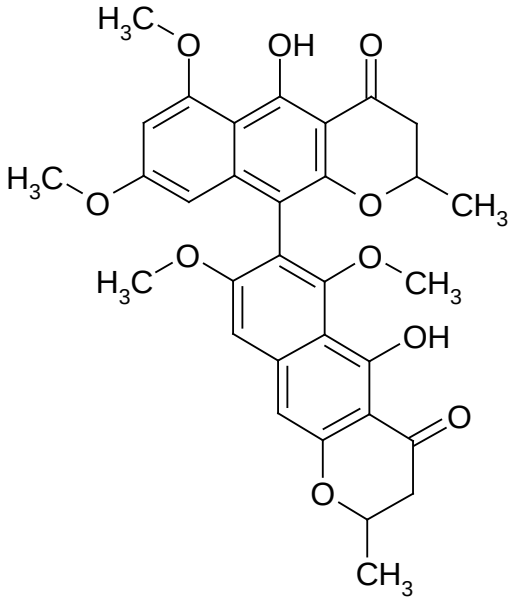


Figure 145: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of aurasperone A (**170**)

Table 34: ^{1}H NMR data of aurasperone A (**170**) in CD_3OD

Position	**δ_H (*J* in Hz)**	**δ_H Literature [358]**
2	–	–
2-CH_3	2.40 (3H, s)	2.42 (3H, s)
3	6.10 (1H, s)	6.06 (1H, s)
4	–	–
4a	–	–
5-OH	–	14.84 (1H, s)
5a	–	–
6	–	–
6-OCH_3	3.43 (3H, s)	3.46 (3H, s)
7	–	–
8	–	–
8- OCH_3	3.80 (3H, s)	3.79 (3H, s)
9	7.08 (1H, s)	6.97 (1H, s)
9a	–	–
10	7.12 (1H, s)	7.16 (1H, s)
10a	–	–
2'	–	–
2'-CH_3	2.13 (3H, s)	2.12 (3H, s)
3'	6.08 (1H, s)	5.98 (1H, s)
4'	–	–
4'-a	–	–
5'-OH	–	15.26 (1H, s)
5'a	–	–
6'	–	–
6'-OCH_3	3.95 (3H, s)	4.03 (3H, s)
7'	6.40 (1H, d, 2.2)	6.41 (1H, d, 2.2)
8'	–	–
8'-OCH_3	3.58 (3H, s)	3.62 (3H, s)
9'	6.12 (1H, d, 2.2)	6.20 (1H, d, 2.2)
9'a	–	–
10'	–	–
10'a	–	–

Aurasperone A (**170**) is a dimeric naphtho-γ-pyrone. It inhibits xanthine oxidase (IC_{50} = 10.9 µmol l^{-1}) [326] and was also shown to be a Taq DNA polymerase inhibitor. [327]



170

8.1.2 Aurasperone C

Compound **171** was isolated as yellow pigment giving a yellow fluorescence under UV at 366 nm. The ^{1}H NMR spectrum exhibited signals of four aromatic methines at δ 6.84, 6.58, 6.38, 6.20; two methylenes at δ 3.43 and 3.58, three methoxy groups at δ 3.80-3.85 and two methyl signals at δ 1.42 and 1.63. From the spectroscopic information obtained and a search in AntiBase, compound **171** was identified as aurasperone C. The structure was further confirmed by comparison with spectra as well as by comparison with reported literature data. [328]

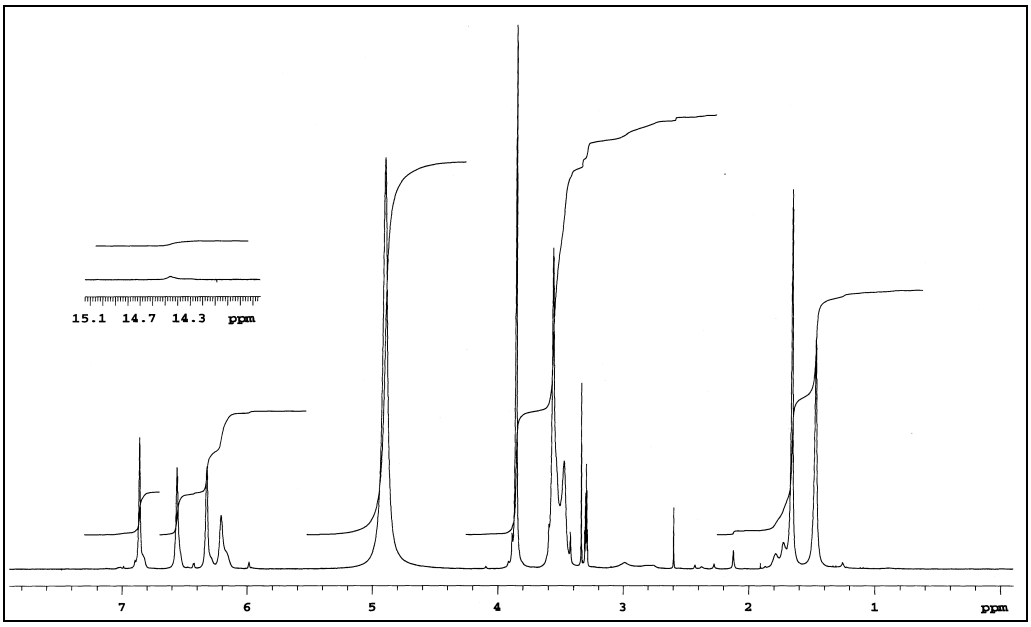


Figure 146: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of aurasperone C (**171**)

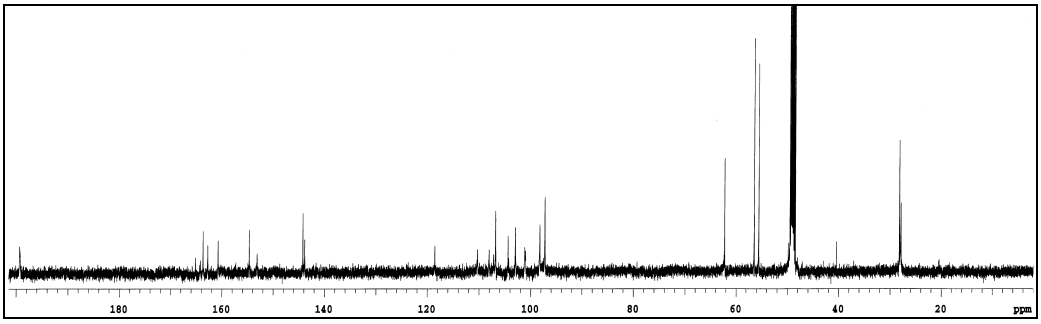


Figure 147: ^{13}C NMR spectrum (CD_3OD, 300 MHz) of aurasperone C (**171**)

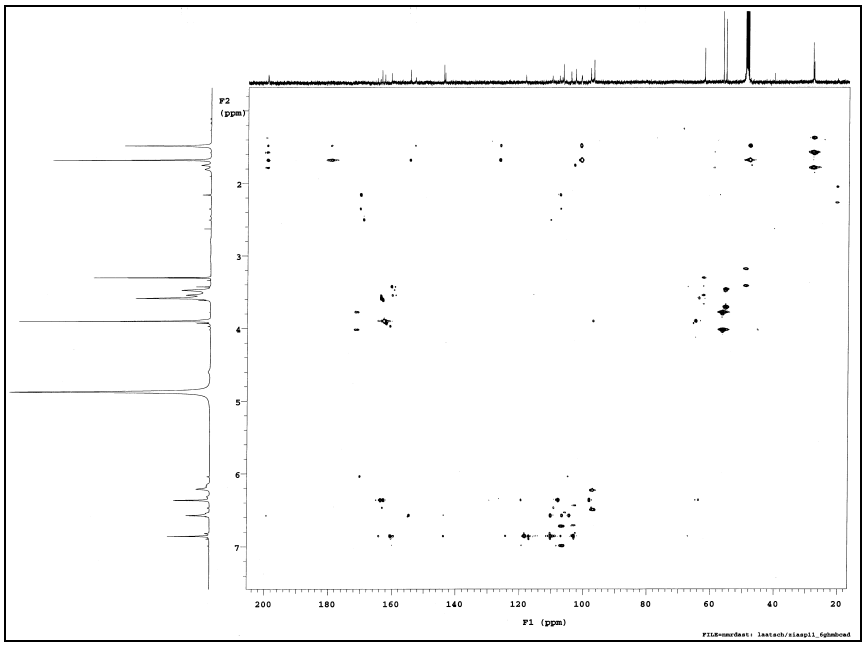
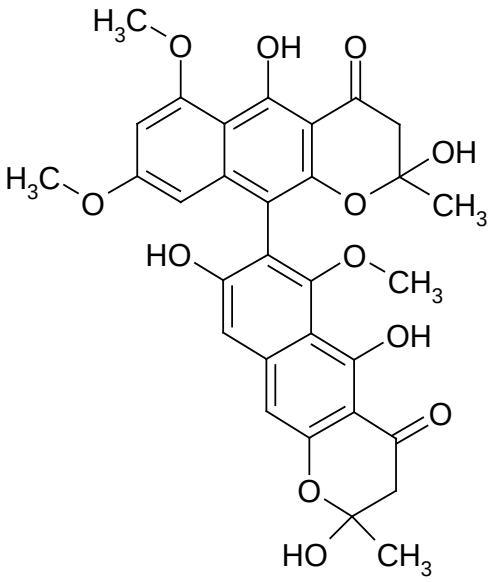


Figure 148: HMBC spectrum (CD_3OD, 600 MHz) of aurasperone C (**171**)

Aurasperone C (**171**) as well as aurasperones A (**170**), and B are dimers of linear naphtho-γ-pyrones or 2,3-dihydronaphthopyrone; they are all yellow pigments which were isolated before from the mycelia of *Aspergillus niger* and *A. awamori.* [329]



171

8.1.3 Lichexanthone and rubrofusarin B

Compounds **172** and **173** were isolated as a mixture. Both were having a yellow colour, were UV absorbing at 254 nm, and showed a yellow fluorescence under UV at 366 nm.

The ^{1}H NMR spectrum showed for compound **172** one methyl singlet at δ 2.50, two methoxy singlets at δ 3.86 and 3.95, four doublets δ 5.98 (1 H, J = 2.1 Hz), 6.25 (1 H, J = 2.1 Hz), 6.58 (1 H, J = 2.0 Hz) and 6.40 (1 H, J = 2.1 Hz), corresponding to two different unsaturated rings and a singlet at δ 12.80 (1 H, OH) due to the presence of a chelated hydroxyl group. From the spectroscopic and spectrometric information obtained and by comparison with literature data, [330,331] it was concluded that the compound isolated was lichexanthone (**172**).

The ^{1}H NMR spectrum of compound **173** showed in the aromatic region, an exchangeable OH proton at δ 14.92, and four methines at δ 6.89 (s, 1H), 6.58 (d, 1H), 6.38 (d, 1H) and 5.98 (s, 1H). It showed in the aliphatic region two methoxy signals at δ 3.98 and δ 3.90, and one methyl at δ 2.37 (s, CH_3-2). According to these data, the structure of compound **173** was assigned as rubrofusarin B.

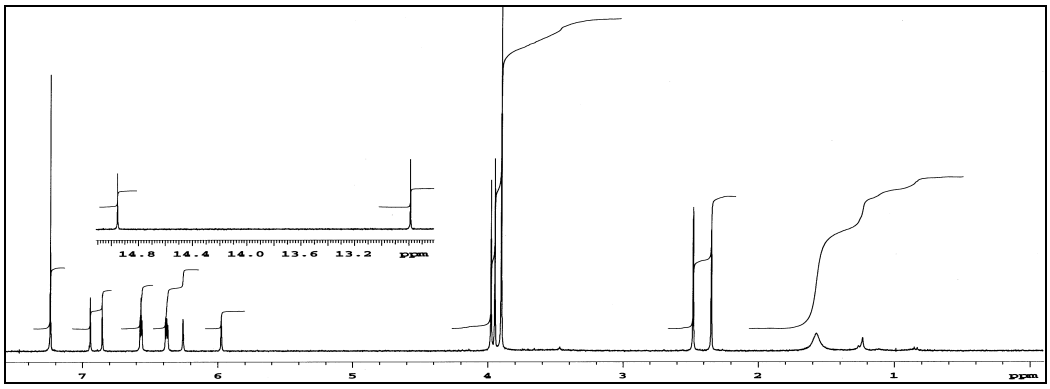
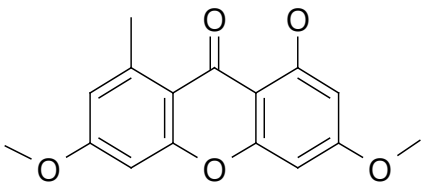


Figure 149: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of lichexanthone (**172**) and rubrofusarin B (**173**)

Table 35: ^{1}H NMR data of lichexanthone (**172**) in $CDCl_3$

Position	δ_H	δ_H Literature [332]
1-OH	12.80	13.39
2-CH	6.25	6.29
3-OMe	3.86	3.89
4	6.40	6.32
4a	–	–
5-CH	6.58	6.65
6-OMe	3.95	3.86
7	6.89	6.67
8-Me	2.50	2.84
8a	–	–
9	–	–
10	–	–
10a	–	–

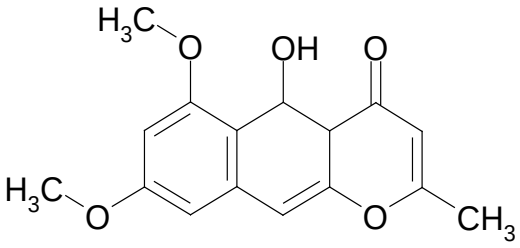


172

Lichexanthone (**172**) was recently isolated from the lichen *Parmotrema* sp.[333] It is the major pigment in the lichen extract of *Laurera benguedensis*. [334]

Table 36: ^{1}H NMR data of rubrofusarin B (**173**) in $CDCl_3$

Position	δ_H	δ_H Literature [335]
1	–	–
2-Me	2.37	2.37
3-CH	5.98	5.94
4	–	–
5-OH	14.92	14.88
6-OMe	3.98	4.02
7-CH	6.38	6.35
8-OMe	3.90	3.92
9-CH	6.58	6.51
10-CH	6.89	6.87

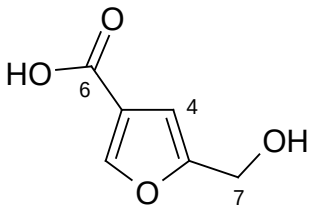


173

Rubrofusarin B (**173**) has shown significant cytotoxicity to the colon cancer cell line SW1116 (IC_{50}: 4.5 µg ml^{-1}). [359] Rubrofusarin B (**173**) and aurasperone A (**170**) are co-inhibitors on xanthin oxidase, the colon cancer cell line SW1116 and the human opportunistic pathogens *C. albican* and *T. rubrum*. [359] Rubrofusarin B (**173**) was able to reverse multi-drug resistance of human epidermal KB carcinoma cells [336] and to inhibit the calmodulin-dependent activity of *c*AMP phosphodiesterase and NAD-kinase in the presence of CaM. [337]

8.1.4 5-Hydroxymethylfuran-3-carboxylic acid

Compound **174** was isolated as colourless needles; it was UV absorbing at 254 nm. The ^{1}H NMR spectrum of **174** showed three signals at δ 7.94 (s, 1H, H-2), 6.49 (br t, 1H; 0.5 Hz, H-4) and 4.40 (br d, 2H, 0.5 Hz, H-7), whereas the ^{13}C NMR spectrum showed six carbon resonances, one carbonyl at δ 176.9 (C-6), four aromatic carbons at δ 141.0 (C-2), 147.4 (C-3), 110.8 (C-4), 170.4 (C-5), and a methylene group attached to oxygen at δ 61.2 (C-7). The chemical shifts were consistent with the presence of a furan ring bearing a carboxyl substituent. The search in AntiBase with of these spectroscopic data resulted in 5-hydroxymethylfuran-3-carboxylic acid, which was further confirmed by comparison with reported literature data.



174

This compound has been previously reported as a synthetic intermediate.[338] Later it was isolated as a natural product from *Polyporus ciliatus*. [339]

8.2 *Aspergillus niger*

The sub-culture of the fungus *Aspergillus niger* was used to inoculate four P-flasks containing each 200 g of rice mixed with 300 ml of M_2 medium and incubated for 30 days at 20 °C to give the mycelium. The mycelium was extracted three times with ethyl acetate, two times with acetone and two times with methanol. The organic phases were evaporated to dryness and combined to afford the crude extract (27.18 g). The extract was evaporated to dryness and separated by silica gel column chromatography to afford kojic acid (**175**), a mixture of stigmasterol (**176**) and γ-sitosterol (**177**), and piperazine (**178**).

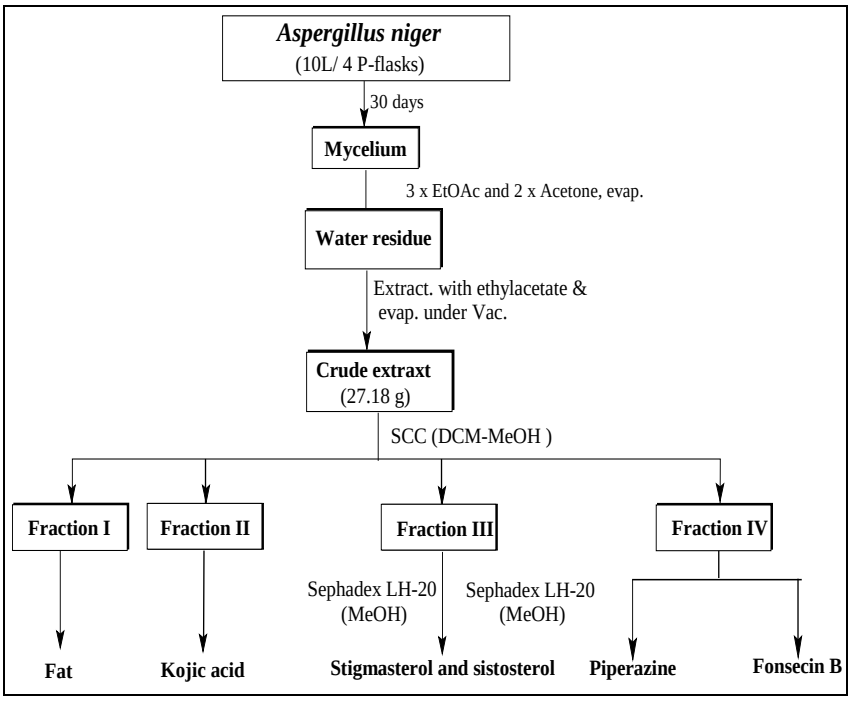


Figure 150: Work up scheme of *Aspergillus niger*

8.2.1 Kojic acid

Compound (**175**) was isolated as colourless solid. On TLC it gave a UV absorbing band, which turned to blue with anisaldehyde/sulphuric acid on heating. The ^{1}H NMR spectrum revealed in the aromatic region two broad signals at δ 8.99 and 5.99 for two acidic D_2O-exchangeable protons and one singlet of a proton attached to an heteroatom at δ 7.99 (1H), and another singlet at δ 6.33 (1H). In the aliphatic region a methylene group attached to a heteroatom appeared at δ 4.29 (2H).

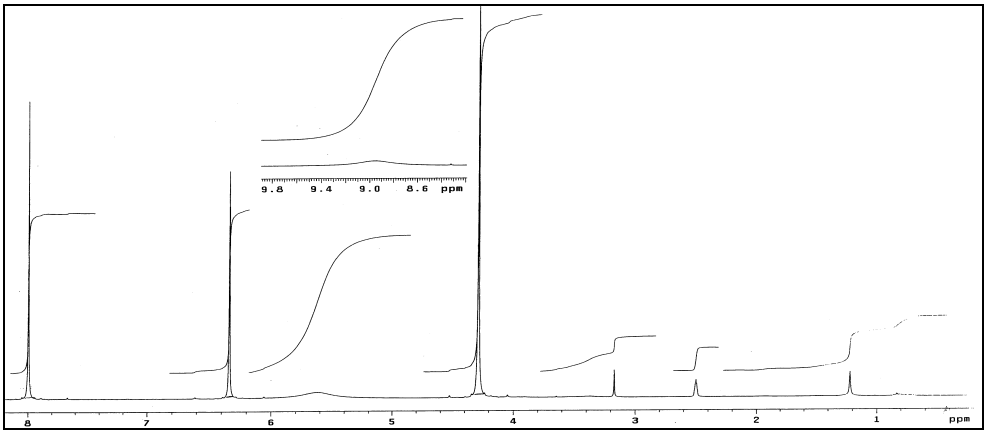


Figure 151: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of kojic acid (**175**)

The ^{13}C NMR spectrum showed six carbon resonances, one carbonyl at δ 174.0 in addition to a quaternary carbon attached to an heteroatom at δ 168.3 and further two carbons at δ 145.9 and at δ 139.0, which represented a double bond fragment in conjugation with a carbonyl group, as well as one signal at δ 110.0 and a methylene group attached to oxygen at δ 59.8. The search in AntiBase with of these spectroscopic data resulted in kojic acid (**175**), which was further confirmed by comparison with reported literature data.

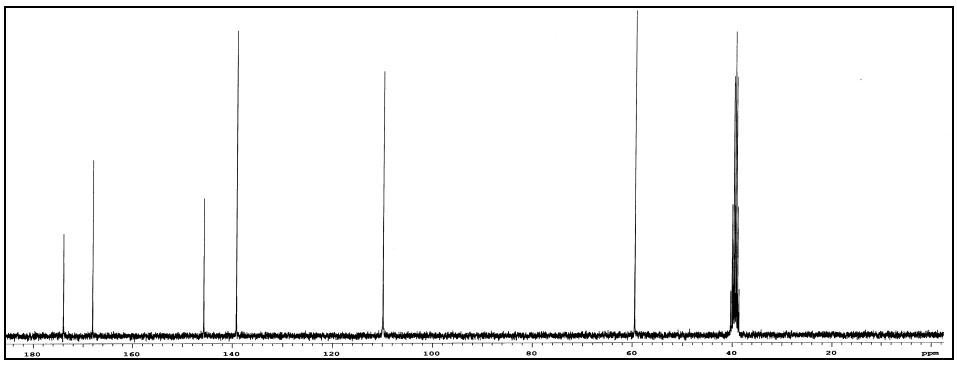
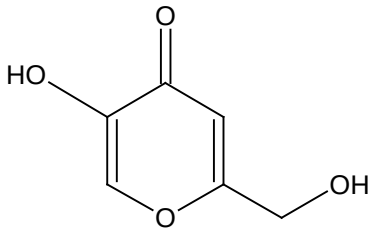


Figure 152: ^{13}C NMR spectrum (DMSO-d_6, 300 MHz) of kojic acid (**175**)

Kojic acid (**175**) was isolated from *Paecilomyces lilacinus*, which was derived from a marine sponge *Petrosia* sp. and from a toxigenic strain of *Aspergillus parasiticus* and a non-toxigenic strain of *Aspergillus flavus*. [340]



175

8.2.2 Stigmasterol and *β*-sitosterol

Stigmasterol (**176**) and γ-sitosterol (**177**) were isolated in mixture as a white crystalline solid, which showed a UV absorbing band and turned to violet with anisaldehyde/sulphuric acid. From EIMS, the molecular weight was determined as *m/z* 414 and 412, and the molecular formulas were obtained by HRESIMS as $C_{29}H_{48}O$ and $C_{29}H_{50}O$, respectively. The ^{1}H NMR spectrum showed two olefinic protons at δ 5.40: one proton for **176** (H-6) and the second one for **177** in addition to two olefinic protons appearing as doublets of doublet at δ 5.16 and 5.00 (H-22) and (H-23), respectively, for **176**. Moreover, the multiplet at δ 3.50 (H-3) is a typical signal for steroids with an oxygenated position C-3. At δ 5.00-5.40 there was evidence of three sp^2 protons or 3 olefinic protons were observed.

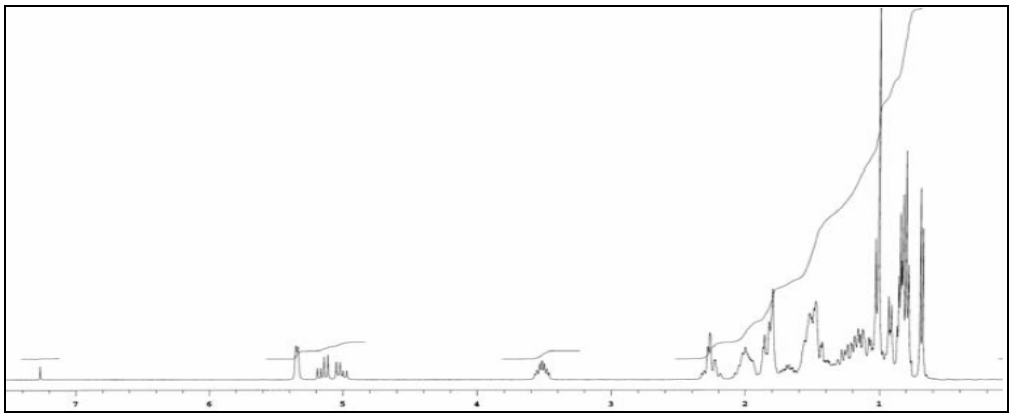


Figure 153: ^{1}H NMR (300 MHz) spectrum of stigmasterol (**176**) and sitosterol (**177**) in $CDCl_3$.

In the ^{13}C NMR spectrum, four carbon signals appeared in the sp^2 region between δ 102-142, which indicated that there were at least two double bonds. There was the signal of an oxygenated methine carbon at δ 77.0. In the sp^3 region, overlapping carbon signals appeared. The NMR data were identical with those of a previously obtained stigmasterol (**176**)/ sitosterol (**177**) mixture.

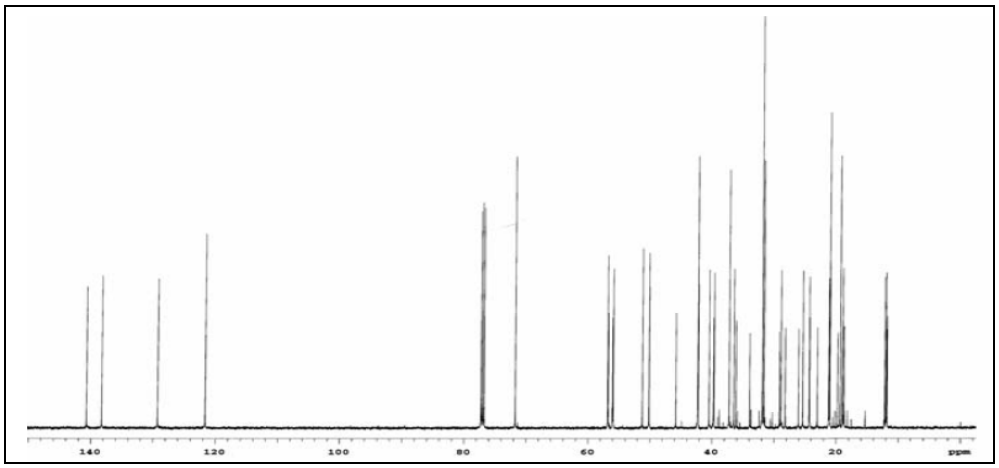
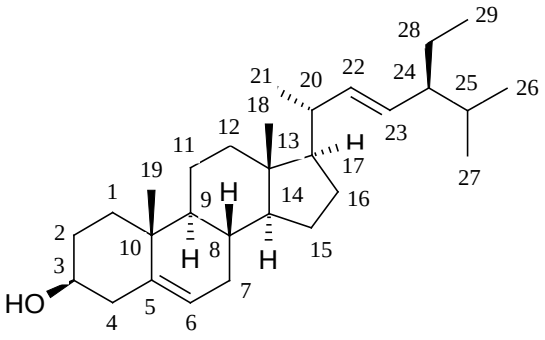
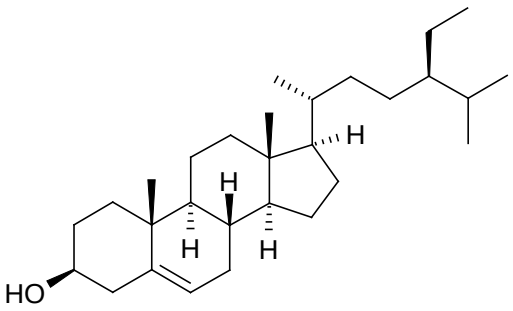


Figure 154: ^{13}C NMR (125 MHz) spectrum of stigmasterol (**176**) and sitosterol (**177**) in $CDCl_3$.



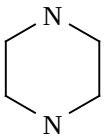
176



177

8.2.3 Piperazine

Compound **178** was isolated as white colourless crystals. The ^{1}H NMR showed one singlet at δ 2.99. By direct comparison, the compound was found to be piperazin (**178**), a compound consisting of a six-membered ring containing two nitrogen atoms in 1,4-position.



178

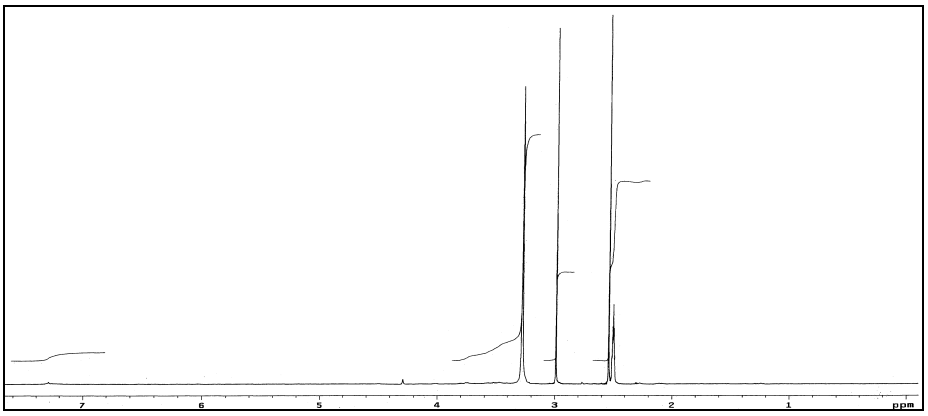


Figure 155: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of piperazine (**178**)

Piperazine (**178**) has a remarkable power to dissolve uric acid and producing a soluble urate, but in clinical experience it has not proved equally successful. Piperazine (**178**) was first introduced as an anthelmintic in 1953. A large number of piperazine derivatives are having anthelmintic activity. Their mode of action is generally by paralysing parasites, which allows the host body to easily remove or expel the invading organisms.

Piperazines and substituted piperazines are important pharmacophores that can be found in many marketed drugs, such as the Merck HIV protease inhibitor Crixivan. [341-342] Recently, piperazine derivatives containing tetrazole nucleus have been reported as antifungal agents. [343-344]

9 Summary

9.1 Results

Phytopathogenic fungi cause large damage to agriculture and its products. Additionally, the extraordinary development of the bacterial multi-resistance to antibiotics and the difficulties encountered by pharmaceutical industry in the synthesis and the production of new or more effective antibiotics generated major problems in the treatment of infectious diseases as well to mankind as also to animals. The discovery of new families of really innovative antibiotics is now a priority in public health.

The search for microorganisms useful in agriculture, agroalimentary and pharmaceutical industries requires the exploration also of ecological niches. Thus, within this background, the rumen (one of the four stomach parts of ruminals like the cow), is such an ecological niche, which is very rich in microorganisms and could be exploited in order to check the antagonistic capacity of the ruminal bacteria against pathogenic bacteria and fungi.

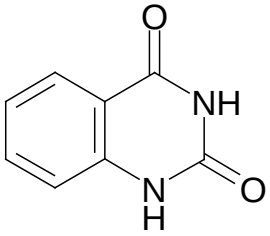
This work thus fits in the biological fight in agriculture against the cryptogamic diseases. Indeed, the use of microorganisms to fight against phytopathogenic fungi is much better for the environment and the human health than the use of pesticides. It avoids the pollution of the ground, water and food. It also fits in the fight against the multiresistant bacteria and fungi.

Sixteen bacteria (seven ruminal, six terrestrial, two marine and one from hospital area) and two fungi were selected and subjected to culture in large scale. The working up and isolation of their metabolic constituents were described.

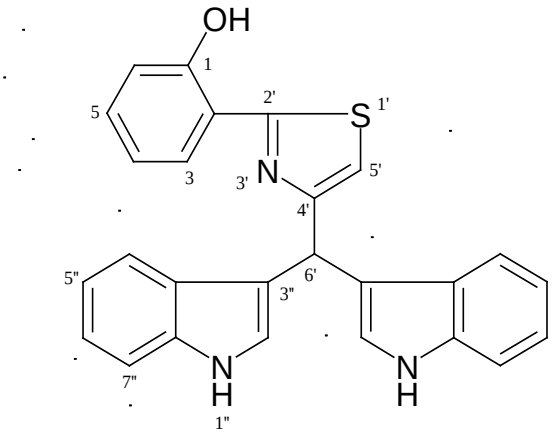
From the extracts of the sixteen bacteria, forty compounds were isolated, among them nine new compounds, of which **92** had a new skeleton. The remaining compounds were classified into indole derivatives, phenazines, quinolones, one quinone, one macrolide, peptides, and one sulphur compound.

The ruminal *Pseudomonas aeruginosa* showed activity against human pathogenic bacteria and fungi, and phytopathogenic fungi. Eight compounds were isolated, among them one (barakacin, **92**) had a new skeleton, together with seven known

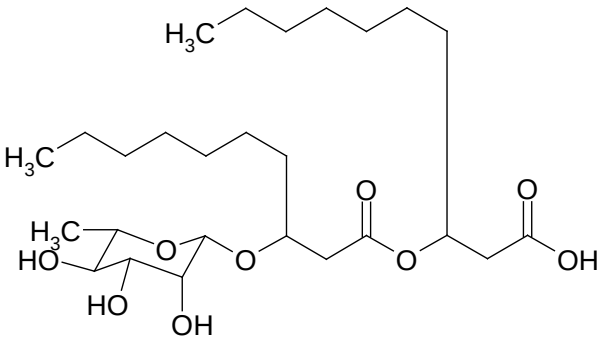
compounds: quinazoline-2,4-dione (**91**), indol-3-carbaldehyde (**96**), 3-(hydroxyacetyl)-indole (**95**), phenazine carboxylic acid (**43**), phenylacetic acid, rhamnolipid A (= glycolipid A) (**94**), and *cyclo*(Phe,Pro) (**97**).



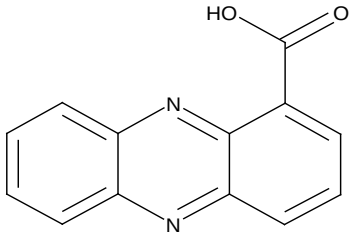
91



92



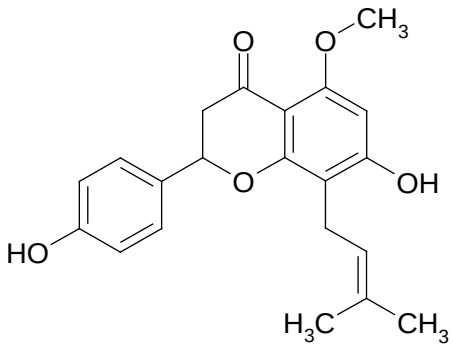
94



43

Barakacin (**92**) showed a weak and unselective cytotoxic activity against human cancer cell lines LXFA 629L, LXFL 529L (lung), MAXF 401NL (breast), MEXF 462NL (melanoma), RXF 944L (kidney) and UXF 1138 (uterus) with a mean IC_{50} value of 2.8 μg/mL (mean IC_{70} = 5.4 μg/mL).

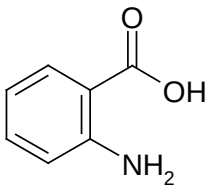
The ruminal bacterium *Citrobacter freundii* was selected due to its biological activity against human pathogenic bacteria such as *Staphylococcus aureus* and *Klebsiella pneumoniae* and phytopathogenic fungi: *Fusarium culmorum*, *Fusarium graminearum* and *Phoma tracheiphila.* Several compounds were isolated and identified as oleic acid (**100**), myristic acid (**101**), palmitoleic acid (**102**), palmitic acid (**103**), 9,10-methanohexadecanoic acid (**104**), isoxanthohumol (**105**), *cyclo*(Tyr,Pro) (**109**), *cyclo*(Pro,Leu) (**107**), phenol (**111**) and adenine (**112**).



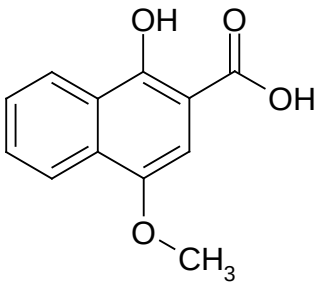
105

The ruminal *Gemella morbillorum* showed inhibition against a collection of phytopathogenic fungi. The supernatant of the culture was assessed by the agar diffusion method and showed inhibition of the fungus *Phoma tracheiphila*. The crude extract

delivered two known compounds: anthranilic acid (**113**), and 1-hydroxy-4-methoxy-2-naphthoic acid (**116**).



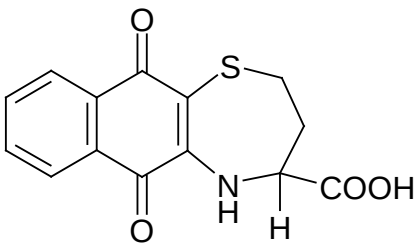
113



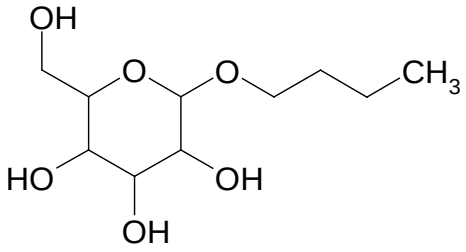
116

The ruminal bacterium *Enterobacter amnigenus* was selected due to its activity against the pathogenic bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae* and against the phytopathogenic fungi *Fusarium culmorum*, *Fusarium graminearum* and *Phoma tracheiphila.*

Eleven compounds were isolated: among them, *n*-butyl-glycoside (**122**) was, whereas the other compounds were known and were identified as 3-(hydroxyacetyl)-indole (**95**), N$_{ß}$-acetyltryptamine (**117**), tyrosol (**118**), phenol **(111)**, tryptophol (**119**), brevinic acid (**120**), indole-3-lactic acid (**121**), uracil (**126**), and adenine (**112**).

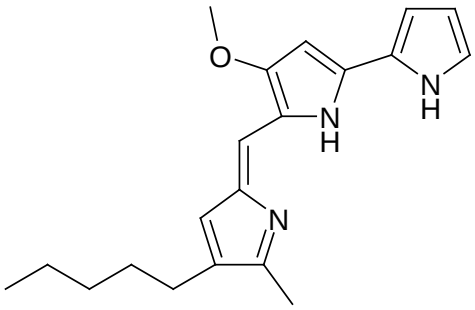


120



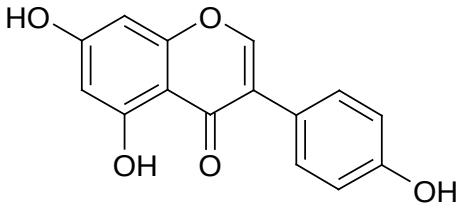
122

From the ruminal *Serratia rubidae*, eight compounds were isolated, of which some had previously been described in this thesis; they were identified as thymine (**123**), adenine (**124**), thymidine (**125**), uracil (**126**), prodigiosin (**127**), indole-3-acetic acid (**128**), 1-acetyl-β-carboline (**129**), actinomycin D (**131**), and 1-hydroxy-4-methoxy-2-naphthoic acid (**134**). From the HPLC-MS of the crude extract, a compound with a molecular weight of *m/z* 1049 was observed. Preliminary ESI-MS/MS studies revealed that the compound was potentially a new cyclopeptide structurally related to surfactin C.



127

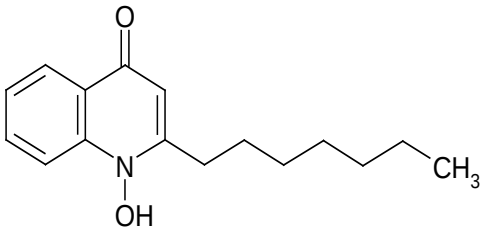
Four known compounds were isolated from the ruminal *Klebsiella pneumoniae* and were identified as adenine (**124**), uracil (**126**), 4',5,7-trihydroxyisoflavone (genistein, **133**), and 4-hydroxybenzoic acid (**134**).



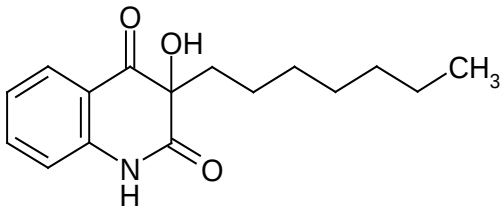
133

The ruminal *Pseudomonas aeruginosa* 210 was selected due to its inhibitory activity against a collection of human pathogenic bacteria. The supernatant of the culture was assessed by the agar diffusion method showed inhibition of a broad spectrum of Gram-negative and Gram-positive bacteria.

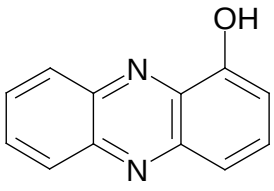
Seven known compounds were isolated from this strain, namely phenazine-1-carboxylic acid (**43**), rhamnolipid A (**94**) and B (**140**), 2-*n*-heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**), 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**), polypropylenglycol (**138**), and 1-phenazinol (**139**).



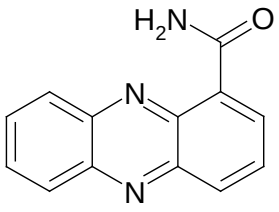
135



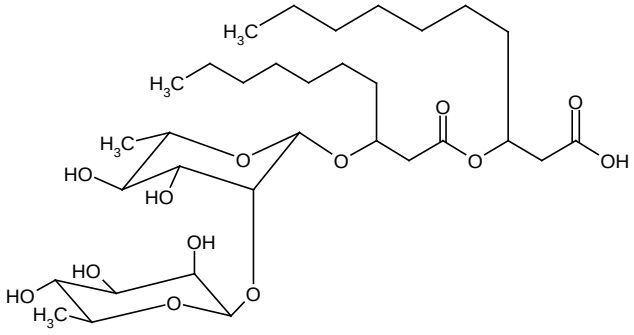
137



139

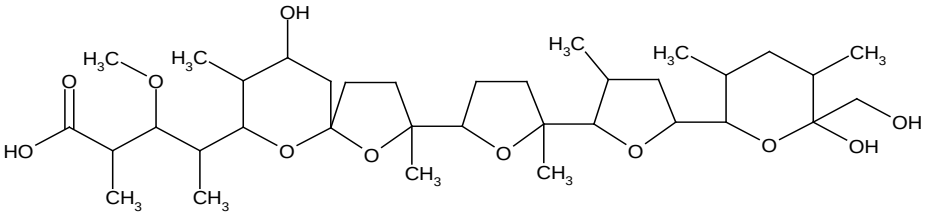


42



140

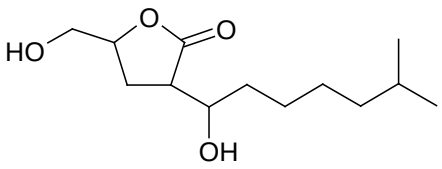
Only one major compound was isolated from the terrestrial *Bacillus* ZIR, namely monensin (**141**).



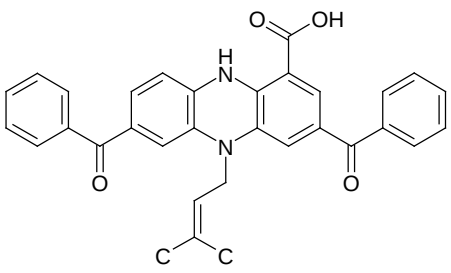
141

Four compounds were isolated from the terrestrial *Pseudomonas* sp. ZIPS; all of them were known and were identified as phenazine-1-carboxylic acid (**43**), linoleic acid (**89**), *cis-cyclo*(Tyr,Pro) (**109**), and anthranilic acid (**113**).

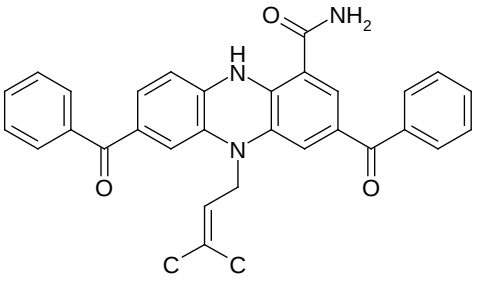
The terrestrial *Streptomyces* sp. 195 yielded only one major compound, 1(1-R,2S,4S)-2-(1-hydroxy-6-methylheptyl)-4-hydroxymethyl-butanolide (**143**).



143

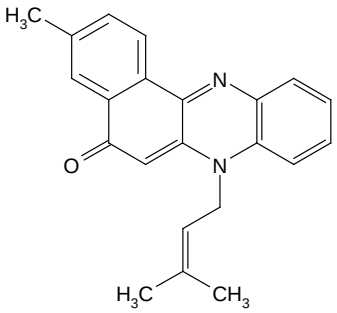


151

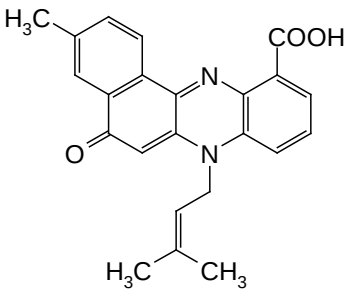


152

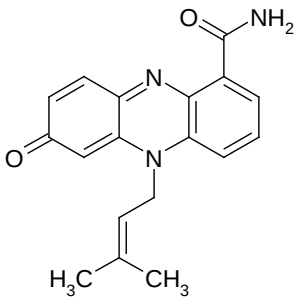
The strain *Streptomyces* sp. Ank 315 was selected due to its biological activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, the fungus *Mucor miehei* (Tü284) and the yeast *Candida albicans.*



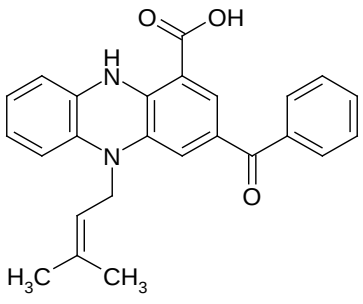
144



145



146

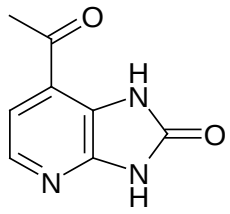


147

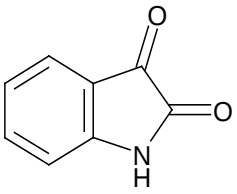
From the strain *Streptomyces* sp. Ank 315, six new compounds were isolated, namely the chromophenazines A-F [9-methyl-5-(3'-methyl-but-2'-enyl)-5*H*-benzo[a]phenazine-7-one (**144**), 9-methyl-5-(3'-methyl-but-2'-enyl)-7-oxo-5,7-dihydrobenzo[a]-phenazine-1-carboxylic acid (**145**), 5-(3'-methyl-but-2'-enyl)-7-oxo-5,7-dihydrophenazine-1-carboxamide (**146**), 3-benzoyl-5-(3'-methylbut-2'-enyl)-5,10-dihydrophenazine-1-carboxylic acid (**147**), 3,7-dibenzoyl-5-(3'-methyl-but-2'-enyl)-5,10-dihydrophenazine-1-carboxylic acid (**151**), and 3,7-dibenzoyl-5-(3'-methyl-but-2'-enyl)-5,10-dihydrophenazine-1-carboxamide (**152**)], together with phenazine-1-carboxylic acid (**43**), anthranilic acid (**113**), tryptophol (**119**), and 1-phenazinol (**139**).

Compounds **144** and **145** were obtained from a 25 L cultivation, whereas compounds **146, 147**, **151** and **152** were isolated from a 60 L cultivation.

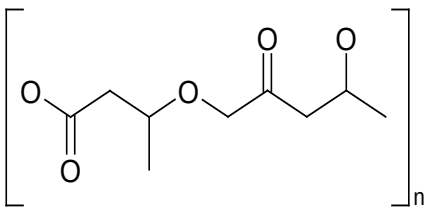
The strain *Streptomyces* sp. Ank 223 was selected due to its biological activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, the fungus *Mucor miehei* (Tü284) and the yeast *Candida albicans*. Five compounds were isolated from *Streptomyces* sp. Ank 223, all of them were known and were identified as 7-acetyl-1,3-dihydroimidazo[4,5,b]pyridine-2-one (**154**)**,** isatin (**155**), polyhydroxybutyric acid (**156**), lumichrome (**157**), and ferroverdin A (**158**).



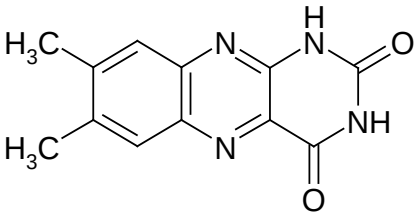
154



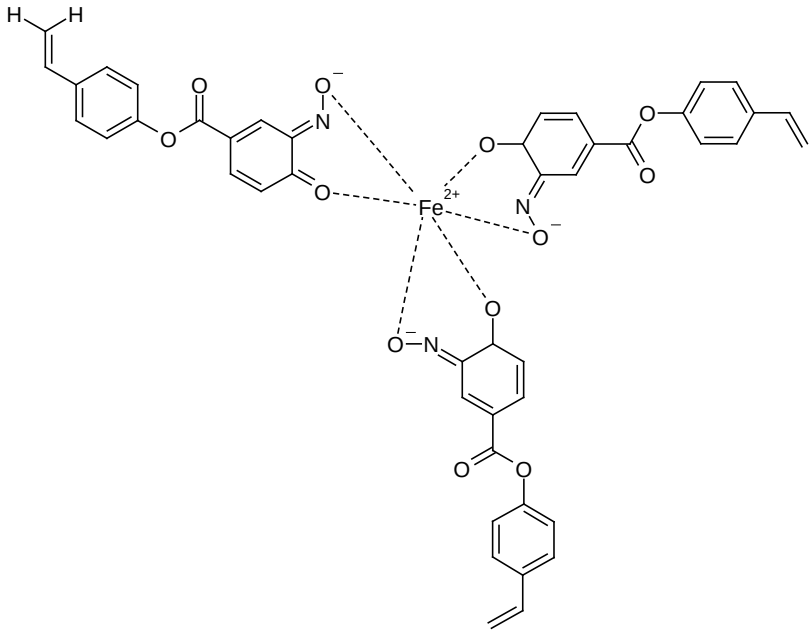
155



156

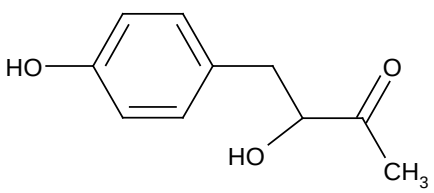


157

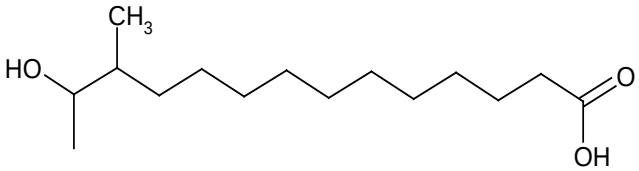


158

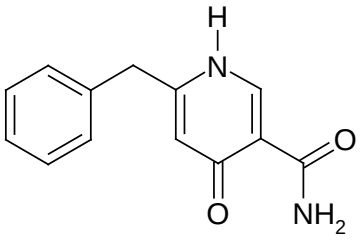
The crude extract of the terrestrial *Streptomyces* sp. WO 668 delivered two new natural products, namely 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**161**) and 13-hydroxy-12-methyl-tetradecanoic acid (**163**), in addition to other known compounds: tryptophol (**119**), indole-3-lactic acid (**121**), indole-3-carbaldehyde (**96**), uracil (**126**), *p*-hydroxybenzoic acid (**134**), and aspernigrin A (**165**).



161

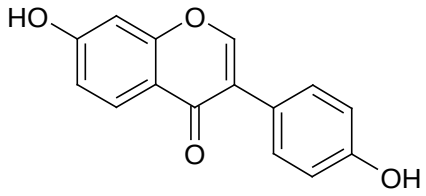


163



165

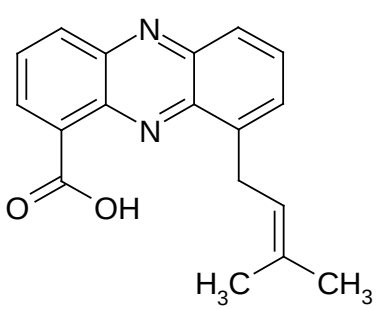
The extract of *Streptomyces* sp. B 909-417 displayed cytotoxic activity with a mortality rate of 100 % at 100 µg/ml against the brine shrimp *Artemia salina* and showed moderate antimicrobial activity. Two compounds were isolated, namely daidzein (**166**) and 4-hydroxybenzoic acid (**134**).



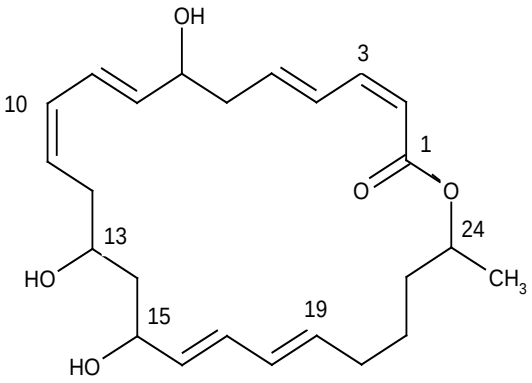
166

The crude extract of the marine *Streptomyces* sp. B7936 exhibited high activity against *Staphylococcus aureus*, *Mucor miehei* (Tü284), *Streptomyces viridochromogenes* (Tü57), moderate activity against *Candida albicans*, *Staphylococcus aureus*, the algae *Chlorella vulgaris*, and *Chlorella sorokiniana*, and weak activity against *Scenedesmus subspicatus*. The chromatographic purification of the extracts led to the isolation of known compounds: *p*-hydroxybenzoic acid methyl ester (**167**), indole-3-acetic acid (**128**), and 1-acetyl-β-carbolin (**129**).

Six compounds were isolated from the hospital area bacterium *Bacillus pumilus* ZIBP1. All of them were known and were identified as endophenazine A (**37**), 3-(hydroxyacetyl)-indole (**95**), N_{β}-acetyltryptamine (**117**), tyrosol (**118**), tryptophol (**119**), uracil (**126**), and macrolactin A (**169**).

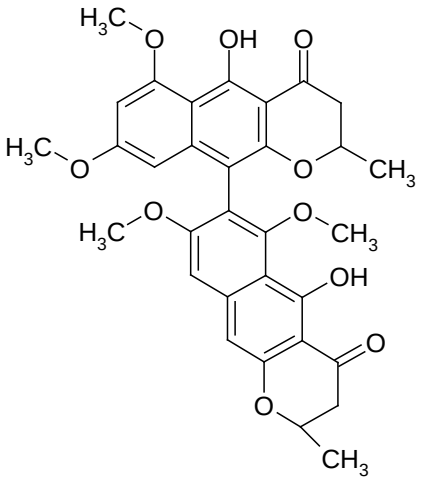


37

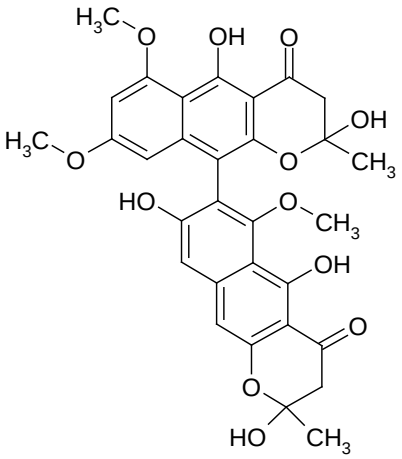


169

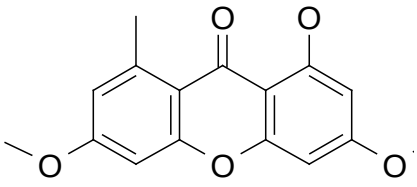
The crude extract of the fungus *Aspergillus flavus* delivered five known compounds, namely aurasperone A (**170**), aurasperone C (**171**), a mixture of lichexanthone (**172**) and rubrofusarin (**173**), and 5-hydroxymethylfuran-3-carboxylic acid (**174**).



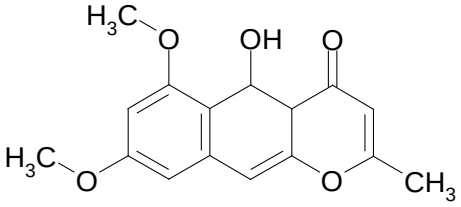
170



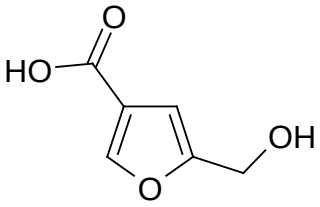
171



172

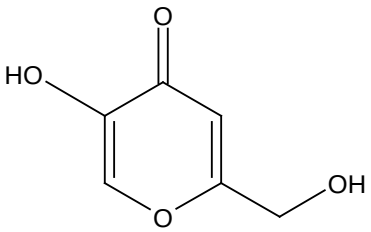


173

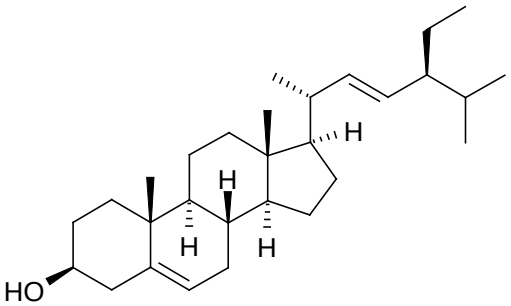


174

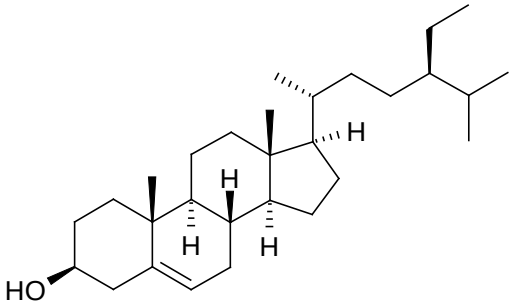
The chromatographic purification of the crude extract of *Aspergillus niger* resulted in the isolation of kojic acid (**175**), a mixture of stigmasterol (**176**) and *β*-sitosterol (**177**) and piperazine (**178**).



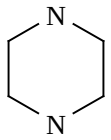
175



176



177



178

Table 37: Total number of isolated compounds from the bacteria and fungi described in this thesis:

Bacteria and Fungi	**Number of strains**	**Number known of compounds**	**Number of new compounds**
Ruminal bacteria	7	40	2
Terrestrial bacteria	6	30	8
Marine-derived *Strep-tomyces* sp.	2	6	0
Hospital area bacteria	1	3	0
Fungi	2	9	0
Total	18	88	10

According to this study, the results can be summarized as following:

- The ruminal bacteria, terrestrial *Streptomyces* and marine *Streptomyces* are an ideal target for discovery of novel potential bioactive compounds.

- The investigation of the terrestrial *Streptomyces* and marine *Streptomyces* should be continued to extend the probability of discovering new compounds with new skeletons.

10 Materials and Methods

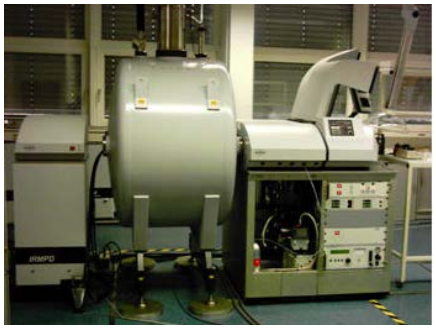
10.1 General

IR spectra: Perkin-Elmer 1600 Series FT–IR; Perkin-Elmer 297 infrared spectrophotometer; Beckman DU-640; Shimadzu FT-IR; (KBr tablet and film). – **UV/Vis spectra:** Perkin-Elmer Lambda 15 UV/Vis spectrometer. – **Optical rotations:** Polarimeter (Perkin-Elmer, model 243); the concentrations were given in [g/100 ml]. – **^{1}H NMR spectra:** Varian Unity 300 (300.145 MHz), Bruker AMX 300 (300.135 MHz), Varian Inova 500 (499.8 MHz), Varian Inova 600 (599.7 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet doublet, t = triplet, q = quartet, m = multiplet, br = broad. – **^{13}C NMR spectra:** Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz), Varian Inova 600 (150.7 MHz). Chemical shifts were measured relative to tetramethylsilane as internal standard. Abbreviations: APT (Attached Proton Test): CH/CH_3 up and C_q/CH_2 down. – **2D NMR spectra:** ^{1}H,^{1}H COSY (^{1}H,^{1}H-Correlated Spectroscopy), HMBC (Heteronuclear Multiple Bond Connectivity), HMQC (Heteronuclear Multiple Quantum Coherence) and NOSY (Nuclear Overhauser Effect Spectroscopy).

– **Mass spectra**: EI MS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perfluorokerosene as standard. DCI-MS: Finnigan MAT 95 A, 200 eV, Reactant gas NH_3. ESI MS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). ESI-HRMS were measured on Micromass LCT mass spectrometer coupled with a HP1100 HPLC with a diode array detector. High-resolution mass spectra (HR-MS) were recorded by ESI MS on an Apex IV 7

Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). ESI MS/MS was performed with normalized collision energy of 35%. EI MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for EI HR-MS, samples were infused with a flow rate of 2 µL/min.

Electrospray ionization mass spectrometry (ESIMS) and high-resolution mass spectra (HR-ESIMS) were recorded on a micrOTOF time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) as well as on an Apex IV 7 Tesla Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA, USA).



High performance liquid chromatography (HPLC): Instrument I: **Analytical**: Jasco multiwavelength detector MD-910, two pumps type Jasco Intelligent Prep. Pump PU-987 with mixing chamber, injection valve (type Rheodyne) with sample loop 20 μl, Borwin HPLC-software. **Preparative**: sample loop 500 µl. **Analytical column:** 1) Eurochrom 4.6 × 125 mm without pre-column: stationary phase: Hypersil, ODS 120 × 5 µm; 2) Vertex 4.6 × 250 mm, stationary phase: Nucleosil NP 100-C-18, particle size 5 µm; **Preparative column**: 1) Vertex 16 × 250 mm with 16 × 30 mm pre-column, stationary phase: Eurospher C-18 RP 100 × 5 μm; 2) Vertex 16 × 250 mm with 16 × 30 mm pre-column, stationary phase: Nucleosil NP 100-C-18, particle size 5 μm, pore diameter 100 Å (Macherey–Nagel & Co.). **Instrument I**: Knauer HPLC equipment containing: spectral-digital-photometer A0293, two pumps type 64 A0307, HPLC software V2.22, mixing chamber A0285, injection valve 6/1 A0263 (type Rheodyne) and sample loop 20 µl. **HPLC solvents:** Acetonitrile/water azeotrop (83.7% acetonitrile, bp. 78.5 °C). The azeotrop was redistilled, filtered

through a membrane filter (pore Ø: 0.45 μm, regenerated cellulose, Sartorius, Göttingen) and then degassed for 15 min by ultrasonic. – **Filter press:** Schenk Niro 212 B40. – **Photo reactor for algal growth:** Cylindrical photoreactor (Ø: 45 cm) with ten vertical neon tubes Philips TLD 15 W/25.

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

10.3 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 and turns yellow with some other N-heterocycles. – **Ninhydrin:** 0.3 g ninhydrin (2,2-dihydroxyindan-1,3-dione) is dissolved in 95 ml *iso*-propanol. The mixture is 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. Ninhydrin in ethanol (0.1 %) was also directly used. – **Chlorine/*o*-dianisidin reaction**: The reagent was prepared from 100 ml (0.032%) *o*-dianisidin in 1 N acetic acid, 1.5 g $Na_2WO_4 \cdot 2\ H_2O$ in 10 ml water, 115 ml acetone

and 450 mg KI. The moistened TLC plate was kept ca. 30 min in a chlorine atmosphere (from 0.5 g $KClO_3$ + 2 ml conc. HCl) and then subjected to drying for ca. 1 h, till the excess of chlorine was evaporated and then dipped into the reagent. The reagent is specific for peptides as universal spraying reagent. – **NaOH** or **KOH:** 2 N NaOH or KOH solutions are used to identify *peri*-hydroxyquinones by deepening of the colour from yellow or orange to violet or blue.

10.4 Microbiological Materials

Fermenter: A 50 l fermenter type Biostat U (B. Braun Dissel Biotech GmbH) consisting of a 70 l metallic container (50 l working volume), propeller stirrer, and culture container for autoclaving, cooling and thermostating (Braun Melsungen, Germany) was used. – **Storage of strains:** Deep-freeze storage in a Dewar vessel, 1'Air liquid type BT 37 A. – **Capillaries for deep-freeze storage:** diameter 1.75 mm, length 80 mm, Hirschmann Laborgeräte Eberstadt. – Soil for soil culture**:** Luvos Heilerde LU-VOS JUST GmbH & Co. Friedrichshof (from the health shop). – **Ultraturrax:** Janke & Munkel KG. – **Shaker:** Infors AG (CH 4103 Einbach) type ITE. – **Laboratory shaker:** IKA-shaker type S50 (max. 6000 Upm). – **Autoclave:** Albert Dargatz Autoclave, volume 119 l, working temperature 121°C, working pressure 1.2 kg/cm^2. – **Antibiotic assay discs:** 9 mm diameter, Schleicher & Schüll No. 321 261. – **Culture media:** agar-agar and soybean fluor was purchased from an Asiatic shop, glucose, bacto peptone, dextrose, and mannit were obtained from Merck, Darmstadt. Yeast extract was bought from Greif Bros. Cooporation, Delaware, Ohio, and malt extract from Aspera, Mülheim an der Ruhr, Germany. – **Antifoam solution:** Niax PPG 2025; Union Carbide Belgium N. V. (Zwiijndrecht). – **Petri dishes:** 94 mm diameter, 16 mm height, Fa. Greiner Labortechnik, Nürtingen. – **Celite:** Celite France S. A., Rueil-Malmaison Cedex. – **Sterile filters:** Midisart 2000, 0.2 μm, PTFE-Filter, Sartorius, Göttingen. – **Laminar-Flow-Box:** Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1. – **Brine shrimp eggs (*Artemia salina*):** SERA Artemia Salinenkrebseier, SERA Heinsberg. – Salinenkrebsfutter: micro cells DOHSE Aquaristik KG Bonn (brine shrimp eggs and food can be obtained from aquaristic shops).

10.5 Recipes

All cultures were autoclaved at 1.2 bar and 120 °C. Sterilisation time for 1 L shaker culture: 33 min, 2 L concentrated medium for fermenter: 50 min.

– Artificial Seawater

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

– Trace element stock solution

H_3BO_3	0.6 11 g
$MnCl_2$	0.3 89 g
$CuSO_4$	0.0 56 g
$ZnSO_4 \cdot 7\ H_2O$	0.0 56 g
$Al_2(SO_4)_3 \cdot 18\ H_2O$	0.0 56 g
$NiSO_4 \cdot 6\ H_2O$	0.0 56 g
$CO(NO_3)_3 \cdot 6\ H_2O$	0.0 56 g

TiO_2	0.056 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.056 g
LiCl	0.028 g
$SnCl_2$	0.028 g
KI	0.028 g
Tap water	add 1 L

– Stock solution

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

10.6 Nutrients

– M_2 medium (without seawater)

malt extract	10 g
glucose	4 g
yeast extract	4 g

Tap water	ad 1 L

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

– M_2^+ medium (M_2 medium with seawater)

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

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

– M_2 100% Seawater + CaCO3

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

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

– $CaCl_2$ Medium

malt extract	4

	0 g
glucose	5 g
$CaCl_2$	4 5 g
tap water	1000 mL

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

– **Luria-Bertani Medium (LB)**

trypton	1 0 g
yeast extract	5 g
NaCl	1 0 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 bacto agar.

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

malt extract	10 g
yeast extract	4 g

glucose	4 g
bacto agar	20 g
demineralised water	1000 mL

The pH was adjusted to 7.8 using 2N NaOH.

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

glucose	40 g
bacto peptone	10 g
agar	20 g
demineralised water	1000 mL

The pH was adjusted to 7.8 using 2N NaOH.

– **Nutritional solution A**

soybean meal (defatted)	30 g
glycerol	30 g
$CaCO_3$	2 g
artificial sea water	750 ml
demineralised water	250 ml

– Nutritional solution B

starch	10 g
NZ-amine	5 g
soybean meal	2g
yeast extract	5 g
KNO_3	3 g
algal extract	2.5 mL
artificial sea water	750 mL
demineralised water	250 mL

Stock Solutions and Media for cultivation of algae

– Fe-EDTA

0.7 g of FeSO4 · 7 H_2O and 0.93 g EDTA (Titriplex III) are dissolved in 80 ml of demineralised water at 60 °C and then diluted to 100 ml.

– Trace element Solution II:

Solution A:

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

Salts are dissolved in 10 ml of demineralised water.

Solution B:

$CuSO_4 \cdot 5H_2O$	5.0 mg

H_3BO_3	10.0 mg
$ZnSO_4 \cdot 7H_2O$	10.0 mg

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

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

$NaNO_3$	0.2 50 g
KH_2PO_4	0.1 75 g
K_2HPO_4	0.0 75 g
$MgSO_4 \cdot 7H_2O$	0.0 75 g
NaCl	0.0 25 g
$CaCl_2 \cdot 2H_2O$	0.0 25 g
Fe-EDTA	1.0 mL
trace element solution II	0.1 mL

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

10.7 Microbiological and Analytical Methods

10.7.1 Storage of Strains

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

10.7.2 Pre-Screening

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

10.7.3 Biological Screening

The crude extracts and pure compounds were dissolved in CH_2Cl_2/MeOH 90:10 and paper disks of the diameter of 9 mm were impregnated with 1 mg of crude extracts or 40 μg of pure compounds, respectively, dried under sterile conditions (flow box) and put on an agar plates inoculated with the Gram-positive bacteria *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57), the Gram-negative *Escherichia coli*; the yeast *Candida albicans*; and the fungus *Mucor miehei* (Tü 284) along with the three microalgae, *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus* as described previously. [345]

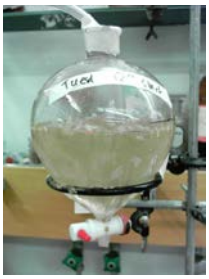
The plates were incubated at 37 °C for bacteria (12 hours), 27 °C for fungi (24 hours), and 24-26 °C under daylight for micro-algae (96 hours). The diameter of inhibition zone was measured in [mm]. Nystatine was used as positive control at 40 μg per paper disk for *C. albicans* giving an inhibition zone diameter of 25 mm, and gentamycine for bacteria, giving a diameter of inhibition zone of 22 mm for *B. subtilis* and *E. coli* and 21 mm for *S. aureus*

10.7.4 Chemical and Pharmacological Screening

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

– Brine shrimp Microwell Cytotoxicity Assay

To a 500 ml separating funnel, filled with 400 ml of artificial seawater, 1 g of dried eggs of *Artemia salina L.* were added (without feed). 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 room temperature 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 formula:

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

M = percent of the dead larvae after 24 h.

A = number of the dead larvae after 24 h.

B = average number of the dead larvae in the blind samples after 24 h

N = number of the dead larvae before starting of the test.

G = total number of brine shrimps

The mortality rate with actinomycin must be 100%.

10.7.5 Production of Zoospores and Bioassy

Sporangia of *Plasmopara viticola* were isolated from the infected leaves of grapevine *Vitis vinifera* cv. Müller-Thurgau. The strain was maintained by regular culturing on the lower surface of young grapevine leaves on Petri dishes containing 1.5% agar at 25 °C and 95% relative humidity. At day 6 of cultivation, the sporangiophores bearing lemon-shaped sporangia were harvested into an eppendorf by a micro-vacuum cleaner. The freshly harvested sporangia were separated from sporangiophores by filtration through 50 μm nylon mesh, washed twice and then incubated in sterilized water (10^4 sporangia/mL) in the dark for 6 h at room temperature (23 °C) to release zoospores. These zoospores remained motile for 10-12 h in sterilized water and were used for the bioassay. For stock solutions, crude extracts and pure compounds were first dissolved in dimethyl sulfoxide (DMSO) and then diluted with distilled water. The concentration of DMSO in the zoospore suspension never exceeded 1% (v/v), a condition that does not affect zoospore motility. The bioassay was carried out as described earlier. [346]

Briefly, 40 μL of sample suspension was directly added to the 360 μL zoospore suspension (*ca.* 10^5/mL) taken in a well of plant tissue culture multi well plate to make a final volume of 400 μL and then quickly mixed with a glass rod. 1% aqueous DMSO was used as a control. The motility of zoospores was observed under a light microscope at 10× magnification. Quantification of time-course changes of motility and lysis of zoospores were carried out as described earlier.[347]

Each treatment was replicated thrice. The mean value (%) ± SE (standard error) of the affected spores in each treatment was calculated.

10.7.6 Antitumor Test

A modified propidium iodide assay was used to examine the antiproliferative activity of the compounds against human tumor cell lines. The test procedure was described elsewhere. [348]

Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice, or obtained from American Type Culture Collection, Rockville, MD, USA, National Cancer Institute, Bethesda, MD, USA, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

10.7.7 Primary Screening

Bases of evaluation

Antibiotic screening (disk diffusion test): The test was performed using paper discs with a diameter of 8 mm under standardized conditions (see above). If the inhibition zone is ranging from 11 to 20 mm, the compound is considered to be weakly active (+), from 21 to 30 mm designated as active (++) and over 30 mm is highly active (+++). – **Chemical screening:** evaluation of the separated bands by the number, intensity and colour reactions with different staining reagents on TLC. – **Toxicity test:** By counting survivors after 24 hrs, the mortality of the extracts was calculated (see above). The extracts, fractions or isolated compounds were considered inactive when the mortality rate was lower than 10% (-), from 10 to 59% as weakly active (+), from 60 to 95% as active (++) and over 95% as strongly active (+++).

11 Metabolites from selected strains

12 Ruminal bacteria

12.1 Ruminal *Pseudomonas aeruginosa* ZIO

12.1.1 Pre-screening

The inhibitory effect of the ruminal bacterium *Pseudomonas aeruginosa* was investigated against a collection of human-pathogenic bacteria. The supernatant of the cul-

ture was assessed by the agar diffusion method and showed inhibition of a broad spectrum of Gram-negative and Gram-positive bacteria (Table 38).

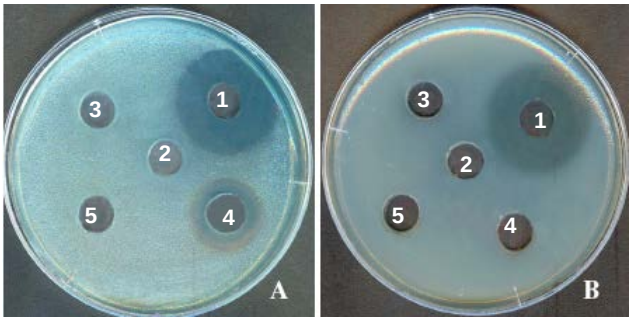


Figure 156: Antimicrobial activity of supernatant from liquid culture of ZIO against *Streptococcus* sp* (R36) (**A**) (30 mm) and *Staphylococcus aureus** (R25) (**B**) (28 mm), tested by the method of diffusion through the wells.

1: supernatant from liquid culture of ZIO; 2: LB liquid medium (positive control); 3: precipitate from liquid culture of ZIO (bacterium cells); 4: supernatant from liquid culture of the ruminal bacterium *Proteus vulgaris* Sa14 (moderate activity, 12 mm, against *Streptococcus* sp* (R36) (**A**) and inactive against *Staphylococcus aureus** (R25) (**B**); 5: precipitate from liquid culture of the ruminal bacterium *Proteus vulgaris* Sa14; *: indicator strains were spread evenly on the agar plates.

Table 38: Antagonistic activity of the ruminal bacterium ZIO against a collection of human pathogenic bacteria.

Targets References	Genera and species	Samples	Department	Activity of strain ZIKSO
R1	*Enterobacter aerogenes*	urine	emergency	++
R2	*Enterobacter aerogenes*	pus	cardiovasculary	-
R3	*Enterobacter aerogenes* BLSE	urine	neonatal	++
R4	*Serratia* BLSE	pus	reanimation	++
R5	*Serratia liquefaciens*	urine	urology	+++
R6	*Staphylococcus aureus*	hemoculture	reanimation	+++
R7	*Providencia* BLSE	visceral sound	visceral chirurgy	++
R8	*Staphylococcus aureus* MRSA	pus	maternity	+++
R9	*Staphylococcus* coagulase-negative	hemoculture	urology	+++
R10	*Staphylococcus aureus* MRSA	pus	dermatology	+++
R11	*Staphylococcus aureus* MRSA	lung	reanimation	+++
R12	*Staphylococcus aureus*	pus	urology	+++
R13	*Staphylococcus aureus*	pus	dermatology	+++
R14	*Staphylococcus aureus*	pus	cardiology	+++
R15	*Staphylococcus aureus*	nose swab	neonatal	+++
R16	*Staphylococcus aureus*	lung	reanimation	-
R17	*Staphylococcus* coagulase-negative	hemoculture	cardiology	+++
R18	*Xanthomonas maltophilia*	lung	neonatal	-
R19	*Staphylococcus* coagulase-negative	hemoculture	reanimation	+++
R20	*Xanthomonas maltophilia*	catheter	neonatal	-
R21	*Xanthomonas maltophilia*	-	reanimation	+++
R22	*Proteus mirabilis* BLSE	catheter	reanimation	+++
R23	*Staphylococcus aureus*	hemoculture	neonatal	+++
R24	*Ochrobacterium anthropi*	anal	neonatal	+++
R25	*Staphylococcus aureus*	hemoculture	neonatal	+++
R26	*Escherichia coli*	pus	urology	+++
R27	*Escherichia coli*	pus	visceral chirurgy	+++
R28	*Escherichia coli*	lung	reanimation	++
R29	*Escherichia coli* (BLSE)	urine	emergency	+
R30	*Staphylococcus* coagulase-negative SCN	hemoculture	reanimation	+++
R31	*Staphylococcus* coagulase-negative SCN	hemoculture	urology	+++
R32	*Escherichia coli*	anal	reanimation	+++
R33	*Escherichia coli*	urine	maternity	+++
R34	*Escherichia coli*	rectal	reanimation	+++
R35	*Klebsiella pneumoniae* BLSE	gastric swab	neonatal	+++
R36	*Streptococcus* A	pus	Dermatology	+++

*Inhibition zone between 0 - 2 mm (-); 2 - 12 mm (+); 12 -20 mm (++); 20-50 mm (+++).

Pseudomonas aeruginosa ZIO showed also inhibitory activity of the pathogenic *Aspergillus flavus* (Figure 157).

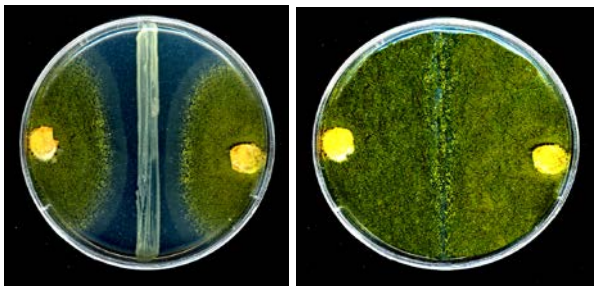


Figure 157: Growth inhibition of human pathogenic *Aspergillus flavus* by ruminal *Pseudomonas aeruginosa* ZIO (left), compared to the standard (*Aspergillus flavus*, right).

12.1.2 Taxonomic characteristics of strain ZIO

The bacterial strain ZIO was isolated from ruminal material from a Tunisan cow and maintained on PYM agar. The strain was taxonomically affiliated on the basis of the 16S rRNA sequence. The strain had a 99.9% gene sequence identity with the *Pseudomonas aeruginosa* strain NCM2.S1 (accession no. AP012280 of the whole genome) and the strain M18 (accession no. CP002496 of the whole genome). It is >98% identical with the type strain of *Pseudomonas aeruginosa* (strain RH815, accession no. X06684). The strain ZIO is deposited in the microbial collection at the Institute of Organic and Biomolecular Chemistry, Georg-August University of Göttingen, Germany, with the voucher number ZIO.

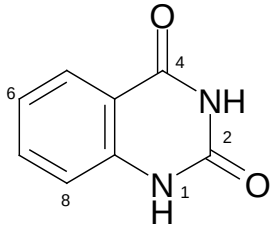
12.1.3 Fermentation and working up

Pseudomonas aeruginosa strain ZIO was pre-cultivated on PYM agar at 37 °C. Pieces (1×1 cm) of well grown agar were used to inoculate 120 of 1-litre Erlenmeyer flasks, each containing 250 mL of PYM broth. The fermentation was carried out at 180 rpm on a linear shaker for 3 days at 37 °C. The brown culture broth was harvest-

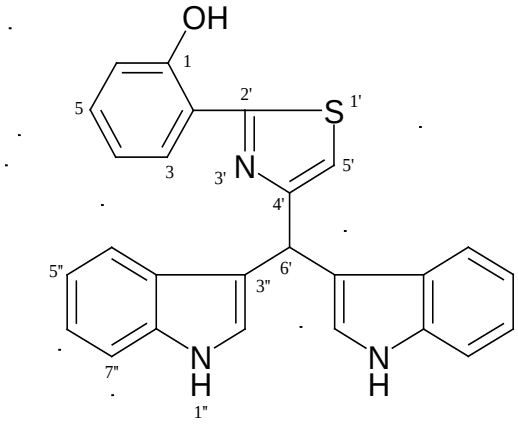
ed and filtered after addition of 1 kg Celite to separate the bacterial biomass, which was extracted with ethyl acetate, while the culture filtrate was passed through Amberlite XAD-16 adsorption resin. The XAD column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluate was concentrated under reduced pressure and the aqueous residue was finally extracted with ethyl acetate. The Celite/biomass mixture was extracted with ethyl acetate (3 times) and acetone (2 times); the combined organic phases were then evaporated to dryness. Both the water and the biomass extracts were combined based on their chromatographic similarity, yielding 10.8 g of a greenish-brown crude extract.

Chromatography on silica gel (column 3 × 100 cm) using a CH_2Cl_2/MeOH gradient (0-60% MeOH) monitored by TLC resulted in six fractions I-IV. Purification of fraction II using PTLC followed by Sephadex LH-20 afforded 25 mg of compound **92** as yellow solid together with 1*H*-quinazoline-2,4-dione (**91**). Fraction III was purified on silica gel column using MeOH/CH_2Cl_2 gradient (0 to 40 % MeOH), followed by Sephadex LH-20 using CH_2Cl_2 /40% MeOH gradient to afford indol-3-carbaldehyde (**96**), 3-(hydroxyacetyl)-indole (**95**), and PTLC using CH_2Cl_2 /5% MeOH gradient gave phenazine carboxylic acid (**43**) and phenyl acetic acid. Fraction IV was separated on a silica gel column using CH_2Cl_2/40% MeOH, then Sephadex LH-20 (CH_2Cl_2 /40% MeOH) and Sephadex LH-20 using MeOH to deliver rhamnolipid A (**94**) and (Phe,Pro) (**97**).

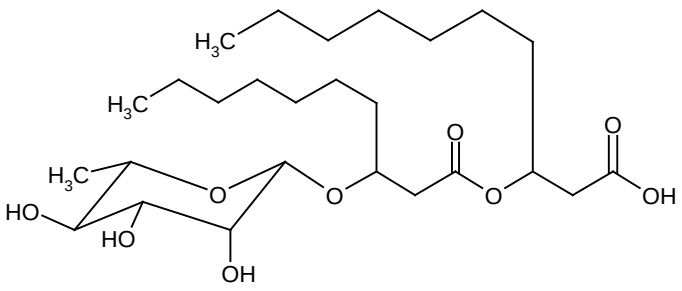
1*H*-Quinazoline-2,4-dione (**91**): Colourless solid, R_f = 0.46 (CH_2Cl_2/5% MeOH), UV absorbing at 254 nm. Colourless with anisaldehyde/sulphuric acid. **– ^{1}H NMR** (DMSO-d_6, 600 MHz): δ 7.97 (dd, 1H, ^3J = 9.2, 1.5, H-8), 7.32 (t, 1H, ^3J = 8.46, 1.5, H-7), 7.06 (dd, ^3J = 9.2, 1.2, H-5), 6.94 (t, 1H, ^3J = 7.9, 0.7, H-6). **– ^{13}C NMR** (DMSO-d_6, 600 MHz): δ 163.8 (C-4), 162.6 (C-2), 155.6 (C-8a), 131.0 (C-5), 127.2 (CH-7), 119.1 (C-4a), 118.0 (CH-6), 116.7 (CH-8).



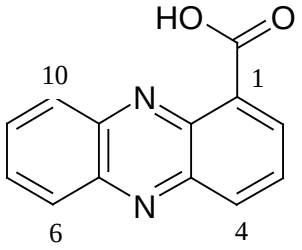
Barakacin (**92**): Yellow solid, R_f = 0.90 (CH_2Cl_2/2% MeOH); 0.61 (CH_2Cl_2), orange with anisaldehyde/H_2SO_4 spraying reagent, red-violet with Ehrlich's reagent. **– ^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.50 (d, 2H, *J* = 7.9 Hz, 4"-CH, 4'"-CH, H-4"), 7.34 (d, 2H, *J* = 8.1 Hz, H-7"), 7.07 (td, 2H, *J* = 7.1, 1.0 Hz, H-5", 5'"), 7.20 (td, 2H, *J* = 7.1, 1.1 Hz, H-6", 6'"), 6.90 (td, *J* = 7.9, 1.1, H-4), 6.82 (d, 2H, *J* = 1.7 Hz, H-2"), 7.94 (s, 2NH, H-1", 1'"), 7.62 (dd, 1H, *J* = 7.8, 1.5 Hz, H-3), 7.29 (td, *J* = 7.3, 1.6, H-5), 7.02 (dd, 1H, *J* = 8.3, 1.1 Hz, H-6), 7.25 (td, *J* = 7.3, 1.6 Hz), 6.87 (td, *J* = 7.9, 1.1 Hz), 6.89 (d, *J* = 0.7 Hz, H-5'), 6.11 (d, *J* = 0.8 Hz, H-6'), 12.43 (br). **– ^{13}C NMR** ($CDCl_3$, 125 MHz): δ 168.7 (-N = C_q-S-, C-2'), 157.9 (C-4'), 156.7 (C-1), 136.5 (C-7"a, C-7'"a), 126.7 (C-3"a, C-3'"a), 117.4 (Cq, C-2), 117.4 (C-3", C-3'"), δ 126.9 (C-3), 131.5 (C-5), 119.5 (C-4", C-4'"), 119.3 (C-4), 123.2 (C-2", C-2'"), 122.0 (C-6", C-6'"), 119.4, 117.6 (C-6), 113.1 (C-5'), 119.4 (C-5", C-5'"), 111.2 (C-7", C-7'"), 36.5 (C-6').



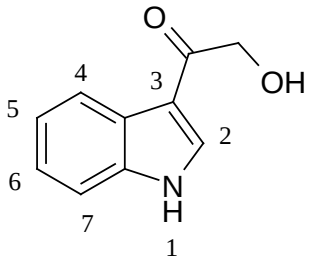
Rhamnolipid A (glycolipid A, 94): Isolated as a polar oily compound, which turned on TLC to dark green after spaying with anisaldehyde/sulphuric acid. ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 4.



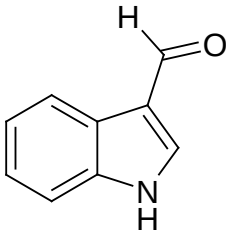
Phenazine-1-carboxylic acid (**43**): Yellow needles (15 mg), UV absorbing at 254 nm, yellow with anisaldehyde/sulphuric acid. **– R_f** = 0.35 (CH_2Cl_2/ 7% MeOH) **– ^{1}H NMR** (300 MHz, $CDCl_3$): δ = 9.00 (dd, ^3J = 7.0 Hz, ^4J = 1.3 Hz, 1H, H-2), 8.58 (dd, ^3J = 8.7 Hz, ^4J = 1.4 Hz, 1H, H-4), 8.40 (m, 1H, H-9), 8.20 (m, 1H, H-6), 8.10-7.98 (m, 3H, H-3,7,8) **– (+)-ESIMS:** *m/z* (%) 247 ([M+Na]+, 80), 471 ([2M+Na]+, 44).



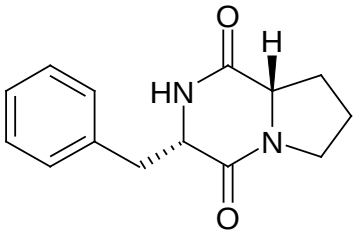
3-(Hydroxyacetyl)-indole (**95**): Purification of fraction III (1.5 g), by Sephadex-LH 20, gave a UV absorbing colourless solid R_f = 0.55 ($CHCl_3$/ MeOH 5%). It exhibited a colour change to orange and pink by anisaldehyde/sulphuric acid and Ehrlich's reagent, respectively. – **^{1}H NMR (**CD_3OD, 300 MHz): δ = 10.30 (s br, 1 H, NH), 7.58 (d, ^3J = 2.1 Hz, 1 H, H-2), 7.38 (m, 1 H, H-4), 7.08 (m, 1 H, H-7), 7.00 (m, 2 H, H-5 and H-6), 3.74 (s, 1 H, OH).



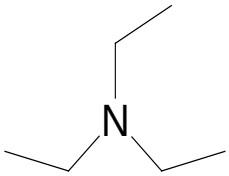
Indole-3-carbaldehyde (**96**): Separation of fraction III on Sephadex LH-20 (CH_2Cl_2/ 40% MeOH) led to a middle polar UV absorbing colourless solid (1.2 mg), which turned to orange by anisaldehyde/sulphuric acid and to pink with Ehrlich's reagent. – R_f = 0.35 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 8.05 (d, 1 H, H-4), 7.95 (s, 1 H, H-2), 7.41 (d, 1H, H-7), 7.17 (m, 2H, H-5,6).



***Cyclo*-(phenylalanyl,prolyl)** (**97**): Isolated from fraction IV on Sephadex LH-20 (CH_2Cl_2/ 40% MeOH) as colourless solid (10 mg). – R_f = 0.45 ($CHCl_3$/5% MeOH), 0.67 ($CHCl_3$/10% MeOH); stained to violet with anisaldehyde/sulphuric acid and pink by Ehrlich's reagent, respectively. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 7.30 (m, 5 H, Ar-H), 6.60 (s, br, 1 H, NH), 4.15 (dd, ^3J = 8.3 Hz, ^3J = 3.0 Hz, 1 H, H-3), 3.98 (m, 1 H, H-6), 3.62 (m, 3 H, H_a-10), δ 3.58-3.45 (9-CH_2), 2.80 (dd, ^2J = 14.1 Hz, ^3J = 8.3 Hz, 1 H, H_b-10), 2.39 (ABX system of a methylene group), 2.10-1.85 (2 m, 1 + 3 H, 7,8-CH_2).



Triethylamine salt (**99**): Oily compound, non-UV absorbing at 254 nm, no colour after spraying with anisaldehyde/sulphuric acid. For the ^{1}H and ^{13}C NMR data (300, 125 MHz) see Table 7.



12.2 Ruminal *Citrobacter freundii* ZIG

12.2.1 Pre-screening

The antimicrobial assay of the ruminal bacterium *Citrobacter freundii* (isolated from a cow) showed high activity against pathogenic bacteria such as *Staphylococcus au-*

reus and *Klebsiella pneumoniae.* Also, it showed activity against the phytopathogenic fungi *Fusarium culmorum*, *Fusarium graminearum*, and *Phoma tracheiphila.*

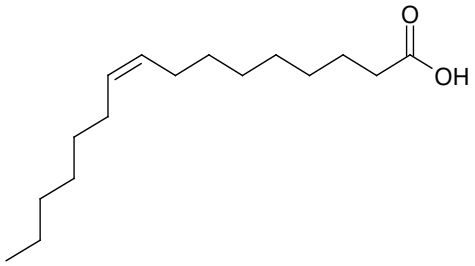
left: TLC of the ethyl acetate extract; middle: TLC of the acetone extract; right TLC of the methanol extract

Nine known compounds were isolated and identified as oleic acid (**100**), isoxanthohumol (**105**), *cyclo*(Pro,Leu) (**107**), *cyclo*(Tyr,Pro) (**109**), phenol (**111**), and adenine (**124**).

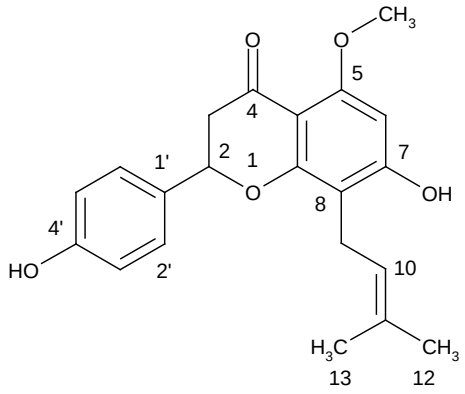
12.2.2 7.2.2 Fermentation and isolation

The ruminal bacterium ZIG was used to inoculate a 20 L shaker culture using LB medium (peptone 8 g, yeast extract 5 g, NaCl 5 g per litre distilled water, adjusted to pH 7), at 37 °C. The culture broth was harvested after three days. Biomass/Celite and filtrate were separated using a filter press. The biomass was extracted three times with ethyl acetate and two times with acetone. The water phase was subjected to a XAD-16 column and extracted with MeOH. Both crude extracts showed identity on TLC and were combined (3.25 g) and chromatographed on silica gel column using a CH_2Cl_2/MeOH gradient (0 to 100 % MeOH). The resulting fractions (Fraction I to III) were purified separately. Fraction I afforded oleic acid (**100**), Fraction II was purified on Sephadex LH-20 with MeOH to afford isoxanthohumol (**105**), *cis*-cyclo(Tyr,Pro) (**109**) and *cis-cyclo*(Prolyl,Leu) (**107**). Fraction III was purified on Sephadex LH-20 with MeOH to give phenol (**111**) and adenine (**124**).

Oleic acid (**100**)**:** Colourless, non-UV absorbing oil, which turned to blue with anisaldehyde/sulphuric acid. – **^{1}H NMR**: δ 11.48 (1H, COOH), 5.36 (m, 2H, H-9, 10), 2.34 (t, CH_2-2), 2.00 (m, CH_2-H-9, 10), 1.28 (CH_2-H-2-8), 0.85 (t, CH_3).

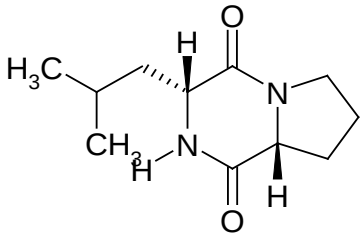


Isoxanthohumol; 4',7-Dihydroxy-5-methoxy-8-prenylflavonone (**105**): Colourless solid, was isolated from fraction II by chromatography on Sephadex LH-20 (MeOH), delivering a UV active pale yellow fluorescent band, which showed no colour reaction by staining with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.14 ($CHCl_3$/10 % MeOH). – **^{1}H NMR** (CH_3OH, 300 MHz): δ 7.30 (d, ^3J = 8.5 Hz, 2 H, 2', H-6'), 6.80 (d, ^3J = 8.3 Hz, 2 H, H-3',5'), 6.04 (s, 1 H, H-6), 5.22 (dd, $J_{2,3a}$ = 12.5, $J_{2,3b}$ = 2.5 Hz, 1 H, H-2), 5.17 (t, ^3J = 7.2 Hz, 1 H-β, H-10), 3.78 (s, 3 H, 5-OCH_3), 3.20 (dd, 9-CH_2), 2.95 (ABX, J_{AB} = 16.92, J_{AX} = 8.25, J_{BX} = 6.23, 1 H, 3-H_A), 2.60 (ABX, J_{AB} = 16.7 Hz, J_{AX} = 8.2 Hz, J_{BX} = 6.1 Hz, 1 H, 3-HB), 1.60 (s, 3 H, 12-CH_3), 1.58 (s, 3 H, 13-CH_3). – **(+)-ESI MS**: *m/z* (%) = 731 ([2 M + Na]$^+$, 100). – **(-)-ESI MS**: *m/z* = 353 ([M-H]$^-$).

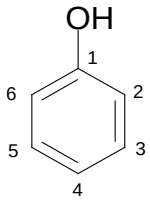


***cis-cyclo*(Leu,Pro)** (**107**): Fraction II (0.41 g) delivered by PTLC (20×20 cm, $CHCl_3$/7% MeOH), and Sephadex LH-20 ($CHCl_3$/MeOH 3:2) a UV absorbing colourless solid (6 mg), as a middle polar substance, which turned to violet by anisalde-

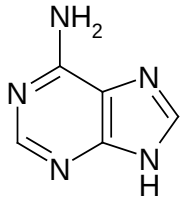
hyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.58 ($CHCl_3$/5 CH_3OH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 6.00 (s br, 1 H, NH), 4.13 (t, ^3J = 8.1 Hz, 1 H, 3-H), 4.02 (dd, 3J = 10.2 Hz, ^3J = 6.2 Hz, 1 H, 6-H), 3.64-3.53 (m, 2 H, 9-CH_2), 2.45-2.28 (m, 1 H, 10-H_A), 2.24-1.98 (m, 3 H, 10-H_B, 7,8-H_A), 1.96 (m, 1H, 8-H_B), 1.61-1.45 (m, 2 H, 7-CH_2), 1.55 (m, 1 H, 11-H), 1.03 (d, ^3J = 8.1 Hz, 3 H, 12-CH_3), 0.96 (d, ^3J = 8.1 Hz, 3 H, 13-CH_3).



Phenol (**111**)**:** Colourless compound with a typical smell. The ^{1}H NMR and ^{13}C NMR (300 MHz, CD_3OD) values were mentioned in Table 8.



Adenine (**112**): Colourless solid, stained to blue by spraying with anisaldehyde/sulphuric acid. It was isolated and purified using PTLC followed by Sephadex LH-20 chromatography. – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 8.1 (s, 1H), 8.05 (s, 1H), 7.05 (br, 2H).



12.3 Ruminal bacterium ZIK

12.3.1 Pre-screening

The inhibitory effect of the ruminal *Gemella morbillorum*, isolated from the rumen of camel, was investigated against a collection of phytopathogenic fungi. When as-

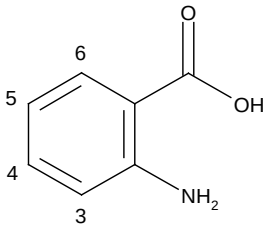
sessed by the agar diffusion method, the supernatant of the culture showed inhibition of the fungus *Phoma tracheiphila*.

12.3.2 Fermentation and isolation

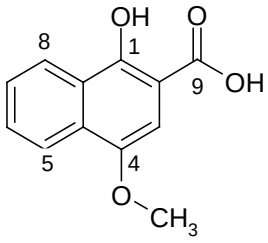
A well-grown sub-culture of the ruminal bacterium ZIK was used for inoculation of a 25 L shaker culture on LB medium; the pH was adjusted to 7.0 before sterilisation. After 7 days of cultivation at 37 °C, the strain showed a brown culture broth. The fermenter broth was filtered with a filter press. The filtrate was passed over XAD-16, which was afterwards extracted with methanol. The methanol phase was evaporated and the remaining water was extracted three times with ethyl acetate. The biomass was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (1.29 g).

Chromatography of the latter on silica gel column using a CH_2Cl_2-MeOH gradient with successively increasing polarity resulted in three fractions I-III according to monitoring by TLC. Fraction II was further purified by Sephadex LH-20 eluted with methanol to give anthranilic acid (**113**). Purification of fraction III afforded 1-hydroxy-4-methoxy-2-naphthoic acid (**116**) and thymine (**123**).

Anthranilic acid (**113**): Isolated from fraction II on Sephadex LH-20 (MeOH) as pale yellow solid; UV absorbing at 254 nm, intensive blue UV fluorescence at 366 nm; $\boldsymbol{R_f}$ = 0.47 (CH_2Cl_2/ 5% MeOH), turned to yellow after spraying with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.95 (dd, ^3J = 8.0, ^4J = 1.0, H-6), 7.37 (td, ^3J = 8.0, ^4J = 1.0, H-4), 6.68 (dd, ^3J = 8.0, ^4J = 1.0, H-3), 6.65 (td, ^3J = 8.0, ^4J = 1.0, H-5).



1-Hydroxy-4-methoxy-2-naphthoic acid (**116**): Colourless powder, blue UV fluorescence at 366 nm. – $\boldsymbol{R_f}$ = 0.66 (CH_2Cl_2 / 5% MeOH). – **^{1}H** and **^{13}C/APT NMR**, see Table 9.



12.4 Ruminal bacterium *Enterobacter amnigenus* ZIH

12.4.1 Pre-screening

Extracts of the ruminal bacterium *Enterobacter amnigenus*, showed activity against the pathogenic bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae* and against the phytopathogenic fungi *Fusarium culmorum*, *Fusarium graminearum* and *Phoma tracheiphila.*

left: TLC of the ethyl acetate extract; middle: TLC of the acetone extract; right TLC of the methanol extract

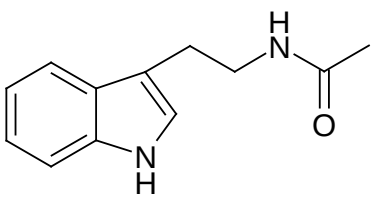
12.4.2 Fermentation and isolation

A 5 l culture of *Enterobacter amnigenus* was grown in 1 l Erlenmeyer flasks filled with 300 ml of LB medium on a linear shaker with 95 rpm at 37 °C. After three days of fermentation, the culture broth was used to inoculate a Braun Biostat U fermenter, filled with 20 l of LB medium. The following conditions were maintained: stirring at

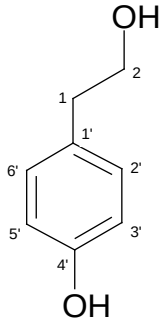
200 rpm, 37 °C, pH 6.5 ± 1.5, aeration 1.5 m^3/h. The culture broth was harvested after 5 days.

The cells phase was filtered off by means of a pressure filter and extracted with ethyl acetate (3 × 5 l) and acetone (2 × 5 l), while the filtrate was passed through an Amberlite XAD-16 column (6 × 120 cm). The latter was washed with 25 l of demineralized water and eluted with 15 l of methanol. After concentration, the aqueous residue was extracted with ethyl acetate (3 times) and acetone (2 times). On basis of their similar chromatograms, both extracts were combined, yielding 3.3 g of crude extract. Chromatography on silica gel by column chromatography (CC; CH_2Cl_2/MeOH, stepwise gradient) under TLC monitoring afforded four fractions I-IV. Fraction II yielded 3-(hydroxy acetyl)-indole (**95**), and N_β-acetyltryptamine (**117**). Fraction III afforded phenol **(111),** tyrosol (**118**) and uracil (**126**). Fraction IV was separated on Sephadex LH-20 into two sub-fractions. The first one was further subjected to Sephadex LH-20 and yielded tryptophol (**119**) and indole-3-lactic acid (**121**). The second subfraction afforded on chromatography on RP-18 column (12 × 1 cm) using 40% aqueous methanol 2-butoxy-6-hydroxymethyl-tetrahydropyran-3,4,5-triol (**122**) and adenine (**112**).

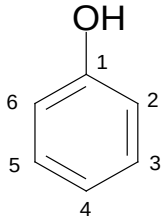
N_β-Acetyltryptamin (**117**): Obtained from fraction II by applying to Sephadex LH-20 (MeOH) as a UV absorbing colourless solid (10 mg), which stained to pink/violet by anisaldehyde/sulphuric acid, and pink (later to blue) by Ehrlich's reagent. – $\boldsymbol{R_f}$ = 0.5 (CH_2Cl_2 / 5 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.30 (s, br, 1 H, 1-NH), 7.60 (d, ^3J = 8.0 Hz, 1 H, H-4), 7.38 (d, ^3J = 7.8 Hz, 1 H, H-7), 7.26 (t, 7.8 Hz, 1 H, H-6), 7.08 (t, 8.0, 1 H, H-5), 7.03 (d, 1.1 Hz, 1 H, H-2), 5.70 (s, br, 1 H, NH), 3.58 (q, ^3J = 6.3 Hz, 2 H, CH_2-2'), 2.98 (t, ^3J = 6.6 Hz, 2 H, CH_2-1'), 1.93 (s, 3 H, CH_3-5'). – **^{13}C NMR** ($CDCl_3$, 50 MHz): δ = 170.5 (C=O, C_q-4'), 136.4 (C_q-7a), 127.2 (C_q-3a), 122.3 (CH-6), 121.7 (CH-2), 119.0 (CH-5), 118.5 (CH-4), 112.3 (C_q-3), 111.5 (CH-7), 40.0 (CH_2-2'), 25.2 (CH_2-1'), 23.1 (CH_3-5').



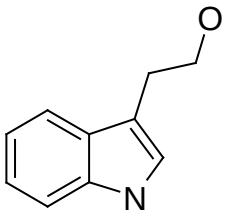
Tyrosol (*ρ*-Hydroxy-2-phenethylalcohol; Salidorol; 118): Colourless oil. – R_f = 0.54 (CH_2Cl_2/ 20% CH_3OH), UV absorbing band at 254 nm, turns red after spraying with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.09 (d, ^3J = 8.88 Hz, 2H, H-2', 6'), 6.74 (d, ^3J = 8.88 Hz, 2H, H-3', 5'), 3.65 (t, ^3J = 7.3 Hz, 2H, CH_2-1), 2.64 (t, ^3J = 7.2 Hz, 2H, CH_2-2).



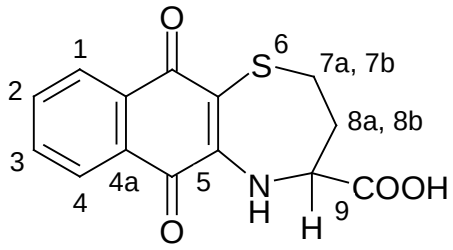
Phenol (**111**): **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.28 (2H, d, J = 8.5 Hz, H-2, 6), 6.87 (1H, t, J = 8.5 Hz, H-4), 6.82 (2H, t, J = 8.5 Hz, H-3, 5), 5.62 (1H, br, s, -OH).



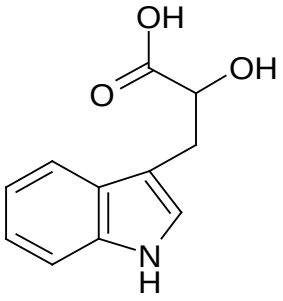
Tryptophol; 3-Indolylethanol (**119**)**:** Obtained from fraction IV by applying to Sephadex LH-20 (MeOH), as an UV absorbing colourless solid (20 mg), turned to intense violet by anisaldehyde/sulphuric acid and pink with Ehrlich's reagent. – R_f = 0.28 ($CHCl_3$/5 % MeOH). – **^{1}H NMR (**$CDCl_3$, 300 MHz): δ = 8.05 (s, br, 1 H, NH), 7.63 (d, ^3J = 8.2 Hz; 1 H, 4-H), 7.39 (d, ^3J = 8.2 Hz, 1 H, 7-H), 7.21 (dd, ^3J = 8.2, ^4J = 1.3 Hz, 1 H, 5-H), 7.19 (dd, ^3J = 8.2 Hz, ^4J = 1.3 Hz, 1 H, 6-H), 7.10 (s, 1 H, 2-H), 3.93 (t, ^3J = 6.6 Hz, 2 H, 9-CH_2), 3.05 (t, ^3J = 6.6 Hz, 2 H, 8-CH_2), the OH signal was invisible. – **EI MS** (70 eV): *m/z* (%) = 161 ($[M]^{\bullet+}$, 29), 130 (100), 103 (11), 77 (16).



Brevinic acid (**120**): Purple pigment; showed UV absorbance under 254 nm. – **^{1}H NMR** (300 MHz, CD_3OD): 7.61 (t, 1H, 2-H), 7.64 (t, 1H, 3-H), 7.91 (d, 1H, 4-H), 7.94 (d, 1H, 1-H); 2.10 (m, 1H, 8-Ha), 2.50 (m, 1H, 8-Hb), 3.09 (dd, *J* = 16 Hz, 6 Hz, 1H, 7-Ha), 3.68 (dq, *J* = 16 Hz, 6 Hz, 1H, 7-Hb), 5.22 (dd, 1H, 9-H). – **^{13}C-NMR** (125 MHz, CD_3OD): δ 32.0 (C-7), 32.5 (C-8), 58.0 (C-9), 114.5 (C-5a), 126.6 (C-1), 128.2 (C-4), 131.6 (C-4a), 133.4 (C-3), 134.2 (C-11a), 135.6 (C-2), 147.9 (C-10a), 180.2 (C-11), 182.5 (C-5).

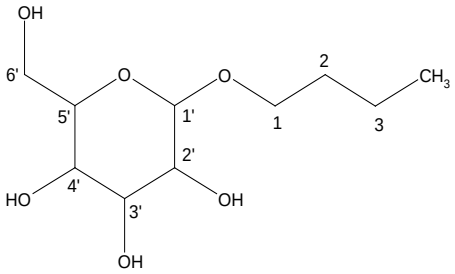


Indole-3-lactic acid (**121**): Colourless oil; it exhibited UV absorption at 254 nm and turned red by anisaldehyde/sulphuric acid. – **^{1}H NMR** (300 MHz, CD_3OD): δ = 7.65 (dd, 1H), 7.37 (dd, 1H), 7.05 (td, 1H), 6.95 (td, 1H), 7.11 (s, 1H), 4.21 (m, CH-OH), 2.90 (1H, CH_2), 2.95 (1H, CH_2).



Butyl glycoside (**122**): Colourless compound, turned to light green after spraying with anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.72 (d, ^{3}J = 1.5 Hz, 1H, H-1'), 3.81-3.39 (m, 9H, 1', 2',3',4',5',6'-H), 1.56 (m, 2H, H-2), 1.41 (m, 2H, H-3), 0.93 (t, ^{3}J = 7.3 Hz, 3H, H-4). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 101.5 (CH-

1'), 74.5 (CH-2'), 72.6 (CH-3'), 72.3 (CH-4'), 68.6 (CH-5'), 68.2 (CH_2-1), 62.9 (CH_2-6'), 32.7 (CH_2-2), 20.5 (CH_2-3), 14.3 (CH_3-4). – $[\alpha]_D^{20}$ = +42.2 (*c* 1.35, MeOH). – **(+)-ESIMS**: *m/z* (%) = 259 ($[M+Na]^+$, 100), 495 ($[2M+Na]^+$, 17). – **(+)-ESI-HRMS**: *m/z* = 259.11536 ($[M+Na]^+$) (calc. 259.11521 for $C_{10}H_{20}O_6Na$).



12.5 Ruminal bacterium *Serratia rubidae* ZIE

12.5.1 Pre-screening

The ruminal bacterium *Serratia rubidae*, isolated from the rumen of sheep, was selected due to its activity against the phytopathogenic fungus *Fusarium culmorum.*

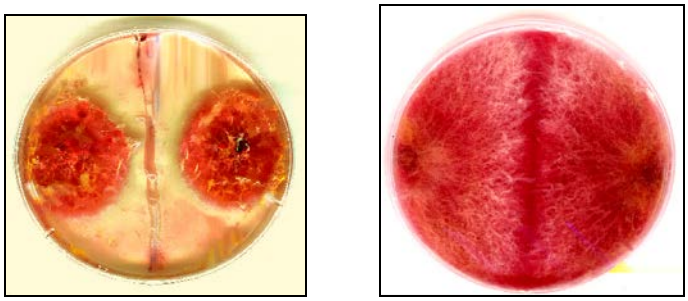


Figure 158: Growth inhibition of phytopathogenic *Fusarium culmorum* by ruminal *Serratia rubidaea* (left), compared to *Fusarium culmorum* as standard (right).

12.5.2 Fermentation, working up and isolation

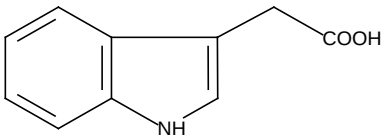
A well-grown sub-culture of the ruminal *Serratia rubidaea* was used for inoculation of a 25 l shaker culture on LB medium; the pH was adjusted to 7.0 before sterilisation. After 7 days of cultivation at 37 °C, the strain showed a pink culture broth. The fermenter broth was filtered with a filter press. The filtrate was passed over XAD-16 and the resin afterwards extracted with methanol. The methanol was evaporated and

the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (6.2 g).

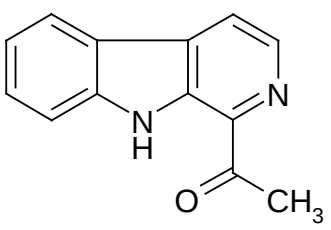
Chromatography of the latter on a silica gel column using a CH_2Cl_2-MeOH gradient with successively increasing polarity resulted in four fractions I-IV according to monitoring with TLC.

Eight compounds were isolated and were identified as thymine (**123**), thymidine (**125**), adenine (**124**), uracil (**126**), 1-hydroxy-4-methoxy-2-naphthoic acid (**134**), prodigiosin (**127**), indole-3-acetic acid (**128**), 1-acetyl-*β*-carboline (**129**), 4-hydroxy-5-methyl-furan-3-one (**130**), and actinomycin D (**131**).

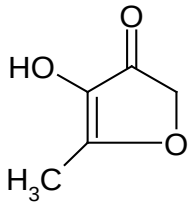
Indole-3-acetic acid (**128**): Isolated from fraction III as colourless solid, showed an UV absorbing band, which turned to orange and pink with anisaldehyde/sulphuric acid and Ehrlich's reagent, respectively. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.53 (d, 1H), 7.37 (d, 1H), 7.09 (t, 1H), 7.01 (t, 1H), 7.17 (s, 1H), 3.94 (s, CH_2).



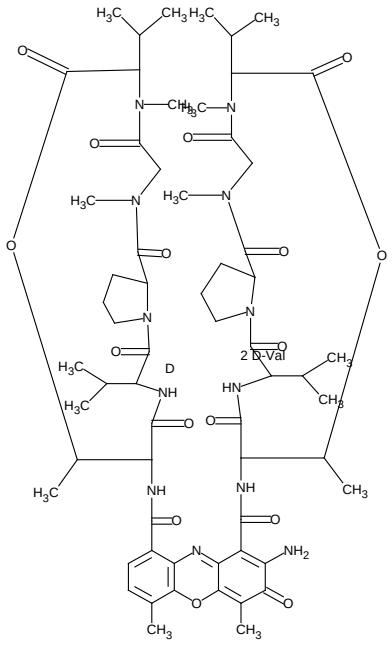
1-Acetyl-*β*-carbolin (**129**): Obtained from fraction III on Sephadex LH-20 (MeOH) as yellow solid. – $\boldsymbol{R}_f$ = 0.90 ($CHCl_3$/10% MeOH), blue UV fluorescence. It turned to yellow with anisaldehyde/sulphuric acid or Ehrlich's reagent. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 10.31 (s, br, 1 H, 9-NH), 8.55 (d, ^3J = 6.1 Hz, 1 H, H-3), 8.16 (d, ^3J = 6.1 Hz, 2 H, H-4), 7.61 (dd, 2 H, H-7,8), 7.34 (m, 1 H, H-6), 2.90 (s, 3 H, 11-CH_3). – **(+)-ESI MS**: *m/z* = 211 ($[M + H]^+$).



4-Hydroxy-5-methyl-furan-3-one (**130**)**:** Oily compound, UV absorbing at 254 nm, turned to violet with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 2.32 (s, 3H), 4.60 (s, 2H). – **(+)-ESIMS:** *m/z* = 115 ($[M+H]^+$). For data, see ref. [254].



Actinomycin D (**131**): Obtained by applying fraction IV to Sephadex LH-20 (MeOH) as orange solid, UV absorbing at 254 nm. It turned to yellow by anisaldehyde/sulphuric acid, no colour reaction with 2 N NaOH, deep red colour reaction with 2N H_2SO_4. – $\boldsymbol{R_f}$ = 0.37 ($CHCl_3$/5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 8.13 (d br; ^3J = 6.4 Hz, 1H, NH-Val$_\alpha$), 7.97 (d br, ^3J = 6.4 Hz, 1H, NH-Val$_\beta$), 7.81 (d br, ^3J = 6.4 Hz, 1H, NH-Thr$_\beta$), 7.21 (d br, ^3J = 6.4 Hz, 1H, NH-Thr$_\alpha$), 7.67 (d, ^3J = 7.9 Hz, 1H, H-8), 7.39 (d, ^3J = 7.9 Hz, 1H, H-7), 6.03 (d, ^3J = 9.1 Hz, 1H, H-2 Pro$_\alpha$), 5.96 (d, ^3J = 9.1 Hz, 1H, H-2 Pro$_\beta$), 5.25-5.15 (m, 2H, H-3 Thr$_{\alpha,\beta}$), 4.81 (d, ^3J = 18.1 Hz, 1H, H_a-2, Sar$_\alpha$), 4.73 (d, ^3J = 18.8 Hz, 1H, H_a-2 Sar$_\beta$), 4.61 (dd, ^3J = 6.4 Hz, ^3J = 2.3 Hz, 1H, H-2 Thr$_\alpha$), 4.49 (dd, ^3J = 6.4 Hz, ^3J = 2.3 Hz, 1H, H-2 Thr$_\alpha$), 4.03-3.40 (m, 8H; H_b-2 Sar$_{\alpha,\beta}$ H-2 Val $_{\alpha,\beta}$ H_2-5, Pro$_{\alpha,\beta}$), 2.93, 2.90, 2.88 (3s, 3H+3H+6H, NCH_3 Me-Val $_{\alpha,\beta}$, NCH_3, Sar$_{\alpha,\beta}$), 2.67-2.57 (m+ s, 6+3, 3-Ha Pro$_{\alpha,\beta}$, 2,3-H, Me-Val $_{\alpha,\beta}$, 6-CH_3), 2.38-1.76+ 2.23 (m+ s, 8 + 3 H, 3-H_b- 4-H_2 Pro $_{\alpha,\beta}$, 3-H Val $_{\alpha,\beta}$, 4-CH_3), 1.28 (m, 6H, CH_3 Thr$_{\alpha,\beta}$), 1.17-1.07 (m, 6H, CH_3 Val $_{\alpha,\beta}$), 0.99-0.82 (m, 12H, CH_3 Val $_{\alpha,\beta}$, CH_3 Me Val$_{\alpha,\beta}$), 0.75 (d, ^3J = 4.9 Hz, 6 H, CH_3 Me Val $_{\alpha,\beta}$). – **(+)-ESIMS:** *m/z* (%) = 1277 ($[M+Na]^+$, 100), 1255 ($[M+H]^+$, 6). – **(-)-ESIMS:** *m/z* = 1254 ($[M-H]^-$).



12.6 Ruminal bacterium *Klebsiella pneumoniae* ZIC

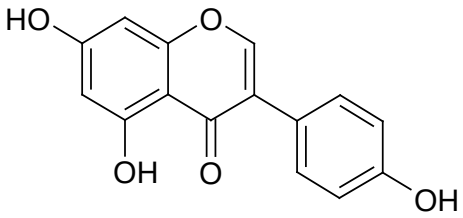
12.6.1 Fermentation and isolation

Klebsiella pneumoniae strain ZIC isolated from the rumen of cow was pre-cultivated on LB agar at 37 °C. Pieces (1×1 cm) of well grown agar plates were used to inoculate 120 of 1L Erlenmeyer flasks, each containing 250 ml of LB broth. The fermentation was carried out at 180 rpm on a linear shaker for 3 days at 37 °C. The culture broth was harvested and filtered after addition of 1 kg Celite to separate the bacterial biomass, which was extracted with ethyl acetate, while the culture filtrate was passed through Amberlite XAD-16 adsorption resin. The XAD column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluate was concentrated under reduced pressure and the aqueous residue was finally extracted with ethyl acetate. The Celite/biomass mixture was extracted with ethyl acetate (3 times) and acetone (2 times); the combined organic phases were then evaporated to dryness. Both the water and the biomass extracts were combined based on their chromatographic similarity, yielding 1.41 g of a brown crude extract.

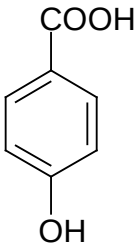
Chromatography on silica gel (column 3 × 100 cm) using a CH_2Cl_2/MeOH gradient (0-60% MeOH) monitored by TLC resulted in three fractions I-III.

Four compounds were isolated and identified as 4', 5,7-trihydroxyisoflavone (genistein, **133**), uracil (**126**), 4-hydroxybenzoic acid (**134**) and adenine (**124**).

4', 5,7-Trihydroxyisoflavone (**genisteine**, **133**): Genisteine (**133**) was isolated from fraction II as white powder, UV absorbing at 254 nm. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.03 (1H, d), 6.38 (1H, dd), 6.19 (1H, d), 7.38 (2H, d) and 6.82 (2H, d).



4-Hydroxybenzoic acid (**134**): Obtained from fraction II as white crystalline solid, which showed UV absorption at 254 nm. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 7.78 (2H, d, J = 8.5 Hz, H-3,5), 6.45 (2H, d, J = 8.5 Hz, H-2,4), 11.42 (1H, br s).



12.7 Ruminal bacterium ZIL

12.7.1 Pre-screening

The producing organism, the ruminal *Pseudomonas aeruginosa* 210, was isolated in Tunisia from the rumen of a cow. A voucher specimen of the strain is deposited in the culture collection at the Institute of Organic and Biomolecular Chemistry, Göttingen, Germany, under the reference ZIO.

P. *aeruginosa* strain 210 was cultured on 20 L of LB medium. The supernatant of the culture was assessed by the agar diffusion method and showed inhibition of a broad spectrum of Gram-negative and Gram-positive bacteria.

12.7.2 Fermentation and isolation

A 5 l culture of *P. aeruginosa* 210 was grown in 1 l Erlenmeyer flasks filled with 300 ml of LB-medium on a linear shaker with 95 rpm at 28 °C. After three days of fermentation, the culture broth was used to inoculate a Braun Biostat U fermenter, filled with 20 l of LB medium. The following conditions were maintained: stirring at 200 rpm, 28 °C, pH 6.5 ± 1.5, aeration 1.5 m^3/h. The culture broth was harvested after 5 days. The mycelium was filtered off by means of a pressure filter and extracted with ethyl acetate (3 × 5 l) and acetone (2 × 5 l), while the filtrate was passed through an Amberlite XAD-16 column (6 × 120 cm); the latter was washed with 25 l of demineralised water and eluted with 15 l methanol. After concentration, the aqueous residue was extracted with ethyl acetate. The extracts from the mycelium and the water phase were similar on TLC and were combined, yielding 3.54 g of a greenish-brown resin. Silica gel column chromatography (CC; CH_2Cl_2/MeOH, stepwise gradient) under TLC monitoring afforded four fractions A-D. Fraction I obtained from (CH_2Cl_2: CH_3OH 9.9:0.1), showed a single spot on TLC which on final purification using silica gel (CH_2Cl_2/ 5 % MeOH) and Sephadex LH-20 (CH_3OH) CC resulted in 1-phenazinol (**139**) (10 mg). Fraction II obtained with 0.2 % MeOH was again subjected to CC on silica gel and finally purified by Sephadex LH-20 (CH_3OH) to provide 2-*n*-heptyl-1-hydroxy-1H-quinolin-4-one (**135**) (100 mg). Fraction III obtained with 0.3 % MeOH afforded 3-*n*-heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**) (15 mg) after Sephadex LH-20 (CH_3OH) and PTLC (CH_2Cl_2: CH_3OH, 9.3:0.7). Phenazine-1-carboxylic acid (**43**) (25 mg) was obtained from fraction IV (0.5 % MeOH) by using Sephadex LH-20 (CH_3OH) and PTLC (CH_2Cl_2: CH_3OH, 8.8:1.2).

2-*n*-Heptyl-1-hydroxy-1*H*-quinolin-4-one (**135**): Colourless crystalline solid; – R_f = 0.40 (CH_2Cl_2/5% CH_3OH; 9.5:0.5). – **UV** (MeOH): λ_{max} (log ε) = 355 (2.88), 254 (3.01), 215 (3.15) nm. – **IR** (KBr) ν_{max} = 3430, 2926, 1656, 1594, 1456, 1121 cm^{-1}. – **^{1}H NMR** (300 MHz, $CDCl_3$) and **^{13}C NMR** (125 MHz, $CDCl_3$) see Table **12**.

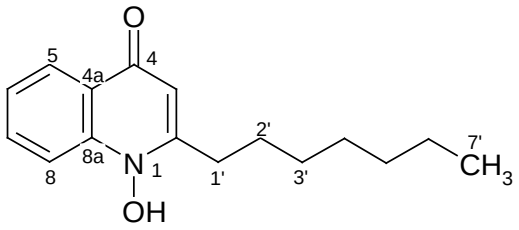
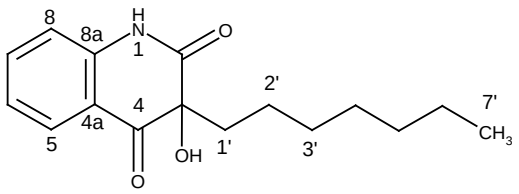


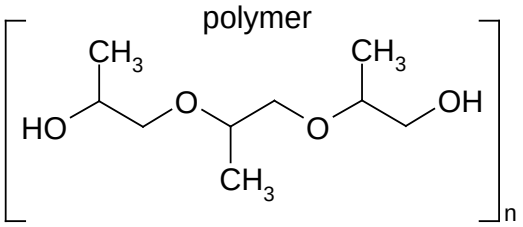
Table 39: Antimicrobial activity of compound **135** in the agar diffusion test at a concentration of 5µg/disk

Test strains	**Ø Inhibition in (mm)**
Staphylococcus aureus	13
Bacillus subtilis	20
Escherichia coli	15
Mucor miehei (Tü 284)	16
Candida albicans	0

3-*n*-Heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione (**137**): Colourless crystalline solid; – R_f = 0.40 (CH_2Cl_2:CH_3OH; 9.5:0.5). – **^{1}H NMR** (300 MHz, $CDCl_3$) and **^{13}C NMR** (125 MHz, $CDCl_3$) see Table 16.



Polypropylenglycol (**Niax, 138**): Colourless oil, 10.5 mg, non UV absorbing zone, turned to pink with anisaldehyde/sulphuric acid spray reagent. – $\boldsymbol{R_f}$ = 0.12 (CH_2Cl_2/ 5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 3.48 (m, CH_2), 1.10 (d, ^3J = 2.3 Hz, CH_3).



Phenazinol (**139**): Green needles, UV absorbing at 254, turned to yellow after spraying with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.37 (CH_2Cl_2/ 5% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 8.22 (dd, 1 H), 8.18 (dd, 1 H), 7.82-7.84 (m, 2 H), 7.22 (d, 1H),7.81 (d, 1H), δ 7.76 (t, 1H). – **^{13}C NMR** ($CDCl_3$, 300 MHz): see Table 17.

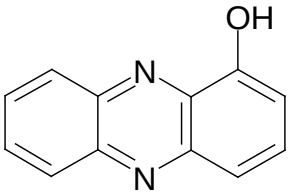


Table 40: *In vitro* antitumor activity of phenazinol (**139**) against tumor cell lines in a monolayer proliferation assay

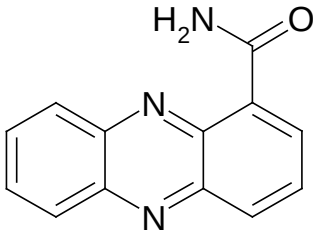
Tumor type	**Tumor cell line N°**	**Test/Control (%) at Drug Concentration (30 µg/ml)**
Lung A Adeno	LXF 529 NL	25 ++
Lung A Adeno	LXF 629 L	13 ++
Breast	MAXF	25 ++
Melanoma Xeno-graft	MEXF 462 NL	28 ++
Prostate	PRXF	23++
Pleuramesothelioma	PXF 1752L	37 +
Renal	RXF 486L	35 +
Uterus body	UXF 1138L	5 +++

- (T/C = 50) + (30< = T/C < 50) ++ (10 < = T/C < 30) +++ (T/C < 10)

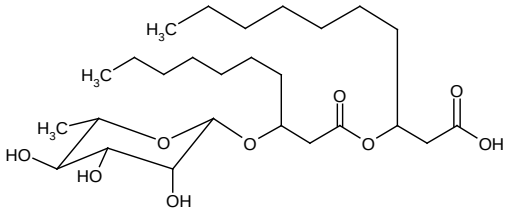
Table 41: *In vitro* antitumor activity of phenazinol (**139**) against tumor cell lines in a monolayer proliferation assay

Tumor type	**Tumor cell line N°**	**IC_{50} µg/ml**	**IC_{70} µg/ml**	**IC_{90} µg/ml**
Lung A Adeno	LXF 529 NL	4.288	8.442	16.618
Lung A Adeno	LXF 629 L	3.582	6.239	10.867
Breast	MAXF	4.545	8.540	16.050
Melanoma Xenograft	MEXF 462 NL	4.849	9.363	18.077
Ovarien Cancer Xeno-graft	OVXF 899L	11.364	26.664	>10.000
Pancreas	PAXF	10.000	23.842	>10.000
Prostate	PRXF	4.316	8.042	14.985
Pleuramesothelioma	PXF 1752L	6.218	12.915	26.826
Renal	RXF 486L	6.017	11.844	23.315
Uterus body	UXF 1138L	3.281	5.384	8.835
	Mean n = 10	6.231	10.590	16.020

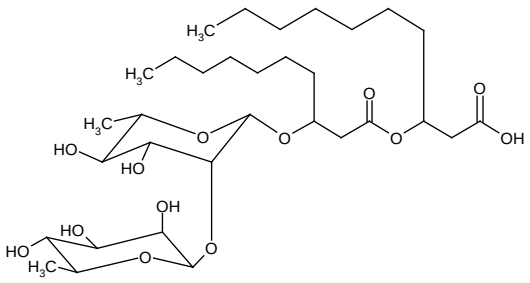
Phenazine-1-carboxamide (**42**)**:** Yellow compound, 16 mg, UV absorbing under 254 nm, turned to yellow with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.31 (CH_2Cl_2/ 5% MeOH). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 10.74 (brs, 1H, 1-NH), 9.02 (dd, ^3J = 7.1, J = 1.5 Hz, 1H, H-2), 8.42 (dd, ^3J = 8.7, J = 1.5 Hz, 1H, H-4), 8.28 (m, 1H, H-6), 8.23 (m, 1H, H-9), 7.97 (dd, ^3J = 7.1, J = 1.5 Hz, 1H, H-3), 7.93 (dd, ^3J = 7.0 Hz, J = 1.6 Hz, 1H, H-7), 7.89 (dd, ^3J = 7.7 Hz, J = 1.0 Hz, 1H, H-8), 6.38 (brs, 1H, 1-NH). – **(+)-ESIMS:** *m/z* (%) = 245 ($[M+Na]^+$, 13), 469 ($[2M+Na]^+$, 100). – **(+)-HRESIMS:** *m/z* = 246.06383 $[M+Na]^+$ (calcd. 246.06378 for $C_{13}H_9N_3ONa$).



Rhamnolipid A (**94**) **and Rhamnolipid B** (**140**): Oily mixture of two compounds, polar, turned to intense green after spray with anisaldehyde/sulphuric acid. – **^{1}H NMR** (300 MHz, CD_3OD): δ = 4.83, 3.80, 3.40, 3.65- 3.75, 1.25, 0.88, 1.15-1.62 and 1.25.



94



140

13 Terrestrial bacteria

13.1 Terrestrial *Bacillus* sp. ZIR

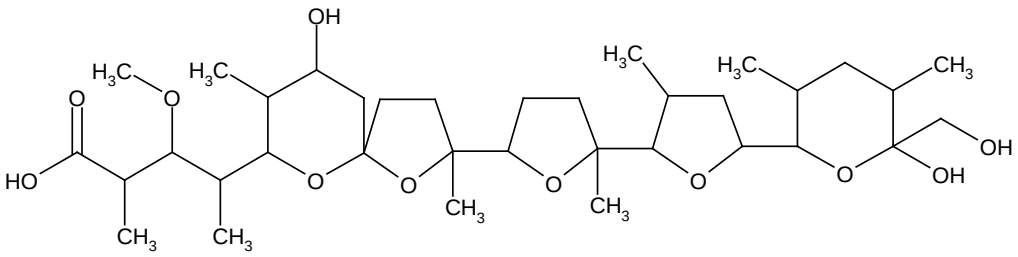
13.1.1 Fermentation and isolation

Bacillus sp. ZIR was pre-cultivated on LB agar at 37 °C. Pieces (1×1 cm) of well grown agar were used to inoculate 40 of 1-litre Erlenmeyer flasks, each containing 250 ml of LB broth. The fermentation was carried out at 180 rpm on a linear shaker for 3 days at 37 °C. The culture broth was harvested and filtered after addition of 1 kg Celite to separate the bacterial biomass, which was extracted with ethyl acetate, while the culture filtrate was passed through Amberlite XAD-16 adsorption resin. The XAD column was washed with 25 L demineralised water and eluted with 15 L methanol. The eluate was concentrated under reduced pressure and the aqueous residue was finally extracted with ethyl acetate. The Celite/biomass mixture was extracted with ethyl acetate (3 times) and acetone (2 times); the combined organic phases were then evaporated to dryness. Both the water and the biomass extracts were combined based on their chromatographic similarity, yielding 4.5 g of a brown crude extract.

Chromatography on silica gel (column 3 × 100 cm) using a CH_2Cl_2/MeOH gradient (0-60% MeOH) monitored by TLC resulted in four fractions I-IV. Fractions I-III were oily and fraction IV afforded monensin (**141**) (500 mg).

left: TLC of the ethyl acetate crude extract; middle: TLC of the acetate crude extract; right: TLC of the crude methanol extract

Monensin-B (**141**)**:** colourless, UV nonabsorbing solid, which turned to orange-red by anisaldehyde/sulphuric acid spraying reagent. – $\boldsymbol{R_f}$ = 0.37 (CH_2Cl_2/5% MeOH). – **^{1}H** and **^{13}C NMR**, see Table 18.



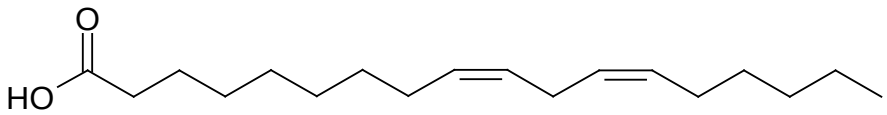
13.2 Terrestrial *Pseudomonas* sp. ZIPS

Fermentation and isolation

For up scaling, a 10 L shaker culture was inoculated with a well-grown sub-culture and cultivated at 28 °C for 5 days on a linear shaker. After 3 days, the culture was harvested and the biomass and water phase were separated with the aid of the filter press. The biomass was extracted with ethyl acetate and acetone until the filtrate was colourless. The water phase was adsorbed on an XAD-16 column and the latter extracted with methanol. Due to a similar composition of both extracts, they were combined and separated by a silica gel column chromatography (CH_2Cl_2/MeOH) into

four fractions. Four compounds were isolated from the terrestrial bacterium *Pseudomonas* sp. ZIPS; all of them were known and were identified as phenazine-1-carboxylic acid (**43**), anthranilic acid (**113**), linoleic acid (**89**) and *cis-cyclo*(Tyr,Pro) (**109**).

Linoleic acid; (9Z, 12Z)-9, 12-octadecanoic acid (**89**): Obtained by chromatography of fraction II on Sephadex LH-20 (MeOH) as a UV absorbing polar colourless oil, which turned to blue by anisaldehyde/sulphuric acid and heating. – R_f = 0.75 (CH_2Cl_2/MeOH, 5%). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 11.2 (s, br, 1 H, COOH), 5.41-5.24 (m, 4 H, 9,10,12,13-CH), 2.78 (t, ^3J = 6.0 Hz, 2 H, 11-CH_2), 2.38 (t, ^3J = 7.2 Hz, 2 H, 2-CH_2), 2.08 (m, 4 H, 8,14-CH_2), 1.63 (m, 2 H, 3-CH_2), 1.40-1.20 (m, 10 H, CH_2), 0.85 (m, 3 H, 18-CH_3).



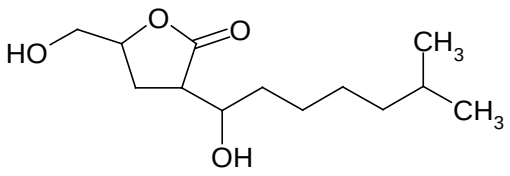
13.3 Terrestrial bacterium *Streptomyces* Ank 195

Fermentation and isolation

The terrestrial *Streptomyces* sp. Ank 195 subculture was used to inoculate a 25 L shake culture using M_2^+ medium at 28 °C (pH 7.8). The culture broth was harvested after ten days. Biomass and filtrate were separated with the aid of the filter press. The biomass was extracted three times with ethyl acetate followed by aceton (2 ×). The water phase was subjected to XAD-16 column and extracted with MeOH. Both crude extracts showed identity on TLC, so they were combined (1.75 g) and chromatographed on silica gel column using CH_2Cl_2/MeOH gradient (0 to 40 % MeOH). Only two fractions were resulting. The first fraction one contained fat and the second fraction was purified on Sephadex LH-20 to afford compound **143**.

5-Hydroxymethyl-3-(1-hydroxy-6-methyl-heptyl)-dihydrofuran-2-one (**143**): **^{1}H NMR** (300 MHz, $CDC1_3$): δ = 0.81 (d, J = 7.2 Hz, 6H, CH_3 isopropyl), 1.05-1.55 (m, 9H, aliphatic side chain), 2.05 (ddd, J = 4Hz, J' = 10 Hz, J'' = 12.8 Hz, lH, H_β-3),

2.35 (ddd, *J* = *J*' = 8 Hz, *J*'' = 12.8 Hz, lH, H_α-3), 2.78 (ddd, *J* = 2.6 Hz, *J*' = 8Hz, *J*'' = 10.5 Hz, lH, H-2), 3.55 (dd, *J* = 7Hz, *J*' = 2.5Hz, lH, H-5), 3.83 (dd, IH, *J* = 7Hz, *J*' = 1.5 Hz, H-5'), 4.08 (m, lH, H-1'), 4.60 (m, 1H, H-4). – **^{13}C NMR** (300 MHz, $CDC1_3$): δ = 179.9 (C-1), 80.0 (C-4), 69.3 (C-l'), 64.4 (C-5), 46.3 (C-2), 34.8 (C-2'), 32.5 (C-3'), 29.2 (C-6'), 27.7 (C-4'), 22.4 (C-5'), 23.0 (C-3), 22.5 (C-7', C-8').



Compound **143** showed a cytotoxic activity against brine shrimps (*Artemia salina*) with an LD_{50} of 2.5 μg/ml.

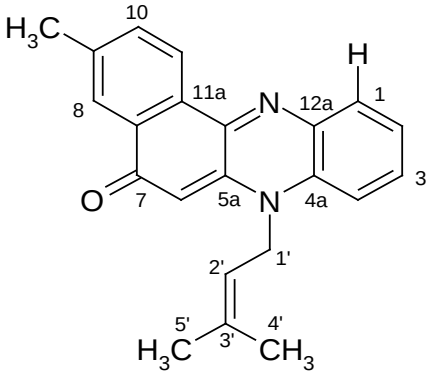
13.4 Terrestrial *Streptomyces* Ank 315

Streptomyces sp. Ank 315 was cultivated on a 25 L and 60 L scale using 1 L Erlenmeyer flasks containing 250 mL of M_2 medium (malt extract 10 g, yeast extract 4 g, glucose 4 g per litre tap water; adjusted to pH 7.8 with 2 N NaOH) at 28 °C for eight days on a linear shaker (250 rpm).

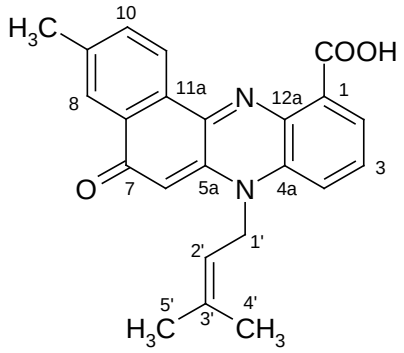
The culture broth was mixed with Celite and filtered with a filter press. The filtrate was passed through an Amberlite XAD-16 column (120 × 5.5 cm), the resin was washed with distilled H_2O, and eluted with MeOH. The methanol phase was concentrated, and the aqueous residue was extracted with EtOAc. The mycelium was extracted sequentially with EtOAc and then acetone. The extracts showed similar compositions on TLC, and were combined. Compounds **144** and **145** were obtained from the 25 L cultivation, whereas compounds **146**, **147, 151** and **152** were isolated from the 60 L cultivation. Additionally, the following known compounds were isolated: phenazine-1-carboxylic acid (**43**, 40 mg), 1-phenazinol (**139**, 25 mg), 1-phenazinecarboxamide (**42**), 15 mg), anthranilic acid (**113**, 12 mg), and tryptophol (**119**, 10 mg).

Chromophenazine A (**9-methyl-5-(3'-methyl-but-2'-enyl)-5*H*-benzo[a]phenazin-7-one, 144):** Orange powder; – R_f = 0.36 (5 % MeOH/CH_2Cl_2); – **UV/Vis** (MeOH): λ_{max} (log ε) = 229 (4.91), 256 (4.20), 271 (4.02), 278 (4.04), 308 (4.16), 372 (3.76),

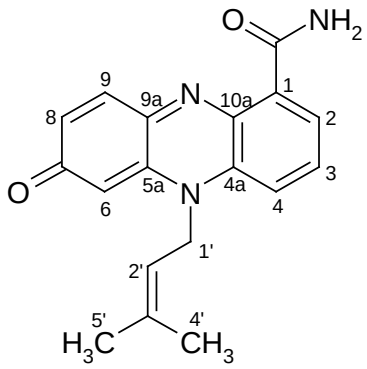
463 sh (3.91), 490 (4.02), 519 sh (3.87) nm; – **IR** (KBr): ν_{max} = 3429, 2923, 2854, 1583, 1544, 1460, 1377, 1321, 1233, 1161, 1055 cm^{-1}; – **^{1}H NMR** (300 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 20; – **(+)-ESIMS:** *m/z* = 329 ($[M+H]^+$); – **(+)-HRESIMS:** *m/z* = 329.16498 $[M+H]^+$ (calcd for $C_{22}H_{21}N_2O$, 329.16484).



Chromophenazine B (**3-methyl-7-(3'-methyl-but-2'-enyl)-5-oxo-5,7-dihydrobenzo[a]phenazine-11-carboxylic acid, 145**)**:** Orange powder; – ***R*$_f$** = 0.28 (5 % $MeOH/CH_2Cl_2$); – **UV/Vis** (MeOH): λ_{max} (log ε) = 247 sh (4.22), 277 sh (4.04), 301 (3.98), 309 (3.98), 363 (3.62), 491 (3.39), 521 sh (3.28) nm; – **^{1}H NMR** (600 MHz, $CDCl_3$) data, see Table 21; – **(+)-ESIMS:** *m/z* (%) = 767 ($[2M + Na]^+$, 25), 395 ($[M+Na]^+$, 100); – **(-)-ESIMS:** *m/z* = 371 ($[M-H]^-$; – **(+)-HRESIMS:** *m/z* = 373.15459 $[M+H]^+$ (calcd for $C_{23}H_{21}N_2O_3$, 373.15467) .



Chromophenazine C (**5-(3'-methyl-but-2'-enyl)-7-oxo-5,7-dihydrophenazine-1-carboxamide, 146**)**:** Violet powder; – R_f = 0.20 (7 % MeOH/CH_2Cl_2); – **UV/Vis** (MeOH): λ_{max} (log ε) = 224 (3.48), 281 (3.34), 361 (2.84), 531 (2.80) nm; – **IR** (KBr): ν_{max} = 3446, 2928, 1736, 1718, 1654, 1541, 1459, 1384, 589 cm^{-1}; – **^{1}H NMR** (300 MHz, DMSO-d_6) and **^{13}C NMR** (125 MHz, DMSO-d_6) data, Table 22; – **(+)-ESIMS:** *m*/*z* (%) = 637 ($[2M+Na]^+$, 95), 330 ($[M+Na]^+$, 100); – **(-)-ESIMS:** *m*/*z* = 306 ($[M-H]^-$); – **(+)-HRESIMS:** *m*/*z* = 308.13960 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_3O_2$, 308.13935).



Chromophenazine D (**3-benzoyl-5-(3'-methylbut-2'-enyl)-5,10-dihydrophenazine-1-carboxylic acid, 147**)**:** Dark red solid; – R_f = 0.13 (5 % MeOH/CH_2Cl_2); – **UV/Vis** (MeOH): λ_{max} (log ε) = 247 (4.20), 301 (4.02), 389 (3.50), 491 (3.62) nm; – **IR** (KBr): ν_{max} = 3447, 2925, 2373, 2080, 1836, 1560, 1495, 1292, 587 cm^{-1}; – **^{1}H NMR** (300 MHz, DMSO-d_6) and **^{13}C NMR** (125 MHz, DMSO-d_6) data, see (Table

23); – **(-)-ESIMS:** *m/z* (%) = 817 ([2M+NaOH]$^-$, 50), 397 ([M-H]$^-$, 100); – **(-)-HRESIMS:** *m/z* = 397.15581 [M-H]$^-$ (calcd for $C_{25}H_{21}N_2O_3$, 397.15577); – **(+)-HRESIMS:** *m/z* = 398.16241 $[M]^{\bullet+}$ (calcd for $C_{25}H_{22}N_2O_3$, 398.16249).

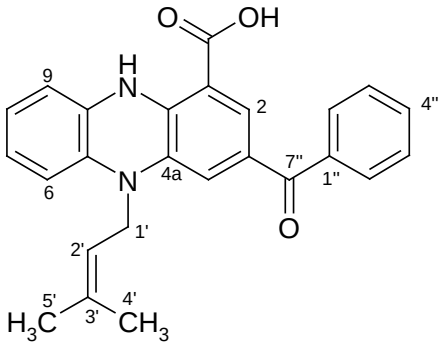


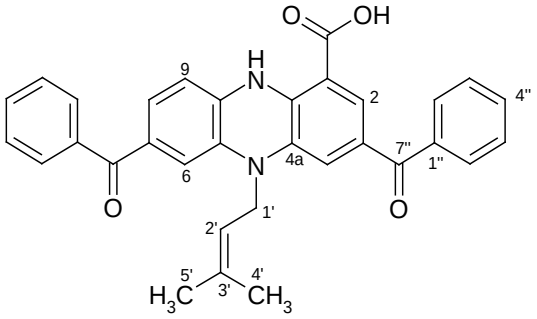
Table 42: Biological activity of Chromophenazine D (**147**)

Test organisms	**Inhibition zone (ø mm)**
Staphylococcus aureus	0
Bacillus subtilis	10
Escherichia coli	10
Streptomyces viridochromogenes (Tü 57)	0
Candida albicans	0
Mucor miehei	10

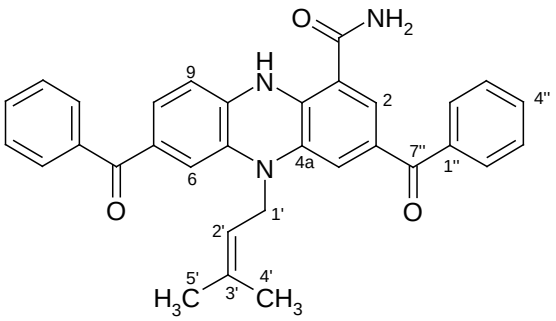
Chromophenazine D (**147**) showed a weak activity of 18 % (+) against *Artemia salina*

Chromophenazine E (**3,7-dibenzoyl-5-(3'-methyl-but-2'-enyl)-5,10-dihydrophenazine-1-carboxylic acid, 151**)**:** Pink solid; – R_f = 0.30 (5% MeOH/CH_2Cl_2); – **UV/Vis** (MeOH): λ_{max} (log ε) = 249 (4.09), 310 (4.16), 387 (3.95), 522 (3.72) nm; – **IR** (KBr): ν_{max} = 3448, 2927, 1653, 1636, 1542, 1497, 1424, 1274, 1118, 890, 722

cm^{-1}; – **^{1}H NMR** (300 MHz, DMSO-d_6) and **^{13}C NMR** (125 MHz, DMSO-d_6) data, see Table 24; – (-)-**ESIMS:** *m/z* (%) = 1025 ($[2M-2H+Na]^-$, 12), 501 ($[M-H]^-$, 40), 432 ($[M-C_5H_{10}]^-$, 100); – **(-)-HRESIMS:** *m/z* = 501.18201 $[M-H]^-$ (calcd for $C_{32}H_{25}N_2O_4$, 501.18198); – **(+)-ESIMS:** *m/z* (%) = 503 ($[M+H]^+$, 33), 502 ($[M]^{\bullet+}$, 100).



Chromophenazine F (3,7-dibenzoyl 5-(3'-methyl-but-2'-enyl)-5,10-dihydrophenazine-1-carboxamide, 152): Red powder; – ***R_f*** = 0.26 (5% $MeOH/CH_2Cl_2$); – **UV/Vis** (MeOH): λ_{max} (log ε) = 251 (3.84), 304 (3.76), 394 (3.15), 511 (3.24) nm; – **IR** (KBr): ν_{max} = 3429, 2923, 2853, 1641, 1544, 1496, 1446, 1384, 1275, 1128, 715 cm^{-1}; – **^{1}H NMR** (300 MHz, DMSO-d_6) and **^{13}C NMR** (125 MHz, DMSO-d_6) data, see Table 27; – **(-)-ESIMS:** *m/z* = 500 ($[M-H]^-$); – **(-)-HRESIMS:** *m/z* = 500.19823 $[M-H]^-$ (calcd for $C_{32}H_{26}N_3O_3$, 500.19797); – **(+)-ESIMS** *m/z* (%) 1025 = ($[2M+Na]^+$, 100), 524 ($[M+Na]^+$, 75); 502 ($[M+H]^+$, 30), 501 ($[M]^{\bullet+}$, 100); – **(+)-HRESIMS:** *m/z* = 524.19472 $[M+Na]^+$ (calcd for $C_{32}H_{27}N_3O_3Na$ 524.19446).



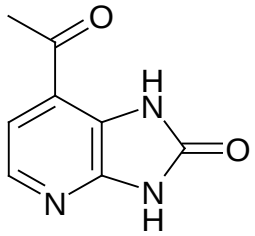
13.5 Terrestrial bacterium *Streptomyces* Ank 223

A well-grown sub-culture of the terrestrial *Streptomyces* sp. Ank 223 was used to inoculate 20 l shaker culture on M_2 medium; the pH was adjusted to 7.80 before ster-

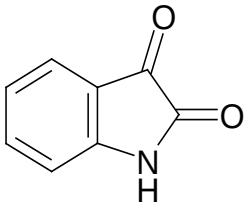
ilisation. After 7 days cultivation at 28 °C, the strain showed a grey culture broth. The fermenter broth was filtered with a filter press. The filtrate was passed through XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in vacuo and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (3.5 g).

Four compounds were isolated from *Streptomyces* sp. Ank 223, all of them were known and were identified as 7-acetyl-1,3-dihydroimidazo [4,5,b] pyridine-2-one, isatin (**155**), polyhydroxybutyric acid (**156**), lumichrome (**157**), ferroverdin A (**158**).

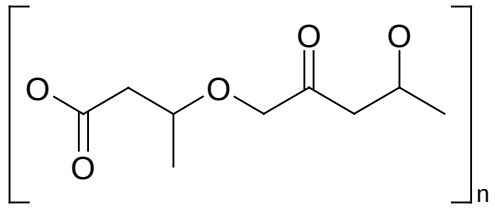
7-Acetyl-1,3-dihydroimidazo[4,5,b] pyridine-2-one (**154**)**:** White solid. – **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.15 (d, 1H), δ 7.15 (d, 1H), δ 2.60 (s, CH_3).



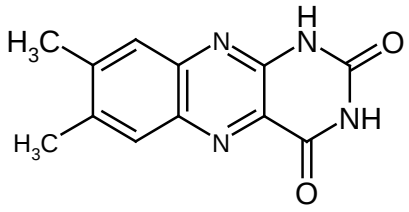
Isatin (**155**)**:** Deep orange powder. – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 7.58 (d, 1H), 6.85 (d, 1H), *7*.55 (t, 1H), 7.08 (t, 1H).



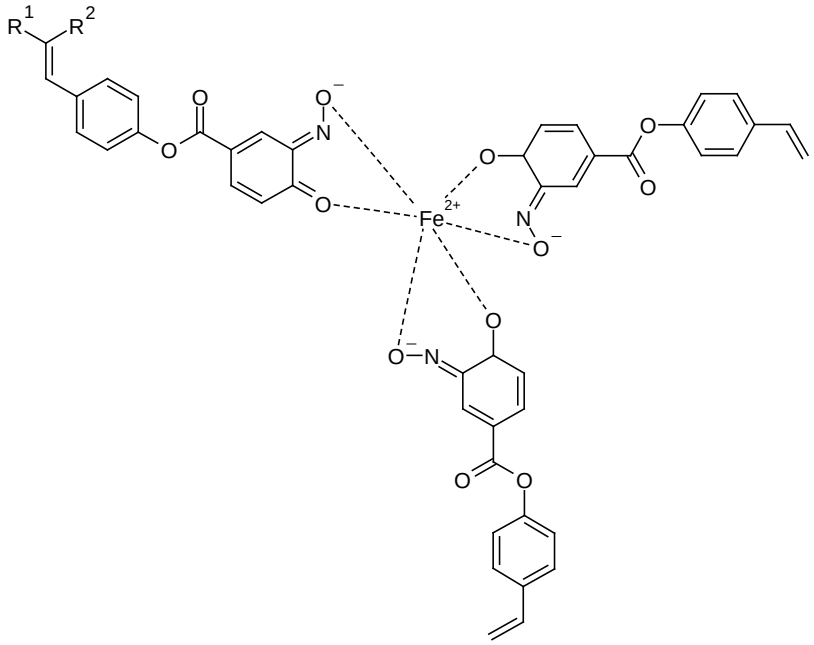
Polyhydroxybutyric acid, PHB (**156**): – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 1.23 (d, CH_3), 2.40 (m), 2.60 (m), 5.23 (m).



Lumichrome (**157**): Yellow solid, UV absorbing at 254 nm, strong white UV fluorescence; – $\boldsymbol{R_f}$ = 0.15 (CH_2Cl_2/5% CH_3OH), gave blue colouration with anisaldehyde/sulphuric acid and heating. – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 13.9 (br s, 1H, -NH), 8.03 (s, 1H, H-4), 7.85 (s, 1H, H-1), 5.01 (br s, 1H,-NH), 2.32 (s, 3H, CH_3-3), 2.25 (s, 3H, CH_3-2).



Ferroverdin A (**158**): Green powder; – R_f = 0.35 (CH_2Cl_2/5% CH_3OH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ = 8.10 (dd, 9.2, 2.0, H-6), 7.74 (d, 2.2, H-2), 7.18 (d, 9.2, H-5), 7.53 (d, 8.6, H-10), 7.22 (d, 8.6, H-9), 6.75 (dd, H-12), 5.26 (d, H-13), 5.82 (d, H-13). – **^{13}C NMR** (DMSO-d_6, 125 MHz) see Table 25 – **HR-ESIMS:** *m/z* = 861.1257 (calcd. 861.1258 for $C_{45}H_{31}N_3O_{12}$ Fe $[M+H]^+$).



13.6 *Streptomyces* sp. WO 668

13.6.1 Pre-screening

The crude extracts showed in the agar diffusion test activity against *Escherichia coli*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus*, *Mucor miehei* and *Artemia salina*.

Table 43: Cytotoxicity activity results of the different fractions obtained from *Streptomyces* sp. WO668 crude extracts.

Sample Reference	Sample Type	Sample amount (mg)	Cytotoxicity assay results %)
ZIWO668EA	Ethyl Acetate Crude Extract	595 (oily)	100
ZIWO668AC	Acetone Crude Extract	265	100
ZIWO668M	Methanol Crude Extract	194	0
ZIWO668M1-2	Fraction MeOH Extract	11	8
ZIWO668M2	Fraction MeOH Extract	13	3
ZIWO668M3	Fraction MeOH Extract	6	4

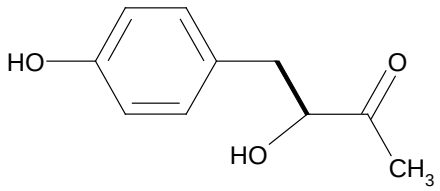
ZIWO668M4	Fraction MeOH Extract	14	4
ZIWO668M5	Fraction MeOH Extract	16	2
ZIWO668M6	Fraction MeOH Extract	17	33
ZIWO668M7	Fraction MeOH Extract	18	0
ZIWO668M8	Fraction MeOH Extract	7	12
ZIWO668M 9	Fraction MeOH Extract	22	9
ZIWO668M10	Fraction MeOH Extract	11	6
ZIWO668M11	Fraction MeOH Extract	11	15
ZIWO668M12	Fraction MeOH Extract	10	8
ZIWO668AC0	Fraction Acetone Extract	88	90
ZIWO668AC1	Fraction Acetone Extract	46	0
ZIWO668AC2	Fraction Acetone Extract	9	5
ZIWO668AC3	Fraction Acetone Extract	2	0
ZIWO668AC4	Fraction Acetone Extract	7	3
ZIWO668AC5	Fraction Acetone Extract	10	100
ZIWO668AC6	Fraction Acetone Extract	15	11
ZIWO668AC7	Fraction Acetone Extract	7	3
ZIWO668AC8	Fraction Acetone Extract	16	100
ZIWO668AC9	Fraction Acetone Extract	10	100
ZIWO668AC10	Fraction Acetone Extract	10	0
ZIWO668EA1	Fraction EtOAc Extract	39	100
ZIWO668EA2	Fraction EtOAc Extract	50	0
ZIWO668EA3	Fraction EtOAc Extract	160	0
ZIWO668EA4	Fraction EtOAc Extract	57	0
ZIWO668EA5	Fraction EtOAc Extract	25	3
ZIWO668EA6	Fraction EtOAc Extract	16	5
ZIWO668EA7	Fraction EtOAc Extract	22	5

All fractions were sent to cooperation partners for further tests on antifungal and insectidal activities.

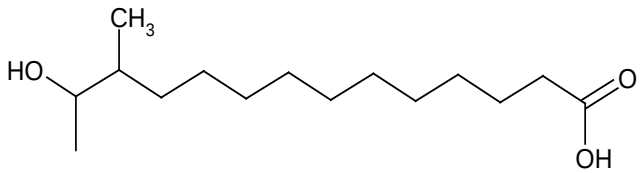
13.6.2 Fermentation and Isolation

A well-grown sub-culture of the terrestrial *Streptomyces* sp. WO 668 was used for inoculation of 60 l shaker culture on M_2 medium; the pH was adjusted to 7.80 before sterilisation. After 10 days cultivation at 28 °C, the strain showed a brown culture broth. The fermenter broth was filtered with a filter press. The filtrate was passed over XAD-16 and the latter afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate 3 times and with acetone 2 times. Three crude extracts were obtained: ethyl acetate extract (2.70 g), acetone extract (3.70 g) and methanol extract (2.32 g).

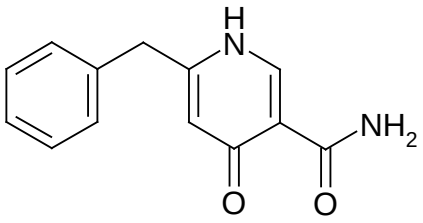
3-Hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**161**): Colourless oily compound, UV absorbing under 254 nm, turned to dark pink after spraying with anisaldehyde/ sulphuric acid; – $\boldsymbol{R_f}$ = 0.28 (CH_2Cl_2/ 7% MeOH). – $[\alpha]_D^{20}$ = +3.3 (*c* 0.18, MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.04 (d, *J* = 8.7 Hz, 2H, H-3, 5), 6.68 (d, *J* = 15.9 Hz; 2H, H-2,6), 4.23 (m, 1H, H-2'), 2.93 (dd,1H, *J* = 18.7 Hz, *J* = 4.6 Hz, Ha-1'), 2.72 (m,1H, 1H, Hb-1'), 2.18.(s, 3H, H-4'). – **^{13}C NMR** (CD_3OD, 125 MHz): see Table 30. – **^{1}H-^{1}H COSY and HMBC** see Figure 130. – **(+)-ESIMS:** *m/z* = 203 $[M+Na]^+$, 383 $[2M+Na]^+$. – **(-)-ESIMS:** *m/z* = 179 $[M-H]^-$, 359 $[2M-H]^-$.



13-Hydroxy-12-methyl-tetradecanoic acid (163): Oily compound; – **^{1}H NMR** (CD_3OD, 300 MHz), **^{13}C NMR** (CD_3OD, 125 MHz): see Table 31.



Aspernigrin A (**165**)**:** White crystals, UV absorbing under 254 nm. – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 8.52 (s, 1H, H-2), 6.34 (s, 1H, H-5), 3.95 (s, 2H, H-7), 7.31 (m, 2H, H-9,13), 7.28 (m, 2H, H-10,12), 7.24 (m, 1H, H-11). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 145.7 (C-2), 117.7 (C-3), 180.6 (C-4), 119.3, C-5), 155.7 (C-6), 37.6 (C-7), 138.1 (C-8), 129.8 (C-9/C-13), 130.1 (C-10/C-12), 128.1 (C-11).



14 Marine bacteria

14.1 Marine bacterium *Streptomyces* sp. B 909-417

14.1.1 Pre-screening

Streptomyces sp. B 909-417 showed grey mycelial colonies when cultivated on M_2^+ medium agar plates at 28°C for 3 days. TLC of the crude extract showed UV absorption bands at 254 nm.

The extract displayed cytotoxic activity with mortality of 100 % against the brine shrimp *Artemia salina* as test organism. It showed moderate antimicrobial activity (Table 44).

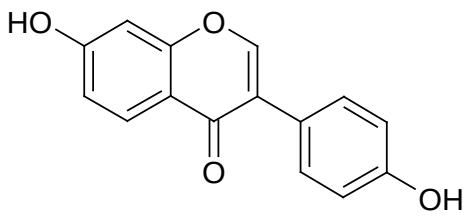
Table 44: Antimicrobial activity of the crude extract of *Streptomyces* sp. B 909-417 on M_2^+ medium [40 µL/paper disk (100 mg/mL)]

Test microorganisms	Inhibition zone Ø (mm)
Bacillus subtilis	16
Mucor miehei	15

14.1.2 Fermentation and work up

The subculture of *Streptomyces* sp. B 909-417 was used to inoculate a 25 L shaker culture using M_2^+ medium at pH 7.8. After 10 days, the culture was harvested and the resulting very dark culture was filtrated over Celite using a filter press. The filtrate was given on XAD-16, and the adsorbed metabolites were eluted with methanol. The methanol was evaporated under reduced pressure and the resulting water residue was extracted by ethyl acetate. The biomass was extracted by ethyl acetate and acetone until the dark brown colour had disappeared. The combined organic solutions were evaporated under vacuum to yield 2.64 g of dark brown crude extract. Separation was performed by flash silica gel column chromatography (CH_2Cl_2/MeOH gradient).

Daidzein (**0**): From fraction II, a white powder was obtained, which gave on TLC a UV absorbing spot at 254 nm. – **^{1}H NMR** (CD_3OD, 600 MHz): δ 7.85 (d, 1H, H-5), 6.73 (dd, 1H, H-8), 6.58 (d, 1H, H-3´), 7.26 (d, 1H, H-2´), 6.82 (d, 1H, H-6), 8.56 (s, 1H, H-2). – **(+)-ESIMS:** *m/z* = 255 $[M+H]^+$. – **(-)- ESIMS:** *m/z* = 253 $[M-H]^-$.



14.2 Marine *Streptomyces* sp B7936

14.2.1 Pre-screening

In the biological screening the crude extract of the marine derived *Streptomyces* sp. B7936 showed antimicrobial activity as summarized in Table 45.

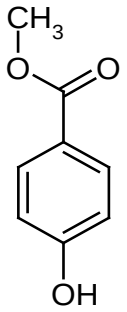
Table 45: Antimicrobial activity of the crude extract of *Streptomyces* sp. B 7936 on M_2^+ medium [40 µl /paper disk (100 mg /mL)]

Test microorganisms	Inhibition zone Ø (mm)
Bacillus subtilis	0
Staphylococcus aureus	21
Streptomyces viridochromogenes (Tü 57)	0
Escherichia coli	0

14.2.2 Fermentation and isolation

Well-grown agar plates were used to inoculate 50 of 1 L Erlenmeyer flasks each containing 300 ml of M_2^+ medium (with 50% sea water) and incubated on the linear shaker culture at 28 °C for 8 days. The culture broth was passed through a filter press to afford biomass and filtrate. The filtrate was passed through XAD-16 resin and the latter extracted with MeOH, while the biomass was extracted with EtOAc three times followed by acetone two times. The organic phases were brought to dryness. The combined crude extract (2.5 g) was defatted by cyclohexane and chromatographed on a silica gel column chromatography using CH_2Cl_2/MeOH gradient (0 to 20 % MeOH) to deliver three fractions I-III. Fraction I contained fat and purifications II and III led to the isolation of compounds **167**, **128** and **129**.

***p*-Hydroxybenzoic acid methyl ester** (**167**)**:** White crystalline solid, UV absorbing at 254 nm, pink with anisaldehyde /sulphuric acid. – $\boldsymbol{R_f}$ = 0.48 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.95 (d, 2H, ^3J = 8.9 Hz, H-2,H-5), 6.87 (dd, 2H, AA'BB', ^3J = 8.9 Hz, H-3, H-4), 3.87 (s, 3H, OCH_3).



15 Hospital area bacterium

15.1 *Bacillus pumilus* 1 ZIBP1

Well-grown agar plates were used to inoculate 10 of 1 L Erlenmeyer flasks, each containing 250 mL LB medium at pH 7. The culture was cultivated on a linear shaker for 3 days. The resulting culture broth was filtrated with the aid of a filter press. The water phase was subjected to an XAD-16 column and extracted with MeOH.

The biomass was extracted with ethyl acetate and acetone. The two combined phases were brought to dryness under reduced pressure to yield crude extract, which was subjected to a silica gel column chromatography using a CH_2Cl_2 /MeOH gradient and separated into fractions I-IV.

Endophenazine A (**37**): **P**ale yellow compound, UV absorbing at 254 nm. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 8.95 (dd, 1H, *J* = 7.5, 1.5 Hz, H-2), 7.85 (dd, 1H, *J* = 8.5, 7.5 Hz, H-3), 8.50 (dd, 1H, *J* = 8.5, 1.5 Hz, H-4), 8.19 (dd, 1H, *J* = 8.5, 1.5 Hz, H-6), 8.00 (dd, 1H, *J* = 8.5, 7.0 Hz, H-7), 7.80 (dd, 1H, *J* = 7.0, 1.5 Hz, H-8), 4.02 (d, 2H, *J* = 7.0 Hz, H-1'), 5.45 (t, 1H, *J* = 7.0 Hz, H-2'), 1.79 (s, 6H, H-4'). – **^{13}C NMR** ($CDCl_3$, 300 MHz): δ = 166.0 (1-COOH), 29.8 (C-1'), 120.3 (C-2'), 135.4 (C-3'), 25.7 (C-4'), 18.0 (C-5'). – **(+)-ESIMS:** *m/z* = 292.1211 $[M+H]^+$ calc. 293.1314 for $C_{18}H_{17}N_2O_2$.

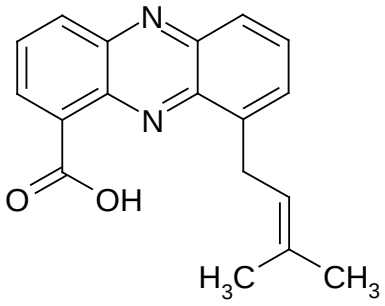


Table 46: Antitumor activity of Endophenazine A (**37**) in a panel of 12 tumor cell lines

	Potency		**Active /total* at**			
	mean IC_{50} [µg/ml]	mean IC_{70} [µg/ml]	1 µg/ml		10 µg/ml	
Endophenazine A (**37**)	1.617	4.467	1/12	8%	9/12	75%
	Tumor selectivity					
	Selectivity[1)] / total	% selectivity	Rating[2)]			
	1/12	8%	+			

1) individual IC_{70} <1/3 mean IC_{70}, for example if mean IC_{70} = 2.1 µM the threshold for above average sensitivity was 2) – (% selective = < 4%); + (4% > % selective > = 10%); ++ (10% > % selective > = 10%); +++ (% selective > 20%)

Table 47: *In vitro* antitumor activity of endophenazine A in a panel of 12 tumor cell lines

	Potency		**Active /total* at**			
	mean IC_{50} [µg/ml]	mean IC_{70} [µg/ml]	1 µg/ml		10 µg/ml	
Endophenazine A (37)	1.166	2.608	12/37	32%	31/37	84%
	Tumor selectivity					
	Selectivity[1)] / total	% selectivity	Rating[2)]			
	10/37	27%	+++			

1) individual IC_{70} <1/3 mean IC_{70}, for example if mean IC_{70} = 2.1 µM the threshold for above average sensitivity was 2) – (% selective = < 4%); + (4% > % selective > = 10%); ++ (10% > % selective > = 10%); +++ (% selective > 20%)

16 Fungal metabolites

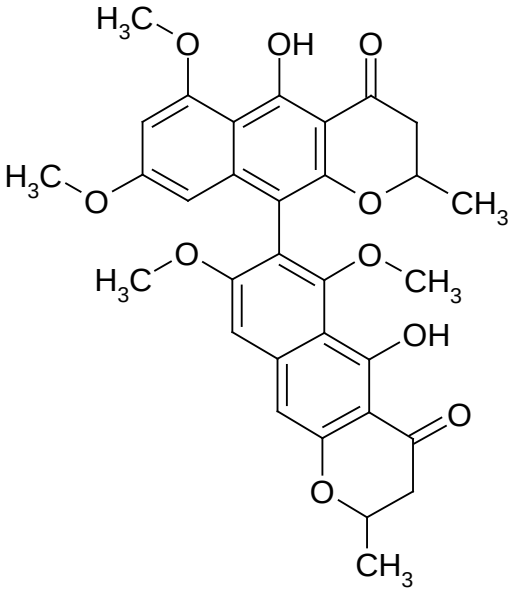
16.1 Aspergillus flavus

16.1.1 Fermentation and Isolation

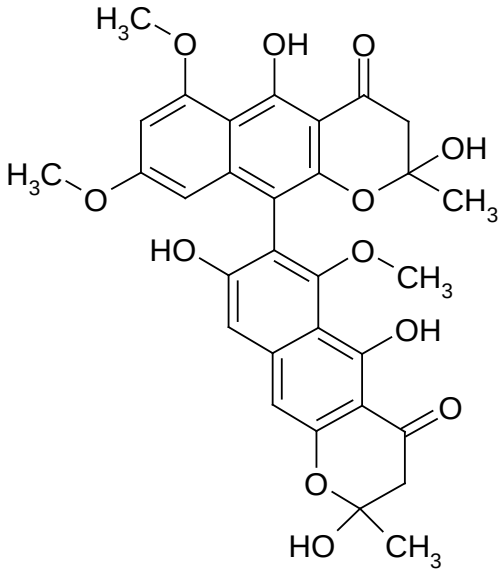
The sub-culture of the fungus *Aspergillus flavus* was used to inoculate 4 P-flasks containing each 200 g of rice mixed with 300 ml of M_2 medium and incubated for 30 days at 20 °C to give the mycelium. The latter was extracted three times with ethyl acetate, two times with acetone and two times with methanol, then filtrated. The organic phases were evaporated to dryness and combined to afford the crude extract

(25.43 g). The filtrate was extracted with ethyl acetate. The extract was evaporated to dryness and separated by silica gel column chromatography (CH_2Cl_2/ MeOH) to afford aurasperone A (**170**), aurasperone C (**171**), a mixture of lichexanthone (**172**) and rubofusarin (**173**), and 5-hydroxymethylfuran-3-carboxylic acid (**174**).

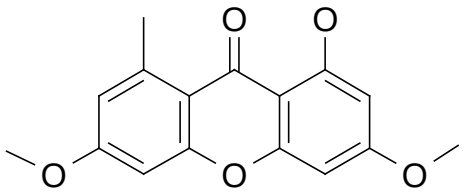
Aurasperone A (170): Yellow pigment, UV absorbing under 254 nm, yellow fluorescence under UV 366 nm. – **^{1}H NMR** and **^{13}C NMR** (CD_3OD, 300 MHz, 125 MHz; respectively) see Table 34.



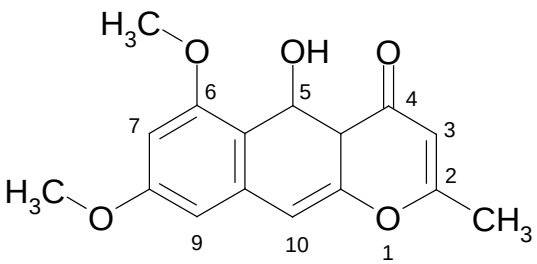
Aurasperone C (**0**): Yellow pigment, yellow fluorescence under UV 366 nm. – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 6.84 (s, 1H, H-10), 6.58 (s, 1H, H-9), 6.38 (d, 1H, H-9), 6.20 (d, 1H, H-7´), 3.43 (s, 3H, 6-OCH_3), 3.58 (s, 3H, , 8´-OCH_3), 3.82 (s, 3H, 6´-OCH_3), 3.30-3.29 (m, 4H, 3,3´-H_2), 1.42 (s, 3H, 2´-CH_3) and 1.63 (s, 3H, 2-CH_3).



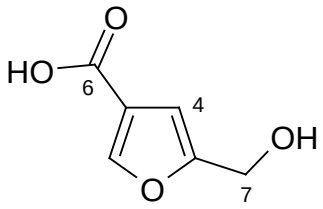
Lichexanthone (**0**): Yellow compound, UV absorbing at 254 nm, and yellow fluorescent under UV 366 nm. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 2.50 (s, 3H, CH_3, H-8), 3.86 (s, 3H, 6-OCH_3), 3.95 (s, 3H, 3-OCH_3), 5.98 (d, J = 2.1 Hz, 1 H, H-2), 6.25 (d, 1 H, J = 2.1 Hz,H-4), 6.58 (d, 1 H, J = 2.04 Hz, H-7), 6.40 (d, J = 2.06 Hz, 1 H, H-5), 12.80 (s, 1 H, 1-OH).



Rubrofusarin B (**0**): Yellow colour, UV absorbing at 254 nm, yellow fluorescent under UV at 366 nm. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ = 14.92 (s, 5-OH), 6.89 (s, 1H-H-10), 6.58 (d, 1H, H-9), 6.38 (d, 1H, H-7), 5.98 (s, 1H, H-3), 3.98 (s, 6-OCH_3), 3.90 (s, 8-OCH_3), 2.37 (s, CH_3-2). Compound **0** was isolated as a mixture with lichexanthone (**0**).



5-Hydroxymethylfuran-3-carboxylic acid (174): Colourless crystals, R_f = 0.26 – **^{1}H NMR** (CD_3OD, 300 MHz): δ = 7.94 (s, 1H, H-2), 6.49 (s, 0.5 Hz, H-4), 4.40 (s, 0.5 Hz, H-7). – **^{13}C NMR** (CD_3OD): δ = 176.9 (C-6), 170.4 (C-5), 147.4 (C-3), 141.0 (C-2), 110.8 (C-4), 61.2 (C-7).



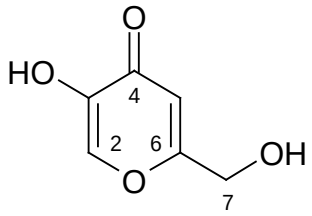
16.2 Aspergillus niger

16.2.1 Fermentation and Isolation

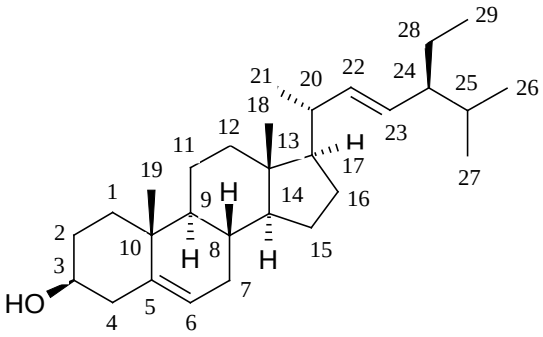
The sub-culture of the fungus *Aspergillus niger* was used to inoculate 4 P-flasks containing each 200 g of rice mixed with 300 ml of M_2 medium and incubated for 30 days at 20 °C to give the mycelium. The latter was extracted three times with ethyl acetate, two times with acetone and two times with methanol. The organic phases were evaporated to dryness and combined to afford the crude extract (27.18 g). The extract was separated on silica gel by column chromatography (CH_2Cl_2/ MeOH) to afford kojic acid (**175**), a mixture of stigmasterol (**176**) and *β*-sitosterol (**177**), piperazine (**178**) and fonsecin B (**178**).

Kojic acid (**175**): Colourless solid, UV absorbing at 254 nm, turned to blue by anisaldehyde/sulphuric after heating. – $\boldsymbol{R_f}$ = 0.75 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ = 8.99 (br, 1H, OH), 5.99 (br, 1H, OH), 7.99 (d, 1H, J = 7.97 Hz, CH-2), 6.33 (s, 1H, CH-5), 4.29 (s, 2H, CH_2-7). – **^{13}C/APT NMR** (DMSO-

d$_6$, 125 MHz): δ = 174.0 (C_q-4), 168.2 (C_q-6), 153.0 (C_q-3), 139.0 (CH-2), 110.0 (CH-5), 59.8 (CH_2-7).

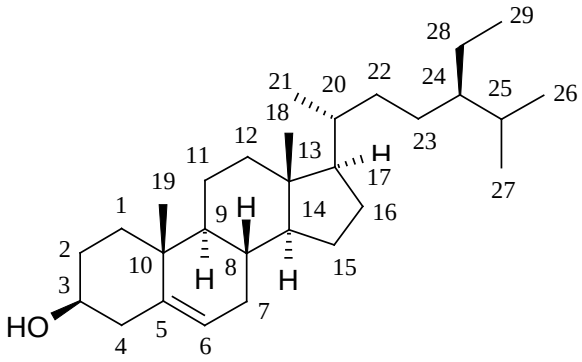


Stigmasterol (**176**): White solid; R_f = 0.23 – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 5.40 (d, ^3J = 5.2 Hz, 1H, H-6), 5.16 (dd, ^3J = 15.0, ^3J = 8.4 Hz, 1H, H-22), 5.00 (dd, ^3J = 15.0, 8.4 Hz, 1H, H-23), 3.50 (m, 1H, H-3), 1.01 (s, 3H, CH_3-19), 0.93 (d, ^3J = 6.4 Hz, 3H, CH_3-21), 0.85 (d, J = 6.0 Hz, 3H, CH_3-29), 0.83 (d, ^3J = 7.6 Hz, 3H, CH_3-27), 0.81 (d, ^3J = 6.8 Hz, 3H, CH_3-26), 0.68 (s, 3H, CH_3-18). – **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 140.7 (C-5), 138.3 (22-C), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 55.9 (C-17), 50.2 (C-9), 45.8 (C-24), 42.3 (C-13), 42.2 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.4 (C-20), 31.9 (C-8), 31.9 (C-7), 31.9 (C-2), 28.9 (C-25), 28.2 (C-16), 24.4 (C-15), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.0 (C-29), 12.2 (C-18). – **EIMS** (70 eV): *m/z* (%) = 412 ([M]$^{\bullet+}$, 18), 255 (100).

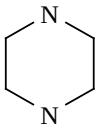


***β*-Sitosterol** (**177**): White solid, R_f = 0.43 – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 5.40 (d, ^3J = 5.2 Hz, 1H, H-6), 3.50 (1H, m, H-3), 1.01 (s, 3H, CH_3-19), 0.93 (d, ^3J = 6.4 Hz, 3H, CH_3-21), 0.85 (d, ^3J = 6.0 Hz, 3H, CH_3-29), 0.83 (d, ^3J = 7.6 Hz, 3H, CH_3-27), 0.81 (d, ^3J = 6.8 Hz, 3H, CH_3-26), 0.68 (s, 3H, CH_3-18). – **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 55.9 (C-17), 50.2 (C-9), 45.8 (C-24), 42.3 (C-13), 42.2 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.4 (C-

20), 33.9 (C-22), 31.9 (C-8), 31.9 (C-7), 31.9 (C-2), 28.9 (C-25), 28.2 (C-16), 26.1 (C-23), 24.4 (C-15), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.2 (C-29), 11.9 (C-18). – **EIMS** (70 eV): *m/z* (%) = 414 ($[M]^{\bullet+}$, 100), 396 (32), 255 (62).



Piperazine (**178**): White colourless crystals. – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 2.99 (s).



17 References

17.1 Citations

[1] D. J. Newman, G. M. Cragg, K. M. Snader, *Nat. Prod. Rep.* **2000**, *17*, 215-234.

[2] G. M. Cragg, D. J. Newman, K. M. Snader, *J. Nat. Prod.* **1997**, *60*, 52-60.

[3] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2007**, *70*, 461-477.

[4] K. Nakanishi, *Nat. Prod. Chem.* **1999**, *8*, xxi-xxxviii.

[5] K. Nakanishi, An historical perspective of natural products chemistry. In S. Ushio (Ed.), *Comprehensive Natural Products Chemistry*, Vol. 1 Elsevier Science B. V. , Amsterdam, pp. 23-40.

[6] D. Baker, U. Mocek, C. Garr. Nicholson, Eds. ; The Royal Society of Chemistry: Cambridge, UK, **2000**; pp 66-72

[7] S. A. Waksman, H. A. Lechevalier, *Science* **1949**, *109*, 305-307.

[8] U. Gräfe, *Biochemie der Antibiotika*, Spektrum Akad. Verlag, Heidelberg, Berlin, New York, **1992**, *42*.

[9] T. Beppu, *Gene* **1992**, *115*, 159-165.

[10] A. Fleming, *Brit. J. Exptl. Pathol.* **1929**, *10*, 226-236.

[11] W. Fenical, *Chem. Rev*. **1993**, *93*, 1673-1683.

[12] D. J. Newman, G. M. Cragg, K. M. Snader *J. Nat. Prod*. **2003**, *66*, 1022-1037.

[13] M. Tomasz, Y. Palom, *Pharmacol. Ther.* **1997**, *76*, 73-87.

[14] H. Matsue, C. Yang, K. Matsue, D. Edelbaum, M. Mummert, A. Takashima, *J. Immunol.* **2002**, *169*, 3555-3564.

[15] H. Hasibeder, H. J. Staab, K. Seibel, B. Heibel, G. Schmidle, W. März, *Eur. J. Clin. Pharmacol.* **1991**, *40* [Suppl 1]: S 91-S 94.

[16] M. C. De Pinto. , A. R. Barcelo, *Phytochemistry* **1996**, *42*, 283-286.

[17] M. A. Moskowitz, M. G. Buzzi, *J. Neurol.* **1991,** *238*, S18-S22.

[18] G. Keller, L. Spatola, D. McCabe, B. Martinell, W. Swain, E. Maliyakal T. John, *Transgenic Research* **1997**, *6*, 385-392.

[19] A. L. Demain, *Nat. Biotechnol.* **1998**, *16*, 3-4.

[20] D. H. Miles, C. L. Nguyen, D. H. Miles, *Curr. Med. Chem.* **1998**, *5*, 421-440.

[21] S. Grabley, I. Sattler, In A. Hillisch, R. Hingenfeld (Eds.), *Modern Methods of Drug Discovery.* Birkhäuser Verlag, Switzerland, **2003** pp. 87-107.

[22] T. J. Little, M. Horowitz, C. Feinle-Bisset, *Obes. Rev.* **2005**, *6*, 297-306.

[23] J. P. Chhatwal, A. R. Gutman, K. A. Maguschak, M. E. Bowser, Y. Yang, M. Davis, K. J. Ressler, *Neuropsychopharmacology* **2009**, *34*, 509-521.

[24] E. Hochgraf, Y. Levy, M. Aviram, J. G. Brook, U. Cogan, *Metabolism* **1994**, *43*, 11-17.

[25] N. Perico, P. Ruggenenti, E. Gotti, F. Gaspari, D. Cattaneo, U. Valente, M. Salvadori, G. Segoloni, D. Donati, S. Sandrini, M. Ganeva, B. D. Dimitrov, G. Remuzzi, Insights from the MY. S. S. Trial. *Kidney Internat.* **2004**, *65*, 1084-1090.

[26] J. Engelberth, T. Koch, G. Schüler, N. Bachmann, J. Rechtenbach, W. Boland *Plant Physiology* **2001**, *125*, 369-377.

[27] S. L. Bernhard, S. P. Lei, D. M. Fishwild, S. F. Carroll, *J. Biol. Chem.* **1992**, *267*, 16712-16718.

[28] B. R. O'Keefe, *J. Nat. Prod.* **2001**, *64*, 1373-1381.

[29] C. G. Shayne, Drug discovery handbook, Wiley Press, **2005**, 1471 pages

[30] K. Gebhardt, J. Schimana, P. Krastel, K. Dettner, J. Rheinheimer, A. Zeeck, H. P. Fiedler, *J. Antibiot.* **2002**, *55*, 794-800.

[31] P. Krastel, A. Zeeck, *J. Antibiot.* **2002**, *55*, 801-806.

[32] H. Budzikiewicz, *FEMS Microbiol. Rev.* **1993**, *10*, 209-228.

[33] A. Geiger, W. Keller-Schierlein, M. Brandl, H. Zähner, *J. Antibiot.* **1988**, 1542-1551.

[34] N. N. Gerber, M. P. Lechevalier, *Biochemistry* **1964**, *3*, 598.

[35] R. Wilson, D. A. Sykes, D. Watson, A. Rutman, G. W. Taylor, P. J. Cole, *Immun.* **1988**, *56*, 2515-2517.

[36] U. Beifuss, M. Tietze, *Curr. Chem.* **2005**, *244*, 77-113.

[37] L. S. Thomashow, D. M. Weller, *J. Bacteriol.* **1988**, *170*, 3499-3508.

[38] L. S. Thomashow, D. M. Weller, R. F. Bonsall, *Appl. Environ. Microbiol.* **1990**, *56*, 908-912.

[39] A. W. T. F. Chin, G. V. Bloemberg, I. H. Mulders, L. C. Dekkers, B. J. Lugtenberg, *Mol. Plant-Microbe Interact.* **2000**, *13*, 1340-1345.

[40] K. Kanthakumar, G. Taylor, K. W. Tsang, D. R. Cundell, A. Rutman, S. Smith, P. K. Jeffery, P. J. Cole, R. Wilson, *Infect. Immun.* **1993**, *61*, 2848-2853.

[41] H. Ran, D. J. Hassett, G. W. Lau, *Proc. Natl. Acad. Sci.* USA **2003**, *100*, 14315-14320.

[42] R. B. Herbert, F. G. Holliman, *J. Chem. Soc.* **1969**, *18*, 2517-2520.

[43] F. G. A. Holliman, *J. Chem. Soc.* **1969**, *18*, 2514-2516.

[44] J. B. Laursen, J. Nielsen, *Chem. Rev.* **2004**, *104*, 1663-1686.

[45] S. Fotso, D. A. Santosa, R. Saraswati, J. Yang, T. Mahmud, T. Mark Zabriskie, P. J. Proteau, *J. Nat. Prod.* **2010**, *73*, 472-475.

[46] M. Kitahara, H. Nakamura, Y. Matsuda, M. Hamada, H. Naganawa, K. Maeda, H. Umezawa, Y. Iitaka, *J. Antibiot.* **1982**, *35*, 1412.

[47] K. H. Michel, M. M. Hoehn, **1982**. U. S. Patent 4, 316, 959.

[48] M. S. Abdelfattah, K. Toume, M. Ishibashi, *J Nat Prod.* **2010**, *27*, 1999-2002.

[49] M. S. Abdelfattah, K. Toume, M. Ishibashi, *Chem. Pharm. Bull.* **2011**, *59*, 508-510.

[50] N. Imamura, M. Nishijima, T. Takadera, K. Adachi, M. Sakai, H. Sano, *J. Antibiot.* **1997**, *50*, 8-12.

[51] S. R. Giddens, D. C. Bean, *Int. J. Antimicrob. Agents* **2007**, *29*, 93-97.

[52] H. Budzikiewicz, *FEMS Microbiol. Rev.* **1993**, *10*, 209-228.

[53] J. R. Kerr, *Infect. Dis. Rev.* **2000**, *2*, 184-194.

[54] J. M. Turner, A. J. Messenger, *Adv. Microb. Physiol.* **1986**, *27*, 211.

[55] J. Gibson, A. Sood, D. A. Hogan, *App. Env. Micr.* **2009**, *75*, 504-513.

[56] A. M. Burja, B. Banaigs, E. Abou-Mansour, J. G. Burgess, P. C. Wright, *Tetrahedron* **2001**, *57*, 9347-9377.

[57] P. J. Scheuer, *J. Nat. Prod. (Lloydia)* **1995**, *58*, 335-343.

[58] I. Botos, A. Wlodawer, *Cell. Mol. Life Sci.* **2003**, *60*, 277-287.

[59] A. M. Burja, E. Abou-Mansour, B. Banaigs, C. Payri, J. G. Burgess, P. C. Wright *Journal of Microbiological Methods* **2002**, *48*, 207-219.

[60] S. J. Shaw, D. Zhang, K. F. Sundermann, D. C. Myles, **2005**. Fragment Assembly: An Alternative Approach to Generating Complex Polyketides. *Synthetic Commun.* , *35*, 1735-1743.

[61] C. Roche, R. L. Roux, M. Haddad, P. Phansavath, J. P. Genet, *Synlett* **2009**, *4*, 573-576.

[62] K. J. Hale, S. Manviazar, *Chem. Asian J.* **2010**, *5*, 704-54.

[63] K. C. Fortner, D. Kato, Y. Tanaka, M. D. Shair, *J. Am. Chem. Soc*. **2010**, *132*, 275-280.

[64] K. L. Rinehart, J. B. Gloer, J. C. Cook, *J. Am. Chem Soc*. **1981**, *103*, 1857-1859

[65] M. J. Amador, J. Jimeno, L. Paz-Ares, H. Cortes-Funes, M. Hidalgo, *Ann. Oncol*. **2003**, *14*, 1607-1615.

[66] R. N. Asolkar, R. P. Maskey, E. Helmke, H. Laatsch, *J. Antibiot.* **2002**, *55*, 893-898.

[67] R. P. Maskey, F. Li, S. Qin, H. H. Fiebig, H. Laatsch, *J. Antibiot*. **2003**, *56*, 622-629.

[68] M. I. Mitova, G. Lang, J. Wiese, J. F. Imhoff, *J. Nat. Prod.* **2008**, *71*, 824-827.

[69] W. M. Abdel-Mageed, B. F. Milne, M. Wagner, M. Schumacher, P. Sandor, W. Pathom-aree, M. Goodfellow, A. T. Bull, K. Horikoshi, R. Ebel, M. Diederich, H. P. Fiedler, M. Jaspars, *Org. Biomol. Chem.* **2010**, *8*, 2352-2362.

[70] B. Haefner, *Drug Discov. Today* **2003**, *8*, 536.

[71] M. Peterka, K. Tepsic, T. Accetto, R. Kostanjsek, A. Ramsak, L. Lipoglavsek, G. Avgustin, *Acta Microbiol Immunol Hungarica* **2003**, *50*, 395-406.

[72] J. Waltera, R. A. Brittonb, S. Roosc, *PNAS* **2011**, *108*, 4645-4652.

[73] B. A. Dehority, P. A. Tirabasso, *Appl. Environ. Microbiol.* **2000,** *66*, 2921-2927.

[74] J. B. Russell, J. L. Rychlik, *Science* **2001**, *292*, 1119-1122.

[75] I. Koppova, F. Lukas, J. Kopeny, *Folia Microbiol* **2006**, *51*, 291-293.

[76] A. H. Smith, J. A. Imlay, R. I. Mackie, *Appl. Env. Micr.* **2003**, *69*, 3406-3411.

[77] S. E. Gilliland *FEMS Microbiol Rev.* **1990**, *87*, 175-188.

[78] A. Gratia, *C. R. Soc. Biol.* **1925**, *93*, 1040-1041.

[79] L. A. Rogers, *J. Bacteriol*. **1928**, *16*, 321-325.

[80] H. R. Whitehead, *Biochem. J.* **1933**, *27*, 1793-1800.

[81] A. T. R. Mattick, A. Hirsch, *Nature* **1944**, *154*, 551.

[82] J. I. Taylor, I. Hirsch, A. T. R. Mattick, *Vet. Rec.* **1949**, *61*, 197-198.

[83] A. Hirsch, E. Grinsted, *J. Dairy Res.* **1951**, *18*, 198-204.

[84] F. Jacob, A. Lwoff, A. Siminovitch, E. Wollman, *Ann. Inst. Pasteur Paris*. **1953**, *84*, 222-224.

[85] J. R. Tagg, A. S. Dajani, L. W. Wannamaker, *Bacteriol. Rev.* **1976**, *40*, 722-756.

[86] T. J. Montville, A. L. Kaiser, D. G. Hoover, L. R. Steenson, eds. Academic Press, San Diego. **1993** p. 1-22.

[87] A. T. R. Mattick, A. Hirsch, *The Lancet*. **1947**, *2*, 5-8.

[88] R. W. Jack, J. R. Tagg, B. Ray, *Microbiol. Rev.* **1995**, *59*, 171-200.

[89] E. Breukink, C. Kraaij, R. A. Demel, R. J. Siezen, B. Kruijff, O. P. Kuipers, *Biochemistry* **1998**, *37*, 8153-8162.

[90] W. Liu, J. N. Hansen, *Appl. Environ. Microbiol* **1990**, *56*, 2551-2558.

[91] G. N. Moll, W. N. Konings, A. J. M. Driessen, *Ant. Van Leeuwenhoek.* **1999**, *76*, 185-198.

[92] A. A. Odenyo, R. I. Mackie, D. A. Stahl, B. A. White, *Appl. Environ. Microbiol.* **1994**, *60*, 3688-3696.

[93] W. W. Chan, B. A. Dehority, *Anim. Feed Sci. Technol.* **1999**, *77*, 61-71.

[94] M. L. Kalmokoff, F. Bartlett, R. M. Teather, *J. Dairy Sci.* **1996**, *79*, 2297-2308.

[95] J. E. Wells, D. O. Krause, T. R. Callaway, J. B. Russell, *FEMS Microbiol. Ecol.* **1997**, *22*, 237-243.

[96] H. C. De Klerk, J. A. Smit, *J. Gen Microbiol.* **1967**, *48*, 309-316.

[97] R. M. Teather, R. J. Forster, *Can. J. Anim. Sci.* **1998**, *78*, 57-69.

[98] A. Laukova, S. Czikkova, *Lett. Appl. Microbiol.* **1998**, *26*, 215-218.

[99] M. F. Whitford, M. A. McPherson, R. J. Forster, R. M. Teather, *Appl. Environ. Microbiol.* **2001**, *67*, 569-574.

[100] H. C. Mantovani, D. K. Kam, J. K. Ha, J. B. Russell, *FEMS Microbial Ecol.* **2001**, *37*, 223-229.

[101] J. B. Russell, H. C. Mantovani *J. Mol. Microbiol. Biotechnol.* **2002**, *4*, 347-355.

[102] H. Laatsch, *AntiBase* **2011**. *A Data Base for Rapid Dereplication Structure Determination of Microbial Natural Products*; Wiley-VCH; Weinheim, Germany; see http//wwwuser. gwdg. de/~ucoc/laatsch/AntiBase. htm.

[103] Dictionary of Natural Products on CD-ROM, Chapman & Hall, Chemical Database, Version 8, *2*, **2004**.

[104] Chemical Abstracts by SciFinder, **2007**.

[105] Institute for Biotechnology and Drug Research, Kaiserslautern University, Germany.

[106] H. Laatsch, Microorganismen als biologische Quelle neuer Wirstoffe, in: Pharmazeutische Biotechnologie (Ed. O. Kayser, R. H. Müller), Wissenschaftliche Verlagsges. , Stuttgart **2000**, p. 13-43.

[107] F. Vanmiddlesworth, R. J. P. Cannell, **1998**. eds. R. J. P. Cannell, Humana Press, Totowa, New Jersey.

[108] J. Bukingham, S. Thompson, *Dictionary of natural products and other information sources for natural products scientists*, Royal Society of Chemistry, London, pp 53-67, **1997**.

[109] G. Bringmann, G. Lang, *Sponges* (*porifers*), Eds. Mueller, E. G. Werner, Springer-Verlag, Berlin, **2003**, pp. 89-116.

[110] G. Bringmann, K. Messer, W. Saeb, E. M. Peters, K. Peters, *Phytochemistry* **2001,** *56*, 387-391.

[111] I. Oka, F. Frauendorf, H. Laatsch, *poster,* 37. Diskussionstagung der Deutschen Gesellschaft für Massenspektrometrie, Leipzig **2004**.

[112] G. Bringmann, S. Busemann, in: *Natural Product Analysis* (eds. : P. Schreier, M. Herderich, H. U. Humpf, W. Schwab), Vieweg, Wiesbaden, **1998**, pp. 195-212.

[113] L. Li, G. Yang, T. Dang, Z. Chen, *Zhongcaoyao* **1996**, *27*, 381-391.

[114] R. P. Maskey, M. Shaaban, I. Gruen-Wolly, H. Laatsch, *J. Nat. Prod.* **2004**, *67*, 1131-1134.

[115] J. E. Francis, W. D. Cash, W. D. Barbaz, P. S. Bernard, R. A. Lovell, G. C. Mazzenga, R. C. Friedmann, J. L. Hyun, A. F. Braunwalder, P. S. Loo, D. A. Bennett, *J. Med. Chem.* **1991**, *34*, 281.

[116] F. Kathawala, G. E. Hardtmann, Ger Offen, *Chem Abstr* **1972**, *77*, 48501.

[117] S. Johne, *Pharmazie* **1982**, *36*, 583.

[118] H. Nagano, M. Takagi, N. Kubodera, I. Matsunaga, H. Nabata, Y. Ohba, K. Sakai, S. Hata, Y. Uchida, Eur Pat 89065 (**1983**) Chugai Pharmaceutical Co. , Ltd. ; *Chem Abstr* **1984**, *100*, 6547.

[119] J. Imagawa, K. Sakai, *Eur. J. Pharmacol.* **1986**, *131*, 257.

[120] Merck Index; Merck: Whitehouse Station, NJ, **1996**, *12*, pp 7897, 1512, 3489.

[121] S. Goto, H. Tsuboi, K. Kagara, *Chem Express* **1993**, *8*, 761.

[122] K. Kagara, S. Goto, H. Tsuboi, *Chem. Abstr*. **1989,** *111*, 972-974.

[123] S. Mohri, *J. Synth. Org. Chem. Jpn.* **2001**, *59*, 514.

[124] G. Pastor, C. Blanchard, C. Montginoul, E. Torreilles, L. Giral, A. Texier, *Bull Soc Chim Fr* **1975**, 1331.

[125] M. Khalifa, A. N. Osman, M. G. Ibrahim, A. R. E. Ossman, M. A. Ismail, *Pharmazie* **1982**, *37*, 115.

[126] M. Michman, S. Patai, Y. Wiesel, *Org. Prep. Proced. Int*, **1978**, *10*, 13.

[127] N. A. Lange, F. E. Sheibley, *Org. Synth.* **1943**, *2*, 79.

[128] H. Vorbrueggen, K. Krolikiewicz, *Tetrahedron* **1994**, *50*, 6549.

[129] A. Zunnundzhanov, I. A. Bessonova, N. D. Abdullaev, D. K. Ogai, *Khimiya Prirodnykh Soedinenii* **1987**, *4*, 553-558.

[130] K. L. Rinehart, A. L. Staley, S. R. Wilson, R. G. Ankenbauer, C. D. Cox, *J. Org. Chem.* **1995**, *60*, 2786-2791.

[131] R. Carmi, S. Carmeli, E. Levy, F. J. Gough, *J. Nat. Prod.* **1994**, *57*, 1200-1205.

[132] K. A. Shaaban, M. Shaaban, H. Rahman, I. Grün-Wollny, H. Laatsch *J. Am. Chem Soc.* Submitted, **2008**.

[133] Z. Lin, R. R. Antemano, R. W. Hughen, M. D. Tianero, O. Peraud, M. G. Havgood, G. P. Conception, B. M. Olivera, A. Light, E. W. Schmidt, *J. Nat. Prod*. **2010**, *73*, 1922-1926.

[134] R. Bell, S. Carmeli, N. Sar, *J. Nat. Prod.* **1994**, *57*, 1587-1590.

[135] I. Zendah, K. A. Shaaban, A. Raies, E. Helmke H. Laatsch. *Z. Naturforsch.* B **2012**, *67b*, 1-4.

[136] T. Roth, A. M. Burger, W. A. Dengler, H. Willmann, H. H. Fiebig In: H. H. Fiebig, A. M. Burger (eds). Relevance of Tumor Models for Anticancer Drug Development. Basel: Karger, **1999**, *54*, 145-156.

[137] H. H. Fiebig, D. P. Berger, W. A. Dengler, E. Wallbrecher, B. Winterhalter In: H. H. Fiebig, D. P. Berger (eds). Immunodeficient Mice in Oncology. Basel: Karger, **1992**, *42*, 321-351.

[138] A. Geiger, W. Keller-Schierlein, M. Brandl, H. Zähner *J. Antibiot.* **1988**, *41*, 1542-1551.

[139] I. Schneemann, J. Wiese, A. L. Kunz, J. F. Imhoff *Mar. Drugs* **2011**, *9*, 772-789.

[140] C. Olano, C. Méndez, J. A. Salas, *Mar. Drugs* **2009**, *7*, 210-248.

[141] K. Isono, K. Anzai, S. Suzuki, *J. Antibiot.* **1958**, *11*, 264-267.

[142] D. Schröder, Untersuchungen zum Sekundärmetabolismus arktischer und antarktischer Meereisbakterien, *Dissertation*, University of Göttingen **2002**.

[143] B. Böhlendorf, E. Forche, N. Bedorf, K. Gerth, H. Irschik, R. Jansen, B. Kunse, W. rowitzsch-Kiensat, H. Reichenbach, G. Hölfle, *Liebigs*, **1996**, *Ann. Chem.* , 49-53.

[144] A. W. Ayer, S. L. Trifonov, *J. Nat. Prod.* **1994**, *57*, 839-841.

[145] M. Bernart, W. H. Gerwick, *Phytochemistry* **1990**, *29*, 3697-3698.

[146] R. L. Dillman, J. H. Cardellina, *J. Nat. Prod.* **1991**, *54*, 1056-1061.

[147] S. Yang, A. G. Cordell, *J. Nat. Prod.* **1997**, *60*, 44-48.

[148] R. Shen, M. Wei, X. Tunhai, L. Xiuping, Y. Hao, Y. Bin, Z. Xue-Feng, Y. Xian-Wen, L. Lijuan, L. Kyung Jin, G. Qipin, L. Yonghong, *The Journal of Antibiotics* **2010**, *63*, 699-701.

[149] M. Adamczeski, *J. Nat. Prod.* **1995**, *58*, 201-208.

[150] Y. S. Chen, *Bull. Agric. Chem. Soc. Jpn.* **1960**, *24*, 372-24377.

[151] K. H. Rhee, K. H. Choi, C. J. Kim, C. H. Kim, *J. Microbiol. Biotechnol*. **2001**, *11*, 469-474.

[152] C. Della Pina, E. Falletta, M. Rossi, *Topics in Catalysis* **2007** *44*, 1-2.

[153] H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512-7515.

[154] S. Rakesh, M. Sarojadevi, *Chemistry Chemical Technology* **2008,** *2*, 239-247.

[155] M. Smith, Organic Chemistry: An Acid-Base Approach, *Dissertation*, **2010**, 1574 pages.

[156] W. Spyra, K. Winkelmann. The conversion of liquid rocket fuels: risk assessment, technology and treatment options for the conversion of abandoned liquid ballistic missile propellants (fuels and oxidizers) in Azerbaijan North Atlantic Treaty Organization Springer, **2004** - 148 pages.

[157] M. Xiea, Z. Zhoua, Z. Wanga, D. Chena, F. Qi. *International Journal of Mass Spectrometry* **2011**, *303*, 137-146.

[158] A. Bruggink, **2001.** Synthesis of β-lactam antibiotics: chemistry, biocatalysis & process integration, 335 pages.

[159] A. Gizir, Nermin Kus, Recep Ozen, *Nato Science Series* **2006**, *162*, 125-133.

[160] F. Dennis Kohn, **1997**. Anesthesia and analgesia in laboratory animals 426 pages.

[161] J. A. Menendez, L. Vellon, R. Colomer, R. Lupu, *Ann. Oncol.* **2005**, *16*, 359-371.

[162] F. Natali, L. Siculella, S. Salvati, G. V. J. Gnoni1, *Lipid Res.* **2007**, *48*, 1966-1975.

[163] A. Bartmanska, E. Huszcza, T. Tronina, *J. Mol. Catal. B*: *Enzymatic* **2009**, *61*, 221-224.

[164] L. Delmulle, A. Bellahcène, W. Dhooge, F. Comhaire, F. Roelens, K. Heyerick, V. Castronovo, D. De Keukeleire, *Phytomedecine* **2006**, *13*, 732-734.

[165] S. R. Milligan, J. C. Kalita, A. Heyerick, H. Rong, L. De Cooman, D. De Keukeleire, *J. Clin. Endocrinol. Metab*. **1999**, *84*, 2249-2252.

[166] M. Verzele, J. Stockx, F. Fontijn, M. Anteunis, *Bull. Soc. Chim. Belg.* **1957**, *66*, 452-475.

[167] J. F. Stevens, A. W. Taylor, J. E. Clawson, M. L. Deinzer, *J. Agric. Food Chem.* **1999**, *47*, 2421-2428.

[168] C. L. Miranda, G. L. Aponso, J. F . Stevens, M. Deinzer, D. Buhler, *Food Chem. Toxicol.* **1999**, *37*, 271-285.

[169] N. Tabata, M. Ito, H. Tomoda, S. Omura, *Phytochemistry* **1997**, *46*, 683-687.

[170] N. G. Coldham, M. J. Sauer, *Food Chem. Toxicol.* **2001**, *39*, 1211-1224.

[171] V. E. Buckwold, R. J. Wilson, A. Nalca, B. B. Beer, T. G. Voss, J. A. Turpin, R. W. Buckheit III, J. Wei, M. Wenzel-Mathers, E. M. Walton, R. J. Smith, M. Pallansch, P. Ward, J. Wells, L. Chuvala, S. Sloane, R. Paulman, J. Russell, T. Hartman, R. Ptak, *Antiviral Res.* **2004**, *61*, 57-62.

[172] C. L. Miranda, J. F. Stevens, V. Ivanov, M. McCall, B. Frei, M. L. Deinzer, D. R. Buhler, *J. Agric. Food Chem.* **2000**, *48*, 3876-3884.

[173] C. Gerhauser, *Eur. J. Cancer* **2005**, *41*, 1941-1954.

[174] L. Delmulle, T. Vanden Berge, D. De Keukeleire, P. Vandenabeele, *Phytother. Res.* **2007**, *22*, 197-203.

[175] W. A. Ayer, L. M. Browne, M. Feng, H. Orszanska, H. Saeedi-Ghomi, *Can. J. Chem.* **1986**, *64*, 904-909.

[176] R. Ki-Hyeong *J. Microbiol. Biotechnol*. **2003**, *13*, 984-988.

[177] S. H. Park, G. A Strobel. *Biochim. Biophys. Acra* **1994**, *1199*, 13-19.

[178] M. Bodansky, G. F. Singler, A. Bodansky *J. Am. Chem. Soc*. **1973,** *95*, 2352-2357.

[179] C. Prasad, *Peptides*, **1995**, *16*, 151-164.

[180] P. Sames *Br. Med. J.* , **1968**, *1*, 56.

[181] A. Svobodová, J. Psotová, D. Walterová, *Biomed. Papers* **2003**, *147*, 137-145.

[182] S. Budavari, ed **1996**. The Merck Index: An Encyclopedia of Chemical, Drugs, Biologicals. Whitehouse Station, NJ: Merck.

[183] T. M. Lin, S. S. Lee, C. S. Lai, S. D. Lin, *Journal of the International Society for Burn Injuries*, **2006**, *32*, 517-521.

[184] J. T. F. Liao, F. W. Oehme, *Toxicol. Appl. Pharmacol.* **1981**, *57*, 220-225.

[185] H. Yüntsen, H. Yonehara, H. Ui, *J. Antibiot*. **1954**, *7*, 133-115.

[186] E. G. Brown, **1998**. Ring Nitrogen and Key Biomolecules: The Biochemistry of N-Heterocycles. Boston: Luwer Academic Publishers.

[187] M. F. Grundon, D. M. Harrison, G. G. Spyropoulos, *J. Chem. Soc. Chem. Commun.* **1974**, 51-52.

[188] J. F. Collings, W. J. Donnelly, M. F. Grundon, K. J. James, *J. Chem. Soc.* Perkin. I. **1974**, 2177-2218.

[189] C. F. Neville, M. F. Grundon, V. R. Ramachandran, G. Reisch, *J. Reisch. J. Chem. Soc. P. I.* **1974,** 2177.

[190] K. B. G. Torssell, *Nat. Prod. Chem.* **1997**, 391-394.

[191] C. Pfefferle, J. Breinholt, H. Gürtler, H. Fiedler, *J. Antibiot.* **1997**, *50*, 1067-1068.

[192] M. Schiebel, Diskriminanzanalyse und HPLC-MS-Techniken im Screening nach neuen Metaboliten aus terrestrischen und marinen Bakterien, *Dissertation*, University of Göttingen, **2002**.

[193] U. Maeda, N. Hara, Y. Fujimoto, A. Srivastava, Y. K. Gupta, M. Sahai, *Phytochemistry* **1993**, *34*, 1633-1635.

[194] A. Trigos, S. Reyna, G. Galindo Ramos, *J. Nat. Prod. Lett.* **1996**, *8*, 199-205.

[195] Q. Huang, Y. Tezuka Hatanaka, Y. Kikuchi, T. Nishi, A. Tubaki, K. , *Chem. Pharm. Bull.* **1995**, *43*, 1035-1038.

[196] W. A. Ayer, L. S. Trifonov, *Phytochemistry* **1995**, *38*, 371-372.

[197] O. Guzmán-López, Á. Trigos, F. J. Fernández, M. Yanez-Morales, G. Saucedo-Castaneda, *World J. Microbiol. Biotechnol.* **2007**, *23*, 1473-1477.

[198] R. M. Lamuela-Raventos, E. Gimeno, M. Fito, A-I Castellote, M. Covas, M. C. De la Torre-Boronat, M. C. López-Sabater, *Biol. Res.* **2004,** *37*, 247-252.

[199] R. Lucas, F. Comelles, D. Alcntara, O. S. Maldonado, M. Curcuroze, J. L. Parra, J. C. Morales, *J. Agric. Food Chem.* **2010**, *58*, 8021-8026.

[200] C. Giovannini, E. Straface, D. Modesti, E. Coni, A. Cantafora, M. De Vincenzi, W. Malorni, R. Masella, *J. Nutr.* **1999**, *129*, 1269-1277.

[201] B. T. Lingappa, M. Prasad, Y. Lingappa, D. F. Hunt, K. Bieman, *Science* **1969**, *163*, 192-194.

[202] W. B. Turner, D. C. Aldridge, *Fungal Metabolites* II; Academic Press: New York, 1983; p 538.

[203] B. E. Cross, R. H. B. Galt, J. R. Hanson, P. J. Curtis, J. F. Grove, A. Morrison, *J. Chem. Soc.* **1963**, 2937-2943.

[204] A. A. William, M. B. Lois, F. Meow-Chen, O. Helena, S. G. Hossein, *Can. J. Chem.* **1996**, *64*, 904-907.

[205] F. Ehrlich, Ber. Dtsch,. *Chem. Ges.* **1912**, *45*, 883-889.

[206] N. Noda, S. Kubota, Y. Miyata, K. Miyahara, *Chem. Pharm. Bull.* **2000**, *48*, 1749-1752.

[207] S. Sprunck, H. J. Jacobsen, T. Reinard, *J. Plant Growth Regul.* **1995**, *14*, 191-197.

[208] J. E. Perley, B. B. Stowe, *Plant Physiol.* **1966**, *41*, 234-237.

[209] H. Garrett Reginald, M. Grisham Charles, Principals of Biochemistry with a Human Focus. United States: Brooks/Cole Thomson Learning, **1997**.

[210] S. T Mashiyama, C. Courtemanche, I. Elson-Schwab, J. Crott, B. L. Lee, C. N. Ong, M. Fenech, B. N. Ames, *Anal. Biochem.* **2004**, *330*, 58-69.

[211] A. Hildalgo, C. Pompei, A. Galli, S. Cazzola, *J. Agric. Food. Chem.* **2005**, *53*, 349-355.

[212] K. Oka, T. Hirano, M. Homma, H. Ishii, K. Murakami, S. Mogami, A. Motizuki, H. Morita, K. Takeya, H. Itokawa, *Chem. Pharm. Bull.* **1993**, *41*, 1000-1002.

[213] H. Itokawa, T. Miyashita, H. Morita, K. Takeya, T. Hirano, M. Homma, K.

Oka, *Chem. Pharm. Bull.* **1994**, *42*, 604-607.

[214] S. Nagai, K. Okimura, N. Kaizawa, K. Ohki, S. Kantomo, *Chem. Pharm. Bull.* **1996**, *44*, 5-10.

[215] N. N. Gerber, *J. Antibiot.* **1971**, *24*, 636-640.

[216] M. Isaka, A. Jaturapat, J. Kramyu, M. Tanticharoen, Y. Thebtaranonth, *Ant. Ag. Chem.* **2002**, *46*, 1112-1113.

[217] C. Hyo Je, K. Kyung-Jin, K. Myung Hee, K. Beom Sik, *Proteins* **2007**, *70*, 257-262.

[218] N. R. Williamson, P. C. Fineran, T. Gristwood, S. R. Chawrai, F. J. Leeper, G. P. Salmond, *Future Microbiol.* **2007**, *6*, 605-618.

[219] F. Huth, Einsatz der Neutronenaktivierungsanalyse und der Elektrospray-Massenspektrometrie im Screening nach Organohalogenverbindungen – Strukturaufklärung cyclischer Peptide durch MS-Methoden. *Dissertation*, Universität Göttingen, **1999**.

[220] H. Bayer, W. Walter, Lehrbuch der organische Chemie, 23 Auflage, S. Hierzel Verlag Stuttgart. Leipzig, **1998**, 779.

[221] D. S. C. Sheila, S. R. Abrams, S. J. Ambrose, A. J. Cutler, M. Loewen, A. Ross, A. R. Kermode, *Plant J.* **2003**, *35*, 405-417.

[222] B. S Joshi, V. N. Kamat, D. H. Gawad, *Heterocycles* **1977**, *7*,193-200.

[223] J. R. F. Allen, B. R. Holmstedt, *Phytochemistry* **1980**, *19*, 1573-1582.

[224] H. -P. Husson. *In the Alkaloids*; Brossi, A. , Ed. , Elsevier Science, **1985**, *26*, 1-51.

[225] F. Bracher, D. Hildebrand, Liebigs *Ann. Chem.* **1992**, 1315-1319.

[226] T. Ohmoto, K. Koike, *Shoyakugaku Zasshi* **1988**, *42*, 160-162, *Chem. Abstr.* **1989**, *110*, 111567z.

[227] M. D. Barker, P. R. Woodward, J. R. Lewis, Patent GB 2155462 A1 19850925 **1985**, *Chem. Abstr. 104*, P207245v, **1986**.

[228] *M. Arfan, K. A. Shaaban, A. Schüffler, Hartmut Laatsch, Nat. Prod. Commun.* **2012**, *7*, 1199-1202.

[229] C. Yong Boo, O. Che Jeon, G. Byeong Lee, W. Kyung Ro, *Eun J. Kim.* US005602259A **1997**.

[230] H. Lackner, *Angew. Chemie* **1975**, *14*, 375-386.

[231] H. Brockmann, Fortschr. *Chem. Org. Naturst.* **1960**, *18*, 1; *Angew. Chem.* **1960**, *72*, 939-947.

[232] E. Katz, in D. Gottlieb and P. D. Shaw. Antibiotics, Biosynthesis. Springer, New York **1967**, Vol. II, P. 276.

[233] J. Guo, T. Wu, J. Bess, L. E. Hederson, J. G. Levin, *J. Virol.* **1998**, *72*, 716-724.

[234] H. Lackner, I. Bahner, N. Shigematsu, L. K. Pannel, A. B. Mauger, *J. Nat. Prod.* **2000**, *63*, 352-356.

[235] D. M. Green, *Eur. J. Cancer*. **1997**, *33*, 409-418.

[236] R. B. Womer, *J. Am. Med. Soc.* **1997**, *33*, 2230-2234.

[237] J. Guo, T. Wu, J. Bess, L. E. Henderson, J. G. Leving, *J. Virol.* **1998**, *72*, 6716-6724.

[238] R. A. Dixon, Ferreira, *Phytochemistry* **2002**, *60*, 205-211.

[239] L. Coward, N. C. Barnes, K. D. R. Setchell, S. Barnes, *J. Agric. Food Chem.* **1993**, *41*, 1961-1967.

[240] S. M. Heinonen, H. K. Waehaelae, Adlercreutz, *Phytochemistry. Rev.* **2002**, 175-182.

[241] K. Komiyama, S. Funayama, Y. Anraku, A. Mita, Y. Takahashi, S. Omura, H. Shimasaki *J. Antibiot.* **1989**, *42*, 1344-1349.

[242] P. Foti, d. Erba, P. Riso, A. Spadafranca, F. Criscuoli, G. Testolin, *Arch. Biochem. Biophys.* **2005**, *33*, 421-427.

[243] R. Changhyun, S. Su-Hyun, C. Kwon-Young, C. Minho, P. Bishnu Prasad, K. June-Hyung, P. Jun-Seong, K. Duck Hee, C. Ih Seop, K. Byung-Gee, *Journal of Bioscience and Bioengineering* **2009**, 41-46.

[244] K. Klus, W. Barz, *Arch. Microbiol.* **1995**, *164*, 428-434.

[245] C. E. Rufer, SE. Kulling, *J. Agric. Food Chem.* **2006**, *54*, 2926-2931.

[246] T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukami, *J. Biol. Chem.* , **1987**, *262*, 5592-5595.

[247] T. S. Chang, H. Y. Ding, H. C. Lin, *Biosci. Biotechnol. Biochem*. **2005**, *69*, 1999-2001.

[248] C. Voss Sepulveda-Broza, F. W. Zillike, *Biochem. Pharm.* **1992**, *44*, 157-162.

[249] L. Coward, N. C. Barnes, K. D. R. Setchell, S. Barnes, *J. Agric. Food. Chem.* **1993**, *41*, 1961-1967.

[250] E. Q. Shyong, Y. Lu, A. Lazinsky, R. N. Saladi, R. G. Phleps, L. M. Austin, M. Lebwohl, H. Wie, *Carcinogenesis* **2002**, *23*, 317-321.

[251] J. Kumi-Diaka, R. Rodiguez, Goudaze, *Biol. Cell*. **1998**, *90*, 349-354.

[252] I. O. Anyanwutaku, E. Zirbes, J. P. N Rosazza, *J. Nat. Prod.* **1992**, *55*, 1498-1504.

[253] W. K. Lutz, O. Tiedge, R. W. Lutz, H. Stopper, *Toxicol. Sci.* 2005, *86*, 318-323.

[254] O. J. Bandele, N. Osheroff, *Biochemistry* 2007, *46*, 6097-6108.

[255] Y. Jin, H. Wu, E. M. Cohen, J. Wei, H. Jin, H. Prentice, J. Y. Wu, *J. Biomed. Sci.* **2007**, *14*, 275-284.

[256] F. Schmidt, C. B. Knobbe, B. Frank, H. Wolburg, M. Weller, *Oncol. Rep.* **2008,** *19*, 1061-1066.

[257] M. López-Lazaro, E. Willmore, C. A. Austin, *J. Nat. Prod.* **2007**, *70*, 763-767.

[258] S. Kitamura, K. Hashizume, T. Iida, E. Miyashita, K. Shirahata, H. Kase, *J. Antibiot.* **1986**, *39*, 1160-1166.

[259] Md. T. Islam, S. Tahara, *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1933-1948.

[260] B. B. Kiefer, M. Riemann, C. Büche, H-H. Kassemeyer, P. Nick, *Planta* **2002**, *215*, 387-393.

[261] Md. T. Islam, Y. Hashidoko, A. Deora, T. Ito, S. Tahara, *Appl. Environ. Microbiol.* **2005**, *71*, 3786-3796.

[262] H. S. Judelson, F. A. Blanco, *Nat. Rev. Microbiol.* **2005**, *3*, 47-58.

[263] E. E. Hays, J. C. Wells, P. A. Katzman, C. K. Cain, F. A. Jacobs, S. A. Thayer, E. A. Doisy, W. L. Gaby, E. C. Roberts, R. D. Muir, C. J. Carroll, L. It. Jones, C. N. J. Wade, *J. Biol. Chem.* **1945**, *159*, 725-749.

[264] F. L. Jackson, J. W. Lightbown, *J. Gen. Microbiol.* **1954,** *11*, iv- v.

[265] J. W. Lightbown, F. L. Jackson, *Biochem. J.* **1956**, *63*, 130- 137.

[266] W. Neuenhaux, H. Budzikiewichz, G. Korth Pilveres, *Z. Naturforsch.* **1979**, *34*b, 313-315.

[267] D. C. Hooper, *Biochimica and Biophysica Acta* **1998**, *1400*, 45-61.

[268] P. Xiao-Su, A. G. Katherine, L. M. Fisher, *Antimicrob. Ag. Chemother.* **2009**, *53*, 3822-3831.

[269] P. Ball, A. Fernald, G. Tillotson, *Exp. Opin. Investig. Drugs* **1998**, *7*, 761-783.

[270] J. M. Domagala, *J. Anmicrob. Chemother.* **1994**, *33*, 685-706.

[271] D. V. Vukomanovic, D. E. Zoutman, J. A. Stone, G. S. Marks, J. F. Brien, K. Nakatsu, *J. Biochem.* **1997**, 322-325.

[272] H. Isoda, D. Kitamoto, H. Shinmoto, M. Matsumura, T. Nakahara, *Biosci. Biotechnol. Biochem.* **1997**, *61*, 609-614.

[273] H. Isoda, H. Shinmoto, D. Kitamoto, M. Matsumura, T. Nakahara. *Lipids* **1997**, *32*, 263-271.

[274] B. S. Kim, B. K. Hwang, *J. Microbiol. Biotechnol.* **1993**, *3*, 12-18.

[275] M. E. Staghelli, R. M. Miller, *Plant diseases* **1997**, *81*, 4-12.

[276] G. Piljac, V. Piljac, **1996**. WP 9602233A1.

[277] F. G. Jarvis, M. J. Johnson, *J. Am. Chem. Soc.* **1949**, *71*, 4124-4126.

[278] P. S. J. Cheetham, **1997**. Combining the technical push and the business pull for natural flavours, *In* T. Scheper (Ed), Biotechnology of aroma compounds, *Advances in Biochem. Eng. / Biotechnol.* **55**. pp. l-49, Springer-Verlag. Berlin.

[279] R. J. Linhardt, R. Bakhit, L. Daniels, F. Mayerl, W. Pickenhagen, *Biotechnol. Bioeng.* **1989**, *33*, 365-368.

[280] A. Fiechter, *Tibtech.* **1992**, *10*, 208-217.

[281] R. S. Makkar, S. Cameotra, *J. Am. Oil Chem. Soc*. **1997**, *74*, 887-889.

[282] M. E. Mercade, M. A. Manresa, *J. Am. Oil Chem. Soc.* **1994**, *71*, 61-64.

[283] F. Vaufrey, A. M. Delort, G. Jeminet, G. Dauphin, *J. Antibiot.* **1990**, *43*, 1189-1191.

[284] L. E. Day, J. W. Chabberlin, E. Z. Gordee, S. Chen, M. Gorman, R. L. Hamill, T. Ness, R. E. Weeks, R. Stroshane, *Antimicrob. Agents Chemother* **1973**, *4*, 410-414.

[285] K. Reynolds, J. Robinson, *J. Chem. Soc. Chem. Commun*. **1985**, 1831-1832.

[286] D. E. Cane, T. C. Liang, H. Hasler, *J. Am. Chem. Soc*. **1981**, *103*, 5962-5965.

[287] A. A. Ajaz, J. A. Robinson, *J. Chem. Soc. Chem. Commun.* **1983**, 679-680.

[288] S. V. Khotimchenko, *Phytochemistry* **1998**, *49*, 2363-2369.

[289] N. M. Carballeira, M. Pagan, F. Shalabi, J. T. Nechev, K. Lahtchev, A. Ivanova, K. Stefanov, *J. Nat. Prod.* **2000**, *63*, 1573-1575.

[290] C. Pathirana, R. Dwight, P. R. Jensen, W. Fenical, *Tetrahedron Lett.* , **1991**, *32*, 7001-7004.

[291] K. Shin-Ya, K. Furihata, Y. Teshima, Y. Hayakawa, H. Seto, *J. Org. Chem*. **1993**, *58*, 4170-4172.

[292] K. Shin-Ya, Y. Hayakawa, H. Seto, *J. Nat. Prod.* **1993**, *56*, 1255-1258.

[293] T. Kunigami, K. Shin-Ya, K. Furihata, Y. Hayakawa, H. Seto, *J. Antibiot.* **1998**, *51*, 880-882.

[294] A. Sugimoto, Y. Yoshino, R. Watanabe, K. Mizuno, K. Uehara, *J. Heterocycl.* Chem. , **1999**, *36*, 1057-1064.

[295] PC Spartan Pro **2000**, Wavefunction Inc. , Irvine, CA, U. S. A. ; http://www. wavefun. com

[296] P. Krastel, A. Zeeck, K. Gebhardt, H. Fiedler, J. Rheinheimer, *J. Antibiot.* **2002**, *55*, 801-806.

[297] G. S. Byng, J. M. Turner, *Biochem. Soc. Trans.* **1975**, *3*, 742-744.

[298] K. Shin-Ya, S. Shimizu, T. Kunigami, K. Furihata, Y. Hayakawa, H. Seto, *J. Antibiot.* **1995**, *48*, 1378-1381.

[299] A. S. Gil-Turnes, M. E. Hay, W. Fenical. *Science* **1989**, *246*, 116-118.

[300] K. Benkendorff, J. B. Bremner, A. R. Davis, *Molecules* **2001**, *6*, 70-78.

[301] F. Bruns, *Z. Naturwiss.* **1954**, *41*, 360.

[302] C. Binda, M. Li, F. Hubalek, N. Restelli, D. E. Edmondson, A. Mattevi, *Proc. Nat. Acad. Sci.* USA, **2003**, *100*, 9750-9755.

[303] E. Epstein, P. G. Miles *Botan. Mag.* **1966**, *79*, 566-571.

[304] S. Karsten, Z. Axel, A. Noemi, F. Peter, *J. Antibiot.* **2005**, *58*, 103-110.

[305] S. Masaoki, T. Hiroshi, O. Katsunori, K. Yukio, Y. Tamotsu, *Nippon Nog. Kag. Kaish.* **1974**, *48*, 569-571.

[306] O. Nakayama, M. Yagi, S. Kiyoto, M. Okuhara, M. Kohsaka, *J. Antibiot.* **1990**, *43*, 1615-1616.

[307] N. Tabata, H. Tomoda, S. Omura, *J. Antibiot.* **1999**, *52*, 1108- 1113.

[308] H. Tomoda, N. Tabata, M. Shinose, Y. Takahashi, H. B. Woodruff, S. Omura, *J. Antibiot.* **1999**, *52*, 1101-1107.

[309] W. Yaling, T. Hongyu, C. Haitao, Y. Honglin, S. Baoguo, *Jingxi Huagong* **2011**, *28*, 6, 560-563.

[310] H. Yong Ye, L. Hai Zhu, C. Yong Song, Y. Jun Liu, X. Ren Tan, *J. Nat. Prod.* **2005**, *68*, 1106-1108.

[311] P. A. Murphy, *Food Technol.* **1982**, *34*, 60-64.

[312] M. Nairn, S. Gestetner Zilkah, Bondi, *Agri. Food Chem.* , **1974**, *22*, 806-810.

[313] A. C. Eldridge, *Agri Food Chem.* **1982**, *30*, 353-355.

[314] K. D. R. Setchell, H. Adlercreutz, I. R. Rowland, ed. (Academic Press, London) pp. 315-345.

[315] H. Ogawara, I. Akiyama, S. Watanabe, N. Itoh, M. Katobi, Y. Seoda, *J. Antibiot.* **1989**, *42*, 340-343.

[316] H. Kim, T. G. Peterson, S. Barnes, *Am. J. Clin. Nutr*. , **1998**, *68*, 1418S-1425S.

[317] K. D. R. Setchell, *Am. J. Clin. Nutr.* , **1998**, *68*,1333S-1346S.

[318] K. D. R. Setchell, A. Cassidy, *Nutr.* , **1999**, *129*, 758S-767S.

[319] S. E. Kulling, D. M. Honing, M. Metzler, *J. Agric. Food Chem.* , **2001,** *49*, 3024-3033.

[320] K. Gebhardt, J. Schimana, P. Krastel, K. Dettner, J. Rheinheimer, A. Zeeck, H. P. Fiedler, *J. Antibiot.* , **2002**, *55*, 794-800.

[321] K. Gustafson, M. Roman, W. J. Fenical, *J. Am. Chem. Soc.* , **1989**, *111*, 7519-7524.

[322] Y. Kobayashi, A. Fukuda, T. Kimachi, M. Ju-ichi, Y. Takemoto, *Tetrahedron Lett.* **2004**, *45*, 677-680.

[323] J. P. Marino, M. S. McClure, D. P. Holub, J. V. Comasseto, F. C. Tucci, *J. Am. Chem. Soc.* **2003**, *124*, 1664-1668.

[324] S. D. Rychnovsky, D. J. Skalitzky, C. Pathirana, P. R. Jensen, W. J. Fenical. *J. Am. Chem. Soc.* **1992,** *114*, 671-677.

[325] F. Ramos Campos, A. Barison, C. Daolio, A. G. Ferreira; E. Rodrigues-Fo, *Magn. Reson. Chem.* **2005**, *43*, 962-965.

[326] Y. C. Song, H. Li, Y. H. Ye, C. Y. Shan, Y. M. Yang, R. X. Tan, *FEMS Microbiol. Lett.* **2004**, *241*, 67-72.

[327] K. Akiyama, S. Teraguchi, Y. Hamasaki, M. Mori, K. Tatsumi, K. Ohnishi, H. Hayashi, *J. Nat. Prod.* **2003**, *66*, 136-139.

[328] H. Tanaka, P. Wang, M. Namiki, *Agr. Biol. Chem.* **1972**, *36*, 2511-2517.

[329] H. Tanaka, P. Wang, O. Yamada. , T. Tamura, *Agr. Biol. Chem.* **1966**, *30*, 107.

[330] A. Carvalho, G. Alcantara, S. Oliveira, A. N. Micheletti, G. Honda Maia, *Electrochim. Acta* **2009**, *54*, 2290-2297.

[331] R. Letcher, *Oms.* **1968**, *1*, 551-562.

[332] A. Buitrago Díat, J. Rojas Vera, V. Cote, J. Bruno-Colmenárez, G. Díaz de Delgado *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **2010**, *9*, 470 - 474.

[333] A. Micheletti, A. Beatriz, D. Lima, N. Honda, C. Pessoa, M. Moraes, L. Lotufo, H. Magalhaes, N. Carvalho, *Quím. Nova* **2009**, *32*, 12-20.

[334] S. Markovic Zoran, T. Manojlovic Nedeljko, *Monatsh. Chem*. **2010**, *141*, 945-952.

[335] H. A. Priestap, *Tetrahedron* **1984**, *40*, 3617-3624.

[336] S. Ikeda, M. Sugita, A. Yoshimura, T. Sumizawa, H. Douzono, Y. Nagata, S. Akiyama, *Int. J. Cancer* **1990**, *45*, 508-513.

[337] R. Mata, A. Gamboa, M. Macias, S. Santilla´n, M. Ulloa, M. C. Gonza´lez, *J. Agric. Food Chem.* **2003**, *51*, 4559-4562.

[338] L. M. Pevzner, V. M. Ignat'ev, B. I. Ionin, *Russ. J. Gen. Chem.* **1999**, *69*, 551.

[339] G. M. Cabrera, M. J. Roberti, J. E. Wright, A. M. Seldes, *Phytochemistry* 2002, *61*, 189-193.

[340] A. Q. Lin, L. Du, Y. C. Fang, F. Z. Wang, T. J. Zhu, Q. Q. Gu, W. M. Zhu, *Chem. Nat. Com.* **2009**, *45*, 677-680.

[341] J. P. Vacca, B. D. Dorsey, W. A. Schleif, R. B. ; Levine, S. L. McDaniel, P. L. Darke, J. Zugay, J. C. Quintero, O. M. Blahy, B. B. Sardana, A. J. Schlabach, P. I. Graham, J. H. Condra, L. Gotlib, M. K. Holloway, J. Lin, I. W. Chen, K. Vastag, D. Ostovic, P. S. Anderson, E. A. Emini, J. R. Huff, *J. R. Proc. Natl. Acad. Sci.* U. S. A. **1994**, *91*, 4096.

[342] K. Rossen, S. A. Weissman, J. Sagar, R. A. Reamer, D. A. Askin, R. P. Volante, P. J. Reider, *Tetrahedron Lett.* **1995**, *36*, 6419.

[343] E. Mishani, C. S. Dence, T. J. McCarthy, M. J. Welch, *Tetrahedron Lett.* **1996**, *37*, 319.

[344] I. A. Cliffe, C. I. Brightswell, A. Fletcher, E. P. Forster, Chaudhary *Bioorg. Med. Chem.* **2006**, *14*, 1819-1826, 1825A.

[345] R. P. Maskey, R. N. Asolkar, E. Kapaun, I. Wagner-Döbler, H. Laatsch, *J. Antibiot.* **2002**, *55*, 643-649.

[346] Md. T. Islam, T. Ito, M. Sakasai, S. J. Tahara, *Agric. Food Chem.* **2002**, *50*, 6697-6703.

[347] Md. T. Islam, A. Tiedemann, *Phytopathology* **2008**, *98*, S72.

[348] W. A. Dengler, J. Schulte, D. P. Berger, R. Mertelsmann, H. H. Fiebig, *Anticancer Drugs* **1995**, *6*, 522-532.

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Prof. Dr. Hartmut Laatsch for providing me the opportunity to do work in his group. I am deeply appreciative for his supervision, for the valuable and constructive discussions, for his guidance, fruitful advices, which enriched the quality of this work. I am very lucky to be one of his students. In addition, I am very grateful for his kindness, and unlimited help in solving my problems during my stay in Germany.

I would like also to thank Prof. Aly Raies, my second supervisor, for registering me at the Faculté des Sciences de Tunis. I highly appreciate his guidance, help, kindness and encouragements during this work.

My thanks and appreciations are extended to Prof. Axel Zeeck for accepting to be my co-supervisor in Germany and for reading this work.

I would like to thank too Prof. Djamel DRIDER for his encouragements and help during my thesis and to be the examiner of this work.

I am also thankful to Prof. Sadok BERREJEB for accepting to Prof. Omrane BEL-HADJ to be president of my thesis committee.

Special thanks go to the Ministry of High Education, Tunisia and the "German Academic Exchange Services" (DAAD) for their financial support during my PhD study.

I appreciate the patience of my parents, brothers, sisters in law, and of my husband.

My great gratitude goes to Mrs. F. Lissy and Mr. A. Kohl for the microbiological work and technical assistance respectively. I am also thankful to Mrs. P. Lappe, Mrs. Peinemann, Dr. (Mrs.) Dietrich for their administrative help and kindness during my study.

I am very thankful to all my laboratory colleagues specially Dr. Khaled Attia Shaaban (Egypt), Dr. Naheed Riaz (Pakistan), Dr. Mohamed Shaaban (Egypt), Dr. Josphat Matasyoh (Kenya), Dr. Petrea Facey (Jamaica), Dr. Clarisse Fondja Yao (Cameroon), for their collaboration and helpful discussions.

Also I would like to thank Dr. Mahmoud Al Refai (Jordania), Dr. Hnin Yu Win (Manymar), Dr. Hafizur Rahman (Bangladesh), Dr. Sayed Ahmed (Egypt), Dr. Yerlan Suleimanov (Kazarestan), Dr. Hari Bhattarai (Nepal), Dr. Babita Paudel (Nepal), Dr. Selvam Masilamani (India) and Dr. Abdelhamid Béji (Tunisia).

Deep thanks to my PhD colleagues Muhammed Bahi (Indonesia), Humaira Naureen (Pakistan), Hongpeng Wang (China), Hamdi Nasr (Egypt), Dame Zarihun (Ethiopia), Michel Kongue (Cameroon), Ferdinand Talontsi (Cameroon) and PhD students guests: Imran Sajid (Pakistan), Muhammed Arfan (Pakistan), Muaaz Al Ajlani (Syria), Nelum Piyasena (Srilanka), Fatemeh Mohammdipana (Iran), Mohamed Osman (Egypt), Nadia Jamil (Pakistan), Salah Eddine Samri (Morocco) and Kumar Konda Shravan (India). I thank them sincerely for their kind cooperation, support and enjoyable work atmosphere.

It was a very nice experience to meet international PhD students and guests from nearly thirty nationalities. I learned a lot about the other cultures, religions, traditions and mentalities.

I am also thankful to all families and friends in Göttingen for friendly behaviour, support and kindness throughout my stay.

Words cannot express my deep gratitude and thanks to my parents, who were supporting, caring and praying for me all the time. I would also like to appreciate their understanding, their great support and their continuous role, which they are playing in my life. Providing me the opportunity to enrich my academic career was the greatest gift they could have given me. Their love, prayers and encouragement, helped me in every stage of my life.

More thanks go to my brothers, sisters in law, my husband and his family for their support. My gratitude goes to my extended family and friends.

CURRICULUM VITÆ

PERSONAL INFORMATION

Last, First Name:	Imène ZENDAH EL EUCH
Date of Birth:	April 28, 1977
Place of Birth:	Bizerte, Tunisia
Civil Status:	Married
E-mail:	Imene.Zendah@gmail.com

EDUCATION

Post-graduate studies

April 2007- July 2012: PhD Student, co-worker in Prof Laatsch's group, Institute of Organic and Biomolecular Chemistry, University Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany

Oct. 2005-Mar. 2007: First year in preparation of Doctorate Thesis in Biology speciality (Biochemistry/ Microbiology) Faculté des Sciences de Tunis. Université d'El Manar II. Tunisia.

Dec.2003- Fev. 2005: "Mastère de Biochimie et Technobiologie" (Master's Degree – Biochemistry and Technobiology) Faculté des Sciences de Tunis. Université Tunis El Manar II. Tunisia.

2002-2003: AEA de Biochimie et Technobiologie. (AEA of Biochemistry and Technobiology)

Graduate studies

1999-2001 "Maîtrise en Sciences Naturelles (Bachelor degree in Natural Sciences) Faculté des Sciences de Bizerte. Université de 7 Novembre à Carthage, Tunisie

1997-1999 "Diplôme des Etudes Supérieurs" (DES) (Degree of Superior Studies). Faculté des Sciences de Bizerte. Université de 7 Novembre à Carthage, Tunisie

High School

1996-1997 "Diplôme de Baccalauréat en Sciences Expérimentales" (Baccalaureate Degree in Experimental Sciences). Lycée Bach Hamba Bizerte,Tunisie.

PARTICIPATION IN SCIENTIFIC MEETINGS:

2004: ZENDAH Imène, FATTOUCH Sami, HAJLAOUI M^{ed} Rabeh, KOURDA Rym, BEN HASSINE Fethy et BELHADJ Omrane. «Isolement et Identification de Bactéries à partir du rumen et étude de leur pouvoir antagoniste contre certains microorganismes pathogènes». 15ème Journées Biologiques de l'Association Tunisienne des Sciences Biologiques «Forum des Sciences Biologiques». (ATSB) (Hammam Sousse- El Kantaoui, 18-21 Mars 2004, Tunisie). (Oral presentation)

2004: ZENDAH Imène, HAJLAOUI M^{ed} Rabeh, BEN HASSINE Fethy, KARCHANI Salma. «Effet antagoniste de *Streptococcus acidomini-*

mus isolée du rumen de dromadaire contre le champignon phytopathogène *Botrytis cineria*». 4ème Colloque «Sciences et Environnement» (Bizerte, 9-10-11 Avril 2004, Tunisie). (Poster)

2004: ZENDAH Imène, HAJLAOUI Med Rabeh, BEN HASSINE Fethy, ROUAISSI Mustapha TAYACHI Lamia. «Antagonisme entre *Serratia rubidaea* isolée du rumen de veau et *Fusarium culmorum*». 4ème Colloque «Sciences et Environnement» (Bizerte, 9-10-11 Avril 2004, Tunisie). (Poster)

2004: Atelier «Bionettoyage dans les établissements de soins» de la IXème Journée Régionale d'Hygiène Hospitalière de Bizerte. Direction de l'Hygiène du milieu et de la Protection de l'Environnement. Direction Régionale de la santé Publique de Bizerte. (Bizerte, 2 Octobre 2004, Tunisie). (Participation)

2004: ZENDAH Imène, BEN HASSINE Fethy, TAYACHI Lamia et HAJLAOUI Med Rabeh. «Effet antagoniste de *Citrobacter freundii*, d'*Enterobacter amnigenus*, de *Burkholderia cepacia* et d'*Enterococcus hirae* contre *Staphylococcus aureus* ATCC 25923». IXème Journée Régionale d'Hygiène Hospitalière de Bizerte. Direction de l'Hygiène du milieu et de la Protection de l'Environnement. Direction Régionale de la santé Publique de Bizerte. (Bizerte 2, Octobre 2004, Tunisie). (Poster)

2004: ZENDAH Imène, BEN HASSINE Fethy, TAYACHI Lamia et HAJLAOUI Med Rabeh. «Antagonisme entre *Burkholderia cepacia* et A*spergillus flavus*». IXème Journée Régionale d'Hygiène Hospitalière de Bizerte. Direction de l'Hygiène du milieu et de la Protection de l'Environnement. Direction Régionale de la santé Publique de Bizerte. (Bizerte, 2 Octobre 2004, Tunisie). (Poster)

2004: ZENDAH Imène, BEN HASSINE Fethy, ROUAISSI Mustapha, KARCHANI Salma et HAJLAOUI Med Rabeh. «Inhibition de croissance de *Phoma tracheiphila* par *Burkholderia cepacia* et *Klebsiella*

spp isolées du rumen de veau». 1er Séminaire de Microbiologie et 4ème Journées Biotechnologiques de l'Association Tunisienne de BioTechnologie (ATBT) (Hammamet, 19-22 Décembre 2004, Tunisie). (Poster)

2004: ZENDAH Imène, BEN HASSINE Fethy et HAJLAOUI Med Rabeh. «Relations d'antagonisme *in vitro* et en aérobiose entre des bactéries ruminales». 1er Séminaire de Microbiologie et 4ème Journées Biotechnologiques de l'Association Tunisienne de BioTechnologie (ATBT) (Hammamet, 19-22 Décembre 2004, Tunisie). (Poster)

2004: ZENDAH Imène, HAJLAOUI Med Rabeh, BEN HASSINE Fethy. «Isolement de quatre souches bactériennes ruminales antagonistes contre le champignon phytopathogène *Fusarium graminearum*». 11èmes Journées Scientifiques sur les Résultats de la Recherche Agricoles. (Hammamet, 9-10 Décembre 2004, Tunisie). (Poster)

2004: ZENDAH Imène, HAJLAOUI Med Rabeh et BEN HASSINE Fethy. «Mise en évidence d'un phénomène d'antagonisme *in Vitro* de bactéries ruminales contre des souches de *Bacillus thurengiensis*». 11èmes Journées Scientifiques sur les Résultats de la Recherche Agricoles. (Hammamet, 9-10 Décembre 2004, Tunisie). (Poster)

2005: ZENDAH Imène, HAJLAOUI Med Rabeh, BEN HASSINE Fethy. «Caractérisation physico-chimique des substances inhibitrices responsables d'antagonisme entre certaines bactéries ruminales». 16èmes Journées des Sciences Biologiques. 2ème Forum des Sciences Biologiques. (ATSB) (Yasmine Hammamet, 20-23 Mars 2005, Tunisie). (Oral presentation)

2005: ZENDAH Imène, BEN HASSINE Fethy et HAJLAOUI Med Rabeh. «Caractérisation physico-chimique des substances inhibitrices sécrétées par des bactéries ruminales antagonistes vis-à-vis de souches de *Bacillus thurengiensis*». XVèmes Journées Nationales de Biologie «Agrobiologie et Environnement» (SSNT) (Hammamet, 19-22 Mars 2005, Tunisie). (Poster)

2005: Journée de sensibilisation «Certification de Personnes en HACCP» organisée par l'Association Africaine de Microbiologie et d'Hygiène Alimentaire et le Centre Européen de Prévention des risques. (Sousse, 24 Juin 2005, Tunisie). (Participation)

2005: Colloque international de la deuxième Journée de Biotechnologie de l'ISBM «Organismes Génétiquement modifiés: Défis Biotechnologiques et enjeux Economiques». (Monastir, 30 Septembre- 1 Octobre 2005, Tunisie). (Participation)

2005: 1er Colloque Euro-Magrébin de Toxicologie Aquatique: «Aspects protéomiques et Génomiques». (Sousse, 3 Octobre 2005, Tunisie). (Participation)

2005: ZENDAH Imène, HAJLAOUI Med Rabeh, BEN HASSINE Fethy.«Caractérisation physico-chimique des substances inhibitrices sécrétées par *Citrobacter freundii*, *Enterobacter amnigenus* et *Burkholderia cepacia* antagonistes de *Staphylococcus aureus* ATCC 25923». Xème Journée Régionale d'Hygiène Hospitalière de Bizerte. Direction de l'Hygiène du milieu et de la protection de l'Environnement. Direction Régionale de la santé Publique de Bizerte. (Bizerte, 3 Décembre 2005, Tunisie). (Poster)

2005: ZENDAH Imène, HAJLAOUI Med Rabeh et RAIES Aly. «Mise en évidence de certaines bactéries ruminales antagonistes de bactéries pathogènes à l'Homme». 12èmes Journées Scientifiques sur les Résultats de la Recherche Agricoles. (Hammamet, 8-9 Décembre 2005, Tunisie). (Poster)

2005: ZENDAH Imène, HAJLAOUI Med Rabeh, SADFI Najla, BEN HASSINE Fethy et RAIES Aly. «Recherche de nouvelles substances antimicrobiennes sécrétées par des bactéries de différentes niches écologiques». 1ères Journées Scientifiques «Métabolites et microbiologie industrielle. Agriculture et Environnement. Entérocoques en microbio-

logie humaine. Microbiologie vétérinaire. Microbiologie alimentaire». (Hammamet, 17-20 Décembre 2005, Tunisie). (Poster)

2006: ZENDAH Imène, NAGHMOUCHI Karim, HAJLAOUI M^{ed} Rabeh, AMOR Ali and RAIES Aly. «Production of antimicrobial substances by ruminal bacteria». Ist Internationnal Symposium on Food, Veterinary and Medical Applications of Antimicrobial peptides. Nantes (ENITIAA), France. June 21st-23rd 2006. (Poster)

2006: 1er Congrès International de Nutrition en Tunisie (Tunis, 27-28 Octobre 2006, Tunisie). (Participation)

2006: ZENDAH Imène, HAJLAOUI M^{ed} Rabeh, BARGUELLIL Farouk et RAIES Aly. «Effet de bactéries du Genre *Bacillus* vis-à-vis de bactéries pathogènes à l'Homme». Société des Sciences Naturelles de Tunisie XVI^{èmes} Journées Nationales de Biologie «Gestion des Bioressources » (Hammamet, 04-07 Novembre 2006, Tunisie). (Poster)

2006: ZENDAH Imène, HAJLAOUI M^{ed} Rabeh, BARGUELLIL Farouk et RAIES Aly. «Les Mycotoxines dans les aliments des ruminants. Effet des bactéries ruminales sur certains champignons». Société des Sciences Naturelles de Tunisie XVI^{èmes} Journées Nationales de Biologie «Gestion des Bioressources» (Hammamet, 04-07 Novembre 2006, Tunisie). (Poster)

2007: ZENDAH Imène, DRIDER Djamel, RAIES Aly, BARGUELLIL Farouk et Lilia Nazef, PREVOST Herve et LAATSCH Hartmut. «Effect of Bacteria isolated from palm rhizosphere against clinical pathogenic bacteria» 1st International Congress on Macromolecular Biochemistry and Genetics (Gafsa, April 12-16, 2007. Tunisia). (Poster)

2007: ZENDAH Imène, DRIDER Djamel, RAIES Aly, BARGUELLIL Farouk et Lilia Nazef, PREVOST Herve et LAATSCH Hartmut. «Isolement et Identification de bactéries ruminales à large spectre antimi-

cobien». Société Francaise de Micobiologie «SFM» (Nantes, 30, 31 Mai et 01 Juin 2007, France). (Poster)

2007: ZENDAH Imène, SHAABAN ATTIA Khaled, BARGUELLIL Farouk, RAIES Aly et LAATSCH Hartmut. «A broad antimicrobial spectrum from ruminal bacteria». Fakultät für Chemie (Göttingen, 29 June 2007, Germany). (Poster)

2007: ZENDAH Imène, SHAABAN ATTIA Khaled, RAIES Aly and LAATSCH Hartmut. «Antagonism effect of palm rhizosphere bacteria against pathogenic microorganisms». Fakultät für Chemie (Göttingen, 29 Juin 2007, Germany). (Poster)

2007: ZENDAH Imène, SHAABAN ATTIA Khaled, RAIES Aly and LAATSCH Hartmut. Participation at Tübinger-Göttinger Gespräche zur Chemie von Mikroorganismen, (Würzburg, 19-21 September 2007, Germany) (Workshop)

2007: ZENDAH Imène, SHAABAN ATTIA Khaled, RAIES Aly and LAATSCH Hartmut. «Screening, Fermentation, Purification et Elucidation de la structure d'un nouveau antibiotique la Barakacine sécrétée par la bactérie ruminale *Burkholderia cepacia* ZIKSO». Société des Sciences Naturelles de Tunisie XVII^{èmes} Journées Nationales de Biologie «Gestion des Bioressources» (Hammamet, 03 - 06 Novembre 2007, Tunisie). (Poster)

2008: ZENDAH Imène, SHAABAN ATTIA Khaled, RAIES Aly and LAATSCH Hartmut. «Barakacin: A First Thiazolyl-indol Alkaloid Isolated from Nature». Fakultät für Chemie (Göttingen, 29 Juin 2008, Germany). (Poster)

2008: ZENDAH Imène, SHAABAN ATTIA Khaled, SHAABAN Mohamed, FACEY Petrea, RAIES Aly and LAATSCH Hartmut. «Neamamycin: A Highly Cytotoxic Glycolipid Produced by a Ruminal Bacterium». Société des Sciences Naturelles de Tunisie XVIII^{èmes}

Journées Nationales de Biologie «Gestion des Bioressources» (Hammamet, 06 - 09 Novembre 2008, Tunisie). (Poster)

2008: ZENDAH Imène, SHAABAN ATTIA Khaled, SHAABAN Mohamed, FACEY Petrea, RAIES Aly and LAATSCH Hartmut. «A new microbial butylglucoside from the ruminal bacterium *Enterobacter amnigenus*». Société des Sciences Naturelles de Tunisie XVIII^{èmes} Journées Nationales de Biologie «Gestion des Bioressources» (Hammamet, 06 - 09 Novembre 2008, Tunisie). (Poster)

2009: ZENDAH Imène, Hamdi Abdel Rahim, Hnin Yu Win, Shaaban Attia Khaled, and Laatsch Hartmut. «New Phenazine Derivative from a Terrestrial *Streptomyces* sp.». Fakultät für Chemie (Göttingen, 3rd July 2009, Germany). (Poster)

2010: Imène Zendah, Naheed Riaz, and Hartmut Laatsch. « New Dihydrophenazines from a Terrestrial *Streptomyces* sp.». 12th JCF-Frühjahrssymposium 17.3.2010 -20.3.2010 in Göttingen.

2010: Imène Zendah, Naheed Riaz, Aly Raies and Hartmut Laatsch. «Chromophenazines E and F from *Streptomyces* sp. Ank 315». SSNT 2010. Société des Sciences Naturelles de Tunisie XIV^{èmes} Journées Nationales de Biologie «Gestion des Bioressources» (Hammamet, Novembre 2010, Tunisie). (Poster)

2011: Frauendorf Holm, Zendah Imène; Laatsch Hartmut. «Chromophenazines – Radical Ion Formation by Electrospray Ionisation and CID-MS/MS Fragmentation». Deutsche Gesellschaft für Massenspectrometrie DGMS 2011. (Poster)

2011: Imène Zendah, Holm Frauendorf and Hartmut Laatsch. «Chromophenazines Ion Formation by ESI-MS and CID-MS/MS Fragmentations». Göttinger Chemie Forum 2011. Fakultät für Chemie (Göttingen, 08 July 2011, Germany). (Poster)

SCIENTIFIC PUBLICATIONS – Articles

Imène Zendah, Naheed Riaz, Hamdi Abdel Rahim, Holm Frauendorf, Anja Schüffler, Aly Raies, and Hartmut Laatsch. «Chromophenazines from the terrestrial *Streptomyces* sp. Ank 315». *J. Nat. Prod.* **2012**, 75, 2-8.

Imène Zendah, Khaled A. Shaaban, Aly Raies, Elisabeth Helmke and Hartmut Laatsch. «Barakacin: The First Thiazolyl-indole Alkaloid Isolated from Nature». *Z. Naturforsch.* B **2012**, *67b*, 1-4.

Imène Zendah, Naheed Riaz, Abdel Rahim Hamdi, Tofazzal Islam, Aly Raies and Hartmut Laatsch. «A quinolone with zoosporicidal activity against the downy mildew pathogen *Plasmopara viticola*, isolated from the ruminal bacterium *Pseudomonas aeruginosa*». *Journal de la SSNT*.

AWARDS

- Grant from the Ministry of High Education Tunisia March 2007- July 2007
- Grant from the Deutscher Akademischer Austauschdienst DAAD (German Academic Exchange Service) August 2007- January 2008
- Grant from the Ministry of High Education Tunisia March 2008- July 2008
- Grant from the Deutscher Akademischer Austauschdienst DAAD (German Academic Exchange Service) August 2008- January 2009
- Grant from the Deutscher Akademischer Austauschdienst DAAD (German Academic Exchange Service) March 2009- August 2009
- Grant from the Deutscher Akademischer Austauschdienst DAAD (German Academic Exchange Service) March 2010- August 2010

TRAINING COURSES

Training of 5 months at polyclinique CNSS of Bizerte.

Training of 9 months at the Military Hospital of Instruction of Tunis. Tunisia.

LANGUAGES

Arabic	mother language. reading, writing and speaking
French	reading, writing and speaking (2nd language)
English	reading, writing and speaking
German	understanding, reading and writing but not speaking