Isolation, biological activity and secondary metabolite investigations of marine-derived fungi and selected host sponges

Von der Gemeinsamen Naturwissenschaftlichen Fakultät
der Technischen Universität Carolo-Wilhelmina
zu Braunschweig
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)
genehmigte
D i s s e r t a t i o n

von Ulrich Höller
aus Braunschweig

1. Referentin: Prof. Dr. G. M. König
2. Referent: Prof. Dr. H.-J. Aust
eingereicht am: 25.02.1999
mündliche Prüfung (Disputation) am: 26.04.1999
1999
(Druckjahr)

Vorveröffentlichungen der Dissertation / In Advance Publications of the Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Gemeinsamen Naturwissenschaftlichen Fakultät, vertreten durch die Mentorin/Betreuerin der Arbeit, in folgenden Beiträgen vorab veröffentlicht

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Publikationen / Research Papers

Höller, U., König, G. M. and Wright, A. D.; Two New Sesterterpene Tetronic Acids from the Marine Sponge *Ircinia oros*. *J. Nat Prod.* **60**, 832-835 (1997).

Höller, U., König, G. M. and Wright, A. D.; Three New Metabolites from Marine-Derived Fungi of the Genera *Coniothyrium* and *Microsphaeropsis*. *J. Nat. Prod.* **62**, 114-118 (1999).

Tagungsbeiträge / Research Presentations

Höller, U., König, G. M. and Wright, A. D.; New Sesterterpene Tetronic Acids from the Marine Sponge *Ircinia variabilis*. Poster presented at the 44th Annual Congress of the Society for Medical Plant Research, Prague September 1996.

Matthée, G. F., Höller, U., Wright, A. D., König, G. M.; Screening of Marine Organisms and Fungi for HIV-1-RT Inhibitory Activity. Poster presented at the 1st Euroconference on Marine Natural Products, Athens November 1997.

Acknowledgements

I wish to express my gratitude to Prof. Dr. G. M. König and Dr. A. D. Wright for introducing me to this project, for providing excellent, both scientific and friendly, working facillities, and for their understanding of my spare time activities. They kindly supported me, always had time for questions and discussions, and enabled me to participate in a collection trip to Fiji in 1998.

The microbiological part of this study has been carried out in cooperation with the research group of Prof. Dr. H.-J. Aust, Institut für Mikrobiologie, TU Braunschweig. I would like to thank Prof. Dr. H.-J. Aust for kindly enabling me to work in his group, and him and all members of his group for a very friendly and synergistic cooperation. Special thanks go to Dr. B. Schulz for indispensable help concerning the isolation of the fungal strains, Dr. S. Draeger for introducing me to the taxonomic identification of fungi, and for identifiing most of the isolates of this study, and Ms. Qunxiu Hu for patiently explaining standard working procedures to me.

This study involved many different specific tasks, e.g., collection and taxonomic identification of the sponges, recording of various spectra of isolated compounds, and biological testing, which were in part performed in cooperation with other research groups. For this work, thanks go to:

Dr. D. Barthel, Institut für Meereskunde Universität Kiel, for taxonomic identification of the sponge samples from Helgoland;
Dr. R. Desqueroux-Faundez, Musée d'Histoire Naturelle Genève, for taxonomic identification of the sponge samples from Australia, Dominica, Malta and Tenerife;
Dr. R. A. Samson, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, for taxonomic identification of the*Microsphaeropsis* isolate from Helgoland;
Dr. C. Schütt and the scientific dive team of the Biologische Anstalt Helgoland, for collection of the sponge samples from Helgoland;

Ms. P. Holba-Schulz and Ms. I. Rübesamen, NMR Abteilung der Chemischen Institute, TU Braunschweig, for recording ^{1}H NMR spectra;
Dr. V. Wray, Ms. B. Jaschok-Kentner and Ms. C. Kokoschke, GBF Braunschweig, for recording ^{1}H and all other NMR spectra;
Dr. R. Christ, GBF Braunschweig, Dr. H.-M. Schiebel, Dr. U. Papke and Ms. D. Döring, Massenspektrometrie der Chemie, TU Braunschweig, for mass spectral measurements;
Dr. L. Witte for GC-MS measurements and for his help concerning the interpretation of some of these spectra;
Prof. Dr. P. G. Jones, Institut für Anorganische und Analytische Chemie, TU Braunschweig, for performing the single crystal X-ray analysis of colletoketol;
Ms. P. Reich and Mr. T. Kroker, Institut für Pharmazeutische Chemie, TU Braunschweig, for recording all IR spectra;
Ms. H. Heil, Institut für Pharmazeutische Chemie, TU Braunschweig, for the combustion analysis of taurine;
Ms. H.-S. Cho, Institut für Pharmazeutische Technologie, TU Braunschweig, for recording the CD spectrum of 46a;

PD Dr. P. Leseberg, Chemie Bibliothek, TU Braunschweig, for conducting CAS online searches;

Ms. G. Matthée, Ms. I. Rahaus and Mr. C. Dreikorn for performing ELISAs and many agar diffusion assays;
Prof. Dr. C. K. Angerhofer, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, USA, for providing cytotoxicity data of extracts towards KB cells;
Prof. Dr. S. Franzblau, U.S. Department of Health and Human Services, GWL Hansen's Disease Center, Baton Rouge, Louisiana, USA and his research group for testing extracts for inhibition of *Mycobacterium tuberculosis*;
Dr. N. Lang-Unnasch, Division of Geographic Medicine, University of Alabama at Birmingham, USA, for determing antimalarial activity of pure compounds;

Mr. M. Busch, Ms. G. Frech, Mr. A. Reimann, Ms. A.-K. Römmert, Ms. S. Sohler, and Mr. M. Vollmert, who worked as undergraduate students at the Institute, for technical assistance with isolation of fungal strains, extraction of fungal cultures, and biological testing of resulting extracts.

Special thanks go to Ms. Ines Rahaus for cultivation, extraction and HPLC separations of most compounds from the extracts of the *Microsphaeropsis* sp. and *Emericellopsis minima*, and Ms. Britta Störmann for separation of the extract of the *Microascus* sp.;

Ms. Erika Christoph for indispensible help facing everyday struggles against Universitary Bureaucracy,

and to all members of the Institut für Pharmazeutische Biologie, TU Braunschweig, present or past, for a friendly and synergistic cooperation, especially Ms. Ines Rahaus, Mr. Olaf Papendorf and Ms. Claudia Wegner, and Dr. Gerson Graser and Mr. Matthias Wessels for the common joy looking after the students of the Phytochemie-Praktikum.

Parts of this work were supported by a grant from the German BMBF together with Bayer AG Leverkusen to my supervisor.

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Abbreviations

°C	Degrees Celsius
1D	One Dimensional
2D	Two Dimensional
[α]	Specific rotatory power
δ	NMR chemical shift [ppm]
λ	wavelenght [nm]
µL	10^{-6} liter
µm	10^{-6} meter
ν	wave number [cm^{-1}]
approx.	approximately
ASW	Artificial Sea Water
B. m.	*Bacillus megaterium*
BIO	Biomalt agar
br	broad
BuOH	Butanol
c	concentration [g/100 mL]
C. f.	*Chlorella fusca*
CD	Circular Dichroism
cf.	confer
CI	Chemical Ionisation
cm	10^{-2} meter
COSY	COrrelated SpectroscopY
CT	Collection Trip
d	dublet
DAD	Diode Array Detector
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
dest.	destilled
DMSO	DiMethylSulphOxide
E. c.	*Escherichia coli*
e.g.	example given
E. r.	*Eurotium repens*
EI	Electron Ionisation
ELISA	Enzyme Linked Immuno Sorbent Assay
EtOAc	Ethyl acetate
EtOH	Ethanol
F. o.	*Fusarium oxysporum*
FAB	Fast Atom Bombardment
g	gram
GC	Gas Chromatography
GPY	Glucose Pepton Yeast extract agar
HIV-1	Human Immunodeficiency Virus 1
HMBC	Hetero nuclear Multiple Bond Correlation
HMQC	Hetero nuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HR	High Resolution
Hz	Hertz

i. D.	inner Diameter
IC	Inhibition Concentration
IR	Infra Red
J	Spin-spin coupling constant [Hz]
KM	Kartoffel-Möhren [potato carrot] agar
L	Liter
m	multiplet (in connection with NMR data)
M. v.	*Microbotryum violaceum*
MeOH	Methanol
MHz	Megahertz
min	minute
mL	10^{-3} liter
mM	10^{-3} Mol
mp.	melting point
MS	(microbiology) Malt extract Soymeal agar
MS	Mass Spectrometry
NB	Nährboullion [Nutrient Broth]
NCI	National Cancer Institute (USA)
nm	10^{-9} meter
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NP	Normal Phase
ppm	parts per million
q	quartet
RI	Refractive Index
RP	Reversed Phase
RT	Room Temperature
RT	Reverse Transcriptase (in connection with HIV-1)
s	singlet (in connection with NMR data)
SCUBA	Self Contained Underwater Breathing Apparatus
SNA	Standard Nutrient Agar
sp.	species
t	triplet
TK	Tyrosine Kinase
TLC	Thin Layer Chromatography
UV	Ultra Violet
VLC	Vacuum-Liquid Chromatography

1 Introduction

1.1 Marine organisms as a source of new and biologically active metabolites

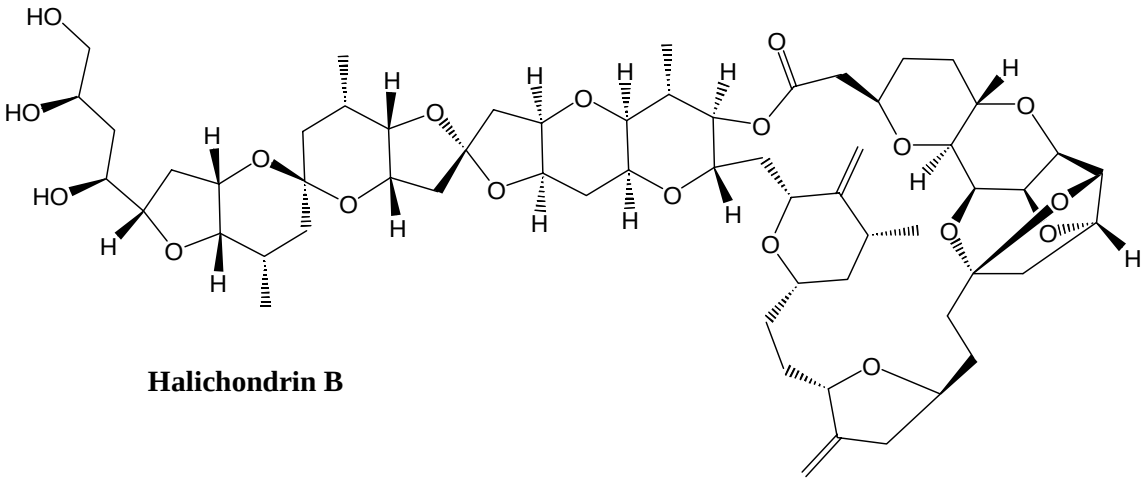
The marine environment comprises 71 % of the earth's surface, and consists of extreme and contrasting habitats, ranging from tropical reefs to ice-shelfs of the polar seas, and to black smokers in the deep sea. The biodiversity in the worlds oceans is immense, e.g., of 33 known animal phyla 15 are exclusively marine, and 32 of them have marine representatives (Norse, 1995). Some habitats are known to be particularly numerous in species, e.g., tropical marine reefs, which represent one of the most diverse ecosystems encountered on earth, comparable in diversity to tropical rain forests.

Nearly all forms of marine life have attracted the attention of natural products chemists, with reports about secondary metabolites from sponges dominating by number (de Vries & Hall, 1994). Marine natural products are distinguished by a great chemical diversity. Metabolites from nearly all groups of natural products have been discovered. Halogenated compounds are particularly numerous due to the natural abundance of chlorine, bromine and to a lesser extent iodine in sea water (Gribble, 1996). The annual reviews on marine natural products by Faulkner (1998, and previous reviews), and the database MARIN LIT (Blunt & Munro, 1999) provide an overview of the impressive variety of the compounds encountered.

Some of these compounds clearly serve an ecological purpose. A laboratory feeding assay with organic sponge extracts for example revealed that 69 % of 71 Caribbean Demosponges contained metabolites which deterred feeding by the wrasse *Thalassoma bifasciatum* (Pawlik *et al.*, 1995). Natural products are also involved in the competition for the limited space on coral reefs. The red alga *Plocamium hamatum* for example could be shown to induce necrosis in the soft coral *Sinularia cruciata* on direct contact, with the algal metabolite chloromertensene being largely responsible for the observed effect (de Nys, Coll & Price, 1991).

1.2 Metabolites with prominent pharmacological activities - example halichondrin B

The reported metabolites from marine organisms are not only interesting from an ecological or chemical point of view. Some of them have been shown to possess prominent biological (pharmacological) activities, e.g., bryostatins (Schaufelberger *et al.*, 1991), didemnins (Rinehart *et al.*, 1988), ecteinascidins (Rinehart *et al.*, 1990). These findings, and the fact that some of these metabolites belong to unique structural types brought them into the focus of the pharmaceutical industry. A small number of compounds are currently being evaluated in clinical trials, many of them as antitumor agents (see current reviews, e.g., König & Wright, 1998). The supply of sufficient amounts of compounds for pharmaceutical development is often problematic. This issue will be discussed using the example of halichondrin B, an antineoplastic compound currently in preclinical evaluation.



Halichondrin B

Halichondrin B was first isolated from the sponge *Halichondria okadai* as the most potent member of a series of related antitumor compounds (Uemura *et al.*, 1985). Later on, it was also reported from other sponge species including *Phakellia carteri* (Pettit *et al.*, 1993), and *Lissodendoryx* sp. (Litaudon *et al.*, 1994). The absolute stereochemistry was deduced from comparisons with the related compound, norhalichondrin A, the structure of which had been established by X-ray crystallographic analysis of its *p*-bromophenacyl ester (Hirata & Uemura, 1986). Halichondrin B showed *in vitro* cytotoxicity against B-16 melanoma cells with an IC_{50}

of 0.093 ng/mL, and also significant *in vivo* activity against B-16, P-388 and L-1210 tumours in mice (Hirata & Uemura, 1986). Mechanism of action studies at the NCI revealed it to bind to the vinca domain of tubulin, and to arrest cells in mitosis (Bai *et al.*, 1991). In the same study, it could be shown to inhibit the *in vitro* polymerisation of purified tubulin, and the microtubule assembly. Such mechanism of action studies, and even more *in vivo* experiments and future clinical trials, depend on sufficient supply of this very complex natural product. Its natural content in sponges ranges from 10^{-5} - 10^{-6} %. Mass collection of biomass is thus not very effective, if ever applicable, and may have massive ecological impacts, even leading to the extinction of a population or species. A total synthesis of halichondrin B has been reported (Aicher *et al.*, 1992), but probably involves too many steps for being an economically valid alternative. Thus, to obtain further amounts of halichondrin B for clinical trials a large-scale culture of the macroorganism, i.e. the sponge *Lissodendoryx* sp., has been established (Munro, 1998). This is the first example of mass cultivation of sponge species for pharmaceutical purposes.

A further possibility to obtain sufficient amounts of a selected compound vividly discussed in the scientific community is the discovery of a microorganism producing the desired metabolite. Microorganisms are in some cases suspected to be the true biosynthetic producers of metabolites isolated from marine macroorganisms. This is especially likely, if the compound in question has been obtained from various macroorganisms not closely related taxonomically, and if the compound belongs to a structural type already known from microorganisms. Although halichondrin B has been found in several sponge species from different origins, and its structure resembles that of polyethers known from dinoflagelates, to date there is no direct evidence for the contribution of microorganism to its biosynthesis (Munro, 1998).

1.3 Marine microorganisms

The example of halichondrin B demonstrates that some marine natural products have such complex structures, that the only reasonable supply is their isolation from the producing organism. Organisms that could easily be cultured in large amounts would

thus be ideal. Such organisms might be marine microorganisms. The fact that some marine microorganisms are easily cultured and that they had long been neglected by many marine natural product chemists, has led to an increased research effort in this area during the last 10 years. Research is focusing mainly on marine bacteria, fungi and microalgae as reflected by the number of natural products described from each group of organisms (Faulkner, 1998; Blunt & Munro, 1999).

1.3.1 Marine fungi

The current project focused on marine fungi. Although the occurrence of fungi in the sea has been known since the middle of the 18th century (see, e.g., Kohlmeyer & Kohlmeyer, 1979), they received broader interest only during the last six decades. The work of Barghoorn & Linder (1944) provided the first key to marine fungi and stimulated further research in this area. Other important milestones in marine mycology include the publications "Marine Mycology: The higher Fungi" (Kohlmeyer & Kohlmeyer, 1979), a comprehensive book dealing with virtually all aspects of marine mycology, and "Illustrated key to the filamentous higher marine fungi" (Kohlmeyer & Volkmann-Kohlmeyer, 1991).

Fungi growing in the sea can be grouped into obligate and facultative marine fungi. According to a definition made by Kohlmeyer (1974), "obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine (brackish water) habitat; facultative marine are fungi from freshwater or terrestrial areas able to grow also in the natural marine environment". In 1991 Kohlmeyer & Volkmann-Kohlmeyer listed 321 filamentous higher marine fungi. Their key included 255 Ascomycetes, 60 mitosporic fungi, and only six Basidiomycetes. Since then, numerous new marine fungi have been described every year. The direct isolation method is most commonly applied (Kohlmeyer & Kohlmeyer, 1979; Hyde, Farrant & Jones, 1987). It includes collection and direct examination of marine substrates under a dissecting microscope for fungi, e.g., visible in the form of ascomata, and the isolation and inoculation of single fungal spores thereof. This procedure allows to show that the fungus isolated was active in the marine environment, and therefore is either an obligate or facultative marine

fungus. In this respect it is important to note that to date there is no other way to prove that an isolated fungal strain is marine other than to observe *in vivo* its ability to grow in the marine environment.

Among the classical substrates for the isolation of marine fungi are marine algae. Apart from lichens, algae-inhabiting fungi consist of parasitic and saprobic species (Kohlmeyer & Kohlmeyer, 1979). Most strains were obtained by direct isolation methods.

While algae have long been known for the occurrence of specifically adapted marine fungi (Kohlmeyer & Kohlmeyer, 1979), sponges as a source of fungi only recently came into focus. In contrast to symbiotic bacteria and cyanobacteria (e.g., Vacelet, 1975; Bergquist, 1978; Rai, 1990), some of which seem to play an important role in the production of biologically active secondary metabolites found in the host animals (Elyakov *et al.*, 1991; Unson, Holland & Faulkner, 1994), the occurrence of fungi in sponges is less well documented. Siepmann & Höhnk (1962) isolated yeasts and two *Fusarium* spp. from the tissue of sponges from the North Atlantic and discussed the possible ecological role of yeasts as producers of vitamins for their host organisms. Höhnk & Ulken (1979) isolated Phycomycetes and members of seven genera of mitosporic fungi from the liquid of squeezed tissue of sponges from the Mediterranean. The relationship between host sponges and fungi is still unknown; only marine Ascomycetes of the genus *Koralionastes* have been reported to be in some way associated with crustaceous sponges (Kohlmeyer & Volkmann-Kohlmeyer, 1990).

As sponges are filter-feeding organisms, spores and mycelium fragments of terrestrial fungi, washed into the sea, are likely to be present in these animals. The presence of these propagules in the sea water all over the world is well established, and they are known to be able to germinate and grow under laboratory conditions (e.g., Roth, Orpurt & Ahearn, 1964; Schaumann, 1974; Miller & Whitney, 1981). Thus, isolates obtained from sponges may represent such terrestrial strains, which is also suggested by the taxonomy of the fungal strains in question, e.g. *Aspergillus niger* and *Trichoderma harzianum* (cf. 1.3.3, Table 2).

1.3.2 Secondary metabolites from marine fungi

Marine-derived fungi have been shown to produce interesting and biologically active metabolites (cf. the reviews of Liberra & Lindequist, 1995; König & Wright, 1996; Pietra, 1997, Biabani & Laatsch, 1998). This is reflected in the number of literature records dealing with secondary metabolites from marine fungi, which have significantly increased over the past five years.

Substrates used for the isolation of marine-derived fungi for chemical investigations are very diverse. Besides marine sponges (see 1.3.3), predominantly algae (e.g., Chen *et al.*, 1996; Belofsky *et al.*, 1998; Numata *et al.*, 1993; Takahashi *et al.*, 1994a), crab shell (e.g., Sugano *et al.*, 1991), fish (e.g., Shigemori *et al.*, 1991), mangroves (e.g., Poch & Gloer, 1991), a sea hare (Numata *et al.*, 1997b), a tunicate (Wang, Borgeson & Crews, 1997), and sediment samples (e.g., Onuki *et al.*, 1998) were used.

Investigations dealing with clearly obligate marine fungi contributed only moderately to the overall number of records. As the number of obligate marine fungi described to date is low, especially when compared to a total of about 71,000 fungal species known (Hawksworth *et al.*, 1995), and many of them are slow-growing and therefore not convenient for the usually applied chemical and biological screening methods, this is not overly surprising.

Reports dealing with the isolation of secondary metabolites from obligate marine fungi are summarised in Table 1. Thus far, this list includes less than 5 % of the known species of higher filamentous marine fungi. Many of the reported metabolites are similar to, and some even identical with compounds previously obtained from terrestrial fungi. For example, the antibiotic siccayne isolated from the marine Basidiomycete *Halocyphina villosa* (Kupka *et al.*, 1981) was already known to be produced by the terrestrial mitosporic fungus *Helminthosporium siccans* (Ishibashi *et al.*, 1968), and isolculmorin isolated from the marine Ascomycete *Kallichroma tethys* (Alam *et al.*, 1996) belongs to a series of similar culmorins, known for example from the terrestrial mitosporic fungus *Fusarium culmorum* (Barton & Werstiuk, 1967). The low percentage of species and genera of obligate marine fungi chemically

investigated to date makes it, however, impossible to oversee the biosynthetic potential of this group of fungi.

Table 1: Reports on secondary metabolites from higher filamentous obligate marine fungi.

Fungus investigated	**Literature citation**
a) Ascomycetes	
Corollospora maritima	Liberra, Jansen & Lindequist (1998)
C. pulchella	Furuya *et al.* (1985) Alvi, Casey & Nair (1998)
Helicascus kanaloanus	Poch & Gloer (1989a)
Hypoxylon oceanicum	Schlingmann *et al.* (1998)
Kallichroma tethys	Alam *et al.* (1996)
Leptosphaeria sp.	Takahashi *et al.* (1994a and b, 1995)
L. obiones	Poch & Gloer (1989b)
L. oraemaris	Schiehser *et al.* (1986) Miller & Savard (1989) Guerriero *et al.* (1991)
Lignincola laevis	Abraham *et al.* (1994)
Zopfiella marina	Kondo *et al.* (1987)
b) Basidiomycetes	
Halocyphina villosa	Kupka *et al.* (1981)
c) Mitosporic fungi	
Asteromyces cruciatus	Shin & Fenical (1987)
Cirrenalia pygmea	Ravishankar *et al.* (1995)
Dendryphiella salina	Guerriero *et al.* (1988, 1989, 1990)

Additionally, many genera of obligate marine fungi have thus far not been investigated at all. The order Spathulosporales for example consists of algae-inhabiting species that are believed to have evolved exclusively in the sea (Kohlmeyer, 1986), in contrast to many other obligate marine fungi, e.g., members of the order Halosphaeriales, which probably have re-migrated to the oceans from the land (Spatafora, Volkmann-Kohlmeyer & Kohlmeyer, 1998). These fungi may have evolved a distinct secondary metabolism, with structural variations yet to be discovered.

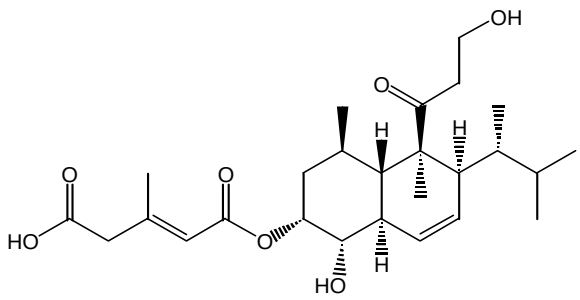
1.3.3 Secondary metabolites from sponge-derived fungi

Many reports on secondary metabolites from marine-derived fungi deal with fungal isolates from sponges. The majority of these fungi belongs to genera of mitosporic fungi typically known from terrestrial habitats, e.g., *Aspergillus* or *Trichoderma*. Biological details, e.g., about the relationship between the isolated strain and its host sponge or the number and taxonomy of fungi obtained from a particular sponge species typically are not discussed in the literature reports. Nevertheless, a growing number of metabolites from sponge-derived fungal strains has been reported in the last years. Table 2 provides an overview of sponge species investigated, taxonomy of isolated fungi, and reported metabolites. The structures of these compounds are given in Figures 1-4. These structures suggest most of the metabolites to be derived from metabolic pathways common also to terrestrial fungi. Such a similarity is, for example, obvious for sesquiterpenes of the hirsutane-type (Figures 1 and 4): Two unidentified sponge-derived fungal strains were found to produce new metabolites of this structural class, that is chloriolins A-C, hirsutanols A-C, and *ent*-gloeosteretriol (Cheng *et al.*, 1994; Wang *et al.*, 1998). One of these isolates additionally yielded coriolin B and dihydrocoriolin C, previously reported from the terrestrial fungus *Coriolus consors* (Cheng *et al.*, 1994). These findings reflect the close phylogenetic relationship between many marine-derived fungi and their terrestrial counterparts.

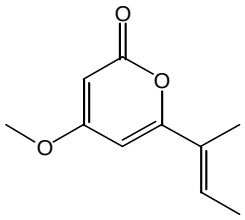
If indeed sponge-derived fungi represent a competitive source for new and biologically active natural products cannot be concluded thus far. The results already published are promising, but future prospect will rely on the isolation of more specifically adapted fungal strains producing secondary metabolites clearly distinct from those known from terrestrial species.

Table 2. Metabolites reported from sponge-derived fungi.

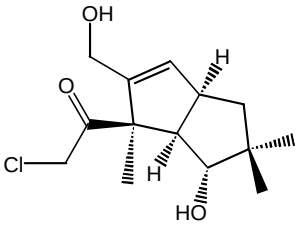
Sponge species investigated	Fungus isolated & investigated	Metabolite(s) isolated	Literature citation
Micale cecilia	*Trichoderma harzianum*	Trichoharzin	Kobayashi *et al.* (1993)
Stylotella sp.	unidentified	Nectriapyrone A-B	Abrell, Cheng & Crews (1994)
Jaspis aff. *johnstoni*	unidentified	Chloriolin A-C, coriolin B, dihydrocoriolin C	Cheng *et al.* (1994)
Taxonomy not given	*Microascus longirostris*	Cathestatin A-C	Yu *et al.* (1996a)
Jaspis cf. *coriacea*	*Aspergillus ?ochraceus*	Chlorocarolide A-B, *R*-mellein, penicillicacid, hexylitaconic acid	Abrell, Borgeson & Crews (1996a)
Agelus sp.	*Microspaeropsis olivacea*	Cerebroside	Keusgen *et al.* (1996)
		10-Methyl-(9*Z*)-octadecenoic acid, 1-*O*-(10-methyl-(9*Z*)-octadecenoyl)-glycerol	Yu *et al.* (1996b)
Mycale adhaerens	*Exophiala pisciphila*	Exophilin A	Doshida *et al.* (1996)
Spirastrella vagabunda	unidentified	Secocurvularin	Abrell, Borgeson & Crews (1996b)
Hyrtios proteus	*Aspergillus niger*	Asperazine	Varoglu *et al.* (1997)
Halichondria japonica	*Gymnascella dankaliensis*	Gymnastatin A-C	Numata *et al.* (1997a)
		Gymnasterone A-B	Amagata, Minoura & Numata (1998b)
Halichondria okadai	*Trichoderma harzianum*	Trichodenone A-C, harzialactone A-B, *R*-mevalonolactone	Amagata *et al.* (1998b)
Taxonomy not given	*Aureobasidium pullulans*	*cyclo*(D-*cis*-Hyp-L-Phe), *cyclo*(D-*cis*-Hyp-L-Leu), orcinotriol	Shigemori *et al.* (1998)
Haliclona sp.	unidentified	Hirsutanol A-C, *ent*-gloeosteretriol	Wang *et al.* (1998)



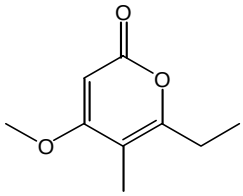
Trichoharzin



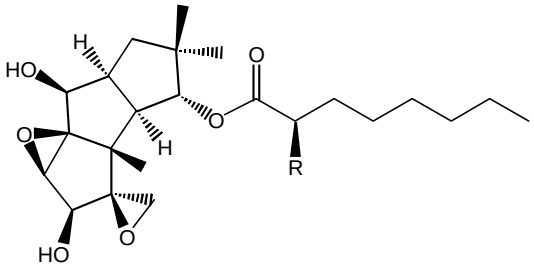
Nectriapyrone A



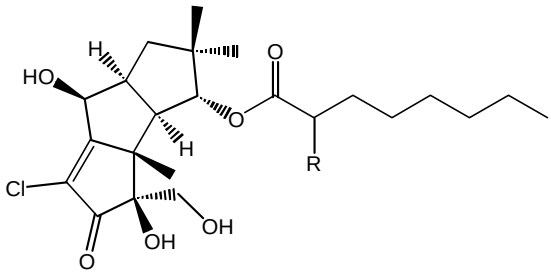
Chloriolin A



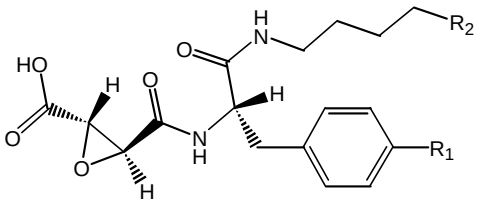
Nectriapyrone B



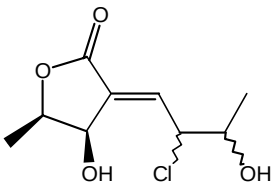
Coriolin B R = H
Dihydrocoriolin C R = OH



Chloriolin B R = OH
Chloriolin C R = H

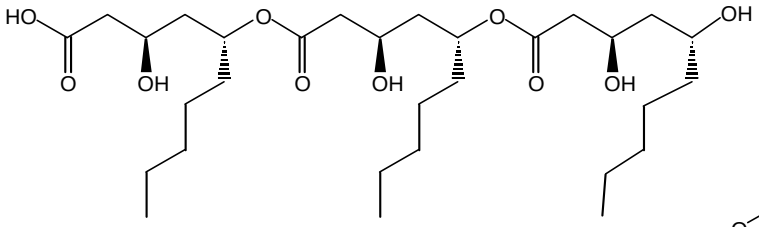


	R_1	R_2
Cathestatin A	H	NH_2
Cathestatin B	OH	NH_2
Cathestatin C	OH	CH_2NH_2

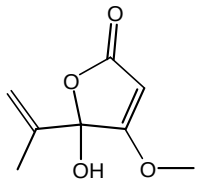


Chlorocarolide A 7*R** 8*S**
Chlorocarolide B 7*S** 8*S**

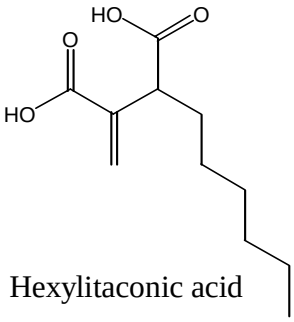
Figure 1: Metabolites from sponge-derived fungi I.



Exophilin A

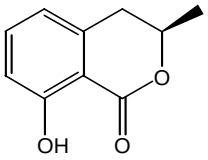


Penicillic acid

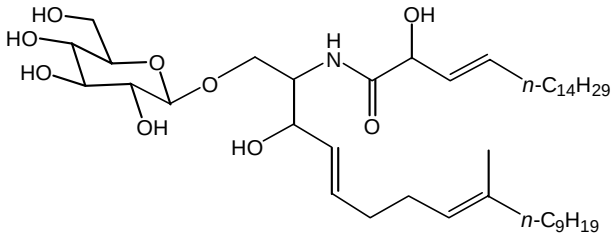


Hexylitaconic acid

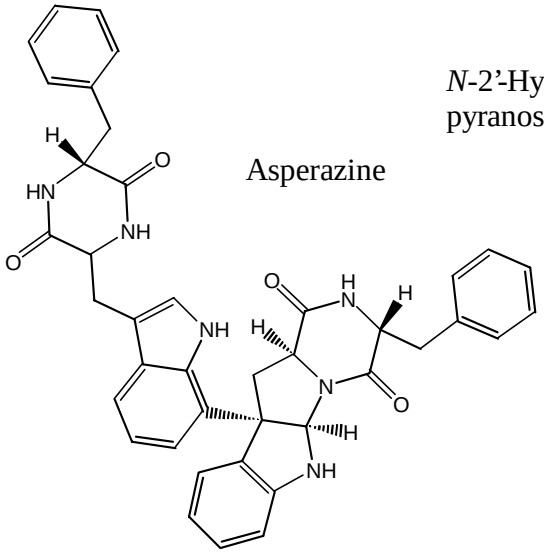
10-Methyl-(9*Z*)-octadecenoic acid R = H
1-*O*-(10-Methyl-(9*Z*)-octadecenoyl)-glycerol
R = $CH_2CH(OH)CH_2OH$



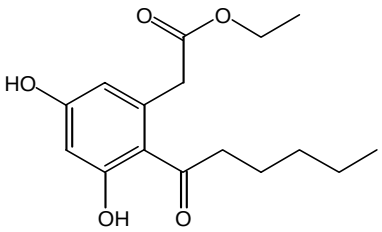
R-Mellein



N-2'-Hydroxy-(3'*E*)-octadecenoyl-1-*O*-β-D-gluco-pyranosyl-9-methyl-(4*E*,8*E*)-sphingadiene

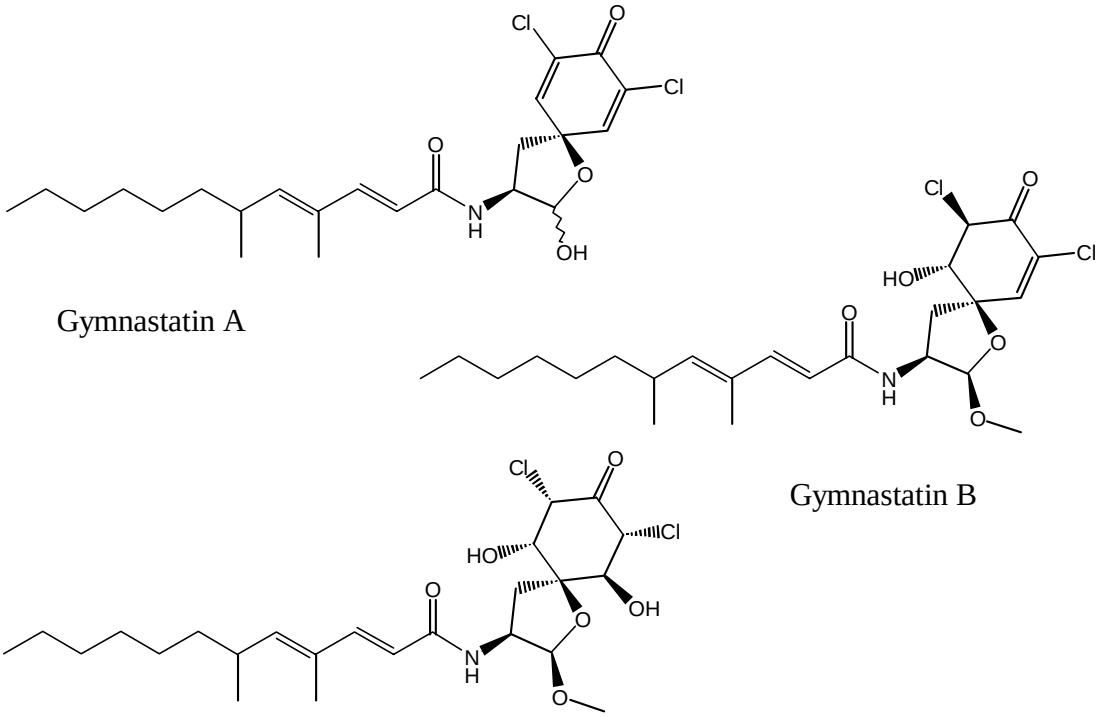


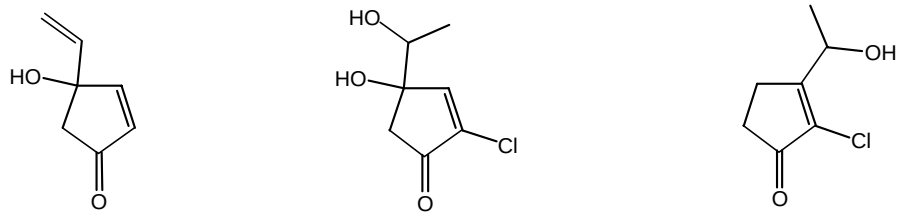
Asperazine



14,15-Secocurvularin

Figure 2: Metabolites from sponge-derived fungi II.





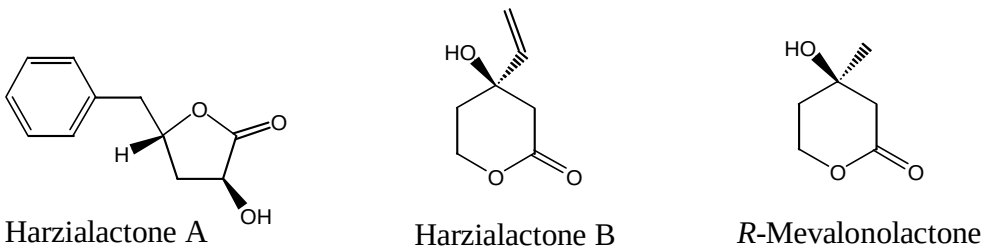
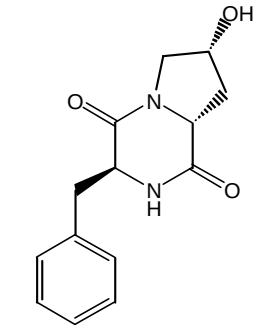
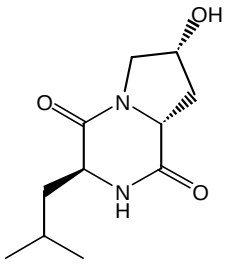


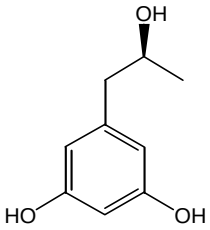
Figure 3: Metabolites from sponge-derived fungi III.



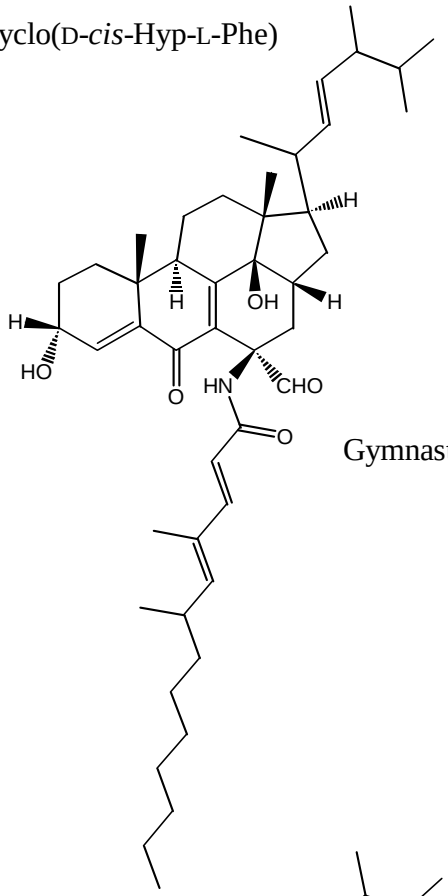
cyclo(D-*cis*-Hyp-L-Phe)



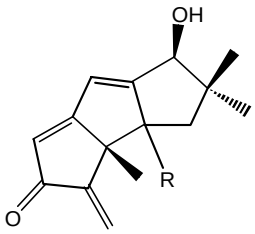
cyclo(D-*cis*-Hyp-L-Leu)



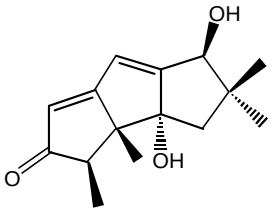
Orcinotriol



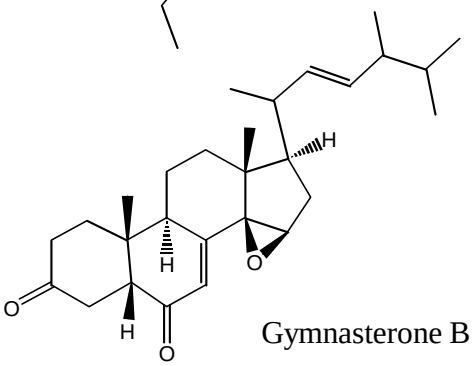
Gymnasterone A



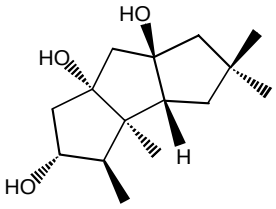
Hirsutanol A R = α-OH
Hirsutanol B R = β-OH



Hirsutanol C



Gymnasterone B



ent-Gloeosteretriol

Figure 4: Metabolites from sponge-derived fungi IV.

2 Scope of the present study

The main goal of the present investigation was the isolation of new and preferably biologically active secondary metabolites from marine organisms, with an emphasis on marine-derived fungi. Substrates for the isolation of fungi were marine sponges, and to a lesser degree algae.

The study can be divided into four main parts:

2.1 Isolation of fungi from marine sponges and algae

This part of the work aimed at finding a suitable isolation method for obtaining fungi in a great taxonomic variety from marine sponges and algae. For subsequent chemical investigations it was attempted to obtain specifically adapted strains, and to exclude terrestrial contaminants of chemically well investigated genera. The isolated fungi then were to be identified taxonomically in order to select promising strains for biological screening and chemical investigations.

2.2 Biological and chemical screening of fungal extracts

The selected strains had to be small scale cultured in order to obtain amounts of extracts suitable for the biological tests. These tests mainly consisted of agar diffusion assays for antibacterial, antifungal and antialgal activity, and ELISA based test systems for inhibition of tyrosine kinase and HIV-1 reverse transcriptase. They aimed at estimating the potential of the isolated fungi to produce biologically active metabolites, and served for the selection of strains for chemical investigations. An additional investigation by TLC should further characterise the fungal culture extracts in order to estimate number and amount of contained metabolites.

2.3 Chemical investigations of selected fungal strains and sponge species

The main purpose of the chemical investigations was to isolate pure and biologically active metabolites from fungal culture extracts. To achieve this, selected strains had to be cultured on a large-scale, extracted, and the extracts separated using diverse chromatographic methods, mainly HPLC. In order to identify biologically active compounds, a bio-assay guided isolation was attempted. The chemical investigations were completed by structure elucidation and physical characterisation of the isolated metabolites.

Additionally, some sponge samples, parts of which had been used for the isolation of fungal strains, were investigated for their secondary metabolite content.

2.4 Biological evaluation of isolated pure compounds

Natural products may serve as lead structures for new pharmaceuticals. Thus, any pure compound obtained in this study was evaluated in a diverse set of bio-assays. This included the same assays as used for the evaluation of the extracts, and additional specific assays, e.g. for antimalarial activity and cytotoxicity, performed in cooperation with other research groups, and Industry high-throughput screening (HTS).

3 Materials and methods

3.1 Animal and plant material

3.1.1 Origin and taxonomy of sponge samples

Marine sponge samples investigated in this study originated from six different locations, including temperate (Helgoland, Germany and Bear Island, Australia), subtropical (Malta and Tenerife, Spain) and tropical (Dominica and The Great Barrier Reef, Australia) regions. All samples were collected by SCUBA divers working at depths between 3 and 15 m and mainly belonged to the subclasses Tetractinomorpha and Ceractinomorpha (Demospongiae).

Samples from Helgoland were collected by the scientific dive team of the Biologische Bundesanstalt Helgoland with support of Dr. C. Schütt, samples from all other locations were collected by Prof. Dr. G. M. König and Dr. A. D. Wright. The taxonomical identification of the sponge samples was performed by Dr. R. Desqueroux-Faundez, Musée d'Histoire Naturelle Genève, (samples from Australia, Dominica, Malta and Tenerife) and Dr. D. Barthel, Institut für Meereskunde Universität Kiel (samples from Helgoland).

Samples from Australia, Dominica and Malta were frozen after collection and stored at -18°C, samples from Tenerife were stored in sterile sea water supplied with 250 mg/L benzylpenicillin and streptomycin sulphate at 4°C for one week until examination, whereas samples from Helgoland were processed immediately after collection.

a) Samples from Australia:

Biemna sp., (CT 902 U), Pelorus Island, Great Barrier Reef; *Callyspongia* sp. *cf. C. flammea* Desqueyroux-Faundez 1984 (CT 293 K), Bear Island, Sydney; *Leucosolenia challengeri* Poleajaeff 1883 (CT 293 T), Kelso Reef (Great Barrier Reef).

b) Samples from Dominica (Lauro Club Reef):

Callyspongia vaginalis Lamarck 1814 (CT 193 A); Ectyplasia perox Duch. & Mich. 1864 (CT 193 H) and *Neofibularia nolitangere* Duch. & Mich. 1864 (CT 193 I).

c) Samples from Germany (Helgoland):

Halichondria panicea Pallas 1766 (H-4); *Myxilla incrustans* Johnston 1842 (H-5); *Leucosolenia* sp., (H-6); *Sycon* sp., (H-7), and *Halichondria panicea* Pallas 1766 (H-8); the two samples of *H. panicea* are from different collection sites.

d) Samples from Malta:

Ircinia oros Schmidt 1864 (CT 912 J), and *Ircinia variabilis* Schmidt 1862 (CT 912 K).

e) Samples from Spain (Tenerife):

Aplysina aerophoba Schmidt 1862 (CT 195 T); *Petrosia ficiformis* Poiret 1789 (CT 195 V), and *Oscarella lobularis* Schmidt 1862 (CT 195 W).

3.1.2 Origin and taxonomy of algal samples

All algal samples were collected by Prof. G. M. König and/or Dr. A. D. Wright in the intertidal zones of Helgoland and Tenerife. Samples from Tenerife were stored in sterile sea water supplied with 250 mg/L benzylpenicillin and streptomycin sulphate at 4°C for one week until examination, whereas samples from Helgoland were processed immediately after collection.

a) Samples from Germany (Helgoland):

Unidentified brown or red alga (H-1); unidentified brown alga (H-2); *Plocamium* sp. (H-3).

b) Samples from Spain (Tenerife):

Cytoseira sp. (CT 195 D); unidentified red alga (CT 195 E); unidentified green or blue-green alga (CT 195 I); *Cytoseira* sp. (CT 195 L); *Hypnea ?pinnosa* (CT 195 M); unidentified brown alga (CT 195 X); unidentified brown alga (CT 195 Y).

3.2 Isolation of fungal strains

3.2.1 Preparation of animal and plant material

Small pieces of inner tissue of fresh sponge material were rinsed three times with sterile artificial sea water (ASW); both fresh and frozen sponges were then aseptically cut into small cubes, approx. $(0.5\ cm)^3$. A total of 50 - 75 cubes of each sample was placed on different isolation media. During the initial investigations, cubes from sponge sample CT 912 J were placed in EtOH (70 %) for various times between 5 and 30 s and subsequently squeezed three times in sterile ASW before inoculation.

Algal samples were rinsed three times with sterile sea water. One half of the sample was then cut aseptically into small pieces (ca. 0.5 cm^2) and placed on different isolation media. The other half was placed in EtOH (70 %) for 30 s (5 s for sample H-1, 5-10 s for samples from Tenerife), rinsed three times with sterile ASW to remove the EtOH, aseptically cut into small pieces and placed on different isolation media.

3.2.2 Media and inoculation conditions

Medium I: Biomalt agar (BIO): biomalt 20 g/L, agar 15g/L, ASW 800 mL/L.

Medium II: Malt extract soymeal agar (MS): malt extract 30 g/L, peptone from soymeal 3 g/L, agar 12 g/L, ASW 800 mL/L, pH adjusted to 5.5 with HCl prior to sterilisation.

Medium III: Potato carrot agar (KM): cooked and mashed potatoes 20 g/L, cooked and mashed carrots 20 g/L, agar 20 g/L, ASW 800 mL/L.

Medium IV: Cellulose agar: cellulose 10 g/L, yeast extract 1 g/L, agar 15 g/L, ASW 800 mL/L.

Medium V: Glucose peptone yeast extract agar (GPY): glucose × H_2O 1 g/L, peptone from soymeal 0.5 g/L, yeast extract 0.1 g/L, agar 15 g/L, ASW 800 mL/L.

Medium VI: Cornmeal agar: 42 g cornmeal was stirred in 500 mL destilled water at 60°C for 12 h, filtered and the filtrate diluted with water to 1 L. To this solution 15 g agar and the salts contained in 800 mL ASW were added.

Artificial sea water (ASW) contained the following salts (g/L): KBr 0.1, NaCl 23.48, $MgCl_2$ × 6 H_2O 10.61, $CaCl_2$ × 2 H_2O 1.47, KCl 0.66, $SrCl_2$ × 6 H_2O 0.04, Na_2SO_4 3.92, $NaHCO_3$ 0.19, H_3BO_3 0.03. All media were supplemented with 250 mg/L benzylpenicillin and 250 mg/L streptomycin sulphate to prevent bacterial growth. To some media (see below) 0.5 mg/L cyclosporine A (Sandoz) was added to inhibit fast growing fungal isolates.

Media for the inoculation of sponge samples were used as follows; samples from Australia: media I-VI, additionally media II and III without ASW; samples from Dominica: media V and VI, media IV and V with added cyclosporine A; samples from Germany: media IV and V, and the same media with cyclosporine A; samples from Malta: media I-IV, as well as media II and III without ASW, and media IV and V with cyclosporine A; samples from Spain: media V and VI, and the same media with cyclosporine A.

Samples were incubated at RT (approx. 20°C) except for those from Helgoland, which were incubated at 14°C, and regularly examined under a dissection microscope for the presence of developing fungal hyphae. Fungal colonies were transferred to one or more of the media for identification. These included media I, II, III, V and VI as above, standard nutrient agar (SNA): KH_2PO_4 1 g/L, KNO_3 1 g/L, $MgSO_4$ × 7 H_2O 0.5 g/L, KCl 0.5 g/L, glucose × H_2O 0.2 g/L, sucrose 0.2 g/L, agar 20 g/L, and starch yeast extract agar (SYA): soluble starch 15 g/L, yeast extract 4 g/L, ASW 800 mL/L, agar

20 g/L. Strains which remained sterile were further cultivated using the "balsa broth shake culture technique" (Hyde, Farrant & Jones, 1987).

Media for the inoculation of algal samples were media I-IV as above for samples from Helgoland, and media V and VI, and the same media with cyclosporine A for samples from Tenerife. Samples were inoculated at RT and further treated as described for the sponge samples.

3.2.3 Identification of fungal strains

Most fungal strains were identified by, or with kind support of, Dr. S. Draeger, Institute for Microbiology, TU Braunschweig. Strain H5-50 was identified by Dr. R. A. Samson, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Because of the great number of isolates, in most cases only the genus of the strains was determined. A species determination was attempted for strains further investigated for their secondary metabolite chemistry.

3.2.4 Origin and taxonomy of fungal strains investigated for their secondary metabolite chemistry

Fungal strains investigated chemically were isolated as described above, and are deposited in the fungal culture collection of both the research group of Prof. G. M. König and Dr. A. D. Wright, and the Institute for Microbiology, TU Braunschweig.

Strain H5-65 was isolated from the sponge *M. incrustans* (Helgoland), and identified as belonging to the genus *Acremonium.*

Strain H5-81 was isolated from the sponge *M. incrustans* (Helgoland), and identified as *Asteromyces cruciatus* Moreau et Moreau ex Hennebert 1962.

Strain 193H77 was isolated from the sponge *E. perox* (Dominica), and identified as belonging to the genus *Coniothyrium*. Morphology on SNA agar: Mycelium

superficial, partly immersed, branched, septate, hyaline. Conidiomata pycnidial, 85 - 125 µm in diameter, globose, brownish black, superficial, unilocular, wall of dark brown textura angularis. Conidiogenous cells annellidic, ampulliform, 1.7 - 2.6 × 2.2 - 6.0 µm, hyaline. Conidia (1.3) - 1.7 × 2.2 - 3.0 µm, brown, thick-walled, smooth, aseptate, cylindrical, apices obtuse.

Strain H7-65 was isolated from an unidentified sponge species of the genus *Sycon* (Helgoland), and identified as*Emericellopsis minima* Stolk 1955.

Strain K14 was isolated from the sponge *I. variabilis* (Malta), and identified as belonging to the genus *Microascus.*

Strain H5-50 was isolated from the sponge *M. incrustans* (Helgoland), and identified as belonging to the genus *Microsphaeropsis*. Morphology on SNA agar: Mycelium superficial, partly immersed, branched, septate, hyaline. Conidiomata pycnidial, 65 - 110 µm in diameter, globose, dark brown, superficial, unilocular, ostiole slightly papillate, wall of olivaceous brown textura angularis. Conidiogenous cells phialidic, cylindrical, 1.5 × 4.5 µm. Conidia 1.3 - 1.7 × 3.0 - 4.3 µm, olivaceous brown, thin-walled, aseptate, smooth, cylindrical, some slightly allantoid, apices obtuse.

Strain 193A20 was isolated from the sponge *C. vaginalis* (Dominica), and identified as belonging to the genus *Monochaetia*. On the occasion of a re-examination of this strain by Dr. R. Emmens, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, no sporulation occurred.

Strain H4-77 was isolated from the sponge *H. panicea* (Helgoland), and identified as belonging to the genus *Phoma*. On the occasion of a re-examination of this strain no sporulation occurred.

Strain H7-19 was isolated from an unidentified sponge species of the genus *Sycon* (Helgoland), and identified as*Scopulariopsis candida* (Guéguen) Vuillemin 1911.

Strain 193A4 was isolated from the sponge *C. vaginalis* (Dominica), and identified as *Ulocladium botrytis* Preuss 1851.

Strain 195-31 was isolated from an unidentified species of brown alga of the genus *Cytoseira* (Tenerife), and identified as *Varicosporina ramulosa* Meyers et Kohlmeyer 1965.

3.2.5 Preparation and maintenance of stock cultures

All fungal strains were kept on two different media in test-tubes in duplicate at 4°C:

Medium 1 (One tube per strain, 7 mL medium, sealed with cotton wool): Biomalt 50 g/L, agar 20 g/L, dest. H_2O 1 L.

Medium 2 (One tube per strain, 7 mL medium, sealed with aluminium cap and parafilm): Biomalt 50 g/L, agar 20 g/L, ASW 800 mL, dest. H_2O 200 mL.

Strains for further chemical investigations were kept in additional five tubes with medium 1 (medium 2 for obligate marine species), sealed with aluminium-cap and parafilm. Slants were prepared and inoculated using standard microbiological techniques.

3.3 Culture and extraction of selected fungal strains

3.3.1 Strains for screening purposes

Isolates were inoculated on two or three (see 8.3 for details) of the following four media (50 mL each) for 14 days at RT (20°C):

Medium A (B): biomalt 20 g/L, agar 6.8 g/L.

Medium B (Bs): biomalt 20 g/L, ASW 800 mL/L, agar 6.8 g/L.

Medium C (Bfl): biomalt 20 g/L, ASW 800 mL/L.

Medium D (MS): Malt extract soymeal agar (see 3.2.2) with 7.6 g/L agar.

Liquid cultures were shaken on an Infors Novotron rotary shaker at 65 rpm. After two weeks, cultures were homogenised using an Ika Ultra-Turrax T 25 at 8000 rpm for 2 min. 50 mL of water were added to solid cultures prior to homogenisation. Resultant mixtures were extracted with EtOAc (3 × 50 mL), the organic fractions were combined, and the solvent removed at reduced pressure and 30°C. Residues were redissolved in 2 mL of a mixture of acetone/MeOH (1:1; v/v) and this solution was then used for the agar diffusion assays and TLC. 28 selected cultures were extracted successively with the same amounts of *n*-BuOH (see 8.3). For the ELISA based assays 500 µg extract was dissolved in 250 µL DMSO to give the appropriate sample solutions.

3.3.2 Strains for chemical investigations

Strains were cultured in penicillium or fernbach flasks containing 0.5 or 0.25 L solid medium respectively. Liquid cultures were grown in 1 L erlenmeyer flasks with indentations containing 0.5 L medium, and shaken on an Infors Novotron rotary shaker at 65 rpm. Media were inoculated with small mycelium plugs from stock culture (liquid media) or a suspension of mycelium from small scale cultivations (one petri dish per five penicillium flasks) in water (solid media).

Media used for cultivation were biomalt agar, malt extract soymeal (MS) agar (3.2.2), and starch yeast extract agar (soluble starch 15 g/L, yeast extract 4 g/L, agar 20 g/L). If not stated otherwise, solid media contained 6.8 g/L (biomalt agar) and 7.6 g/L (MS agar) agar; media with ASW contained 80 % ASW.

The isolate of *A. cruciatus* (H5-85) was cultured on solid medium (Biomalt agar with 20 g/L agar) and starch yeast extract agar (20 g/L agar), both with ASW; 2.5 L each at RT for three months.

The *Coniothyrium* strain (193H77) was cultured in liquid biomalt medium with ASW (7.5 L) at RT for 16 days.

The isolate of *E. minima* (H7-65) was cultured on solid medium (biomalt agar 4.25 L and MS agar 4.5 L) at RT for three months.

The *Microascus* strain (K14) was cultured on solid medium (biomalt agar 0.75 L and MS agar 0.75 L, both with ASW) at RT for six weeks.

The *Microsphaeropsis* strain (H5-50) was cultured on solid biomalt medium (4.5 L) at RT for 40 days.

The *Monochaetia* strain (193A20) was cultured on solid medium (MS agar with ASW, 5.75 L) at RT for five weeks.

The *Phoma* strain (H4-77) was cultured on solid medium (Biomalt agar with ASW, and MS agar with ASW; 1.5 L each) at RT for 9 weeks.

The isolate of *S. candida* (H7-19) was cultured in liquid biomalt medium with ASW (7.5 L) at RT for one month.

The isolate of *U. botrytis* (193A4) was cultured on solid biomalt medium (1 L) at RT for 14 days.

The isolate of *V. ramulosa* (195-31) was cultured on solid biomalt medium with ASW (6.5 L) at RT for five weeks.

If not stated otherwise (see 4.4) medium and mycelium were homogenised together using a Waring blender. To cultures on solid medium, 500 mL water were added to 1 L medium prior to homogenisation. Resultant mixtures were subsequently extracted with EtOAc (3×300 mL per 1 L medium) to yield crude extracts.

3.4 Biological testing

3.4.1 Agar diffusion assays

Agar diffusion assays were carried out according to Schulz *et al.*, (1995). Test organisms were the bacteria *Bacillus megaterium* de Bary (gram positive) and *Escherichia coli* (Migula) Castellani & Chambers (gram negative), the fungi *Microbotryum violaceum* (Pers.) Roussel (Ustomycetes), *Mycotypha microspora* Fenner (Zygomycetes), *Eurotium repens* Corda (Ascomycetes) and *Fusarium oxysporum* Schltdl. (mitosporic fungi), and the alga *Chlorella fusca* Shih Krauss (Chlorophyceae).

If not stated otherwise, sample solutions contained 5 mg/mL extract or 1 mg/mL pure compound. Samples were prepared by taking 50 µL of each solution and pipetting it onto a sterile antibiotic filter disk (Schleicher & Schuell 2668), which was then placed onto the appropriate agar medium and sprayed with a suspension of the test organism. Growth media, preparation of spraying suspensions, and conditions of incubation were as employed by Schulz *et al.*, (1995). The radii of the resultant zones of inhibition were measured from the edge of the filter disks. For extracts, a growth inhibition zone ≥ 3 mm and/or a complete inhibition ≥ 1 mm were regarded as a positive result; growth inhibition: growth of the appropriate test organism was significantly inhibited compared to a negative control; complete inhibition: no growth at all in the appropriate zone.

3.4.2 Tyrosine kinase inhibitory activity

The DMSO sample solution (see 3.3.1) of the appropriate extract/pure compound was diluted with H_2O (1:1 v/v) to yield corresponding sample solutions. Pure compounds, if active, were tested at various concentrations.

TK inhibitory activity was determined using a commercial test kit (Tyrosine Kinase Assay Kit, non-radioactively, Boehringer Mannheim, Cat. No. 1 534 513), modified by Ms. G. F. Matthée. Assays were carried out by either Ms. G. F. Matthée or

Ms. I. Rahaus using T cell tyrosine kinase $p56^{lck}$ (Upstate Biotechnology). Sample solutions were incubated with 1 µM TK substrate II, biotin-labelled (Boehringer Mannheim), 1 mM ATP, 10 mM $MgCl_2$, 1 U TK $p56^{lck}$, 20 µL dilution buffer, and 20 µL assay buffer for 1 hour at 30°C. The resultant concentration of the extract/compound in the test mixture was 200 µg/mL. Dilution buffer (pH 7.0) contained 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 10 % glycerol, and 0.1 % ethylphenolpoly(ethylene-glycolether)$_n$ (NP-40). Assay buffer (pH 7.5) contained 250 mM Tris, 25 mM NaF, 2.5 mM EDTA-Na_2, 4.0 mM [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA), 5 mM DTT, and 150 µM Na_3VO_4. The enzyme activity was determined as described in the TK Assay Kit, except the measuring wavelength was 415 nm instead of 405 nm. In each test series 3 mM piceatannol (Boehringer Mannheim) was included as a positive control. Samples which reduced the enzyme activity by 60 % or more relative to a negative control were regarded as active.

3.4.3 HIV-1 reverse transcriptase inhibitory activity

The assay was performed by Mr. C. Dreikorn or Ms. G. F. Matthée according to a protocol established by the latter. DMSO standard solutions (see 3.3.1) of the appropriate extracts/compounds were diluted with lysis buffer (1:10 v/v) to yield corresponding sample solutions.

HIV-1 RT inhibitory activity was measured using 20 µL of the sample solution, 20 µL lysis buffer, and 20 µL reaction mixture which were then incubated for 1 hour at 37°C. The resultant concentration of the extract in the test mixture was 66 µg/mL. Lysis buffer (pH 7.8) contained 1 ng recombinant HIV-1 RT (Boehringer Mannheim), 50 mM Tris, 80 mM KCl, 2.5 mM dithiothreitol (DTT), 0.75 mM ethylendiamintetra-acetic acid (EDTA), and 0.5 % Triton-X100. The reaction mixture contained template/primer hybrid poly(A)*oligo(dT)15 (600A260nm/mL), 8 µM thymidine-5'-triphosphate (dTTP), 8 µM digoxigenin- and biotin-labeled-2'-deoxy-(uridine-5'-

triphosphate) (dUTP), 40 µM TrisHCl, 230 mM KCl, 24 mM $MgCl_2$, and 8 mM DTT (all Boehringer Mannheim).

Enzyme activity was measured following the ELISA protocol described by Eberle & Seibl (1992) at λ = 415 nm versus λ = 490 nm using an ELISA reader (Rainbow, SLT Labinstruments Deutschland GmbH). As a positive control, 10 µM of phosphonoformic acid (Sigma) was included in each test series. Extracts which reduced the enzyme activity by 20 % or more relative to a negative control were regarded as active.

3.4.4 Inhibition of *Mycobacterium tuberculosis*

Activity of extracts against *M. tuberculosis* H_{37}Rv (ATCC 7294) was tested in cooperation with the research group of Prof. S. Franzblau, U.S. Department of Health and Human Services, GWL Hansen's Disease Center, Baton Rouge, Louisiana, USA, in the BATEC 460 system (Collins & Franzblau, 1997). Percent inhibition was calculated as [1-(growth index of test sample/growth index of control)] × 100.

3.4.5 Cytotoxicity

Cytotoxicity data of extracts towards KB cells were provided by Prof. Dr. C. K. Angerhofer, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, USA. Tests were performed as previously published (Likhitwiayawuid*et al.*, 1993).

3.4.6 Antimalarial activity

Antimalarial activity of pure compounds was determined by Dr. N. Lang-Unnasch, Division of Geographic Medicine, University of Alabama at Birmingham, USA. Tests were performed as previously published (Angerhofer*et al.*, 1992).

3.5 Chromatography

3.5.1 Thin layer chromatography (TLC)

TLC was carried out using either TLC aluminium sheets silica gel 60 F_{254} (Merck 5554) or pre-coated TLC plates SIL RP-18W / UV 254 (Macherey-Nagel).

Standard chromatograms of fungal extracts were prepared by applying 20 µL solution to a silica gel TLC plate and developing it with DCM/MeOH (98/2; v/v) under saturated conditions.

Chromatograms were detected under UV-light (254 and 366 nm), and with vanillin-H_2SO_4 reagent (1 g vanillin dissolved in 100 ml H_2SO_4, heated at 100°C after spraying).

3.5.2 Vacuum-liquid chromatography (VLC)

Sorbents for VLC were either silica gel 60 (70-230 mesh ASTM, Merck 7734; normal-phase) or Polyprep 60-50 C18 (Machery-Nagel 71150; reversed-phase). Columns were filled with the appropriate sorbent soaked with hexane or MeOH. Before applying the sample, the column was equilibrated with the first designated eluent.

3.5.3 High performance liquid chromatography (HPLC)

HPLC was carried out either using a Merck-Hitachi system equipped with a L-6200A Intelligent Pump, a L-4500A diode array detector, a D-6000A interface with D-7000 HSM software, a Rheodyne 7725i injection system, or a system equipped with a Waters associates chromatography pump, a Knauer Differential refractometer, a Rheodyne 7725i injection system and a Linseis L 200 E recorder. If not stated otherwise, the system with differential refractometer as detector was used.

Columns used were either a Knauer Spherisorb S ODS 2 (5 µm, 250 × 8 mm), a Merck LiChrospher Si 60 (5 µm, 250 × 7 mm), or similar columns. Typical flow rates were 1.5 mL/min (reversed-phase) and 2.0 - 3.0 mL/min (normal-phase).

All solvents, except H_2O, were distilled prior to use. The eluents were degassed under reduced pressure. If not stated otherwise, samples were diluted in the eluent to yield solutions of 20 - 40 mg/mL. Injected amounts usually equalled 10 mg of extract/run.

3.6 Structure elucidation

Structures were elucidated mainly using one and two dimensional NMR techniques and various MS methods. If necessary, additional parameters such as optical rotation, UV and IR properties were determined. Identity of isolated compounds with compounds previously published ("known compounds") was judged, if not stated otherwise, based on ^{1}H and ^{13}C NMR spectroscopic data, and specific optical rotation. For some known compounds, mainly those first reported before 1980, no or no complete NMR data have been published. Furthermore, in some cases the published values were found to be insufficient to prove the identity of an isolated metabolite with the published compound in question. In these cases, a complete structure elucidation was performed. Literature searches were done using Chapman & Hall Natural Products on CD-ROM (Buckingham, 1996) and Beilstein on-line databases. Structures were designated as new, if they could not be found in Chemical Abstracts.

3.6.1 NMR spectroscopy

^{1}H NMR spectra of extracts and pure compounds as a purity check were recorded at the Department of Chemistry, TU Braunschweig, on a Bruker AC-200 spectrometer operating at 200 MHz or a Bruker AM-400 spectrometer operating at 400 MHz (with CD_3OD as solvent) by Ms. P. Holba-Schulz and Ms. I. Rübesamen.

^{1}H NMR spectra of pure compounds and all other NMR measurements were performed at the GBF Braunschweig by Dr. V. Wray, Ms. B. Jaschok-Kentner and

Ms. C. Kokoschke on Bruker DPX-300, ARX-400 or DMX-600 NMR spectrometer operating at 300, 400 or 600 MHz (^{1}H), and 75.5, 100 or 125 MHz for ^{13}C, respectively.

Spectra of pure compounds were processed using Bruker 1D WIN-NMR or 2D WIN-NMR software. They were calibrated using solvent signals (^{13}C: $CDCl_3$ 77.00 ppm, CD_3OD 49.00 ppm, $\underline{C}D_3CO\underline{C}D_3$ 30.50 ppm, DMSO-d6 39.70 ppm) or a signal of the portion of the partly or not deuterated solvent (^{1}H: $CHCl_3$ in $CDCl_3$ δ 7.26, HDO in D_2O δ 4.79, CH_3OD in CD_3OD δ 3.35, DMSO in DMSO-d6 δ 2.50, acetone in acetone-d6 δ 2.05). Multiplicity for ^{13}C was deduced from DEPT experiments; s = C, d = CH, t = CH_2, q = CH_3. Structural assignments were based on spectra resulting from one or more of the following NMR experiments: ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C direct correlation (HMQC), ^{1}H-^{13}C long-range correlation (HMBC), ^{1}H-^{1}H ROESY and ^{1}H difference NOE.

3.6.2 Mass spectrometry

Mass spectral measurements were performed by Dr. H.-M. Schiebel, Dr. U. Papke and Ms. D. Döring (all Department of Chemistry, TU Braunschweig) using a Finnigan MAT 8430 spectrometer (EI, 70 eV; CI; FAB, Xe). Selected measurements were performed by Dr. R. Christ, GBF Braunschweig, on a Finnigan MAT 95 spectrometer: DCI-MS of compound **8**, HREIMS of compound **9**, CIMS (NH_3) of compound **17**.

3.6.3 UV and CD measurements

UV spectra were recorded on a Shimadzu UV-200S double beam spectrophotometer coupled with a Kipp & Zonen BD-40 recorder using 1.000 cm quartz cells. The CD-spectrum of **46a** was recorded by Ms. H.-S. Cho, Institute for Pharmaceutical Technology, TU Braunschweig, on a Jasco J-715 spectropolarimeter using EtOH as solvent. Cell length was 0.500 cm.

3.6.4 IR spectroscopy

IR spectra were recorded by Ms. P. Reich and Mr. T. Kroker, Institute for Pharmaceutical Chemistry, TU Braunschweig, on an ATI Mattson Genesis Series FTIR. Samples were measured on NaCl (film).

3.6.5 GC and GC-MS methods

Qualitative determination of D-arabitol, D-mannitol and D-trehalose from *A. cruciatus* was performed after derivatisation with MSTFA using an Intersmat IGC 120 FL GC-system, equipped with a 15 m DB-1 capillary column and a Shimadzu C-R1B integrator. GC-conditions: injection block 250°C, detector 300°C, He 75 kPa, oven temperature 100-300°C (6°C/min), split 1:20. To < 250 µg of each hydrocarbon sample 50 µL MSTFA was added, mixture kept at 80°C for 30 min and directly injected; injected volume ~0.5 µL. Retention times of authentic material: meso-erythritol 8.6 min, D-arabitol 12.7 min, D-xylose 12.8 min, D-mannitol 13.7 min, D-trehalose 28.2 min.

GC-MS analysis were performed by Dr. L. Witte using a Carlo-Erba HRGC 5160 linked to a Finnigan MAT 4515 spectrometer (40 eV). The GC was equipped with a DB1-30W column (30 m × 0.32 mm i.D., stationary phase 0.25 µm; split 1:20). Oven temperature program was modified for each individual sample but usually started at 100°C, increasing 6°C/min.

3.6.6 Optical rotation

Optical rotations were measured using a Perkin Elmer 241 Polarimeter equipped with an 1 mL cell, cell length 10.000 cm.

3.6.7 Melting point

Melting points were recorded on a Wena thermoblock apparatus heated by a small Bunsen burner and are not corrected.

3.6.8 Single crystal X-ray analysis

The single crystal X-ray analysis of colletoketol (**43**) was performed by Prof. P. G. Jones, Institute for Inorganic and Analytical Chemistry, TU Braunschweig. Crystals were grown from EtOAc.

3.7 Chemical derivatisations

3.7.1 Methylation of variabilins

Methylation was carried out in a MNNG diazomethane-generating apparatus (Aldrich Z10,100-1) using the conditions described by Ngan & Toofan (1991). The sesterterpene tetronic acids containing fraction (250 mg) was dissolved in 5 mL diethyl ether/EtOAc 75/25 in the outer tube. In the inner tube 1 mL diethyl ether, 1 mL diethylene glycol monoethyl ether and 0.4 g *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald®, Aldrich D2,800-0) were placed. The lower part of the apparatus (up to the solvent level in the outer tube) was immersed in an ice water bath. Reaction was started by injecting dropwise 1.5 mL 37% KOH through the septum into the inner tube. The apparatus was gently shaken every 5 min by hand. After a total reaction time of 30 min, surplus Diazald® was destroyed by gently injecting 2 mL 30% acetic acid into the inner tube. Diethyl ether and surplus diazomethane were evaporated out of the opened apparatus in a waterbath at 40 °C in a fume hood. Methylation occurred to 100% as monitored by normal-phase TLC (acetone/hexane 30/70).

3.7.2 *p*-Bromo-benzoylation of 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol

5 mg **46**, 15 mg *p*-bromobenzoyl chloride (Fluka 16450) and 3 mg 4-(dimethyl-amino)pyridine (Schuchard 820499) were dissolved in 2 mL DCM in a pear shaped flask sealed with a plastic stopper. The mixture was kept in an ultrasonic bath for 4 h; a warming up to 35°C occurred during this time. The resulting mixture was separated from excess *p*-bromobenzoyl chloride by normal-phase VLC (hexane/EtOAc 50/50), followed by normal-phase HPLC (hexane/EtOAc 80/20) to yield 9,10-dihydro-11,12-di-*p*-bromobenzoate-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46a**, 8.8 mg) as a clear oil.

3.7.3 Hydrogenation of macrodiolides

31 mg (6*R*,11*R*,12*R*,14*R*)-colletodiol (**44**) was dissolved in 5 mL EtOAc and hydrogenated with H_2 after addition of 5 mg 10% Pd/C under stirring for 2 h. Pd/C was removed by normal-phase VLC (acetone). The reaction mixture was further separated by normal-phase HPLC (acetone/petrol ether 50/50) to yield 3,4,9,10-tetrahydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (8.0 mg) and (5*R*)-((1'*R*,3'*R*)-dihydroxybutyl)-dihydrofuran-2-one (8.9 mg).

9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46**, 5.2 mg) was hydrogenated analogously to yield 4.8 mg 3,4,9,10-tetrahydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol after VLC. Decomposition started during the reaction and proceeded almost completely in $CDCl_3$ solution in the NMR tubes during three weeks. The decomposition products were separated by HPLC (as above) to yield 1.1 mg (5*R*)-((1'*R*,3'*R*)-dihydroxybutyl)-dihydrofuran-2-one.

3.7.4 Hydrolysis of colletoketol

To 43 mg colletoketol (**43**), 20 ml 0.05 n NaOH were added. The mixture was stirred for 24 h at RT, gently acidified with conc. HCl and extracted with diethyl ether (5×50 ml). The combined organic layers were dried over Na_2SO_4, the solvent evaporated and the residue subjected to preparative TLC (Si-60, three plates,

EtOAc/petrol ether/acetic acid 45/50/5). A blue zone (UV 254 nm) at Rf 0.45 was extracted with EtOAc to yield (5*R*)-hydroxy-(2*E*)-hexenoic acid (10.5 mg).

3.8 Chemicals

Media components:

Agar (Fluka 05040)
Biomalt (Villa Natura, Kirn)
Malt extract (Merck 5391)
Peptone from casein, tryptic digest (Fluka 70172)
Peptone from meat, enzymatic digest (Fluka 70175)
Peptone from soymeal, papain-digested (Merck 7212)
Yeast extract (Fluka 70161)
Benzylpenicillin (Fluka 13750)
Streptomycin sulfate (Fluka 85880)
Carrots, potatoes and cornmeal were supplied by local food stores.
Water used was de-ionised using a Millipore (milli-Q®) system.

All other chemicals/components were research grade, and if not stated otherwise in the text, supplied by Merck.

Solvents:

EtOH for UV-measurements was from Merck (Uvasol® 980). All other solvents were research grade and supplied by Merck, except *n*-BuOH (Roth). Water used was deionised using a Millipore (milli-Q®) system. Acetone, $CHCl_3$, DCM, EtOAc, hexane, MeOH and petrol ether were distilled prior to use.

Aceton-d6 (Chemotrade 97509, 99.8 % D)
Chloroform-d (Chemotrade, 99.87 % D)
Deuteriumoxid (Merck 518, 99.75 % D)
Dimethylsulphoxid-d6 (Merck Sharp & Dohme MD-1313, 99.5 % D)
Methanol-d4 (ICB 0844-25, 99.8 % D)

4 Results

4.1 Isolation and taxonomy of fungal strains

4.1.1 Fungal isolates from sponges

The main purpose for the isolation of fungi from marine sponges in this study was their subsequent investigation for the production of new and biologically active metabolites. It was thus aimed to obtain a great variety of preferably adapted strains, and to exclude terrestrial contaminants of ubiquitous fungal genera which are well investigated chemically. This was attempted either by destroying contaminants with surface sterilisation or by the use of media inhibiting the growth of these fungi.

Surface sterilisation with EtOH (70 %) was attempted with the sponge *Ircinia variabilis*, and showed that this method is not suitable for sponges. The EtOH could not be removed completely in a defined time, and obviously damaged the sponge tissue after about 10 s. As a sterilisation time of 10 s is surely not sufficient to destroy all fungal spores on a sponge surface, surface sterilisation was not further applied to sponge samples.

During the initial investigation with sponge samples from Malta, small pieces of the inner tissue of the sponges were incubated on the nutrient rich media I-IV. The first fungal colonies could be detected after four to five days as they developed on individual sponge segments. The isolation media quickly became dominated by fast growing fungi such as *Aspergillus* and *Penicillium* spp., even on media supplemented with ASW. To reduce the growth of such fungi, nutrient poor media V and VI, suitable for the isolation of marine fungi, were used and supplemented with cyclosporine A (Dreyfuss, 1986). With these media the resulting fungal colonies remained discrete and did not overgrow the entire agar medium, thus allowing slow growing strains of different genera to be detected. To ensure that as many slow growing fungi as possible were obtained, the size of the sponge pieces was reduced until some remained from which no fungal colonies developed. This protocol was then used for all sponge samples.

From 16 sponge samples a total of 681 fungal strains were isolated. Due to the great number of isolates and the difficulties of taxonomic identification for some species of the genera of mitosporic fungi (e.g., the genus *Phoma*), in most cases only the genus was determined. A different morphology on the same growth medium suggested the isolates of many genera to represent several species. An overview of the genera obtained and the number of isolates from each sponge sample is given in Table 3.

Of the employed media, none selectively inhibited the growth of ubiquitous fungi such as *Aspergillus* and *Penicillium* spp. Since the use of different isolation media resulted only in a quantitative difference in fungal isolates obtained, the results in Table 3 are not differentiated as to the media used.

The isolated fungi were identified as belonging to 13 genera of Ascomycetes, two genera of Zygomycetes and 38 genera of Mitosporic fungi; 37 strains remained sterile even though diverse media and conditions of culture were used to induce sporulation. The only clearly obligate marine fungi (Kohlmeyer & Volkmann-Kohlmeyer, 1991) isolated were *Phialophorophoma litoralis* Linder from the sponge *Aplysina aerophoba* from Tenerife and *Asteromyces cruciatus* Moreau et Moreau ex Hennebert from the sponges *Halichondria panicea and Myxilla incrustans* from Helgoland. Since some genera of mitosporic fungi, e.g., *Cladosporium, Coniothyrium* and *Phoma* are known to have marine representatives (Kohlmeyer & Volkmann-Kohlmeyer, 1991), it is likely that the real number of obligate marine fungi obtained was somewhat higher. This was supported by the finding that 11 *Phoma* sp. originating from sponge samples from Helgoland sporulated only on media supplemented with salt.

Many genera of fungi were found in samples from only one or two collection sites (Table 3), e.g. fungi of the genera *Arthrinium* and *Niesslia* were almost exclusively isolated from sponges from Helgoland and those of the genus *Eurotium* from sponge samples from Malta. In contrast, fungi of the genera *Acremonium, Cladosporium, Fusarium* and *Penicillium* were isolated from sponges from almost every location. Fungi of other genera, e.g., *Aplosporella, Monochaetia,* and *Myrothecium* were obtained exclusively from single sponge samples.

Table 3. Genera and total number of fungal isolates obtained from marine sponge samples by incubation of sponge cubes on different media.

Sponge sample[1] ---------- **Fungal genera**	***Callyspongia* sp. *cf. C. flammea* (A)**	***Callyspongia vaginalis* (D)**	***Ectyplasia perox* (D)**	***Neofibularia nolitangere* (D)**	***Halichondria panicea*, H-4 (H)**	***Myxilla incrustans* (H)**	***Leucosolenia* sp. (H)**	***Sycon* sp. (H)**	***Halichondria panicea*, H-8 (H)**	***Ircinia oros* (M)**	***Ircinia variabilis* (M)**	***Aplysina aerophoba* (T)**	***Petrosia ficiformis* (T)**	***Oscarella lobularis* (T)**	**total number of fungal isolates**
a) Ascomycetes															
Chaetomium	-	-	-	-	4	-	-	1	-	1	1	-	-	-	7
Emericella	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Emericellopsis	-	-	-	-	1	3	-	2	-	-	-	-	-	-	6
Eupenicillium	1	-	-	-	-	-	-	1	-	-	-	-	-	-	2
Eurotium	-	-	-	-	-	-	-	-	-	8	4	-	-	-	12
Leptosphaeria	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Microascus	-	-	-	-	-	-	1	1	-	-	1	-	-	-	3
Monascus	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Myxotrichum	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Niesslia	-	-	-	-	4	1	2	3	1	-	-	-	-	-	11
Preussia	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Sporormiella	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Talaromyces	-	-	1	-	-	-	-	-	-	1	-	-	-	-	2
b) Zygomycetes															
Mucor	-	-	-	-	4*	5*	-*	6*	-*	-	-	-	-	-	15
Syncephalastrum	-	-	-	-	-	-	-	-	-	2	1	-	-	-	3
c) Mitosporic fungi															
Acremonium	1	4	3	9	8*	15*	24*	11*	6*	-	1	1	1	4	88
Alternaria	-	1	-	1	-	-	-	1	1	-	-	-	-	-	4
Aplosporella	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2
Arthrinium	-	-	-	-	13*	13*	7*	7*	10*	2	-	-	-	-	52
Aspergillus	-	-	-	-	-	-	-	-	1	26	24	-	1	-	52
Asteromyces	-	-	-	-	8	1	-	-	-	-	-	-	-	-	9
Beauveria	-	-	1	-	-	1	-	-	1	-	-	-	-	-	3
Botrytis	1	-	-	-	-	-	1	-	-	-	-	-	-	-	2
Cladosporium	-	15	3	-	-	2	-	2	-	3	-	1	-	2	28
Clamnopygnis	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Coniothyrium	-	-	8	1	8	9	-	8	10	-	-	-	-	-	44
Doratomyces	-	1	2	-	1	1	3	-	-	-	-	-	-	-	8
Drechslera	-	-	-	-	2	1	-	-	1	-	-	-	-	-	4

Table 3 continued

Sponge sample[1] ---------- **Fungal genera**	***Callyspongia* sp. *cf. C. flammea* (A)**	***Callyspongia vaginalis* (D)**	***Ectyplasia perox* (D)**	***Neofibularia nolitangere* (D)**	***Halichondria panicea*, H-4 (H)**	***Myxilla incrustans* (H)**	***Leucosolenia* sp. (H)**	***Sycon* sp. (H)**	***Halichondria panicea*, H-8 (H)**	***Ircinia oros* (M)**	***Ircinia variabilis* (M)**	***Aplysina aerophoba* (T)**	***Petrosia ficiformis* (T)**	***Oscarella lobularis* (T)**	**total number of fungal isolates**
Epicoccum	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Fusarium	2	1	1	-	13*	3*	5*	5*	6*	-	-	-	2	-	38
Geomyces	-	-	-	-	1	1	-	-	1	1	-	-	-	-	4
Geotrichum	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Gliocladium	-	-	1	-	2	-	-	1	1	-	-	-	-	-	5
Gonatobotrys	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Microsphaeropsis	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Moniliella	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Monochaetia	-	3	-	-	-	-	-	-	-	-	-	-	-	-	3
Myrothecium	-	3	-	-	-	-	-	-	-	-	-	-	-	-	3
Oidiodendron	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Paecilomyces	-	-	1	-	-	6	11	-	2	-	-	-	1	2	23
Penicillium	4	1	3	-	-*	3*	4*	4*	1*	9	13	1	2	-	45
Phialophorophoma	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Phoma	-	12	37	5	7*	14*	6*	2*	1*	-	-	-	-	-	83
Scolecobasidium	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Scopulariopsis	-	-	-	-	-	1	1	3	-	8	-	-	-	-	13
Sporothrix	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Stachybotrys	-	-	-	-	-	-	-	1	-	1	-	-	-	-	2
Stachylidium	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Tolypocladium	-	-	-	-	-	1	1	1	1	-	-	-	-	-	4
Trichoderma	-	3	3	-	-*	5*	-*	1*	4*	-	-	-	-	-	16
Ulocladium	-	1	-	3	-	-	-	-	-	-	-	-	-	-	4
Verticillium	-	1	1	-	4*	3*	4*	4*	5*	3	-	-	-	-	25
Mycelia sterilia	3	3	12	11	3	-	1	2	2	-	-	-	-	-	37

[1] Origin of the sponge samples: (A) Australia, (D) Dominica, (H) Helgoland, (M) Malta, (T) Tenerife; no fungal isolates were obtained from the sponges *Leucosolenia challengeri* and *Biemna* sp. from Australia.

*Due to the great number of isolates from the genera *Acremonium*, *Arthrinium*, *Fusarium*, *Mucor*, *Penicillium*, *Phoma*, *Trichoderma* and *Verticillium* from the samples from Helgoland, numbers in the table marked with an asterisk represent only those species which were retained in pure culture; - no isolates obtained; -* no isolates retained.

The taxonomic spectrum of fungal isolates, and the number of isolates showed some correlations for sponge samples of the same location. Sponge samples from Australia in general yielded very few fungi. The sponge *Callyspongia* sp. cf. *C. flammea* contained a few fungi, but *Leucosolenia challengeri* and *Biemna* sp. did not. Isolates from the sponge samples from Dominica mainly belonged to the genera *Acremonium* and *Phoma*, and to a lesser extent to the genera *Cladosporium* and *Coniothyrium*. From these samples no *Aspergillus* spp., and only few *Penicillium* spp., were isolated, whereas the number of Mycelia sterilia (16 %) was high compared to other locations. Sponge samples from Helgoland yielded the greatest number of isolates and the highest diversity of genera: 27 genera of mitosporic fungi, six genera of Ascomycetes, and one genus of Zygomycetes. The dominating genera were *Acremonium*, *Arthrinium*, *Fusarium*, *Mucor*, *Penicillium*, *Phoma*, *Trichoderma* and *Verticillium*. Due to the large number of isolates from each genus, and the fact that these genera (with the exception of the genus *Phoma*) were not planned to be further investigated chemically, not all colonies were isolated and registered. Hence, the numbers given in Table 3 do not represent the absolute abundance of these genera in the samples investigated. With the exception of the genera *Asteromyces* and *Chaetomium*, the two samples of the sponge *H. panicea* from different collection sites from Helgoland yielded an almost identical spectrum of fungal isolates. Sponge samples from Malta were dominated by the presence of *Aspergillus* (mostly of the *A. niger* and *A. ochraceus* group), *Eurotium* and *Penicillium* spp. Sponge samples from Tenerife yielded only few isolates, all being mitosporic fungi.

4.1.2 Fungal isolates from algae

Algal samples were cut into small pieces and placed on the different isolation media. About half of the sample was surface sterilised using EtOH prior to its inoculation. Development of fungal colonies visible under the dissecting microscope started about four days after inoculation on the agar plates. From 10 algal samples a total of 105 fungal strains belonging to 25 genera (4 genera of Ascomycetes, 1 genus of Zygomycetes, 19 genera of Mitosporic fungi) and 7 Mycelia sterilia were obtained (Table 4).

Table 4. Genera and total number of fungal strains obtained from algal samples.

Alga sample[1] / Fungal genera	**H-1**	**H-2**	**H-3**	**CT 195 D**	**CT 195 E**	**CT 195 I**	**CT 195 L**	**CT 195 M**	**CT 195 X**	**CT 195 Y**	**total no. of isolates**
a) Ascomycetes											
Achaetomium	-[2]	-	-	-	-	1(1)[3]	-	-	-	-	1(1)
Chaetomium	-	-	-	1(1)	-	1(1)	-	-	-	-	2(2)
Gymnoascus	-	-	1(1)	-	-	-	-	-	-	-	1(1)
Leptosphaeria	-	-	-	-	-	-	-	-	-	1(-)	1(-)
Westerdykella	-	-	2(1)	-	-	-	-	-	-	-	2(1)
b) Zygomycetes											
Mucor	-	-	1(-)	-	-	-	-	-	-	-	1(-)
c) Mitosporic fungi											
Acremonium	2(-)	-	2(-)	-	-	6(2)	1(-)	-	-	3(1)	14(5)
Alternaria	-	-	-	-	-	1(1)	-	-	-	-	1(1)
Arthrinium	-	-	1(1)	-	-	-	-	-	-	-	1(1)
Aspergillus	-	-	1(1)	1(1)	1(-)	6(2)	-	-	-	4(-)	13(5)
Cladosporium	-	-	-	-	-	-	-	1(-)	1(1)	3(2)	5(3)
Chrysosporium	-	-	-	-	-	-	1(-)	-	-	-	1(-)
Coniothyrium	-	-	2(1)	-	-	-	-	-	-	-	2(1)
Curvularia	-	-	-	-	-	1(1)	-	-	-	2(-)	3(1)
Drechslera	-	-	-	-	-	-	-	-	-	1(-)	1(-)
Fusarium	4(1)	-	9(6)	-	-	3(3)	-	-	-	5(3)	21(13)
Paecilomyces	-	-	-	-	-	-	1(-)	-	-	1(1)	2(1)
Penicillium	-	-	1(-)	-	1(-)	2(-)	-	-	-	1(1)	5(2)
Phoma	1(1)	-	3(1)	-	-	2(2)	-	-	-	2(2)	8(6)
Scolecobasidium	-	-	-	-	1(-)	-	-	-	-	1(1)	2(2)
Scopulariopsis	-	-	-	1(1)	-	-	-	-	-	2(-)	3(1)
Trichoderma	1(-)	-	2(-)	1(-)	-	1(-)	-	-	-	-	5(-)
Ulocladium	-	-	-	-	-	-	-	-	-	1(1)	1(1)
Varicosporina	-	-	-	-	-	-	1(-)	-	-	-	1(-)
Verticillium	-	-	-	-	-	1(1)	-	-	-	-	1(1)
Mycelia sterilia	1(-)	1(1)	-	-	-	2(1)	-	-	2(-)	1(-)	7(1)

[1] Origin of algal samples: H-1 to H-3 Helgoland, CT 195 D to CT 195 Y Tenerife; for details about the taxonomy see 3.1.2.
[2] - no isolates obtained.
[3] Numbers in paragraphs indicate portion of isolates obtained after surface sterilisation.

The results are not differentiated as to the media used, because no significant selectivity was observed. The greatest number of isolates (> 25) and the greatest diversity of genera (> 12) was obtained from the samples H-3, CT 195 I and CT 195 Y. Most other samples yielded only single representatives of one to four fungal genera. Only one obligate marine fungus, *Varicosporina ramulosa* from a

sample of a *Cytoseira* sp., was obtained. If the taxonomy of the fungi obtained from sponges and algae from the same geographic origin is compared, some correlations are obvious. This is most significant for the fungal strains obtained from samples from Helgoland. Representatives of all genera of Mitosporic fungi which were encountered in the algal samples were also isolated from sponges (see 4.1.1).

About half of the fungal strains (47.6 %) was obtained after employing a surface sterilisation. Some algae proved to be very sensitive against EtOH, e.g. the red alga H-3 which lost colour within several seconds. Therefore sterilisation time had to be reduced for some samples, and was not sufficient to destroy fungal spores adhaerent to the surface of the algae. In general, the numbers of fungal strains isolated were too low to reveal significant differences as to the pattern and number of genera/species isolated from a selected sample with or without surface sterilisation.

4.2 Biological activity of extracts of selected fungal strains

4.2.1 Biological activity of extracts of fungal isolates from sponges

4.2.1.1 Antimicrobial activity in agar diffusion assays

Of the 681 fungal strains isolated, only some could be further evaluated for the production of biologically active metabolites. The major criteria for selection were the taxonomy of the isolates, and the diversity of secondary metabolite chemistry of the relevant genera, as found in the Chapman & Hall database. Strains of ubiquitous genera with well known secondary metabolism were not further investigated, isolates of less well investigated genera and genera known to possess marine representatives were prefered. Thus, 92 strains were selected and grown on two, and in some cases three different media (see 8.3 for details).

Table 5. Antimicrobial activity of sponge-derived fungal strains, monitored by the activity of EtOAc extracts from fungal cultures in agar diffusion assays.[1]

Genus	Number of strains tested	*E. c.*[2]	*B. m.*	*M. v.*	*E. r.*	*F. o.*	*M. m.*	*C. f.*	total no. of active strains
Aplosporella (D)[3]	2	-[4]	1	1	2	-	1	1	2
Asteromyces (H)	1	-	-	-	-	-	-	1	1
Beauveria (D)	1	-	-	-	-	-	-	-	-
Beauveria (H)	2	-	1	-	-	-	-	-	1
Cladosporium (D)	1	-	-	-	-	-	-	-	-
Coniothyrium (D)	8	1	3	6	8	2	2	6	8
Coniothyrium (H)	9	-	2	8	5	-	-	6	9
Drechslera (H)	2	-	-	2	2	1	1	2	2
Emericellopsis (H)	1	-	1	1	1	-	-	1	1
Gliocladium (D)	1	1	1	1	1	1	1	1	1
Gonatobotrys (H)	1	-	-	-	-	-	-	-	-
Leptosphaeria (D)	1	-	1	1	-	-	1	-	1
Microascus (H)	1	-	-	1	1	-	1	-	1
Microascus (M)	1	-	1	1	1	-	-	-	1
Microsphaeropsis (H)	1	-	1	1	1	-	-	1	1
Monascus (M)	1	-	-	1	1	-	-	1	1
Monochaetia (D)	3	-	1	1	1	1	1	-	1
Myrothecium (D)	3	-	3	2	1	-	2	1	2
Myxotrichum (M)	1	-	1	-	1	1	1	1	1
Niesslia (H)	1	-	1	1	1	-	-	-	1
Phoma (D)	10	-	1	4	4	-	2	2	7
Phoma (H)	11	2	3	6	8	-	3	7	11
Preussia (H)	1	-	1	1	1	-	-	1	1
Scopulariopsis (H)	1	-	1	1	1	-	1	-	1
Sporormiella (A)	1	-	-	-	-	-	-	1	1
Sporothrix (D)	1	-	1	1	1	-	-	-	1
Stachylidium (A)	1	-	-	-	1	-	-	-	1
Ulocladium (D)	2	-	2	1	-	-	1	-	2
Mycelia sterilia (A)	4	1	1	2	2	2	1	-	2
Mycelia sterilia (D)	18	1	5	7	5	2	5	4	13
total	**92**	**6**	**33**	**51**	**50**	**10**	**24**	**37**	**75**

[1] Extracts from fungal cultures on two or three different media were tested. If one of the extracts was active, the corresponding strain was regarded as active.

[2] Test organisms: *E.c. Escherichia coli, B.m. Bacillus megaterium, M.v. Microbotryum violaceum, E.r. Eurotium repens, F.o. Fusarium oxysporum, M.m. Mycotypha microspora, C.f. Chlorella fusca*.

[3] Origin of the sponge samples from which the strains were isolated: (A) Australia, (D) Dominica, (H) Helgoland, (M) Malta.

[4] Isolates whose extracts produced an inhibition zone ≥ 3 mm or a complete inhibition ≥ 1 mm were regarded as active; - no such activity.

Resultant cultures were extracted with EtOAc to yield a total of 256 extracts. In agar diffusion assays for antibacterial, antifungal and antialgal activity, 161 extracts (62.9 %) resulting from 75 strains (81.5 %) proved to be active (growth inhibition zone ≥ 3 mm, or complete inhibition ≥ 1 mm) against at least one test organism (Table 5).

Antifungal activity was the most common inhibitory effect: at least one extract of 63 % of the fungal strains tested exhibited antifungal properties. The sensitivity of fungi used as test organisms in the agar diffusion assays varied greatly. The growth of *E. repens* and *M. violaceum* was inhibited by extracts of 54 and 55 % of the tested strains, respectively, whereas *M. microspora* was inhibited by extracts of 26 %, and *F. oxysporum* by extracts of only 11 % of the tested strains.

Antibacterial activity was found for extracts of 40 % of the selected strains. Extracts of only six strains (7 %) were found to be active against the gram-negative bacterium *E. coli*, whereas extracts of 33 strains (36 %) were found to be active against the gram-positive bacterium, *B. megaterium*. Extracts of 37 strains (42 %) were found to be active against the green alga, *C. fusca*.

The largest number of strains investigated for antimicrobial activity belonged to the genera *Coniothyrium* (17 strains) and *Phoma* (21 strains), or to the Mycelia sterilia (22 strains). The agar diffusion assays demonstrated that all 17 strains of the genus *Coniothyrium*, 18 of 21 tested strains of the genus *Phoma*, and 15 of 22 tested strains of the Mycelia sterilia showed antimicrobial activity. For the genera *Coniothyrium* and *Phoma*, the collection site, e.g., Dominica or Helgoland, appeared to have negligible influence on the activity of the strains.

Extracts of 19 strains selectively inhibited only one test organism (Table 6). A broad spectrum of antimicrobial activity towards all test organisms was observed for extracts of one *Coniothyrium* and one *Gliocladium* strain, both from Dominica. The extracts of most strains, however, showed antimicrobial activity towards only some of the test organisms of each group or towards organisms of one or two groups of test organisms. Extracts from 19 strains were found to be both antibacterial and antifungal,

extracts from 22 strains were found to be antifungal and antialgal, extracts from two strains were found to be antibacterial and antialgal. The extracts of 16 strains inhibited members of all three groups of test organisms.

Table 6: Selectivity of the antimicrobial activity of culture extracts of sponge-derived fungi in the agar diffusion assays.

Number of strains of which the extracts were found to be exclusively active against:[1]	
E. coli	1 (1.2 %)
B. megaterium	6 (7.2 %)
E. repens	2 (2.4 %)
M. violaceum	5 (6.0 %)
C. fusca	5 (6.0 %)
Number of strains of which the extracts were found to be active against two or more groups of test organisms:	
antibacterial and antifungal	19 (22.9 %)
antifungal and antialgal	22 (26.5 %)
antibacterial and antialgal	2 (2.4 %)
antibacterial, antifungal and antialgal	16 (19.3 %)

[1] Extracts of none of the strains were found to be selectively active against *M. microspora* or *F. oxysporum*; extracts which produced an inhibition zone with a radius ≥ 3 mm or a complete inhibition≥ 1 mm were regarded as active.

4.2.1.2 Inhibition of tyrosine kinase and HIV-1 reverse transcriptase

Inhibition of HIV-1 reverse transcriptase and tyrosine kinase was measured using ELISA based systems (Eberle & Seibl, 1992). For lack of other criteria, the selection of strains to be tested was mainly based on their TLC profile and on their antimicrobial activity (growth inhibition zone > 10 mm or prominent complete inhibition zone) in the agar diffusion assays. These criteria for the choice of isolates assume that a strain is likely either to produce a large number of metabolites, some of which are active in one, and others in another assay, or that a metabolite is likely to be inhibitory in more than one assay system. Additionally, the two obligate marine fungi were tested. Thus, 27 strains, representing 15 genera and four Mycelia sterilia were cultured and extracted for the enzyme assays (strains tested and media used are indicated in section 8.3).

From the resulting 48 EtOAc and *n*-BuOH extracts, eight were found to be active in the HIV-1 RT assay and seven in the TK assay (Table 7), with extracts from five strains inhibiting both enzymes. With the exception of two *n*-BuOH extracts from a *Coniothyrium* and a *Phialophorophoma* strain, respectively, the enzyme inhibiting activity was always associated with the EtOAc extracts. From the three *Phoma* strains tested, two extracts were active. Only one extract from the six *Coniothyrium* strains investigated was active.

Table 7. Culture extracts of sponge-derived fungi with significant activity in the enzyme assays for HIV-1 reverse transcriptase and tyrosine kinase inhibition.

Fungal strain and code number	Extract	HIV-1 reverse transcriptase activity[1]	Tyrosine kinase activity[1]
Ulocladium botrytis (193A4, D)[2]	EtOAc	7	4
Mycelia sterilia (193I36, D)	EtOAc	36	16
Phoma sp. (H4-77, H)	EtOAc	77	21
Preussia sp. (H5-73, H)	EtOAc	79	23
Leptosphaeria sp. (195-43, T)	EtOAc	n.a.[3]	37
Coniothyrium sp. (193H44, D)	*n*-BuOH	n.a.	37
Microsphaeropsis sp. (H5-50, H)	EtOAc	77	38
Phoma sp. (H6-51, H)	EtOAc	54	n.a.
Phialophorophoma litoralis (195-40, T)	*n*-BuOH	64	n.a.
Microascus sp. (K14, M)	EtOAc	80	n.a.

[1] Percentage of enzyme activity observed relative to a negative control (100 % HIV-1 RT or TK activity). Reduction of enzyme activity to 80% or less (HIV-1 RT) or 40% or less (TK) was regarded as a significant inhibition. Piceatannol (3 mM, rest activity of TK 2.3 %) and phosphonoformic acid (10 µM, rest activity of HIV-1 RT 23.5 %) were used as positive controls.
[2] Origin of the sponge samples from which the strains were isolated: (D) Dominica, (H) Helgoland, (M) Malta, (T) Tenerife.
[3] n.a. not active.

4.2.1.3 Cytotoxicity and inhibition of *Mycobacterium tuberculosis*

Extracts of selected strains were tested for cytotoxic effects towards KB cells and inhibitory activity against *M. tuberculosis*. In these assays the same fungal strains as

outlined in 4.2.1.2 were investigated. The results for the active extracts are given in Table 8.

Table 8. Activity of culture extracts from sponge-derived fungi towards KB cells and *M. tuberculosis*.

Fungal strain and code number	Culture medium	Extract	IC_{50} against KB cells (μg/mL)[1]	Inhibition (%) of *M. tuberculosis* (300 μg/mL)[2]	Inhibition (%) of *M. tuberculosis* (100 μg/mL)[2]
Gliocladium sp. 193H15	BIO	*n*-BuOH	3.9	n.a.[3]	n.a.
	BIO	EtOAc	0.23	n.a.	n.a.
	MS	EtOAc	6.0	n.a.	n.a.
Mycelia sterilia 193H60	BIO	*n*-BuOH	n.a.	98	96
Mycelia sterilia 293K9	BIO	EtOAc	2.7	99	100
	BIO	*n*-BuOH	n.a.	99	99
Phoma sp. H4-77	BIO	EtOAc	1.4	n.a.	n.a.
	MS	EtOAc	12.6	n.a.	n.a.
Drechslera sp. H5-28	BIO	EtOAc	n.a.	96	89
	BIO	*n*-BuOH	n.a.	96	85
	MS	EtOAc	n.a.	96	n.a.
Microsphaeropsis sp. H5-50	BIO	*n*-BuOH	n.a.	92	5
Gonatobotrys sp. H6-51	BIO	EtOAc	n.a.	99	93
	BIO	*n*-BuOH	n.a.	95	73
Emericellopsis minima H7-65	BIO	*n*-BuOH	7.5	n.a.	n.a.
	MS	*n*-BuOH	15.6	91	81
	MS	EtOAc	2.2	98	94
Coniothyrium sp. H8-57	BIO	EtOAc	13.4	99	100
	BIO	*n*-BuOH	n.a.	99	99
	MS	EtOAc	n.a.	95	89
Microascus sp. K14	BIO	EtOAc	8.8	n.a.	n.a.
	MS	EtOAc	18.7	n.a.	n.a.

[1] An $IC_{50} \geq 20$ μg/mL was not determined, such extracts were regarded as not active.
[2] Results are shown only for extracts which caused an inhibition of 90 % or more at 300 μg/mL and/or 25 % or more at 100 μg/mL.
[3] n.a. not active.

4.2.2 Biological activity of extracts of fungal isolates from algae

Of the 105 strains obtained from the different algae, 22 were selected for further investigation. Selection criteria were the same as those for the sponge-derived isolates. Strains obtained after surface sterilisation were preferred. Cultivation and extraction was performed as for the sponge isolates. For an overview of all strains tested and the media used, see section 8.3. Extracts from cultures of 14 strains (63.6 %) showed a significant activity against at least one test organism (Table 9).

Table 9. Antimicrobial activity of algal-derived fungal strains, monitored by the activity of EtOAc extracts from fungal cultures in agar diffusion assays.[1]

Genus	No. of strains tested	*E. c.*[2]	*B. m.*	*M. v.*	*E. r.*	*F. o.*	*M. m.*	*C. f.*	total no. of active strains
Alternaria (T)[3]	1	-[4]	-	1	1	-	-	-	1
Curvularia (T)	1	-	-	-	-	-	-	-	-
Chrysosporium (T)	1	-	-	-	-	-	-	-	-
Drechslera (T)	1	-	-	-	1	-	1	-	1
Gymnoascus (H)	1	-	1	1	-	-	1	1	1
Leptosphaeria (T)	1	-	-	1	1	-	1	1	-
Phoma (H)	2	-	-	1	1	-	-	1	2
Phoma (T)	2	-	1	1	-	-	-	-	2
Scolecobasidium (T)	2	-	-	-	-	-	-	-	-
Ulocladium (T)	1	-	-	-	-	-	-	-	-
Varicosporina (T)	1	-	-	-	1	-	-	-	1
Westerdykella (H)	1	-	1	-	-	-	-	-	1
Mycelia sterilia (H)	2	-	-	1	1	-	-	-	1
Mycelia sterilia (T)	5	1	4	-	1	-	1	-	4
total	22	1	7	6	7	-	4	3	14

[1] Extracts from fungal cultures on two or three different media were tested. If one of the extracts was active, the corresponding strain was regarded as active.

[2] Test organisms: *E.c. Escherichia coli*, *B.m. Bacillus megaterium*, *M.v. Microbotryum violaceum*, *E.r. Eurotium repens*, *F.o. Fusarium oxysporum*, *M.m. Mycotypha microspora*, *C.f. Chlorella fusca*.

[3] Origin of the algal samples from which the strains were isolated: (H) Helgoland, (T) Tenerife.

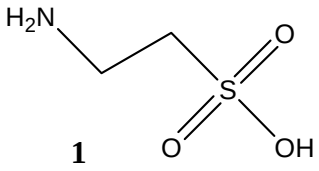
[4] Isolates whose extracts produced an inhibition zone ≥ 3 mm or a complete inhibition ≥ 1 mm were regarded as active; - no such activity.

With the exception of inhibitory activity against *B. megaterium*, the percentage of strains with active extracts was lower compared to the sponge-derived strains. These antibacterial extracts mainly resulted from Mycelia sterilia from Tenerife. Extracts of about 30 % of all isolates inhibited *M. violaceum* and *E. repens*, 18.1 % *M. microspora* and 13.6 % *C. fusca*, whereas *F. oxysporum* was inhibited by none of the extracts.

4.3 Chemical investigation of sponge samples

Three sponge samples, *Callyspongia* sp. cf. *C. flammea*, *Ircinia oros* and *Leucosolenia challengeri*, parts of which had been used for the isolation of fungal strains, were investigated for secondary metabolites. This resulted in the isolation of eight pure compounds, including 22-*O*-methyl derivatives of the two new sesterterpene tetronic acids (8*Z*,13*Z*,18*S*,20*Z*)-strobilinin (**2a**) and (7*E*,12*Z*,18*S*,20*Z*)-variabilin (**3a**) from *I. oros*. The isolated secondary metabolites proved to be typical sponge metabolites, and were distinct from known fungal metabolites.

4.3.1 *Callyspongia* sp. cf. *C. flammea* (CT 293 K)



1

Frozen sponge tissue was freeze-dried and exhaustedly extracted with DCM followed by MeOH. While the MeOH was removed under reduced pressure, compound **1** precipitated as colourless crystals (11.6 mg). DCM and MeOH extracts are currently under investigation for their biological properties. Both extracts were not further investigated chemically. The crystals precipitated from the MeOH extract could be identified as taurine by combined elemental analysis, FAB-MS, and ^{1}H and ^{13}C NMR spectroscopy.

Taurine (1): colourless crystals (11.6 mg); IR (film) ν_{max} 3435, 3210, 3050, 2984, 2970, 1615, 1585, 1510, 1460, 1425, 1390, 1345, 1305, 1250, 1215, 1180, 1110, 1100, 1045, 1040, 960, 895, 740, 600, 535, 525, 465 cm^{-1}; ^{1}H NMR (DMSO-d6, 200 MHz) δ 2.71 (2H, t, J = 6.1 Hz), 3.04 (2H, t, J = 6.1 Hz), 7.67 (2H, brs); ^{13}C NMR (D_2O, 75.5 MHz) δ 38.3 (t), 50.4 (t); FAB-MS (glycerol, negative mode) *m/z* $[M-H]^-$ 124; anal. C 18.75%, H 5.47%, N 10.71%, S 24.46%, calcd for $C_2H_7NO_3S$, C 19.20%, H 5.64%, N 11.19%, S 25.62%.

4.3.2 *Ircinia oros* (CT 912 J)

Frozen sponge tissue was freeze-dried (88.0 g) and successively extracted with DCM (5 x 1 L) and MeOH to give 2.0 g (DCM extract) and 12 g (MeOH extract),

respectively. The DCM extract was fractionated by normal-phase VLC (gradient hexane/EtOAc/MeOH) to yield five fractions. Fraction 2 (hexane/EtOAc 50/50) contained sesterterpene tetronic acids as monitored by ^{1}H NMR spectroscopy. These compounds are known for their instability, especially in the presence of oxygen (Barrow, Blunt & Munro, 1989). To avoid their decomposition, as well as to improve chromatographic behaviour through prevention of keto-enol-tautomerism, the fraction (250 mg) was methylated using diazomethane (Ngan & Toofan, 1991). Separation of the products by reversed-phase (C-18) VLC (MeOH), followed by normal-phase HPLC (EtOAc/hexane 20/80) gave 173 mg of the 22-OCH_3 and 50 mg of 24-OCH_3 derivatives. The 22-OCH_3 derivatives were further purified using reversed-phase (C-18) HPLC (CH_3CN/MeOH/H_2O 42/43/15, photodiode array detection 210-400 nm) to give compound **4** (44.4 mg) and two mixtures. The latter were separated using reversed-phase (C-18) HPLC (MeOH/H_2O 83/17, photodiode array detection 210-400 nm) to yield compounds **2** (4.1 mg), **3** (7.0 mg) and **5** (8.7 mg), and 3.0 mg of a mixture containing **6** and another unidentified sesterterpene tetronic acid. The other fractions from the DCM extract, and the MeOH extract proved to be not interesting as judged by their ^{1}H NMR spectra and were not further investigated.

The molecular formula of **2** was established as $C_{26}H_{36}O_4$ by accurate mass measurement. ^{1}H NMR spectroscopic measurements revealed signals at δ 6.26 (brs), 7.21 (brs) and 7.34 (m) for a 3-substituted furan moiety, four resonances for methyl groups (δ 1.65, d, *J* = 1 Hz; 1.67, s; 1.03, d, *J* = 6.5 Hz and 2.06, s), a signal for a methoxyl function (δ 4.11, s), and three resonances for olefinic protons (δ 5.13, m; 5.09, t, *J* = 7.0 Hz, and δ 5.15, d, *J* = 9.7 Hz). These data together with the observed UV maximum at 263 nm, IR absorptions at 1640 and 1760 cm^{-1} for carbon-carbon double-bonds and a lactone moiety suggested, when compared with literature data, that **2** was a sesterterpene tetronic acid (Rothberg & Shubiak, 1975; Gonzáles, Rodríguez & Barrientos, 1983; Barrow *et al.*, 1988). After assignment of all protons to their directly bonded carbon atoms via a ^{1}H-^{13}C 2D NMR shift correlated measurement (HMQC), it was possible to deduce two major molecular fragments from the ^{1}H-^{1}H COSY spectrum of **2**. Thus, coupling was observed from H-1 (δ 7.34, m) to H-2 (δ 6.26, brs), H-2 to H-4 (δ 7.21, brs) and H-4 to H_2-5 (δ 2.39, dd, *J* = 7.6,

8.1 Hz). Further, H_2-5 coupled to H_2-6 (δ 1.63, m), which in turn coupled with H_2-7 (δ 2.05, m), clearly delineating the first molecular fragment; the furan moiety through to C-7. Coupling of H_3-19 (δ 1.03, d, *J* = 6.5 Hz) to H-18 (δ 2.77, m), H-18 to H-20 (δ 5.15, d, *J* = 9.7 Hz) and to H_2-17 (δ 1.37, brm), H_2-17 to H_2-16 (δ 1.94, m), and H_2-16 to H-15 (δ 5.09, tq, *J* = 7.0, 1.0), which further coupled to H_3-14 (q, *J* = 1.0 Hz), characterised a second major fragment of the molecule; C-20 to C-13. ^{1}H-^{1}H couplings from other proton resonances could not be interpreted unambiguously due to signal overlapping.

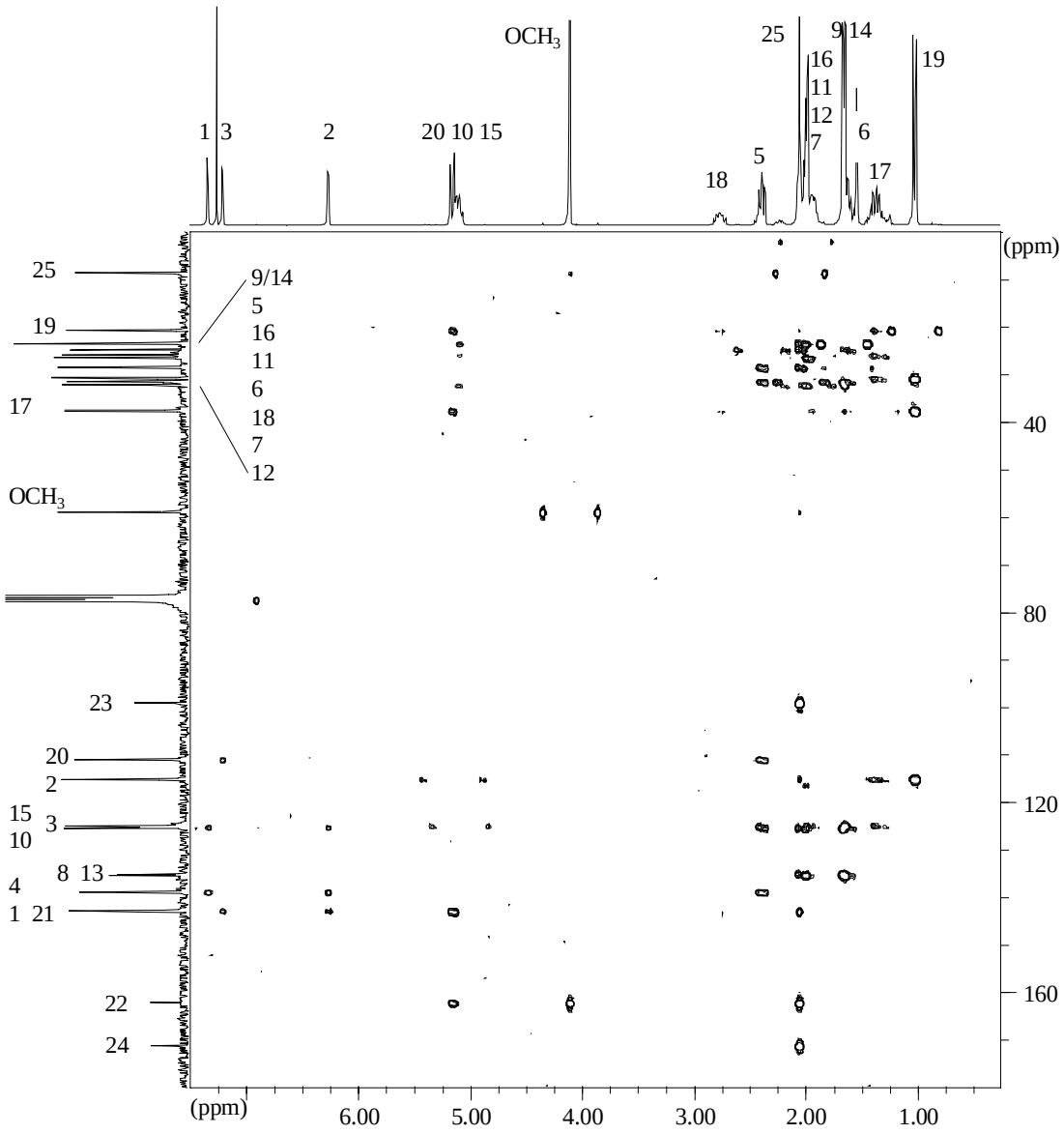


Figure 5: ^{1}H-^{13}C long-range (HMBC) NMR spectrum of 2; $CDCl_3$, 400 MHz for ^{1}H, 100 MHz for ^{13}C.

A long-range ^{1}H-^{13}C 2D NMR shift correlated measurement (HMBC) made with **2** permitted the molecule to be further elaborated. Thus, long-range couplings from C-21 (142.8 ppm) to H-20 and to H_3-25 (δ 2.06, s), and between C-22 (162.0 ppm) and H-20, the methoxyl protons (δ 4.11, s), and H_3-25, as well as between C-24 (171.0 ppm) and H_3-25 clearly established, together with the C-20 to C-13 fragment, the C-25 to C-13 part of **2**. Remaining to be accounted for were two methylene groups, CH_2-11 and CH_2-12, both with identical chemical shift in the ^{1}H NMR spectrum (δ 2.00, m), and a double-bond (δ 5.13, m), substituted with a methyl group (δ 1.67, s). Through detailed analysis of all homo- and heteronuclear NMR correlation spectra it was evident that the two methylene groups are adjacent, as are H_2-11 and H-10. With the major part of the molecule established the C-9 to C-12 fragment had thus to be located between C-7 and C-13, giving rise to a the basic structure of a strobilinin derivative.

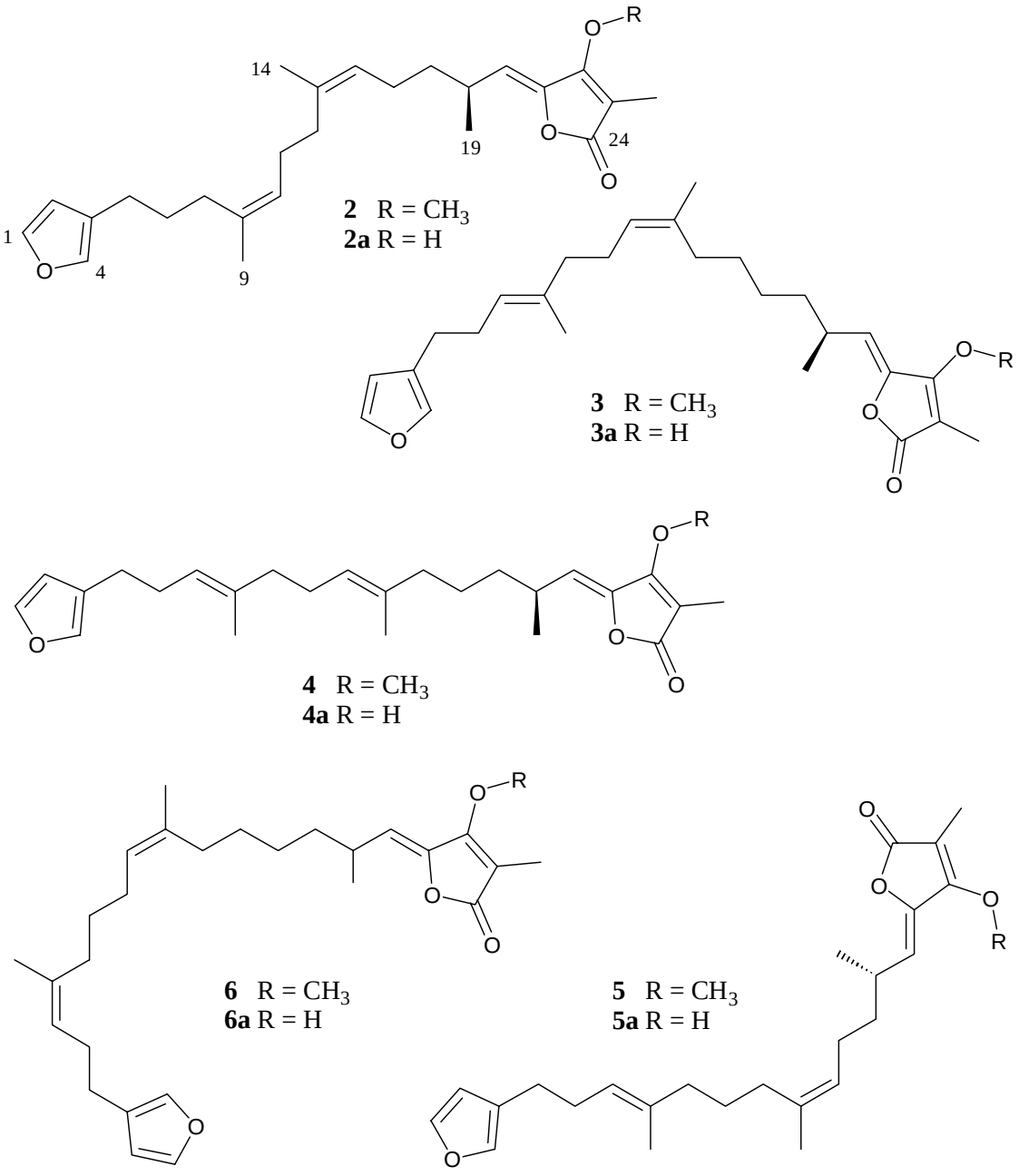
Depending on the geometry of the double-bond, methyl groups on isolated double-bonds in sesterterpene tetronic acids show characteristic ^{1}H NMR chemical shifts: δ > 1.6 for the *Z*- and δ < 1.6 for the *E*-isomer, the corresponding ^{13}C NMR chemical shifts being > 20 ppm and < 20 ppm, respectively, for the equivalent carbon atoms (Gonzáles, Rodríguez & Barrientos, 1983; Barrow *et al.*, 1988). Thus, ^{1}H NMR shifts of δ 1.65 and δ 1.67 for H-14 and H-9 and 23.4 ppm for C-9 and C-14 established the *Z*-configuration at both $\Delta^{8,10}$ and $\Delta^{13,15}$. NOE difference measurements (600 MHz), with irradiation at the resonances for the olefinic protons (δ 5.1), led to the enhancement of the signals for both CH_3-9 and CH_3-14, clearly confirming the 8*Z*,13*Z* geometry of both $\Delta^{8,10}$ and $\Delta^{13,15}$. The geometry of $\Delta^{20,21}$ was also determined as *Z* by comparison of the ^{13}C chemical shifts for C-17 to C-23 with published data for (7*E*,12*E*,20*Z*)-22-*O*-methylvariabilin (Barrow*et al.*, 1988).

The absolute configuration at C-18 in related sesterterpene tetronic acids was determined by degradation studies and shown to be *S* in (7*E*,12*E*,20*Z*)-variabilin from an *Amphimedon* sp., and *Ircinia variabilis* and found to be *R* in (8*E*,13*Z*,20*Z*)- and (8*Z*,13*E*,20*Z*)-strobilinin (Ishibashi *et al.*, 1993; Capon, Dargaville & Davies, 1994). Although literature data differ markedly, a negative optical rotation, e.g., -36° for

(18*S*)-variabilin (Capon, Dargaville & Davies, 1994) seems to be typical for the 18*S* isomers, with a positive rotation, e.g., +36° for (18*R*)-strobilinin acetates (Capon, Dargaville & Davies, 1994) for the 18*R* isomers. In the case of **2** an optical rotation of -43.7° suggests it to be 18*S*. Compound **2** is thus (8*Z*,13*Z*,18*S*,20*Z*)-22-*O*-methylstrobilinin, indicating **2a** to be the new natural product (8*Z*,13*Z*,18*S*,20*Z*)-strobilinin.

Compound **3** was shown by accurate mass measurement to have the identical molecular formula as **2**, $C_{26}H_{36}O_4$. The ^{1}H-^{13}C short-range (HMQC) spectrum of **3** permitted correlation of all proton and carbon resonances of directly bonded atoms. By comparing these NMR data of **3** with those for **2**, it was evident that the C-16 to C-25 part of **3** was identical to that of **2**. Strobilinin derivatives such as **2** show characteristic chemical shifts for the C-5 and C-6 methylene groups in their ^{1}H NMR spectra at δ 2.4 and δ 1.6, whereas these protons in variabilins resonate at δ 2.4 and δ 2.2. The characteristic ^{1}H NMR chemical shifts for the methylene groups H_2-5 and H_2-6 at δ 2.45 (dd, J = 7.1, 7.6 Hz) and δ 2.24 (ddd, J = 7.1, 7.1, 7.6 Hz) respectively, thus suggested **3** to be, in contrast to **2**, a variabilin derivative. ^{1}H-^{1}H COSY and ^{1}H-^{13}C heteronuclear long-range (HMBC) measurements established the connectivities between H_2-5 and H_2-6, and between H_2-6 and C-7. From the ^{1}H-^{1}H COSY it was also clear that H-7 coupled with H_3-9 (δ 1.58, s), the resonance of which showed a cross peak to C-10 (39.9 ppm) in the HMBC spectrum. C-10 in turn had an HMBC correlation to H-12 (δ 5.08, brt, J = 6.1 Hz), which further coupled to H_2-11 (δ 2.00, m) and H_3-14 (δ 1.64, s). From H_3-14 heteronuclear coupling to C-13 (135.1 ppm), and to C-15 (31.7 ppm) was seen in the HMBC spectrum. H-15 (δ 2.00, m) then coupled to H_2-16 (δ 1.35, m), as is evidenced by the relevant cross-peaks in the ^{1}H-^{1}H COSY of **3**. The geometry of double-bonds was determined as outlined for **2**. Chemical shifts of δ 1.58 for H_3-9 and of 16.0 ppm for C-9, and δ 1.64 for H_3-14 and 23.3 ppm for C-14 suggested the 7*E*,12*Z* configuration. This was confirmed by NOE difference measurements: irradiation at δ 5.16 (H-7) did not affect the intensity of the signal for H_3-9, whereas irradiation at δ 5.08 resulted in an enhancement of the ^{1}H NMR signal of H_3-14. On the basis of almost identical ^{13}C NMR chemical shifts of

compounds **2** and **3** in the region of the tetronic acid moiety it was concluded that **3** also has the 20*Z*-configuration. The optical rotation of **3** (-33.1°) suggested the absolute configuration at C-18 to be *S* on the same grounds as discussed for **2**. Thus, compound **3** is (7*E*,12*Z*,18*S*,20*Z*)-22-O-methylvariabilin, indicating **3a** to be the new natural product (7*E*,12*Z*,18*S*,20*Z*)-variabilin.



Compounds **4**, **5** and **6** were identified as the 22-*O*-methyl derivatives of (7*E*,12*E*,18*S*,20*Z*)-variabilin (**4a**), (7*E*,13*Z*,18*S*,20*Z*)-variabilin (**5a**) and (7*Z*,12*Z*,20*Z*)-

variabilin (**6a**) by comparison of their ^{1}H and ^{13}C NMR spectroscopic data and their optical rotations with published values (Gonzáles, Rodrígues & Barrientes, 1983; Barrow *et al.*, 1988).

After completion of the present investigation, five sesterterpene tetronic acid acetates, derivatives of (8*Z*,13*Z*,20*Z*)-strobilinin, (7*Z*,13*Z*,20*Z*)-felixinin, (8*E*,13*Z*,20*Z*)-strobilinin, (7*E*,13*Z*,20*Z*)-felixinin and (7*E*,12*E*,20*Z*)-variabilin, have been reported from *Ircinia felix*, *I. strobilina* and *I. campana* from the Colombian Caribbean (Martinez *et al.*, 1997). Despite derivatisation only one of the compounds was obtained in a pure form, the others were analysed as mixtures. In this work the C-18 was tentatively assigned as having the *R* absolute configuration based on the optical rotations recorded from the mixtures (ratio 1:1), +34.8° and +45.6°, respectively. This assignment may indeed be correct since the stereo- and regiochemistry of the carbon-carbon double bonds and/or methylation or acetylation of the tetronic acid moiety appears to have only a modest affect on the optical rotation of this class of compounds (Ishibashi *et al.*, 1993; Capon, Dargaville & Davies, 1994). Compound **2** of the present study has the same basic structure as (8*Z*,13*Z*,18*R*,20*Z*)-strobilinin acetate isolated by Martinez *et al.* (1997), as part of a mixture. Optical rotation comparisons, however, indicated **2a** to be the enantiomer of (8*Z*,13*Z*,18*R*,20*Z*)-strobilinin.

(8*Z*,13*Z*,18*S*,20*Z*)-22-*O*-Methyl-strobilinin (2): Colourless oil (4.1 mg, 0.005 %); $[\alpha]^{20}_{D}$ -43.7° (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} 263 nm (ε 19900); IR (film) ν_{max} 2927, 1760, 1640, 1453, 1356, 1060, 982 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.03 (3H, d, *J* = 6.5 Hz, H-19), 1.37 (2H, brm, H-17), 1.63 (2H, m, H-6), 1.65 (3H, d, *J* = 1.0 Hz, H-14), 1.67 (3H, s, H-9), 1.94 (2H, m, H-16), 2.00 (4H, m, H-11, H-12), 2.05 (2H, m, H-7), 2.06 (3H, s, H-25), 2.39 (2H, dd, *J* = 7.6, 8.1 Hz, H-5), 2.77 (1H, m, H-18), 4.11 (3H, s, OCH_3), 5.09 (1H, tq, *J* = 7.0, 1.0 Hz, H-15), 5.13 (1H, m, H-10), 5.15 (1H, d, *J* = 9.7 Hz, H-20), 6.26 (brs, H-2), 7.21 (brs, H-3), 7.34 (m, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.6 (q, C-25), 20.6 (q, C-19), 23.4 (q, C-9, C-14), 24.7 (t, C-5), 25.8 (t, C-16), 26.3 (t, C-11), 28.3 (t, C-6), 30.7 (d, C-18), 31.4 (t, C-7), 32.1 (t, C-12), 37.5 (t, C-17), 58.8 (q, OCH_3), 99.0 (s, C-23), 110.9 (d, C-2), 115.1 (d, C-20), 124.9 (d, C-15), 125.1 (s, C-3), 125.2 (d, C-10), 135.0 (s, C-8), 135.3 (s, C-13), 138.7 (d, C-4), 142.6 (d, C-1), 142.8 (s, C-21), 162.0 (s, C-22), 171.0 (s, C-24); EIMS *m/z* $[M]^+$ 412 (6), 397 (1), 223 (13), 205 (10), 167 (17), 149 (100), 135 (19), 111 (16),109 (10); HREIMS *m/z* 412.261 (calcd for $C_{26}H_{36}O_4$, 412.261).

(7*E*,12*Z*,18*S*,20*Z*)-22-*O*-Methyl-variabilin (3): Colourless oil (7.0 mg, 0.008 %); $[\alpha]^{20}_{D}$ -33.1° (*c* 0.7, $CHCl_3$); UV (EtOH) λ_{max} 263 nm (ε 12100); IR (film) ν_{max} 2926, 1759, 1638, 1451, 1356, 1060, 1028, 981 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.04 (3H, d, *J* = 6.6 Hz, H-19), 1.35 (4H, m, H-16, H-17), 1.58 (3H, s, H-9), 1.64 (3H, s, H-14), 2.00 (6H, m, H-10, H-11, H-15), 2.06 (3H, s, H-20), 2.24 (2H, ddd, *J* = 7.1, 7.1, 7.6 Hz, H-6), 2.45 (2H, dd, *J* = 7.1, 7.6 Hz, H-5), 2.77 (1H, m, H-18), 4.11 (3H, s, OCH_3), 5.08 (1H, brt, *J* = 6.1 Hz, H-12), 5.15 (1H, d, *J* = 9.7 Hz, H-20), 5.16 (1H, t, *J* = 7.1 Hz, H-7), 6.27 (1H, brs, H-2), 7.21 (1H, brs, H-4), 7.33 (1H, brs, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.6 (q, C-25), 16.0 (q, C-9), 20.7 (q, C-19), 23.3 (q, C-14), 25.0 (t, C-5), 25.7 (t, C-16), 26.4 (t, C-11), 28.4 (t, C-6), 30.9 (d, C-18), 31.7 (t, C-15), 36.9 (t, C-17), 39.9 (t, C-10), 58.8 (q, OCH_3), 99.1 (s, C-23), 111.1 (d, C-2), 115.1 (d, C-20), 123.8 (d, C-7), 125.0 (s, C-3), 125.0 (d, C-12), 135.1 (s, C-13), 135.7 (s, C-8), 138.8 (d, C-4), 142.5 (d, C-1), 142.7 (s, C-21), 162.0 (s, C-22), 171.0 (s, C-24); EIMS *m/z* $[M]^+$ 412 (24), 397 (7), 331 (3), 318 (4), 207 (15), 204 (19), 193 (24), 181 (33), 167 (100), 149 (42), 141 (61), 135 (60), 123 (46), 109 (34), 81 (80); HREIMS *m/z* 412.261 (calcd for $C_{26}H_{36}O_4$, 412.261).

(7*E*,12*E*,18*S*,20*Z*)-22-*O*-Methyl-variabilin (4): Colourless oil (44.4 mg, 0.05 %); $[\alpha]^{20}_{D}$ -40.8° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.03 (3H, d, *J* = 6.6 Hz, H-19), 1.35 (4H, m, H-16, H-17), 1.55 (3H, s, H-14), 1.58 (3H, s, H-9), 1.94 (2H, m, H-15), 1.99 (2H, m, H-10), 2.06 (2H, m, H-11), 2.06 (3H, s, H-20), 2.24 (2H, ddd, *J* = 7.1, 7.1, 7.6 Hz, H-6), 2.44 (2H, t, *J* = 7.6 Hz, H-5), 2.76 (1H, m, H-18), 4.11 (3H, s, OCH_3), 5.07 (1H, brt, *J* = 6.6 Hz, H-12), 5.15 (1H, d, *J* = 10.2 Hz, H-20), 5.16 (1H, m, H-7), 6.27 (1H, brs, H-2), 7.20 (1H, brs, H-4), 7.33 (1H, brs, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.5 (q, C-25), 15.8 (q, C-14), 16.0 (q, C-9), 20.7 (q, C-19), 25.0 (t, C-5), 25.7 (t, C-16), 26.5 (t, C-11), 28.4 (t, C-6), 30.8 (d, C-18), 36.7 (t, C-17), 39.5 (t, C-10), 39.7 (t, C-15), 58.8 (q, OCH_3), 99.0 (s, C-23), 111.1 (d, C-2), 115.2 (d, C-20), 123.7 (d, C-7), 124.3 (d, C-12), 125.0 (s, C-3), 134.9 (s, C-13), 135.7 (s, C-8), 138.8 (d, C-4), 142.5 (d, C-1), 142.7 (s, C-21), 162.0 (s, C-22), 171.1 (s, C-24); EIMS *m/z* $[M]^+$ 412 (44), 397 (12), 331 (7), 203 (26), 181 (38), 174 (34), 167 (96), 149 (42), 141 (52), 136 (74), 135 (70), 123 (55), 109 (30), 81 (100).

(7*E*,13*Z*,18*S*,20*Z*)-22-*O*-Methyl-variabilin (5): Colourless oil (8.7 mg, 0.01 %); $[\alpha]^{20}_{D}$ -47.2° (*c* 0.9, $CHCl_3$); UV (EtOH) λ_{max} 263 nm (ε 13600); IR (film) ν_{max} 2926, 1760, 1640, 1453, 1356, 1060, 982 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.04 (3H, d, *J* = 7.1 Hz, H-19), 1.40 (4H, m, H-11/17), 1.58 (3H, s, H-9), 1.66 (3H, d, *J* = 1.5 Hz, H-14), 1.94 (6H, m, H-10/12/16), 2.06 (3H, s, H-25), 2.24 (2H, ddd, *J* = 7.1, 7.1, 7.6 Hz, H-6), 2.45 (2H, dd, *J* = 7.1, 7.6 Hz, H-5), 2.78 (1H, m, H-18), 4.11 (3H, s, OCH_3), 5.09 (1H, dd, *J* = 6.6, 7.1 Hz, H-15), 5.16 (1H, m, H-7), 5.16 (1H, d, *J* = 10.2 Hz, H-20), 6.27 (1H, brs, H-2), 7.21 (1H, brs, H-4), 7.33 (1H, t, *J* = 1.5 Hz, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.6 (q, C-25), 15.9 (q, C-9), 20.6 (q, C-19), 23.4 (q, C-14), 25.1 (t, C-5), 25.7 (t, C-16), 26.3 (t, C-11), 28.4 (t, C-6), 30.7 (d, C-18), 31.4 (t, C-12), 37.5 (t, C-17), 39.6 (t, C-10), 58.8 (q, OCH_3), 99.0 (s, C-23), 111.1 (d, C-2), 115.1 (d, C-20), 123.7 (d, C-7), 124.6 (d, C-15), 125.0 (s, C-3), 135.7[a] (s,

C-13), 135.8[a] (s, C-8), 138.8 (d, C-4), 142.5 (d, C-1), 142.8 (s, C-21), 162.0 (s, C-22), 171.0 (s, C-24), [a] assignments may be interchanged; EIMS *m/z* $[M]^+$ 412 (35), 397 (13), 331 (10), 318 (10), 208 (44), 207 (43), 203 (44), 181 (38), 167 (81), 141 (39), 135 (86), 122 (38), 109 (33), 81 (100).

(7*Z*,12*Z*,20*Z*)-22-*O*-Methyl-variabilin (6): Colourless oil (approx. 0.005 %); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.6 (q, C-25), 20.7 (q, C-19), 23.3 (2C, q, C-9 and 14), 25.3 (t, C-6), 25.8 (t, C-16), 26.3 (t, C-11), 28.3 (t, C-5), 30.9 (d, C-18), 31.6 (t, C-15), 32.3 (t, C-10), 37.0 (t, C-17), 58.8 (q, OCH_3), 99.1 (s, C-23), 111.1 (d, C-2), 115.1 (d, C-20), 124.0 (d, C-7), 124.6 (d, C-12), 125.0 (s, C-3), 135.2 (s, C-13), 135.8 (s, C-8), 138.8 (d, C-4), 142.5 (d, C-1), 142.8 (s, C-21), 162.0 (s, C-22), 171.0 (s, C-24).

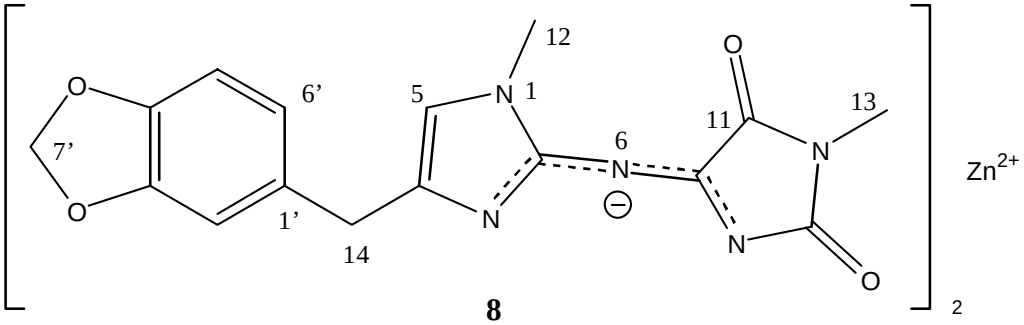
4.3.3 *Leucosolenia challengeri* (CT 293 T)

Frozen sponge tissue was freeze-dried (125 g) and subsequently extracted with DCM and MeOH. The DCM fraction (2.5 g of a brown oil) was fractionated by normal-phase VLC (gradient hexane-EtOAc-MeOH) to yield five fractions. Fraction **3** (EtOAc/hexane 75/25, 46 mg) was separated by normal-phase HPLC (EtOAc/hexane 75/25) to yield compound **7** (5 mg). Fraction 4 (EtOAc, 109 mg) was separated with the same HPLC system to yield compound **8** (5.5 mg). All other fractions did not contain metabolites in amounts worthwhile further investigation as monitored by ^1H NMR spectroscopy.

Compound **7** was obtained as a slightly yellow solid (5.5 mg). The molecule was found to consist of two, or a multitude of two, adjacent methylene groups both substituated with one electronegative atom or moiety, by ^1H and ^{13}C NMR spectroscopy. Unfortunately, no reasonable MS data was obtained by EIMS, CIMS (NH_3), and GC-MS. Finally, compound **7** decomposed and its structure thus remained unresolved.

Compound **8** was an orange-yellow solid. From its DCI-MS (*i*-butane) spectrum the presence of one zinc atom within the molecule could be deduced from the characteristic molecular ion cluster. A literature search (Chapman & Hall database) revealed natural products containing zinc to be quite rare. A molecular ion at *m/z* $[M]^+$ 745 suggested **8** to be a known clathridine zinc complex, which could be confirmed by comparison of its ^1H and ^{13}C NMR data with literature values (Ciminiello

et al., 1989; He *et al.*, 1992). In this respect, it is interesting to note that the ^{1}H NMR signal of the protons of the dioxymethylene-moiety (δ 5.89, 2H, dd, *J* = 1.1, 8.3 Hz, H-7'), is not the expected singlet. This effect might be due to the spatial orientation of the two clathridine molecules forming the complex, resulting in both hydrogen atoms not being equivalent.



8

Unknown 1 (7): slightly yellow solid (5 mg); UV (EtOH) no significant adsorption above 210 nm; IR (film) ν_{max} 3310, 3050, 3020, 2990, 1415, 1335, 1300, 1250, 1210, 1175, 1155, 1110, 960, 760, 670, 615, 465 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.89 (2H (?), ddd, *J* = 4.1, 7.1, 7.1 Hz, J_{CH} = 155 Hz), 4.30 (2H (?), dt, *J* = 2.0, 7.1 Hz, J_{CH} = 149 Hz), 5.26 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.2 (t), 61.0 (t).

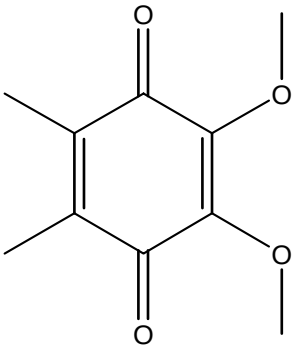
Clathridine Zn complex (8): orange-yellow solid (5.5 mg); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.03 (3H, s, H-13), 3.36 (1H, d, *J* = 16.6 Hz, H-14a), 3.50 (1H, d, *J* = 16.6 Hz, H-14b), 3.80 (3H, s, H-12), 5.89 (2H, dd, *J* = 1.1, 8.3 Hz, H-7'), 6.24 (1H, s, H-2'), 6.26 (1H, d, *J* = 7.5 Hz, H-6'), 6.50 (1H, d, *J* = 7.5 Hz, H-5'), 6.63 (1H, s, H-5); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 24.6 (q, C-13), 32.6 (q, C-12), 33.1 (t, C-14), 101.1 (t, C-7'), 107.7 (d, C-5'), 108.4 (d, C-2'), 117.5 (d, C-5), 121.1 (d, C-6'), 131.1 (s, C-1'), 136.3 (s, C-4), 146.1 (s, C-4'), 147.6 (s, C-3'), 148.6 (s, C-2), 154.5 (s, C-7), 161.4 (s, C-11), 164.6 (s, C-9); DCI-MS (*i*-butane) *m/z* 752 (1), 751 (4), 750 (14), 749 (42), 748 (30), 747 (62), 746 (38), 745 (100) [M(^{64}Zn)+H]$^{+}$, 744 (6), 342 (28), 246 (12).

4.4 Chemical investigation of extracts from selected fungal strains

Extracts of ten sponge-derived and one algal-derived fungi, including the two obligate marine mitosporic fungi *Asteromyces cruciatus* and *Varicosporina ramulosa*, were investigated chemically. Most strains were selected because of prominent activity of their extracts in the biological screening assays. This led to the isolation of 30 pure secondary metabolites. Six compounds, (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde (**10**) from *A. cruciatus*, (3*S*)-(3',5'-dihydroxyphenyl)butan-2-one (**16**) and 2-((1'*E*)-propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol (**17**) from a *Coniothyrium* sp., microsphaeropsisin (**26**) from a *Microsphaeropsis* sp, ulocladol (**39**) from *Ulocladium botrytis* and 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46)** from *V. ramulosa*, proved to be new structures, and further three compounds, (3*S*,5*R*)-dimethyl-dihydrofuran-2-one (**11**) from *A. cruciatus*, 5,7-dimethoxy-4,6-dimethylphthalide (**33**) from a *Monochaetia* sp. and 9,10-dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**45**) from *V. ramulosa* were for the first time obtained as natural products.

4.4.1 *Acremonium* sp. H5-65

The stock cultures of strain H5-65 produced orange-yellow crystals in the initial test tubes at 4ºC. They could be separated from the attached agar and turned out to be pure **9** (7.1 mg).



9

Compound **9** was shown to possess the molecular formula $C_{10}H_{12}O_4$ by HREIMS, indicating five elements of unsaturation within the molecule. Its ^{1}H NMR spectrum consisted of only two singlets (δ 2.01 and 3.99), most probably representing two methyl groups and two methoxyl groups, respectively. The ^{13}C NMR spectra (^{1}H decoupled and DEPT) revealed additionally to the signals expected for the carbon atoms of the methyl (12.1 ppm) and the methoxyl (61.1 ppm) groups three signals representing sp^2-hybridized carbon atoms (138.9,

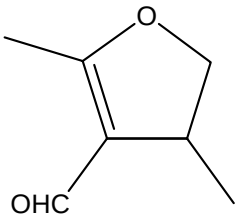
144.4 and 184.3 ppm) one of them possessing a chemical shift typical for carbon atoms of the carbonyl moieties in quinones (184.3 ppm). Together with the molecular formula deduced from the HREIMS, this meant that all signals observed in the NMR spectra accounted for two moieties or atoms, implying a high degree of symmetry within the molecule. These findings corresponded best with tetra-substituted quinones, leaving three possible structures: 2,6-dimethoxy-3,5-dimethylquinone, 2,5-dimethoxy-3,6-dimethylquinone and 2,3-dimethoxy-5,6-dimethylquinone. A literature search (Beilstein data base) revealed these three quinones all to be known; compound **9** could then shown to be 2,3-dimethoxy-5,6-dimethylquinone (aurantiogliocladin) by comparison of its spectroscopic data with published values (Perri & Moore, 1990).

Aurantiogliocladin (9): orange-yellow crystals (7.1 mg); mp 63°C; UV (EtOH) λ_{max} 270 nm; IR (film) ν_{max} 2965, 2665, 2650, 1610, 1455, 1385, 1330, 1270, 1205, 1090, 1050, 940, 925, 865, 735, 545 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.01 (6H, s, 2 × CH_3), 3.99 (6H, s, 2 × OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.1 (2C, q, 2 × CH_3), 61.1 (2C, q, 2 × OCH_3), 138.9 (2C, s, 2 × $\underline{C}$-OCH_3), 144.4 (2C, s, 2 × $\underline{C}$-CH_3), 184.3 (2C, s, 2 × C=O); EIMS *m/z* $[M]^+$ 196 (100), 181 (50), 173 (25), 167 (14), 153 (33), 151 (71), 125 (30), 123 (64), 97 (40); HREIMS *m/z* 196.0716 (calcd for $C_{10}H_{12}O_4$, 196.0736).

4.4.2 *Asteromyces cruciatus* H5-81

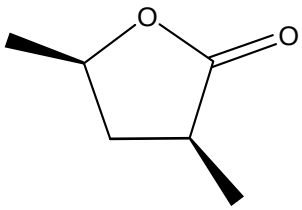
A. cruciatus was one of the obligate marine fungi obtained during this study. On all media tested, it produced abundant brownish-black conidia, which could easily be separated together with the mycelium from the growth medium by scraping them off. Thus, fungus and medium were investigated separately. The two different growth media were combined for extraction and yielded 473 mg of a brown oil (EtOAc extract). Subsequent normal-phase VLC (gradient hexane-EtOAc-MeOH) yielded four fractions. Fractions 1 (hexane/EtOAc 50/50, 352 mg) and 2 (EtOAc, 31 mg) were further separately purified by normal-phase HPLC (hexane/EtOAc 75/25) to give compounds **10** (6.1 mg), **11** (5.3 mg) and **12** (6.1 mg, fraction 2). Mycelium and conidia were freeze-dried (23 g), crushed in a mortar and subsequently extracted with hexane (500 ml) and MeOH (3 × 500 ml). The MeOH extract was suspended in EtOAc and the residue subjected to reversed-phase VLC (MeOH) giving 4.09 g of a

gum. Further normal-phase VLC (EtOAc/MeOH/H_2O/HOAc 60/15/15/10) yielded compounds **13-15**.



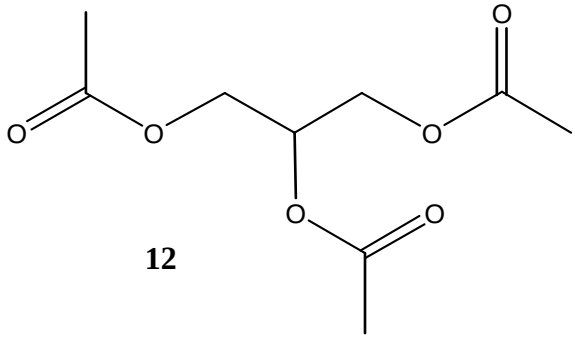
10

Compound **10** was obtained as a colourless oil, and proved to be too volatile for direct EIMS analysis. GC-EIMS however, resulted in the detection of its molecular ion $[M]^+$ at *m/z* 126. The ^{13}C NMR spectra of **4** (^1H decoupled and DEPT) revealed signals for seven carbon atoms, including one aldehyde moiety (185.0 ppm, d, CHO-3), two quaternary sp^2-hybridized carbon atoms (120.5 ppm, s, C-3; 174.6 ppm, s, C-2), two methyl groups (12.5 ppm, q, CH_3-2; 19.3 ppm, q, CH_3-4), one methylene group adjacent to an oxygen (79.3 ppm, t, C-5), and one methine group (34.8 ppm, d, C-4). From the ^1H NMR spectrum the molecular fragment from C<u>H</u>$_3$-4 (δ 1.21, d, *J* = 6.6 Hz) to H-4 (δ 3.34, m) and H_2-5 (δ 4.06, dd, J = 5.1, 9.2 Hz and δ 4.54, dd, *J* = 9.2, 9.7 Hz) could be established. Thus, CH_3-2, CHO-3, C-4 and OCH_2-5 (*via* the oxygen) had to be connected to the carbon-carbon double bond. ^1H-^{13}C long-range (HMBC) correlations between C<u>H</u>O-3, and both C-3 and C-4, and both C<u>H</u>$_3$-4 and H-4, and C-3, respectively, connected CHO and C-4 to C-3. CH_3-2 and the oxomethylene group had therefore to be the other substituents on the double bond. **10** is the new compound (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde.



11

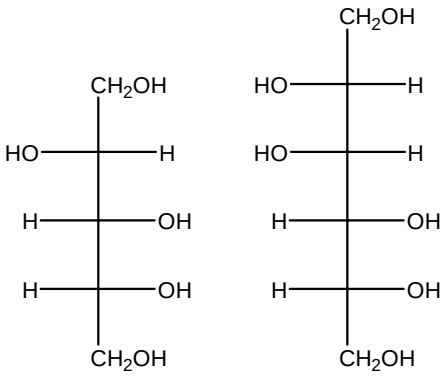
From the ^1H and ^{13}C NMR spectra of compound **11** the presence of two methyl groups, two methine groups, one of which had to be further connected to an oxygen atom, one methylene group, and one carboxyl moiety could be deduced. The ^1H-^1H COSY NMR spectrum revealed the connectivity of all hydrogen-bearing moieties. GC-MS resulted in a molecular ion at *m/z* $[M]^+$ 114, suggesting a molecular formula of $C_6H_{10}O_2$. Thus, the carboxyl group had to be part of a lactone moiety, completing the basic structure of **11**. By comparison of the NMR data and its optical rotation of +3.8° with published values (Davies, Polywka & Warner, 1990; Chu, Negrete & Konopelski, 1991) **11** was found to be (3*S*,5*R*)-dimethyl-dihydrofuran-2-one. This is the first report of this compound as a natural product.



12

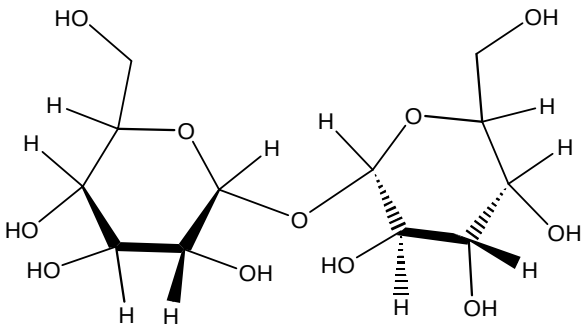
The ^{1}H NMR spectrum of compound **12** revealed signals typical for the glycerol-part of triglycerides (δ 4.14, 2H, dd, *J* = 5.8, 12.0 Hz, H-1a/H-3a; δ 4.29, 2H, dd, *J* = 4.3, 12.0 Hz, H-1b/H-3b, and δ 5.25, m, H-2). Additionally to the two expected signals due to the three carbon atoms of the glycerol-moiety (62.3 and 69.1 ppm), the ^{13}C NMR spectrum revealed for the acid-part of the molecule only signals attributable to the carbon atoms of three methyl groups and three ester moieties. Thus, **12** was presumed to be tri-*O*-acetyl-glycerol. This could be confirmed by comparison of the spectroscopic data with literature values (Numata *et al.*, 1990).

Investigation of the conidia and mycelium extract by ^{1}H NMR spectroscopy revealed it to consist mainly of sugars and polyols. Three main compounds could be separated, and were identified as D-arabitol (**13**), D-mannitol (**14**) and D-trehalose (**15**) by GC and NMR spectroscopy by comparison with authentic material. Not all of the extract was separated, total amounts were estimated to be 3.7 g D-arabitol, 2.8 g D-mannitol and 10.8 g D-trehalose per 100 g freeze-dried mycelium.



13

14



15

(+)-2,4-Dimethyl-4,5-dihydrofuran-3-carbaldehyde (10): colourless oil (6.1 mg; 1.2 mg/L medium), $[\alpha]^{20}_{D}$ +38.9° (*c* 0.61, $CHCl_3$); UV (EtOH) λ_{max} 272 nm (ε 2570); IR (film) ν_{max} 2930, 2740, 2730, 1730, 1635, 1625, 1455, 1395, 1240, 1120, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.21 (3H, d, *J* = 6.6 Hz, CH_3-4), 2.16 (3H, brs, CH_3-2), 3.34 (1H, m, H-4), 4.06 (1H, dd, *J* = 5.1, 9.2 Hz, H-5), 4.54 (1H, dd, *J* = 9.2, 9.7 Hz, H-5), 9.71 (1H, s, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.5 (q,

CH_3-2), 19.3 (q, CH_3-4), 34.8 (d, C-4), 79.3 (t, C-5), 120.5 (s, C-3), 174.6 (s, C-2), 185.0 (d, CHO); GC-EIMS *m/z* $[M]^+$ 126 (100), 111 (80), 83 (97), 55 (68), 43 (98); HRMS, sample decomposed prior to this measurement.

(3*S*,5*R*)-Dimethyl-dihydrofuran-2-one (11): colourless oil (5.3 mg; 1.1 mg/L medium); $[\alpha]^{20}_D$ +3.8° (*c* 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) d 1.26 (3H, d, *J* = 7.1 Hz, CH_3-3), 1.41 (3H, d, *J* = 6.1 Hz, CH_3-5), 1.46 (1H, m, H-4a), 2.51 (1H, m, H-4b), 2.67 (1H, m, H-3), 4.46 (1H, m, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) d 15.1 (q, CH_3-3), 20.9 (q, CH_3-5), 36.4 (d, C-3), 39.1 (t, C-4), 74.9 (d, C-5), 179.5 (s, C-2); GC-EIMS *m/z* $[M]^+$ 114 (3), 70 (66), 55 (96), 45 (22), 42 (100).

Tri-*O*-acetyl-glycerol (12): colourless oil (6.1 mg;1.2 mg/L medium); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.08 (6H, s, 2 × CH_3), 2.09 (3H, s, CH_3), 4.14 (2H, dd, *J* = 5.8, 12.0 Hz, H-1a/H-3a), 4.29 (2H, dd, *J* = 4.3, 12.0 Hz, H-1b/H-3b), 5.25 (1H, m, H-2); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.7 (q, 2 × CH_3), 20.9 (q, CH_3-2'), 62.3 (t, C-1/C-3), 69.1 (d, C-2), 170.1 (s, C=O), 170.5 (s, 2 × C=O)

D-Arabitol (13): $[\alpha]^{20}_D$ +9.5° (*c* 0.26, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 3.64 (1H, m), 3.72 (2H, m), 3.81 (1H, m), 3.86 (1H, m), 3.91 (1H, m), 3.99 (1H, m); ^{13}C NMR (D_2O, 100 MHz) δ 63.3 (t), 63.5 (t), 70.7 (d), 70.9 (d), 71.4 (d).

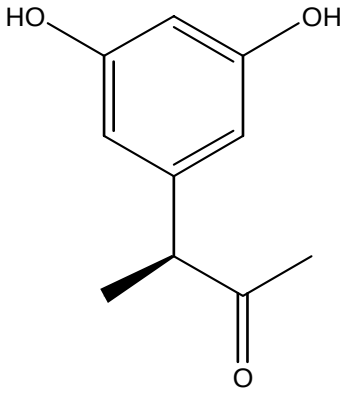
D-Mannitol (14): $[\alpha]^{20}_D$ +10.9° (*c* 0.11, H_2O); ^{1}H NMR (D_2O, 200 MHz) δ 3.50 - 3.95 (8H, m).

D-Trehalose (15): $[\alpha]^{20}_D$ +129.1° (*c* 0.44, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 3.49 (2H, brt, *J* = 9.2 Hz), 3.69 (2H, brdd, *J* = 3.6, 10.2 Hz), 3.81 (2H, m), 3.88 (6H, m), 5.24 (2H, brd, *J* = 4.1 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 60.9 (t), 70.1 (d), 71.4 (d), 72.5 (d), 72.9 (d), 93.6 (d).

4.4.3 *Coniothyrium* sp. 193H77

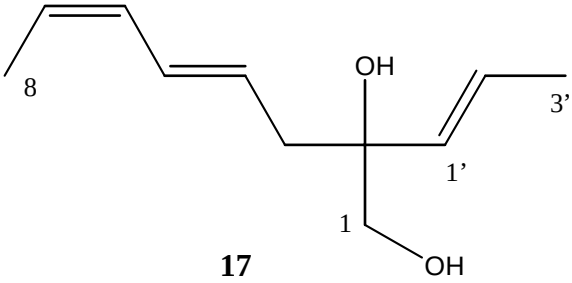
Culture extracts of *Coniothyrium* strain 193H77 showed prominent inhibition of several test-organisms in the initial screening for antimicrobial activity in agar diffusion assays: inhibition zones (extract from culture in liquid Biomalt-ASW-medium) against *B. megaterium* 0.2 cm, *M. violaceum* and *E. repens* 1.0 cm, *M. microspora* 0.5 cm and *C. fusca* 0.8 cm. These results, together with the presence of 7-10 major compounds in the extract as monitored by TLC, promoted further chemical investigation. The EtOAc extract (1.75 g of a brown oil) of a new, upscaled cultivation was fractionated by normal-phase VLC (gradient hexane-EtOAc) to yield five fractions. Fractions 3 (hexane/EtOAc 75/25, 250 mg) and 4 (hexane/EtOAc 50/50,

149 mg) were further separated by normal-phase HPLC (hexane/EtOAc 80/20) to yield **18** (5 mg), **21** (12 mg), and **23** (17 mg). Further purification of one previous HPLC fraction with reversed-phase (C-18) HPLC (MeOH/H_2O 70/30) yielded **20** (5.1 mg). Fraction 5 (EtOAc, 75 mg) was separated by normal-phase HPLC (hexane/EtOAc 70/30) to yield **16** (1.2 mg), **17** (1.3 mg), **19** (7.0 mg) and **22** (3.0 mg).



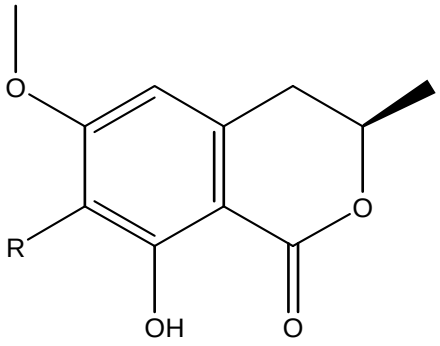
16

The molecular formula of **16** was established as $C_{10}H_{12}O_3$ by accurate mass measurement, indicating the molecule to have five elements of unsaturation. ^{1}H NMR measurements revealed the presence of two methyl groups (δ 1.35, d, J = 6.8 Hz, H_3-4 and δ 2.08, s, H_3-1), the first with an adjacent methine group (δ 3.63, q, J = 6.8 Hz, H-3), and the other connected to a double bond, two hydroxyl groups (δ 5.19, brs), and three aromatic hydrogens (δ 6.27, m). From the ^{13}C NMR and DEPT spectra a carbonyl group (209.3 ppm, s, C-2) and three quaternary aromatic carbons (143.2 and 2 × 157.3 ppm), were deduced. These findings, together with UV maxima at 217 and 280 nm suggested the presence of a 1,3,5-trisubstituted aromatic ring. The ^{13}C NMR chemical shifts of the ring carbons, ranging from 101.8 to 157.3 ppm, showed the typical influence of electronegative ring substituents. Thus, the two OH-groups had to be phenolic, accounting for two of the three substituents on the aromatic ring. The ^{1}H NMR signal for the methyl group H3-1 (δ 2.08) appeared as a singlet, indicating it to be adjacent to the carbonyl group C-2, leaving the methine group to be positioned between it and the aromatic ring, thus giving **16**. Comparison of the ^{1}H NMR shifts of **16** with published values for the di-methoxy-derivative clearly substantiated the structure proposed for **16** (Cristol, Mahfuza & Sankar, 1989). The absolute configuration at C-3 was determined as *S* by comparison of the optical rotation of **16** (+124.0°) to those of similar 3-phenylbutan-2-ones, e.g., -350° for (3*R*)-phenylbutan-2-one, +340° for (3*S*)-phenylbutan-2-one (Fuganti *et al.*, 1984), and +218° for (3*S*)-(*p*-methoxyphenyl)butan-2-one (Collins & Hobbs, 1970). Thus, **16** is the new compound (3*S*)-(3',5'-dihydroxyphenyl)butan-2-one.



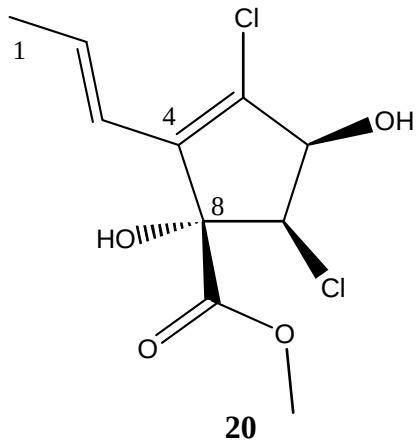
17

Compound **17** was obtained as a clear oil. CIMS with NH_3 gave ions at *m/z* 182 $[M]^+$ and 200 $[M + NH_4]^+$. CIMS with *i*-butane resulted in ions at *m/z* 182 $[M]^+$ and 165 $[MH - H_2O]^+$, while GC-EIMS yielded fragment ions at *m/z* 101 $[M - C_6H_9]^+$ and 151 $[M - CH_2OH]^+$; the molecular formula of **17** is $C_{11}H_{18}O_2$. From a ^1H-^{13}C 2D NMR shift correlated measurement (HMQC) 16 hydrogens could be assigned to their directly bonded carbon atoms, revealing the presence of two methyl groups (13.3 ppm, C-8 and 17.8 ppm, C-3'), one methylene group (41.2 ppm, C-3), one methylene group adjacent to an oxygen (68.8 ppm, C-1), six olefinic methine groups (125.6 ppm, C-7; 126.5 ppm, C-2'; 127.4 ppm, C-4; 129.0 ppm, C-6; 129.7 ppm, C-5 and 133.3 ppm, C-1') and one quaternary carbon atom (74.8 ppm, C-2) adjacent to oxygen. Thus, two hydroxyl groups had to be present within the molecule. From the ^1H-^1H COSY spectrum the molecular fragments from C-3 to C-8 and C-1' to C-3' could be established. Coupling was observed between H-1' (δ 5.47, dd, J = 1.6, 15.7 Hz) and H-2' (δ 5.77, dq, J = 6.8, 15.7 Hz), H-2' and H_3-3' (δ 1.75, brd, J = 6.8 Hz). Further, H_2-3 (δ 2.40, m) coupled with H-4 (δ 5.62, ddd, J = 7.6, 7.6, 15.2 Hz), H-4 with H-5 (δ 6.45, dd, J = 11.4, 15.2 Hz), H-5 with H-6 (δ 5.99, brdd, J = 10.8, 11.4 Hz), H-6 with H-7 (δ 5.45, dq, J = 6.8, 10.8 Hz) and H-7 with H_3-8 (δ 1.75, brd, J = 6.8 Hz). HMBC ^1H-^{13}C long-range correlations observed between H_2-3, H-4, H-1' and C-2 linked both these fragments to the quaternary carbon atom C-2. The hydrogens of the remaining hydroxymethyl group C-1, showed long-range correlations to C-3, thus, also had to be connected to C-2. The configurations of the double bonds were determined to be 4*E*,6*Z*,1'*E* on the basis of the ^1H-^1H spin coupling constants (J = 15.2, 10.8 and 15.7 Hz, respectively), and confirmed by NOE difference measurements. Irradiation at the ^1H NMR frequency of H_3-8 and H_3-3' enhanced the signals of both H-5 and H-1', confirming the *Z*-configuration at $\Delta^{6,7}$ and the *E*-configuration at $\Delta^{1',2'}$. Thus, **17** is the new compound 2-((1'*E*)-propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol.



18 R = H
19 R = Cl

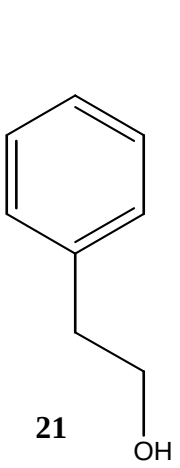
The ^{1}H NMR spectra of compounds **18** and **19** revealed signals typical for mellein derivatives (cf. 4.4.6; compounds **27-29**): one methyl group (δ 1.54, d, *J* = 6.4 Hz) adjacent to a methine group (around δ 4.75, m) substituted with oxygen and a methylene group (around δ 2.85); and one hydroxyl group with a hydrogen atom (around δ 11.5, s) deshielded due to a hydrogen bond to a carbonyl moiety. The ^{1}H NMR spectra of **18** and **19** additionally contained signals due to one methoxyl group and two, respectively one (**19**), hydrogen atom(s) on an aromatic ring. In compound **18** these two hydrogen atoms were located *meta* to each other as judged by the value of their ^{1}H-^{1}H spin coupling constant (*J* = 2.3 Hz), suggesting the methoxyl group to be positioned in between, and thus giving 6-methoxymellein. In compound **19** the remaining substituent of the aromatic ring was obviously a chlorine atom, the presence of which could be clearly derived from the molecular ion cluster in the EIMS spectrum. By comparison of the spectroscopic data with published values, **19** could be shown to be 6-methoxy-7-chloromellein (MacGahren & Mitcher, 1968). The stereochemistry at C-3 of both compounds was established as *R* by comparison of their optical rotations with published values (McGahren & Mitcher, 1968).



20

EIMS revealed for compound **20** a molecular ion at *m/z* $[M]^{+}$ 266, and proved two chlorine atoms to be present within the molecule. From the ^{1}H NMR spectrum the presence of one methyl group (δ 1.81, d, *J* = 4.9 Hz) and one methoxyl group (δ 3.91, s) could be deduced. Additionally, two broad singlets (δ 2.97 and δ 3.97, 1H each), presumably accounting for the hydrogen atoms of two OH-groups, and two multiplets at δ 4.47 and δ 6.20, each accounting for two hydrogen atoms, were observed. The ^{13}C NMR spectrum revealed signals due to 10 carbon atoms, including five sp^{2}-hybridised carbon atoms of double-bonds, one of

them involved in a carboxyl moiety. Further structure elucidation by ^{1}H-^{1}H COSY and ^{1}H-^{13}C long-range (HMBC) correlation NMR measurements was hindered due to the overlapping signals of four hydrogen atoms. A literature search (Chapman & Hall database) for molecules containing two chlorine atoms and possessing a molecular mass around 266 resulted in cryptosporiopsinol as the most probable structure for **20**. This could then be confirmed by comparison of ^{1}H and ^{13}C NMR data and optical rotation with published values (Giles & Turner, 1969; Holker & Young, 1975).



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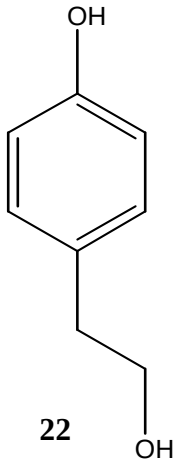

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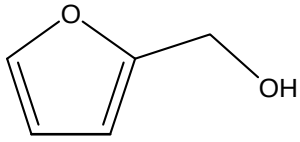


23

Compounds **21-23** were identified as 2-phenylethanol, 2-(*p*-hydroxyphenyl)ethanol and 2-(hydroxymethyl)furan by NMR spectroscopy and MS.

(3*S*)-(3',5'-Dihydroxyphenyl)butan-2-one (16): colourless oil (1.2 mg, 0.16 mg/L medium); $[\alpha]^{20}_{D}$ +124.0° (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} 217 nm (ε 27900) and 280 nm (ε 1170); IR (film) ν_{max} 3250, 2920, 2850, 1670, 1605, 1455, 1155 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.35 (3H, d, *J* = 6.8 Hz, H-4), 2.08 (3H, s, H-1), 3.63 (1H, q, *J* = 6.8 Hz, H-3), 5.19 (2H, brs, OH-3'/5'), 6.27 (3H, m, H-2'/H-4'/H-6'); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 16.9 (q, C-4), 28.3 (q, C-1), 53.5 (d, C-3), 101.8 (d, C-4'), 107.4 (d, C-2'/C-6'), 143.2 (s, C-1'), 157.3 (s, C-3'/C-5'), 209.3 (s, C-2); EIMS *m/z* $[M]^{+}$ 180 (55), 149 (45), 137 (100); HREIMS *m/z* 180.078 (calcd for $C_{10}H_{12}O_3$, 180.079).

2-((1'*E*)-Propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol (17): colourless oil (1.3 mg, 0.17 mg/L medium); $[\alpha]^{20}_{D}$ -13.8° (*c* 0.13, $CHCl_3$); UV (EtOH) λ_{max} 235 nm (ε 12600); IR (film) ν_{max} 3385, 2925, 2855, 1675, 1450, 1440, 1375, 1055, 1035, 985, 970 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.75 (6H, brd, *J* = 6.8 Hz, H-8/H-3'), 2.40 (2H, m, H-3), 3.48 (2H, m, H-1), 5.45 (1H, dq, *J* = 6.8, 10.8 Hz, H-7), 5.47 (1H, dd, *J* = 1.6, 15.7 Hz, H-1'), 5.62 (1H, ddd, *J* = 7.6, 7.6, 15.2 Hz, H-4), 5.77 (1H, dq, *J* = 6.8, 15.7 Hz, H-2'), 5.99 (1H, brdd, *J* = 10.8, 11.4 Hz, H-6), 6.45 (1H, dd, *J* = 11.4, 15.2 Hz, H-5);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.3 (q, C-8), 17.8 (q, C-3'), 41.2 (t, C-3), 68.8 (t, C-1), 74.8 (s, C-2), 125.6 (d, C-7), 126.5 (d, C-2'), 127.4 (d, C-4), 129.0 (d, C-6), 129.7 (d, C-5), 133.3 (d, C-1'); CIMS (NH_3) *m/z* 200 (100) $[M + NH_4]^+$, 182 (15) $[M]^+$; CIMS (*i*-butane) *m/z* 182 (15) $[M]^+$, 165 (100) $[MH - H_2O]^+$; GC-EIMS *m/z* 151 (8) $[M - CH_2OH]^+$, 101 (100) $[M - C_6H_9]^+$, 83 (92), 69 (62), 55 (86); HRMS, sample decomposed prior to this measurement.

(3*R*)-6-Methoxymellein (18): colourless oil (5 mg, 0.67 mg/L medium); $[\alpha]^{20}_D$ -18° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.54 (3H, d, *J* = 6.4 Hz, CH_3-3), 2.88 (2H, m, H-4), 3.87 (3H, s, OCH_3), 4.80 (1H, m, H-3), 6.23 (1H, d, J = 2.3 Hz), 6.43 (1H, d, *J* = 2.3 Hz), 11.33 (1H, s, OH).

(3*R*)-6-Methoxy-7-chloromellein (19): white solid (7 mg, 0.93 mg/L medium); $[\alpha]^{20}_D$ -47° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.53 (3H, d, *J* = 6.0 Hz, CH_3-3), 2.91 (2H, m, H-4), 3.96 (3H, s, OCH_3), 4.70 (1H, m, H-3), 6.33 (1H, s, H-5), 11.69 (1H, s, OH-8); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.6 (q, CH_3-3), 34.8 (t, C-4), 56.5 (q, OCH_3), 75.7 (d, C-3), 101.1 (d, C-5), 102.9 (s, C-8a), 108.3 (s, C-7), 138.9 (s, C-4a), 159.2[a] (s, C-6), 160.8[a] (s, C-8), 169.5 (s, C-1), [a] assignments may be interchanged; EIMS *m/z* 244 (32), 242 (100) $[M]^+$, 226 (10), 224 (30), 200 (23), 198 (66).

Cryptosporiopsinol (20): white solid (5.1 mg, 0.68 mg/L medium); $[\alpha]^{20}_D$ -25.5° (*c* 0.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.81 (3H, d, *J* = 4.9 Hz, H-1), 2.97 (1H, brd, *J* = 11.0 Hz, OH-6), 3.91 (3H, s, OCH_3), 3.97 (1H, brs, OH-8), 4.47 (2H, m, H-7/H-8), 6.20 (2H, m, H-2/H-3); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 19.4 (q, C-1), 54.5 (q, OCH_3), 66.0 (d, C-7), 75.5 (d, C-6), 87.2 (s, C-8), 120.6 (d, C-3), 133.1 (s, C-5), 134.4 (d, C-2), 137.0 (s, C-4), 172.1 (s, COO); EIMS *m/z* 270 (<1), 268 (5), 266 (9) $[M]^+$, 252 (2), 250 (11), 248 (16), 215 (16), 213 (48), 209 (64), 207 (100), 197 (19), 195 (29), 187 (6), 185 (20), 173 (15), 171 (35), 161 (10), 159 (19), 145 (10), 143 (30).

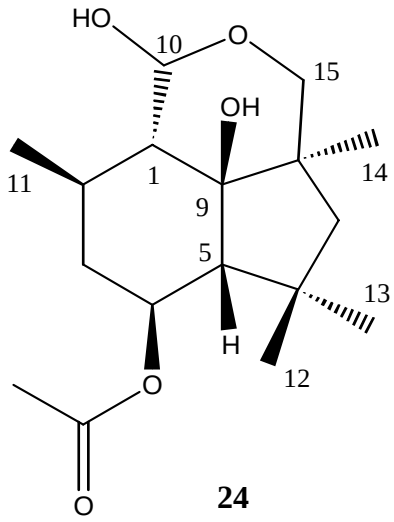
2-Phenylethanol (21): colourless oil (12 mg, 1.6 mg/L medium); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.87 (2H, t, *J* = 6.5 Hz), 3.86 (2H, t, *J* = 6.5 Hz), 7.25 (5H, m); GC-EIMS *m/z* $[M]^+$ 112 (100), 97 (7), 84 (20), 83 (25), 69 (39), 55 (37).

2-(*p*-Hydroxyphenyl)ethanol (22): colourless oil (3.0 mg, 0.4 mg/L medium); ^{1}H NMR (acetone-d6, 200 MHz) δ 2.65 (2H, t, *J* = 7.1 Hz), 3.62 (2H, brt, *J* = 7.1 Hz), 6.68 (2H, m), 6.99 (2H, m); EIMS *m/z* $[M]^+$ 138 (34), 107 (100).

2-(Hydroxymethyl)furan (23): colourless oil (17 mg, 2.3 mg/L medium); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.61 (2H, brs), 6.29 (1H, dd, *J* = 0.7, 3.4 Hz), 6.34 (1H, dd, *J* = 1.9, 3.4 Hz), 7.40 (1H, dd, *J* = 0.7, 1.9 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 57.5 (t), 107.8 (d), 110.3 (d), 142.6 (d), 153.9 (s).

4.4.4 *Emericellopsis minima* H7-65

The isolate of *E. minima* was chosen for further chemical investigation because of prominent antimicrobial activity of its extracts as observed in preliminary agar diffusion assays: inhibition zones (extract from culture on Biomalt medium) against *B. megaterium* 0.6 cm, *M. violaceum* 1.5 cm, *E. repens* 0.8 cm, *F. oxysporum* 0.2 cm, *C. fusca* 0.5 cm. Although the EtOAc extract obtained from the up-scaled recultivation of the strain proved to be inactive in the same assays, it was further investigated chemically. Thus, the extract (1.06 g of a brown oil) was fractionated by normal-phase VLC (gradient hexane-EtOAc-MeOH) to yield 14 fractions. Fraction 4 (hexane/EtOAc 70/30, 67.7 mg) was further separated by normal-phase HPLC (hexane/EtOAc 75/25) to yield compound **24** (19.6 mg).



24

The ^{13}C NMR spectrum of **24** revealed signals for 16 aliphatic carbon atoms and one carbon atom of a carboxyl moiety (170.5 ppm, s). By DEPT and ^{1}H-^{13}C shift correlated (HMQC) NMR measurements, the 16 aliphatic moieties could be shown to consist of five methyl groups, three methylene groups, one of them presumably connected to an oxygen atom, five methine groups (two of them adjacent to an oxygen atom) and three quaternary carbon atoms (one of them adjacent to an oxygen atom). The ^{1}H NMR spectrum additionally revealed the presence of two hydroxyl groups. By ^{1}H-^{1}H COSY NMR measurements, the molecular part from CH_3-11 and CHOH-10 to CH-5 could be established. The structure was further elaborated by interpretation of the ^{1}H-^{13}C long-range (HMBC) NMR spectrum of **24**. Long-range correlations observed between both H-4 and the hydrogens (δ 2.02) of one methyl group, and the carbon atom of the carboxyl moiety revealed an acetate as substituent at C-4. Further long-range correlations between both C$\underline{H}_3$-12 and C$\underline{H}_3$-13, and C-5, C-6 and C-7, and between C$\underline{H}_3$-14 and C-7, C-8, C-9 and C-15 established the molecular part from CH-5 to C-9 and to CH_2-15. Additional long-range correlations between both H-5 and H-1, and C-9, and between C$\underline{H}_2$-15 and C-10 connected the molecular fragments to a three ring

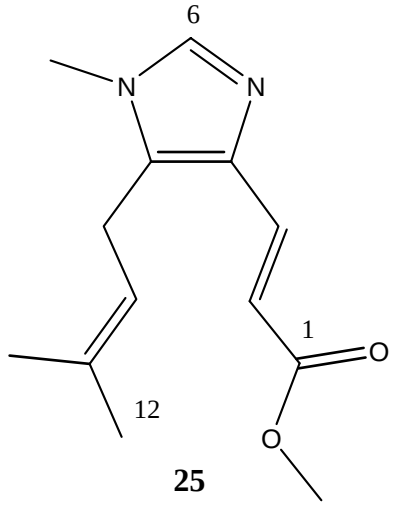
system, leaving the remaining OH-group to be positioned at C-9. A literature search (Chapman & Hall database) revealed compound **24** to possess an identical basic structure as dihydrobotrydial (Fehlhaber *et al.*, 1974). It could be shown to be identical with this metabolite by comparing the NMR data and optical rotation with published values.

Dihydrobotrydial (24): colourless crystals (19.6 mg; 2.2 mg/L medium); mp. 164°C; $[\alpha]^{20}_{D}$ +36.7° (*c* 0.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98 (3H, d, *J* = 6.1 Hz, H-11), 1.07 (1H, m, H-3a), 1.11 (3H, s, H-12 or H-13), 1.17 (1H, d, *J* = 11.7 Hz, H-7a), 1.26 (3H,s, H-12 or H-13), 1.28 (3H, s, H-14), 1.64 (1H, d, *J* = 12.7 Hz, H-1), 1.80 (1H, m, H-2), 1.88 (1H, d, *J* = 11.7 Hz, H-7b), 1.90 (1H, d, *J* = 10.7 Hz, H-5), 2.02 (3H, s, CH_3CO), 2.06 (1H, m, H-3b), 3.25 (1H, d, *J* = 10.7 Hz, H-15a), 3.52 (1H, brs, OH-9 or OH-10), 3.60 (1H, s, OH-9 or OH-10), 4.19 (1H, d, *J* = 10.7 Hz, H-15b), 5.08 (1H, ddd, *J* = 5.1, 10.7, 11.2 Hz, H-4), 5.33 (1H, brs, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.1 (q, C-11), 21.4 (q, $\underline{C}H_3CO$), 25.3 (q, C-14), 27.3 (q, C-12 or C-13), 28.7 (d, C-2), 35.9 (q, C-12 or C-13), 38.8 (s, C-6), 39.9 (t, C-3), 45.4 (s, C-8), 50.4 (t, C-7), 55.0 (d, C-1), 60.0 (d, C-5), 67.6 (t, C-15), 72.7 (d, C-4), 83.4 (s, C-9), 92.5 (d, C-10), 170.5 (s, $CH_3\underline{C}O$); EIMS *m/z* $[M - H_2O]^+$ 294 (11), 252 (9), 235 (16), 234 (20), 175 (52), 86 (67), 84 (100).

4.4.5 *Microascus* sp. K14

The *Microascus* strain K 14 had been chosen for further chemical investigation because of prominent antifungal activity observed in agar diffusion assays: inhibition zones (extract from culture on Biomalt medium) against *B. megaterium* 0.1 cm, *M. violaceum* 0.2 cm, *E. repens* 1.3 cm. 0.5 g of the EtOAc extract of a recultivation was fractionated by normal-phase VLC (gradient petrol ether-EtOAc-MeOH) to yield four fractions. Fraction 3 (MeOH/EtOAc 50/50) was further fractionated by normal-phase HPLC (petrol ether/acetone 50/50). The major compound remained on the column and was eluted with pure acetone to give **25** (5 mg).

The ^{1}H NMR spectrum of **25** revealed the presence of two methyl groups on double bonds (δ 1.72, s, 3H and δ 1.75, s, 3H), two methyl groups attached to electro-negative atoms such as oxygen or nitrogen (δ 3.62, s, 3H and δ 3.72, s, 3H), one *E* substituted double bond (δ 6.70 and δ 7.56; 1H each, *J* = 15.4 Hz), one methylene group (δ 3.42, d, *J* = 6.8 Hz), and two methine groups (δ 5.01, m and δ 7.99, brs).



25

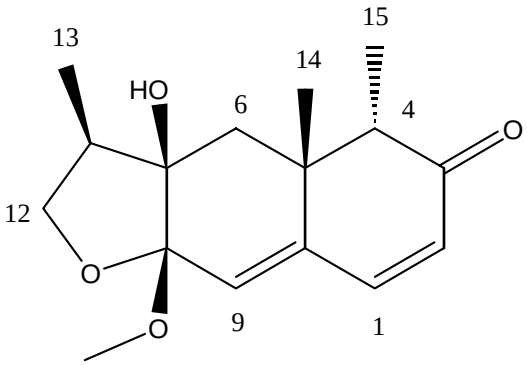
From the ^{13}C NMR spectra (^{1}H decoupled and DEPT) the additional presence of four sp^2-hybridised quaternary carbon atoms could be deduced, one of them most probably part of an ester moiety. After assignment of all protons to their directly bonded carbon atoms via a ^{1}H-^{13}C 2D NMR shift correlated measurement (HMQC), the structure of **25** was further elaborated by interpretation of its ^{1}H-^{13}C longe-range (HMBC) NMR spectrum. Long-range coupling was observed between both the hydrogen atoms of the methoxyl group and and those of the *E* substituted double-bond, and the carbon atom of the ester moiety, establishing the molecular part from the methoxyl group to C-3. Further long-range correlations between both C$\underline{H}_3$-12, C$\underline{H}_3$-13 and C$\underline{H}_2$-9, and both C-10 and C-11 established a second fragment of the molecule. Both fragments were connected via the two sp^2-hybridised carbon atoms C-4 and C-8: thus long-range correlations between both H-3 and C$\underline{H}_2$-9, and C-4 and C-8 were observed. This left only one methyl group and the methine group C-6 to be positioned. The chemical shifts of this methyl group (δ 3.62 and 32.2 ppm) suggested it to be attached to a nitrogen atom. The corresponding protons showed long-range correlations only with C-8 and C-6, thus the N-methyl group had to be positioned in between these two carbon atoms. The methine group CH-6 had further to form a double-bond with a hetero-atom due to the absence of additional carbon atoms, and thus giving an *N*-methylimidazole structure for the central part of **25**. The positioning of the N-methyl group and the assignment of C-4 and C-8 was so far only tentative, it could have been proven by difference NOE measurements. A literature search (Beilstein database), however, revealed both possible isomers to be known. A closer inspection showed them to be isolated from two different *Fusarium* spp., and to be published with almost identical NMR data (the 5-NCH_3 isomer as visoltricin: Visconti & Solfrizzo, 1994; the 7-NCH_3 isomer as fungerin: Kato *et al.*, 1996), both data sets being in good agreement with the data obtained in this study. A recent total sythesis (Benhida, Lezama & Fourrey, 1998) confirmed the structure proposed by Kato *et al.* Thus, **25** is fungerin.

Fungerin (25): colourless solid (5 mg; 3.3 mg/L medium); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.72 (3H, brs, H-12), 1.75 (3H, brs, H-13), 3.42 (2H, d, *J* = 6.8 Hz, H-9), 3.62 (3H, s, NCH_3), 3.76 (3H, s, OCH_3), 5.01 (1H, m, H-10), 6.70 (1H, d, *J* = 15.4 Hz, H-2), 7.56 (1H, d, *J* = 15.4 Hz, H-3), 7.99 (1H, brs, H-6); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 18.0 (q, C-13), 22.3 (t, C-9), 25.5 (q, C-12), 32.2 (q, NCH_3), 51.5 (q, OCH_3), 116.4 (d, C-2), 118.5 (d, C-10), 132.0 (s, C-4), 132.9 (d, C-3), 134.1 (s, C-8), 135.1 (s, C-11), 138.1 (d, C-6), 168.0 (s, C-1).

4.4.6 *Microsphaeropsis* sp. H5-50

The *Microsphaeropsis* strain H5-50 had been chosen for further chemical investigation because of prominent antimicrobial activity of its extracts as observed in preliminary agar diffusion assays: inhibition zones (extract from culture on Biomalt medium) against *B. megaterium* 0.3 cm, *M. violaceum* 1.0 cm, *E. repens* 0.5 cm, *C. fusca* 0.3 cm). TLC investigation of this extract revealed at least five main components. The EtOAc extract of a recultivation (0.93 g of brown oil) was fractionated by normal-phase VLC (gradient hexane-EtOAc) to yield 4 fractions. Fraction 2 (145 mg) was further fractionated by normal-phase HPLC (hexane/EtOAc 70/30) to yield **26** (1.3 mg), **27** (20.8 mg), **28** (425 mg), and **29** (51 mg). Further purification of one previous HPLC fraction with reversed-phase (C-18) HPLC (MeOH/H_2O 75/25) yielded **30** (5 mg) and **31** (0.5 mg).

The molecular formula of **26** was determined as $C_{16}H_{22}O_4$ by accurate mass measurement. ^{13}C NMR spectroscopy (^{1}H decoupled and DEPT) showed that three of the six elements of unsaturation, as indicated by the molecular formula of **26**, could be attributed to two carbon-carbon double bonds (126.3 ppm, d, C-2; 126.9 ppm, d, C-9; 140.9 ppm, s, C-10 and 143.9 ppm, d, C-1), and a carbonyl group (204.2 ppm, s, C-3); the molecule is thus tricyclic. After association of all ^{1}H NMR resonances with the ^{13}C NMR resonances of the directly bonded carbon atoms via a 1H-13C 2D NMR shift-correlated measurement (HMQC), the presence of a single OH-group was evident (δ 3.03, d, *J* = 1.9 Hz). ^{1}H and ^{13}C NMR spectra further revealed the presence of one methoxyl group (48.3 ppm), two methylene groups (42.1 ppm, t, C-6 and 72.8 ppm, t, C-12), one of which had to be attached to oxygen, two methine groups (δ 2.10, m, H-11 and δ 2.27, q, *J* = 7.3 Hz, H-4) with adjacent methyl groups (δ 0.98, d,



26

J = 7.3 Hz, H3-15 and δ 1.05, d, *J* = 7.3 Hz, H_3-13), one quaternary methyl group (δ 1.39, s, H_3-14), and three quaternary carbon atoms (39.7 ppm, s, C-5; 78.2 ppm, s, C-7 and 101.3 ppm, s, C-8), including one substituted with an oxygen containing functionality and one as part of an acetal moiety. From the ^{1}H-^{1}H COSY of 26 three fragments of the molecule could be deduced. Thus, coupling was observed between H_3-15 and H-4, H_3-13 and H-11, and further to H_2-12. The only other ^{1}H-^{1}H spin system involved the methine protons H-1 (δ 6.89, d, *J* = 9.9 Hz) and H-2 (δ 5.90, d, *J* = 9.9 Hz), and left C-9 and C-10 to form the second carbon-carbon double bond. An UV maximum at λ = 280 nm suggested the double bonds and the carbonyl group to be conjugated. This deduction was confirmed via a ^{1}H-^{13}C HMBC measurement which revealed the carbonyl group (C-3) and the $\Delta^{9,10}$ double bond both to be connected to the $\Delta^{1,2}$ double bond. Long-range correlations between both H-4 and H_3-15 and C-3 established the connectivity between C-3 and C-4. Correlations between H_3-14 and C-4, C-5, C-6 and C-10 showed that C-14 was bound to C-5, and further C-5 to C-4, C-10 and C-6, thus establishing the first of the three rings.

Further, long-range correlations between the hydrogens of the hydroxyl and the methoxyl group and C-7 and C-8, respectively, located the hydroxyl group at C-7 and the methoxyl group at C-8. As C-8, evidenced by its ^{13}C NMR chemical shift (101.3 ppm) is the carbon atom involved in the acetal moiety, it had to be further connected to the methylene group C-12 via the remaining oxygen atom. Further long-range correlations between the 7-OH and C-6, and between both H_3-13 and H-11 and C-7 connected both C-6 and C-11 to C-7, leaving the acetal moiety (C-8) to be bound to both C-7 and C-9, thus completing the planar structure of **26**.

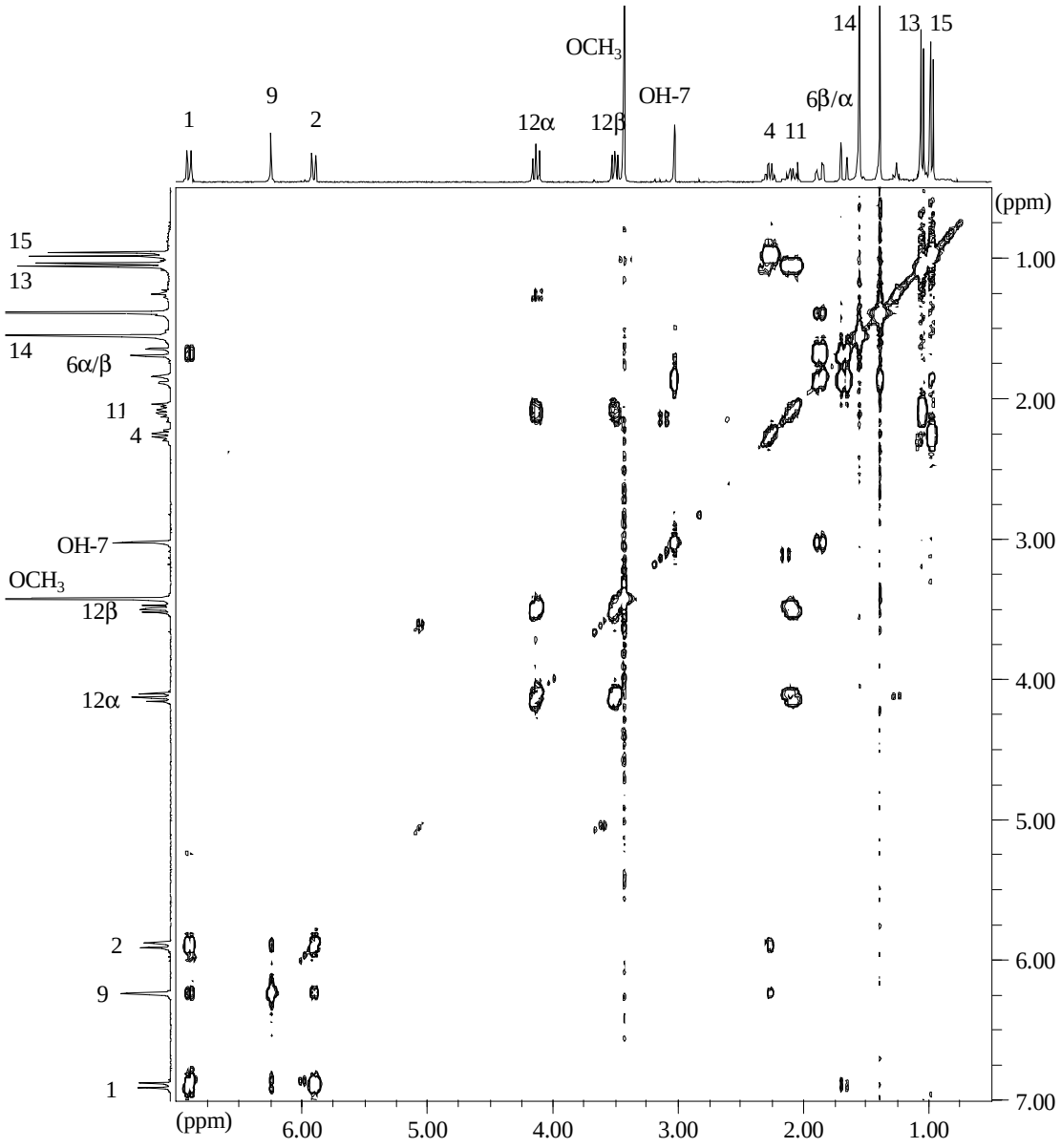
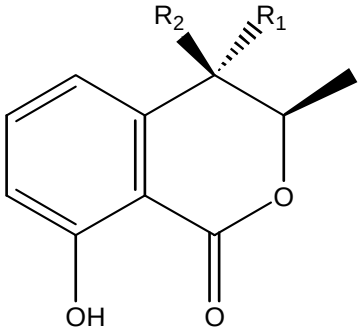


Figure 6: ^{1}H-^{1}H COSY NMR spectrum of 26; $CDCl_3$, 300 MHz.

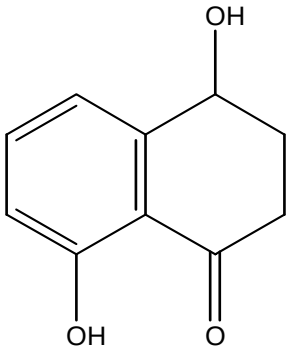
The relative configurations at the five chiral centres were established by NOE difference measurements. Irradiation at the resonance frequency of H_3-15 (δ 0.98) only enhanced the signal of H-6α, whereas irradiation at δ 1.39 (H_3-14) enhanced the signals of H-4, H-6β and 7-OH. Irradiation at δ 1.87 (H-6α) enhanced the signals of H-11 and H3-15; irradiation at δ 3.03 (7-OH) enhanced the signals of H_3-13, H_3-14 and the methoxyl group. Further, irradiation at the resonance frequency of H_3-13 (δ 1.05) enhanced the signals of H-12β and 7-OH. Thus, H-4, H-6β, 7-OH, H-12β,

H_3-13, H_3-14 and the methoxyl group had to be on the same side of the molecule, and H-6α and H_3-15 on the other side. Finally, irradiation at δ 6.24 (H-9) enhanced the signal of H-1, confirming the double bond assignments. Compound **26** is thus a new natural product of the eremophilane type for which the trivial name microsphaeropsisin is suggested.



27 $R_1 = R_2 = H$
28 $R_1 = OH$, $R_2 = H$
29 $R_1 = H$, $R_2 = OH$

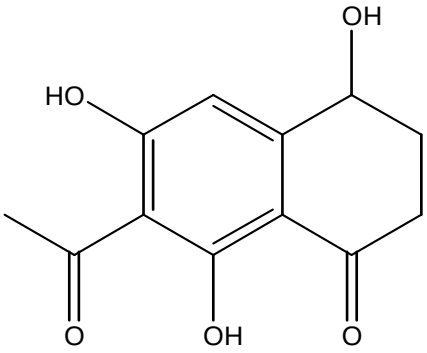
The ^{1}H NMR spectrum of compound **27** showed signals typical for the hydrogen atoms of a 1,2,3-trisubstituted aromatic ring (δ 6.68, 1H, dd, *J* = 0.9, 7.4 Hz, δ 6.88, 1H, d, *J* = 8.3 Hz, and δ 7.42, 1H, dd, *J* = 7.4, 8.3 Hz), a methyl group (δ 1.54, d, *J* = 6.3 Hz) adjacent to a methine group (δ 4.73, dq, *J* = 6.3, 7.3 Hz) substituted with an oxygen atom, a methylene group (δ 2.92, d, *J* = 7.3 Hz) adjacent to the aromatic ring and a hydroxyl group (δ 11.07, s) involved in a hydrogen bond to a carbonyl moiety. Together with its characteristic smell, compound **27** was presumed to be the widespread fungal metabolite mellein. The ^{1}H NMR spectra of compounds **28** and **29** revealed in comparison to mellein, that one hydrogen atom of the methylene group was substituted for an oxygen, the rest of the molecule seemed to be unaltered. Compounds **27-29** were then identified by comparison of their spectroscopic data and optical rotations with published values as the known fungal metabolites *R*-mellein (Holker & Simpson, 1981; Garson, Staunton & Jones, 1984), (3*R*,4*S*)-hydroxymellein (Devys *et al.*, 1992; Izawa *et al.*, 1989), and (3*R*,4*R*)-hydroxymellein (Izawa *et al.*, 1989; Aldridge *et al.*, 1971).



30

Compound **30** appeared to be similar to **28** and **29** as judged by interpretation of its ^{1}H NMR spectrum, except for the methyl group and one aliphatic methine group, which were obviously replaced in **30** by two methylene groups. EIMS revealed a molecular ion at *m/z* $[M]^+$ 178. With this information, a literature search (Chapman & Hall database) showed compound **30** to be 4,8-dihydroxy-3,4-dihydro-2*H*-

naphthalene-1-one (Findlay & Kwan, 1973; Talapatra *et al.*, 1988). The observed optical rotation of zero for **30** suggested the isolate to be racemic.



31 (tentative structure)

The ^{1}H NMR spectrum of compound **31** was similar to that of **30**. A corresponding CH_2-CH_2-CHO-fragment could be deduced. Only one signal due to a hydrogen atom on an aromatic ring was present, instead a signal due to one additional methyl group on a double bond was observed, and the signal of the hydrogen atom of the OH-group at δ 12.41 was replaced by two signals further downfield (δ 14.34 and δ 14.98), each representing one hydrogen atom. Unfortunately, compound **31** was too scarce for recording a ^{13}C NMR spectrum. NOE difference measurements with irradiation at the resonance frequency of the hydrogen atom on the aromatic ring resulted in an enhancement of the signal of the hydrogen atom of the other methine group, whereas irradiation at the resonance frequency of the hydrogens of the methyl group gave no positive NOE. EIMS revealed a molecular ion at *m/z* $[M]^+$ 236, suggesting in combination with the NMR data a molecular formula of $C_{12}H_{12}O_5$. A fragment ion at *m/z* 221 most probably resulted from the loss of the methyl group. Taking these results into account and presuming for **31** a basic structure similar to that of **30**, the only differences were likely to be the substituents at C-6 and C-7. These substituents may consist of one acetyl moiety and one OH-group. Unfortunately, compound **31** decomposed before further measurements could be performed. A literature search (Chapman & Hall and Beilstein databases) with the confirmed structural fragments revealed no structure to fit the described data.

Microsphaeropsisin (26): colourless solid (1.3 mg, 0.29 mg/L medium); mp 152-154°C; $[\alpha]^{20}_{D}$ -97.7° (*c* 0.13, $CHCl_3$); UV (EtOH) λ_{max} 223 nm (ε 2250) and 280 nm (ε 8560); IR (film) ν_{max} 3515, 2925, 1665, 1245, 1205, 1180, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.98 (3H, d, *J* = 7.3 Hz, H-15), 1.05 (3H, d, *J* = 7.3 Hz, H-13), 1.39 (3H, s, H-14), 1.68 (1H, d, *J* = 14.2 Hz, H-6β), 1.87 (1H, brd, *J* = 14.2 Hz, H-6α), 2.10 (1H, ddq, *J* = 6.5, 7.3, 8.4 Hz, H-11), 2.27 (1H, q, *J* = 7.3 Hz, H-4), 3.03 (1H, d, *J* = 1.9 Hz, OH-7), 3.43 (3H, s, OCH_3), 3.50 (1H, dd, *J* = 6.5, 8.8 Hz, H-12β), 4.13 (1H, dd, *J* = 8.4, 8.8 Hz, H-12α), 5.90 (1H, d, *J* = 9.9 Hz, H-2), 6.24 (1H, s, H-9), 6.89 (1H, d, *J* = 9.9 Hz,

H-1); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.4 (q, C-13), 14.6 (q, C-15), 27.4 (q, C-14), 39.7 (s, C-5), 42.1 (t, C-6), 42.9 (d, C-11), 48.3 (q, OCH_3), 53.5 (d, C-4), 72.8 (t, C-12), 78.2 (s, C-7), 101.3 (s, C-8), 126.3 (d, C-2), 126.9 (d, C-9), 140.9 (s, C-10), 143.9 (d, C-1), 204.2 (s, C-3); EIMS *m/z* $[M]^+$ 278 (100), 247 (56), 221 (56), 203 (94), 162 (54), 134 (70); HREIMS *m/z* 278.151 (calcd for $C_{16}H_{22}O_4$ 278.152).

***R*-Mellein (27):** white solid (20.8 mg, 4.6 mg/L medium); mp. 52-54°C; $[\alpha]^{20}_D$ -76° (*c* 1.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.54 (3H, d, *J* = 6.3 Hz), 2.92 (2H, d, *J* = 7.3 Hz), 4.73 (1H, dq, *J* = 6.3, 7.3 Hz), 6.68 (1H, dd, *J* = 0.9, 7.4 Hz), 6.88 (1H, brd, *J* = 8.3 Hz), 7.42 (1H, dd, *J* = 7.4, 8.3 Hz), 11.07 (1H, s).

(3*R*,4*S*)-Hydroxymellein (28): white crystals (425 mg, 94 mg/L medium); mp. 132-136°C; $[\alpha]^{20}_D$ -13° (*c* 0.53, $CHCl_3$); IR (film) ν_{max} 3400, 3200, 2985, 1655, 1465, 1425, 1400, 1365, 1310, 1280, 1235, 1215, 1110, 1090, 1070, 1050, 985, 955, 820, 790, 745, 695, 590, 470 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.52 (3H, d, *J* = 6.1 Hz), 2.35 (1H, brs), 3.49 (1H, brs), 4.61 (2H, m), 6.99 (1H, d, *J* = 7.5 Hz), 7.02 (1H, d, *J* = 6.5 Hz), 7.56 (1H, dd, *J* = 6.5, 7.5 Hz), 11.01 (1H, brs); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 17.9 (q), 69.1 (d), 80.0 (d), 106.7 (s), 116.3 (d), 117.8 (d), 136.9 (d), 141.1 (s), 161.9 (s), 168.5 (s); EIMS *m/z* $[M]^+$ 194 (60), 150 (100), 122 (73), 121 (97).

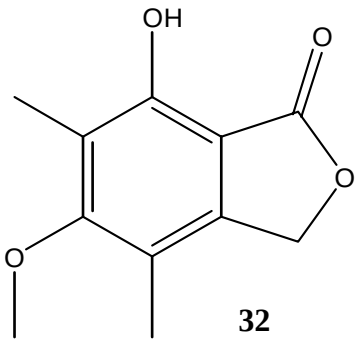
(3*R*,4*R*)-Hydroxymellein (29): white crystals (51 mg, 11 mg/L medium); mp. 113-114°C; $[\alpha]^{20}_D$ -39.6° (*c* 0.59, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.61 (3H, d, *J* = 6.6 Hz), 2.04 (1H, brs), 4.58 (1H, d, *J* = 1.8 Hz), 4.72 (1H, dq, *J* = 1.8, 6.6 Hz), 6.94 (1H, d, *J* = 7.4 Hz), 7.04 (1H, dd, *J* = 1.0, 8.5 Hz), 7.54 (1H, dd, *J* = 7.4, 8.5 Hz), 11.02 (1H, brs); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 16.0 (q), 67.2 (d), 78.2 (d), 106.8 (s), 118.3 (d), 118.5 (d), 136.8 (d), 140.5 (s), 162.0 (s), 169.2 (s).

4,8-Dihydroxy-3,4-dihydro-2*H*-naphthalene-1-one (30): white solid (5 mg, 1.1 mg/L medium); $[\alpha]^{20}_D$ 0° (*c* 0.13, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.84 (1H, brs, OH-4), 2.19 (1H, m, H-3a), 2.35 (1H, m, H-3b), 2.65 (1H, ddd, *J* = 4.9, 8.3, 17.9 Hz, H-2a), 3.01 (1H, ddd, *J* = 4.9, 8.3, 17.9 Hz, H-2b), 4.92 (1H, m, H-3), 6.93 (1H, d, *J* = 8.3 Hz), 7.02 (1H, d, *J* = 7.2 Hz), 7.50 (1H, dd, *J* = 7.2, 8.3 Hz, H-6), 12.41 (1H, s, OH-8); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 31.2 (t), 34.6 (t), 67.7 (d), 115.3 (s), 117.3 (d), 117.8 (d), 137.0 (d), 145.8 (s), 162.7 (s), 204.2 (s); EIMS *m/z* $[M]^+$ 178 (100), 160 (12), 150 (30), 122 (39), 121 (87).

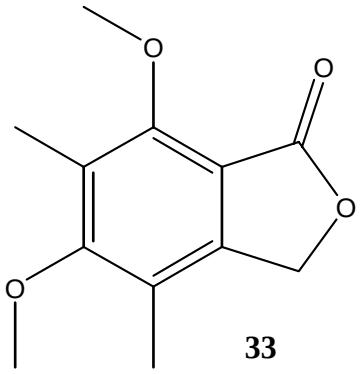
Unknown 2 (31): white solid (0.5 mg, 0.1 mg/L medium); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.13 (1H, m), 2.32 (1H, m), 2.65 (1H, ddd, *J* = 4.9, 9.8, 18.1 Hz), 2.77 (3H, s), 2.92 (1H, ddd, *J* = 4.9, 6.8, 18.1 Hz), 4.80 (1H, dd, *J* = 3.8, 8.7 Hz), 6.63 (1H, brs), 14.34 (1H, brs), 14.98 (1H, s); EIMS *m/z* $[M]^+$ 236 (64), 221 (100).

4.4.7 *Monochaetia* sp. 193A20

Extracts of small scale cultures of *Monochaetia* strain 193A20 possessed significant antifungal, and to a lesser degree, antibacterial activity: inhibition zones (extract from fungal culture on MS-ASW medium) against *B. megaterium* 0.3 cm, *M. violaceum* 0.8 cm, *E. repens* 1.4 cm, *F. oxysporum* 0.4 cm, *M. microspora* 0.4 cm). These findings together with the fact that this genus had not been investigated chemically thus far as judged by a literature search (Chapman & Hall and Beilstein databases) seemed to warrant further chemical investigation. The EtOAc extract of an up-scaled recultivation (4.6 g of brown oil) was fractionated by normal-phase VLC (gradient hexane-EtOAc-MeOH) to yield five fractions. Agar diffusion assays revealed the previously observed antimicrobial activity against *B. megaterium* and *M. violaceum* to be concentrated in fractions 3 and 4. Both fractions were separated by normal-phase HPLC; only fraction 3 yielded pure compounds in amounts allowing further structure elucidations. Thus, fraction 3 (EtOAc/hexane 75/25, 200 mg) was separated by normal-phase HPLC (petrol ether/acetone 70/30) to give compounds **32** and **33**, and a mixture of fatty acid monoglycerides. This mixture was further separated by reversed-phase HPLC (MeOH/H_2O 81/15) to yield compounds **34** and **35**.



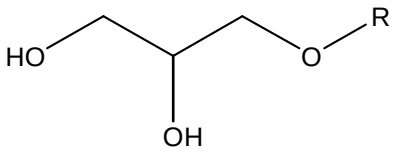
32



33

Compound **32** had a molecular formula of $C_{11}H_{12}O_4$ as determined by HREIMS, indicating six elements of unsaturation within the molecule. The ^{13}C NMR spectrum revealed the presence of seven quaternary sp^2-hybridised carbon atoms (106.1, 116.5, 118.5, 143.4, 153.8, 163.8 and 173.0 ppm), probably part of an aromatic ring and one carboxyl moiety. In combination with the ^1H NMR spectrum two methyl (δ 2.16, s and δ 2.21, s), one methoxyl (δ 3.78, s) and one methylene group (δ 5.22, s) adjacent to

oxygen could be deduced, representing four of the six substituents on the aromatic ring. Thus, the signal in the ^{1}H NMR spectrum at δ 7.70 was due to the hydrogen atom of an OH group, deshielded due to a weak hydrogen bond to the carboxyl moiety, suggesting both groups to be adjacent substituents on the aromatic ring. The carboxyl moiety then further had to form a lacton via the methylene group, the structure of **32** being a substituted phthalide. The substitution pattern of the two methyl and the one methoxyl group on the aromatic ring was established by comparing the spectroscopic data of **32** with literature values for different phthalides (see below). The ^{1}H and ^{13}C NMR spectra of compound **33** were closely similar to those of **32** except for the lack of a signal due to a hydrogen atom of an OH group, and the presence of a signal due to an additional methoxyl group. This suggested **33** to be the methoxyl derivative of **32**. Thus, **32** turned out to be the known fungal metabolite 7-hydroxy-5-methoxy-4,6-dimethylphthalide, first isolated from a *Hansfordia* sp. (Schneider, Anke & Sterner, 1997), and **33** to be 5,7-dimethoxy-4,6-dimethylphthalide, previously only reported as a methylation product of 5,7-dihydroxy-4,6-dimethylphthalide (Achenbach, Mühlenfeld & Brillinger, 1985).



34 R = oleyl
35 R = linoleyl

The HPLC separations further resulted in the isolation of two fatty acid monoglycerides (**34** and **35**), as judged by their ^{1}H NMR spectra. The signal due to hydrogen atom at position 2 of the glycerol-part of the molecules had a chemical shift of δ 3.93. Thus, the corresponding OH-2 group was not esterified, because this would result in a deshielding of the hydrogen atom at C-2, in which case it resonates around δ 5.0. Therefore the esterification had to be at position 1. A structure elucidation of long-chain fatty acids by NMR spectroscopy is often hindered due to overlapping resonance signals of methylene groups. The structure of the fatty acids was therefore further elucidated by EIMS. Typical fragment ions at *m/z* 264 (for compound **34**) and 262 (for compound **35**), resulting from the cleavage of the ester between the carbon atom of the carboxyl moiety and the oxygen atom of the ester bond, revealed both fatty acids to possess 18 carbon atoms, and one respectively two double bonds within the chain. Compounds **34** and **35** could then be determined as 1-*O*-oleyl-glycerol and

1-*O*-linoleyl-glycerol by comparison of the spectroscopic data with literature values (Mistry & Min, 1987; Zhang, Xing & Zhang, 1997). An optical rotation of zero, respectively, suggests both compounds to be racemic.

7-Hydroxy-5-methoxy-4,6-dimethylphthalide (32): white solid (3 mg; 0.5 mg/L medium); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.16 (3H, s, CH_3-4), 2.21 (3H, s, CH_3-6), 3.78 (3H, s, OCH_3), 5.22 (2H, s, H-3), 7.70 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 8.5 (q, CH_3-6), 11.4 (q, CH_3-4), 60.3 (q, OCH_3), 70.0 (t, C-3), 106.1 (s, C-7a), 116.5 (s, C-4), 118.5 (s, C-6), 143.4 (s, C-3a), 153.8 (s, C-7), 163.8 (C-5), 173.0 (s, C-1); EIMS *m/z* $[M]^+$ 208 (100), 193 (14), 190 (22), 179 (75); HREIMS *m/z* 208.0735 (calcd for $C_{11}H_{12}O_4$, 208.0736).

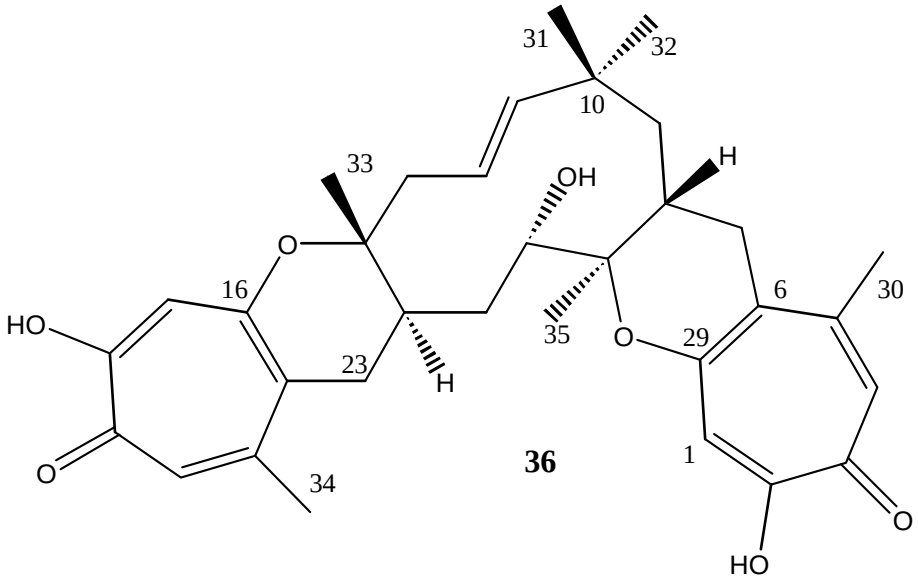
5,7-Dimethoxy-4,6-dimethylphthalide (33): white solid (2 mg; 0.3 mg/L medium); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.19 (3H, s, CH_3-4), 2.25 (3H, s, CH_3-6), 3.78 (3H, s, OCH_3-5), 4.05 (3H, s, OCH_3-7), 5.15 (2H, s, H-3); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 9.3 (q, CH_3-6), 11.3 (q, CH_3-4), 60.2 (q, OCH_3-5), 62.2 (q, OCH_3-7), 68.3 (t, C-3), 112.5 (s, C-7a), 119.9 (s, C-4), 125.3 (s, C-6), 146.1 (s, C-3a), 156.7 (s, C-7), 162.9 (s, C-5), 169.0 (s, C-1); EIMS *m/z* $[M]^+$ 222 (100), 207 (21), 204 (38), 193 (64), 176 (73).

1-*O*-Oleyl-glycerol (34): colourless oil (5.3 mg; 0.9 mg/L medium); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, *J* = 6.9 Hz), 1.29 (20H, m), 1.63 (2H, m), 2.01 (4H, m), 2.35 (2H, m), 3.60 (1H, dd, *J* = 5.6, 11.4 Hz), 3.69 (1H, dd, *J* = 4.1, 11.4 Hz), 3.93 (1H, dddd, *J* = 4.1, 4.6, 5.6, 6.1 Hz), 4.15 (1H, dd, *J* = 6.1, 11.7 Hz), 4.21 (1H, dd, *J* = 4.6, 11.7 Hz), 5.34 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (q), 22.7 (t), 24.9 (t), 27.1 (t), 27.2 (t), 29.07 (2C, t), 29.13 (t), 29.3 (2C, t), 29.5 (t), 29.7 (t), 29.8 (t), 31.9 (t), 34.1 (t), 63.3 (t), 65.2 (t), 70.3 (d), 129.7 (d), 130.0 (d), 174.3 (s); EIMS *m/z* $[M]^+$ 356 (5), 338 (10), 264 (75), 55 (100).

1-*O*-Linoleyl-glycerol (35): colourless oil (6.5 mg; 1.1 mg/L medium); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, t, *J* = 6.9 Hz), 1.31 (14H, m), 1.63 (2H, m), 2.05 (4H, m), 2.35 (2H, m), 2.77 (2H, m), 3.59 (1H, dd, *J* = 6.1, 11.7 Hz), 3.69 (1H, dd, *J* = 4.1, 11.7 Hz), 3.93 (1H, dddd, *J* = 4.1, 4.6, 6.1, 6.1 Hz), 4.15 (1H, dd, *J* = 6.1, 11.7 Hz), 4.20 (1H, dd, *J* = 4.6, 11.7 Hz), 5.35 (4H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (q), 22.6 (t), 24.9 (t), 25.6 (t), 27.17 (t), 27.20 (t), 29.08 (2C, t), 29.13 (t), 29.3 (t), 29.6 (t), 31.5 (t), 34.1 (t), 63.3 (t), 65.2 (t), 70.3 (d), 127.9 (d), 128.1 (d), 130.0 (d), 130.2 (d), 174.3 (s); EIMS *m/z* $[M]^+$ 354 (4), 352 (7), 336 (4), 262 (30), 55 (100).

4.4.8 *Phoma* sp. H4-77

Strain H4-77 was selected for further chemical investigations because of the significant activity of its culture extracts in both agar diffusion and ELISA based assays. The EtOAc extract of a recultivation of the strain (0.2 g of a green-brown oil) was subjected to VLC (sephadex, MeOH) to yield five fractions. Fractions 2 (50 mg) and 3 (70 mg) were further separated by reversed-phase HPLC (MeOH/H_2O 85/15, UV-detection 210-400 nm) to yield **36** as pale yellow crystals.



36

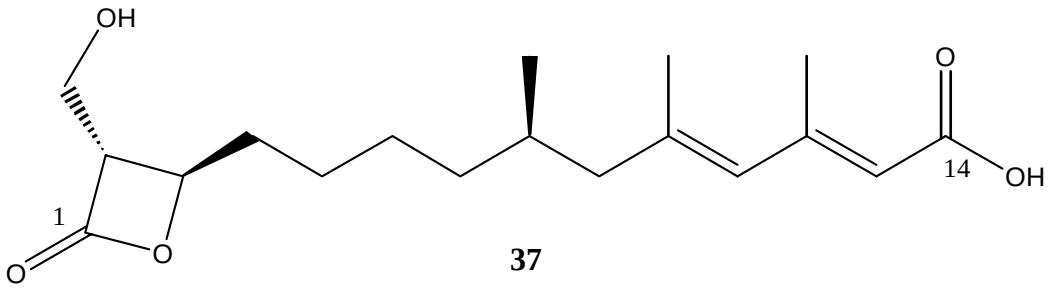
Compound **36** seemed to represent a relatively large molecule as judged by its complex ^{1}H NMR spectrum. Additionally, the signals in the downfield region (δ 6.5 to 7.5) underwent some slight shifts during the separation process, as obvious when comparing ^{1}H NMR spectra from the crude extract, the fractions and the pure compound. ^{13}C NMR spectroscopy revealed the presence of 33 carbon atoms, and by EIMS a molecular ion at $[M]^+$ *m/z* 548 was observed. A literature search (Chapman & Hall database) for natural products with a corresponding mass showed such compounds to be quite rare and revealed two possible structures, pycnidione (Harris *et al.*, 1993) isolated from a *Phoma* sp., and its C-8 epimer eupenifeldin (Mayerl *et al.*, 1993) isolated from *Eupenicillium brefeldianum*. Comparison of ^{1}H and ^{13}C NMR data and the optical rotation of **36** with published values for these two compounds revealed it to be pycnidione.

Pycnidione (36): pale yellow crystals (9.3 mg; 6.2 mg/L medium); mp. 190°C (decomp.); $[\alpha]^{20}_{D}$ +207.7° (*c* 0.31, DCM/MeOH 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (3H, s), 1.10 (3H, s), 1.10 - 1.35 (2H, m), 1.22 (3H, s), 1.27 (3H, s), 1.65 (2H, m), 1.85 (1H, m), 2.15-2.55 (4H, m), 2.39 (6H, 2s), 2.69 (2H, m), 3.00 (1H, dd), 3.65 (1H, d), 5.41 (2H, m), 6.94 (1H, s), 7.00 (1H, s), 7.10 (1H, s), 7.12 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.9 (q, C-35), 18.6 (q, C-33), 21.9 (q, C-31), 27.1 (q, C-30), 27.2 (q, C-34), 31.2 (q, C-31), 31.4 (d, C-8), 33.5 (t, C-25), 33.9 (t, C-23), 34.1 (t, C-7), 36.7 (s, C-10), 39.5 (d, C-24), 46.1 (t, C-9), 48.7 (t, C-13), 76.2 (d, C-26), 81.6 (s, C-14), 83.8 (s, C-27), 112.8 (d, C-1), 113.7 (d, C-17), 120.3 (s, C-6), 121.9 (d, C-22), 122.3 (s, C-12), 124.0 (d, C-20), 124.5 (d, C-4), 143.7 (d, C-11), 149.5 (s, C-21), 149.7 (s, C-5), 159.9 (s, C-29), 160.6 (s, C-16), 163.5 (s, C-2), 164.0 (s, C-18), 171.5 (s, C-19), 172.4 (s, C-3); EIMS *m/z* $[M]^+$ 548 (100).

4.4.9 *Scopulariopsis candida* H7-19

The isolate of *S. candida* was chosen for further chemical investigations because of prominent antifungal activity of extracts of small scale cultivations (Biomalt medium, inhibition zones against *M. violaceum* 2.5 cm, *E. repens* 2.0 cm, *M. microspora* 1.3 cm). The EtOAc extract of a recultivation (6.15 g of a yellow oil) was extracted with MeOH to separate the more polar components from the dominating fat fraction. After removing the MeOH under reduced pressure, the residue was fractionated by normal-phase VLC (gradient hexane-EtOAc-MeOH) to give five fractions. Fraction 3 (EtOAc) was further separated by normal-phase HPLC (EtOAc/hexane 50/50; DAD detection 210-400 nm) to yield 14 fraction. After removing the eluent, each fraction was redissolved in 3 mL eluent and subjected to agar diffusion assays for inhibition of *E. repens* and *M. violaceum*. Antifungal activity could be shown to be most pronounced in fraction 7 (2.7 mg of a white solid). During the structure elucidation, this fraction was found to be a 2:1 M/M mixture of **37** and 2-(*p*-hydroxyphenyl)ethanol (**22**), which was further separated by reversed-phase HPLC (MeOH/H_2O 65/35; UV detection 272 nm) to yield pure **37** (2.3 mg). Fraction 4 (EtOAc/MeOH 50/50) was further separated by reversed-phase VLC (MeOH/H_2O 75/25) to yield five fractions; fractions 4 and 5 were subjected to reversed-phase HPLC with the same eluent (DAD detection 210-400 nm) to yield 11 further fractions. The corresponding fractions 2-4 of each separation, as judged by reversed-phase TLC with the same eluent, were subjected to preparative normal-phase VLC (eluent a: acetone, 16.5 cm; eluent b: acetone/MeOH containing 2% NH_3 50/50, 12.5 cm) yielding four bands

(UV 254 nm). Bands 1 and 2 (6.5 - 10.5 cm; 17.8 mg) were purified by reversed-phase HPLC (MeOH/H_2O 25/75, DAD 210-400 nm) to yield **38** (6.1 mg).

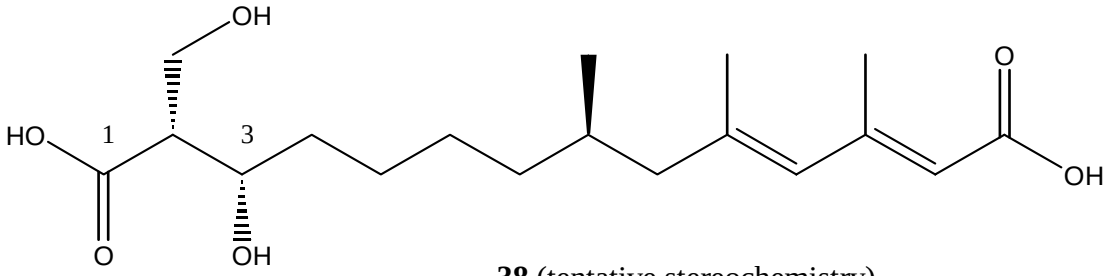


37

By ^1H and ^{13}C NMR spectroscopy (^1H decoupled and DEPT), compound **37** could be shown to contain two carboxyl moieties, further two sp^2-hybridised quaternary carbons, two olefinic methine groups, three methyl groups, two of them located on double bonds and one adjacent to a methine group, six methylene groups, including one hydroxymethyl group, and three aliphatic methine groups, one of which had to be attached to an oxygen atom. By interpretation of the ^1H-^1H COSY NMR spectrum of **37**, the molecular part from the CH_2OH group to CH_2-9 could be established. An UV maximum at λ = 260 nm suggested the two double bonds and one carboxyl group to be conjugated, forming the molecular part from C-10 to C-14 (without stereochemistry at the double-bonds). By interpretation of the ^1H-^{13}C long-range (HMBC) NMR spectrum, a connectivity between C-9 and C-10 could be established (correlation between C$\underline{H_2}$-9 and C-10). Additionally, the second carboxyl moiety had to be connected to C-2 and formed a lactone via C-3, as obvious by the downfield shift of the signal of the hydrogen atom at C-3, thus giving the basic structure of **37**. **37** could then be identified as the known fungal metabolite 1233A by comparison of its spectral data and optical rotation with published values (Aldridge, Giles & Turner, 1971; Kumagai, Tomoda & Omura, 1992).

Compound **38** appeared to possess a structure similar to that of **37** as judged by comparison of their ^1H NMR spectra. Nevertheless, **38** was substantially more polar than **37**, suggesting it to be an "open lactone" derivative of **37**. This could be confirmed by comparing ^{13}C NMR data of both compounds, and by interpretation of the ^1H-^1H COSY NMR spectrum of **38**. A literature search (Beilstein database) revealed two compounds with this basic structure to be reported. One of them

(1233B) had been isolated from cultures of the same fungus which produced 1233A, the other had been obtained by alkaline hydrolysis of 1233A (Aldridge, Giles & Turner, 1971). Both compounds were presumed to differ in the stereochemistry at C-3, with 1233A possessing a stereochemistry at C-3 opposite to that of 1233B. Unfortunately no ^{13}C NMR spectroscopic data were reported for both isomers, the ^{1}H NMR values did not allow to determine the identity of the metabolite isolated in this study with one of the compounds described in literature. The production of **37** and **38** by the same fungal strain, however, suggested the stereochemistry at C-2 and C-8 to be identical with that of the lactone (1233A), and the stereochemistry at C-3 to be identical with that of 1233B.



38 (tentative stereochemistry)

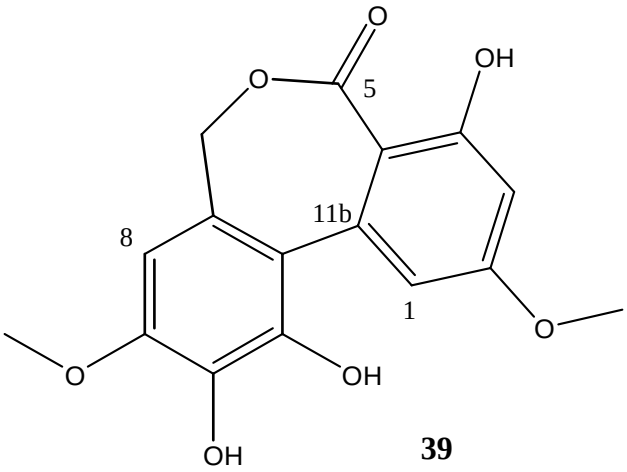
1233A (37): white solid (2.3 mg; 0.3 mg/L medium); mp. 62-63°C; $[\alpha]^{20}_{D}$ +24.3° (*c* 0.23, $CHCl_3$); UV (EtOH) λ_{max} 260 nm; IR (film) ν_{max} 3400, 2930, 1810, 1695, 1615, 1515, 1455, 1375, 1245, 1170, 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.84 (3H, d, *J* = 6.8 Hz, CH_3-8), 1.12 (1H, m, H-7a), 1.32 (1H, m, H-7b), 1.38 (2H, m, H-6), 1.42 (2H, m, H-5), 1.67 (1H, m, H-8), 1.78 (1H, m, H-4a), 1.81 (3H, s, CH_3-10), 1.86 (1H, dd, *J* = 9.0, 13.5 Hz, H-9a), 1.91 (1H, m, H-4b), 2.09 (1H, dd, *J* = 6.8, 13.5 Hz, H-9b), 2.24 (3H, s, CH_3-12), 3.40 (1H, ddd, *J* = 4.1, 4.1, 4.9 Hz, H-2), 3.89 (1H, dd, *J* = 4.1, 11.7 Hz, CH_2O), 4.05 (1H, dd, *J* = 4.9, 11.7 Hz, CH_2O), 4.58 (1H, ddd, *J* = 4.1, 6.0, 10.1 Hz, H-3), 5.69 (1H, s, H-12), 5.73 (1H, s, H-13); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 18.5 (q, CH_3-10), 19.4 (q, CH_3-8), 20.0 (q, CH_3-12), 25.2 (t, C-5), 26.6 (t, C-6), 30.9 (d, C-8), 34.0 (t, C-4), 36.6 (t, C-7), 49.0 (t, C-9), 58.1 (t, CH_2O), 58.6 (d, C-2), 74.9 (d, C-3), 116.5 (d, C-13), 129.5 (d, C-11), 142.1 (s, C-10), 157.1 (s, C-12), 169.8 (s, C-1), 171.0 (s, C-14); CIMS (NH_3, negative mode) *m/z* $[M]^+$ 324 (100), 308 (9), 280 (34).

(38): colourless solid (6.1 mg; 0.8 mg/L medium); ^{1}H NMR (CD_3OD, 300 MHz) δ 0.88 (3H, d, *J* = 6.3 Hz, CH_3-10), 1.18 (1H, m, H-7a), 1.40 (1H, m, H-7b), 1.40 (2H, m, H-6), 1.49 (2H, m, H-5), 1.57 (2H, m, H-4), 1.71 (1H, m, H-8), 1.83 (3H, d, *J* = 1.2 Hz, CH_3-10), 1.85 (1H, m, H-9a), 2.13 (3H, d, *J* = 1.0 Hz, CH_3-12), 2.15 (1H, m, H-9b), 2.47 (1H, m, H-2), 3.84 (3H, m, H-3, CH_2O), 5.71 (1H, brs, H-13), 5.74 (1H, brs, H-11); ^{13}C NMR (CD_3OD, 100 MHz) δ 18.3 (q, CH_3-10), 19.6 (q, CH_3-8), 19.8 (q, CH_3-12), 27.5 (t, C-5), 28.2 (t, C-6), 32.2 (d, C-8), 37.1 (t, C-4), 38.2 (t, C-7), 49.9

(t, C-9), 56.2 (d, C-2), 62.7 (t, CH_2OH), 70.9 (d, C-3), 125.2 (d, C-13), 131.0 (d, C-11), 139.6 (s, C-10), 146.7 (s, C-12), 175.5 (s, C-14), 181.1 (s, C-1).

4.4.10 *Ulocladium botrytis* 193A4

In the preliminary screening for inhibition of tyrosine kinase, extracts of strain 193A4 showed a complete inhibition at 200 µg/mL. In an attempt to isolate the active component(s) the fungus was recultivated. The EtOAc extract (366 mg of a brown oil) was separated by normal-phase VLC (gradient hexane-EtOAc-MeOH) to give 6 fractions. ELISA's showed that the TK inhibitory activity was concentrated in fractions 4 (EtOAc/hexane 75/25) and 5 (EtOAc). These fractions were further purified by normal-phase HPLC (hexane/EtOAc 75/25) to give **39** (5.4 mg), **41** (6.0 mg), and a fraction presumably containing**40**.



39

The molecular formula of **39** was determined as $C_{16}H_{14}O_7$ by accurate mass measurement, indicating 10 elements of unsaturation within the molecule. From the ^{1}H NMR spectrum of **39** signals for two methoxyl groups (δ 3.87, s, OCH_3-2 and δ 3.95, s, OCH_3-9), two meta-coupled aromatic protons (δ 6.55, d, J = 2.5 Hz, H-3 and δ 6.92, d, J = 2.5 Hz, H-1), one not further coupled aromatic proton (δ 6.57, s, H-8), three phenolic hydroxyl groups (δ 5.68 and 5.77, each brs, OH-10/OH-11) including one (δ 10.29, s, OH-4) deshielded due to a hydrogen bonding with a carbonyl moiety, and one methylene group attached to oxygen (δ 4.79 and 5.11, each 1H, d, J = 11.7 Hz, H_2-7) could be deduced. The ^{13}C NMR spectrum revealed signals for 13 sp^2-hybridized carbon atoms, (six carbon-carbon double bonds, one carbon-oxygen double bond), accounting for 7 of the 10 elements of unsaturation, and suggesting the presence of two separate

aromatic rings. The third ring had thus to be a lactone due to signals in the IR spectrum at ν 1650 and 1660 cm^{-1}, and involving the only methylene group.

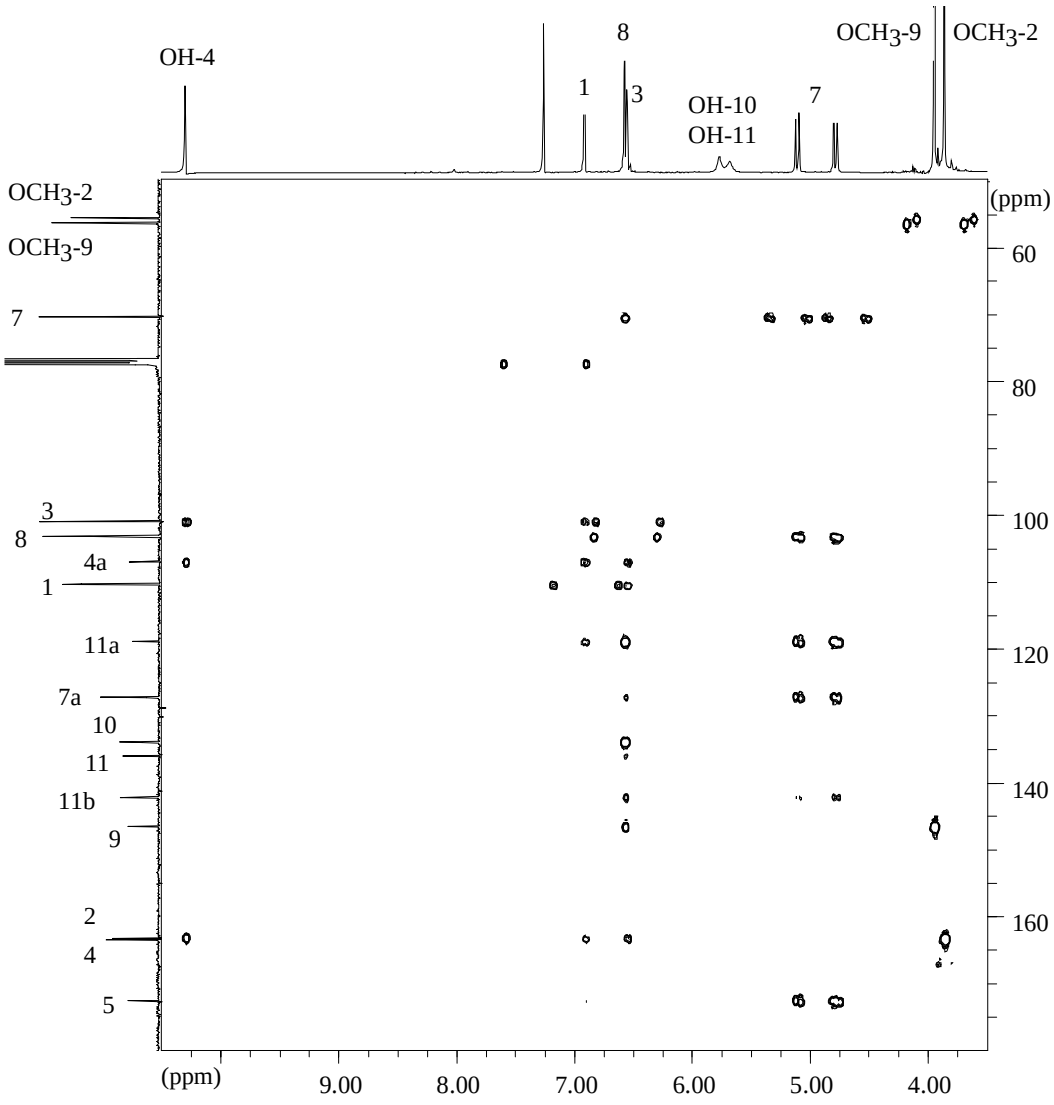
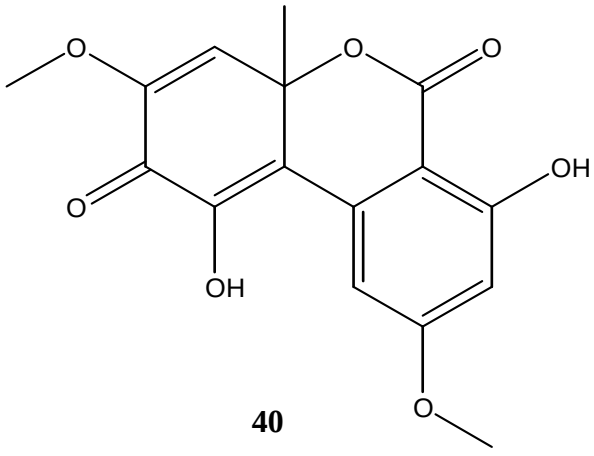


Figure 7: ^{1}H-^{13}C long-range (HMBC) NMR spectrum of 39; $CDCl_3$, 300 MHz for ^{1}H, 75.5 MHz for ^{13}C.

The three hydroxyl groups, two methoxyl groups, and one lactone moiety together with three olefinic hydrogens accounted for 10 of the possible 12 substituents on the aromatic rings, both rings thus had to be directly connected *via* a carbon-carbon bond. After correlating all carbons with their directly bonded hydrogens *via* a ^{1}H-^{13}C shift correlated NMR measurement (HMQC), the structure was further elaborated by interpretation of the ^{1}H-^{13}C long-range (HMBC) spectrum.

Long-range correlations between each H-1, H-3 and O$C\underline{H}_3$-2 to C-2 connected each C-1, C-3 and the methoxyl group to C-2. Further O$\underline{H}$-4 showed long-range

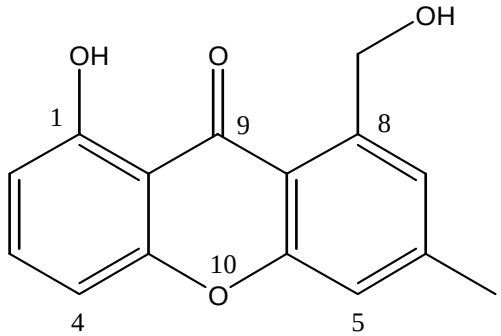
correlations to C-3, C-4 and C-4a establishing the molecular fragment from C-1 to C-4a. The signal of O<u>H</u>-4 appeared as a singlet at δ 10.29, deshielded due to a hydrogen bond to a carbonyl moiety, thus the carbon of the lactone moiety C-5 had to be connected to C-4a. Long-range correlations between OC<u>H</u>$_2$-7 and each C-5, C-7a, C-8 and C-11a established a further major part of the molecule, revealing that the two aromatic rings had to be connected *via* the two quaternary carbon atoms C-11a and C-11b.The remaining two hydroxyl groups, and one methoxyl group were the other substituents, still to be placed. The regio-chemistry of the molecular fragment from C-8 to C-11 was established by difference NOE measurements. Irradiations at the resonance frequencies of OC<u>H</u>$_3$-9 (δ 3.95, s) and OC<u>H</u>$_2$-7 (δ 4.79 and 5.11, each 1H, d, J = 11.7 Hz) respectively, both enhanced the signal of H-8 (δ 6.57, s). Thus, OCH_2-7 and OCH_3-9 had to be attached to carbon atoms adjacent to C-8, leaving the two hydroxyl groups to be positioned at C-10 and C-11. Compound **39** is the new compound 4,10,11-trihydroxy-2,9-dimethoxy-7*H*-dibenzo[c,e]oxepin-5-one for which the trivial name ulocladol is proposed.



40

It is interesting to note that a similar compound, botrallin (**40**), formally derived from a common polyketide precursor by closure of the lactone at different sites, has been reported from *Botrytis allii* (Kameda *et al.*, 1974). It was tentatively identified in a fraction of the current investigation by ^{1}H NMR spectroscopy and EIMS, but material was too scarce to be further characterised.

The ^{1}H NMR spectrum of compound **41** revealed two spin systems characteristic for 1,2,3 and 1,3 respectively substituated aromatic rings. Additionally, one methyl group on an aromatic ring, one methylene group adjacent to oxygen, and one phenolic OH-group, deshielded due to a hydrogen bond to a carbonyl moiety could be deduced.



41

The DEPT spectrum of **41** revealed the expected five signals for the carbon atoms of the aromatic methine groups; no other methine group was present. Thus, the triplet in the ^{1}H NMR spectrum at δ 4.35 had to account for an aliphatic OH-group, and revealed the presence of a hydroxylmethylene moiety. From the ^{13}C NMR spectrum the additional presence of one carbonyl moiety (184.3 ppm), and seven sp^{2}-hybridised quaternary aromatic carbon atoms could be deduced. These NMR spectroscopic data suggested the presence of a xanthone derivative. The phenolic OH group and the CH_2OH group had then to be adjacent to the carbonyl moiety at positions 1 and 8, leaving the methyl group to be positioned either at position 3 or 6. By comparing its ^{1}H NMR data with published values (Ayer & Taylor, 1976) **41** was identified as 1-hydroxy-6-methyl-8-hydroxymethylxanthone, first reported from the Basidiomycete *Cyathus intermedius*. ^{13}C NMR data were not reported, all carbon atoms could be assigned by interpretation of the ^{1}H-^{13}C long-range (HMBC) NMR spectrum of **41**.

Ulocladol (39): white solid (5.4 mg; 5.4 mg/L medium); mp 110-111°C; UV (EtOH) λ_{max} 251 (ε 12900), 299 (ε 3500) nm; IR (film) ν_{max} 3440, 2930, 1660, 1650, 1615, 1345, 1155, 1095 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.87 (3H, s, OCH_3-2), 3.95 (3H, s, OCH_3-9), 4.79 (1H, d, *J* = 11.7 Hz, H-7a), 5.11 (1H, d, *J* = 11.7 Hz, H-7b), 5.68 (1H, brs, OH-10 or 11), 5.77 (1H, brs, OH-10 or 11), 6.56 (1H, d, *J* = 2.5 Hz, H-3), 6.57 (1H, s, H-8), 6.92 (1H, d, *J* = 2.5 Hz, H-1), 10.29 (1H, s, OH-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.5 (q, OCH_3-2), 56.3 (q, OCH_3-9), 70.3 (t, C-7), 100.8 (d, C-3), 103.1 (d, C-8), 106.9 (s, C-4a), 110.2 (d, C-1), 118.8 (s, C-11a), 127.1 (s, C-7a), 133.8 (s, C-10), 135.9 (s, C-11)[a], 142.1 (s, C-11b)[a], 146.3 (s, C-9), 163.0 (s, C-4), 163.2 (s, C-2), 172.5 (s, C-5), [a] assignments may be interchanged; EIMS *m/z* [M]$^{+}$ 318 (100), 300 (70), 282 (20), 254 (30); HREIMS *m/z* 318.073 (calcd for $C_{15}H_{14}O_7$, 318.074).

Botrallin (40): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.76 (3H, s, CH_3), 3.80 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.13 (1H, s), 6.53 (1H, d, *J* = 2.4 Hz), 7.34 (1H, d, *J* = 2.4 Hz), 11.32 (1H, s).

1-Hydroxy-6-methyl-8-hydroxymethylxanthone (41): white crystals (6.0 mg; 6.0 mg/L medium); mp. 175-177°C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.50 (3H, s, CH_3-6), 4.35 (1H, t, *J* = 7.6 Hz, $CH_2O\underline{H}$), 4.96 (2H, d, *J* = 7.6 Hz, $C\underline{H}_2OH$), 6.81 (1H, dd, *J* = 1.0, 8.1 Hz, H-2), 6.92 (1H, dd, *J* = 1.0, 8.7 Hz,

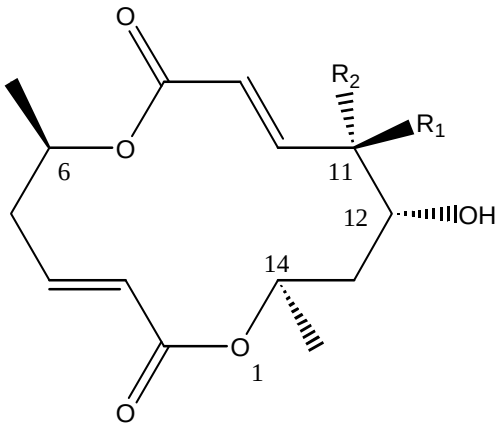
H-4), 7.16 (1H, d, *J* = 1.0 Hz, H-5), 7.27 (1H, d, *J* = 1.0 Hz, H-7), 7.60 (1H, dd, *J* = 8.1, 8.7 Hz, H-3), 12.64 (1H, s, OH-1); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.9 (q, CH_3), 65.2 (t, CH_2OH), 106.6 (d, C-4), 109.4 (s, C-9a), 110.6 (d, C-2), 116.5 (s, C-8a), 118.0 (d, C-5), 127.2 (d, C-7), 136.9 (d, C-3), 142.6 (s, C-8), 147.5 (s, C-6), 155.7 (s, C-4a), 158.1 (s, C-10a), 161.9 (s, C-1), 184.3 (s, C-9); EIMS *m/z* $[M]^+$ 256 (100), 238 (58), 227 (43), 210 (75).

4.4.11 *Varicosporina ramulosa* 195-31

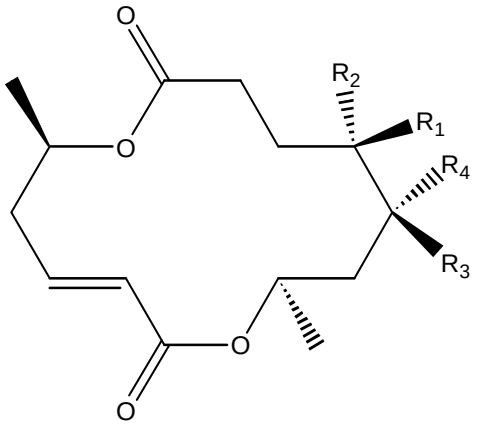
The isolate of *V. ramulosa* represented one of the few obligate marine fungi isolated in this study, and was therefore selected for further chemical investigations. The EtOAc extract (3.85 g of a yellow oil) of a recultivation was subjected to normal-phase VLC (gradient hexane-EtOAc) to give six fractions, fraction 5 (hexane/EtOAc 50/50) containing pure **44** (680 mg). Fraction 3 (hexane/EtOAc 25/100, 65 mg) was purified by normal-phase HPLC (hexane/EtOAc 90/10) to yield **47** (8 mg). Fraction 4 (hexane/EtOAc 50/50, 380 mg) was purified by normal-phase HPLC (EtOAc/hexane 25/75) to give **42** (24 mg) and **43** (168 mg), fraction 6 was purified by normal-phase HPLC (EtOAc/hexane 50/50) to give **45** (23 mg) and **46** (12.7 mg).

Compounds **42-46** seemed to possess similar basic structures as judged by their ^{1}H and ^{13}C NMR spectra. Structure elucidation started with the most abundant compound **44**. By interpretation of the ^{1}H-^{1}H COSY NMR spectrum of **44**, the two major molecular fragments from C-3 to CH_3-6 and from C-9 to CH_3-14 could be established. Both fragments were further connected via ester moieties, thus forming a di-lactone, as deduced from the ^{1}H-^{13}C long-range (HMBC) NMR spectrum, which revealed correlations between H-6 and C-8, and H-14 and C-1, respectively. A literature search (Chapman & Hall database) revealed a couple of such macrodiolides to be known. Compounds **42**, **44** and **45** were then identified by comparing their spectroscopic data and optical rotations with published values as grahamimycin A_1 (**42**, Bestmann & Schobert, 1987), (6*R*,11*R*,12*R*,14*R*)-colletodiol (**44**, MacMillan & Simpson, 1973; Amstutz, Hungerbühler & Seebach, 1981; Keck, Boden & Wiley, 1989) and 9,10-dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**45**, Ohta *et al.*, 1993). Compound **45** was originally described under its systematic name (3*E*,6*R*,11*S*,12*S*,14*R*)-11,12-dihydroxy-6,14-dimethyl-1,7-dioxa-3-cyclotetradecene-2,8-tetrone (Ohta *et al.*, 1993). To show

the relationship of **45** and **46** to (6*R*,11*R*,12*R*,14*R*)-colletodiol (**44**), here they are referred to as 9,10-dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol and 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol. **45** had originally been obtained during a total synthesis of **44**, but as yet not as a fungal metabolite.



43 R_1, R_2 = O
44 R_1 = OH; R_2 = H



42 R_1, R_2 = O; R_3, R_4 = O
45 R_1 = R_4 = H; R_2 = R_3 = OH
46 R_1 = R_4 = OH; R_2 = R_3 = H
46a R_1 = R_4 = *p*-bromobenzoate; R_2 = R_3 = H

Compound **43** was found to have the same basic structure as both colletoketol (MacMillan & Simpson, 1973) and grahamimycin A (Gurusiddaiah & Ronald, 1981; Gurusiddaiah *et al.*, 1981) on the ground of extensive 1D and 2D NMR studies. The configuration of colletoketol is 6*R*,12*R*,14*R*, with that of grahamimycin A being 6*R*,12*R*,14*S*. The ^{13}C NMR data for **43** were found to be in good agreement with values published for grahamimycin A, even though the ^{1}H NMR data differed (Gurusiddaiah *et al.*, 1981). There appeared to be no reported ^{13}C NMR data for colletoketol, and other physical properties as melting point and optical rotation proved in this investigation to be insufficient to determine unambigouasly if **43** was identical with one of the two compounds in question. To resolve these issues a single crystal X-ray analysis of **43** was performed, which established the relative stereochemistry (Figure 8).

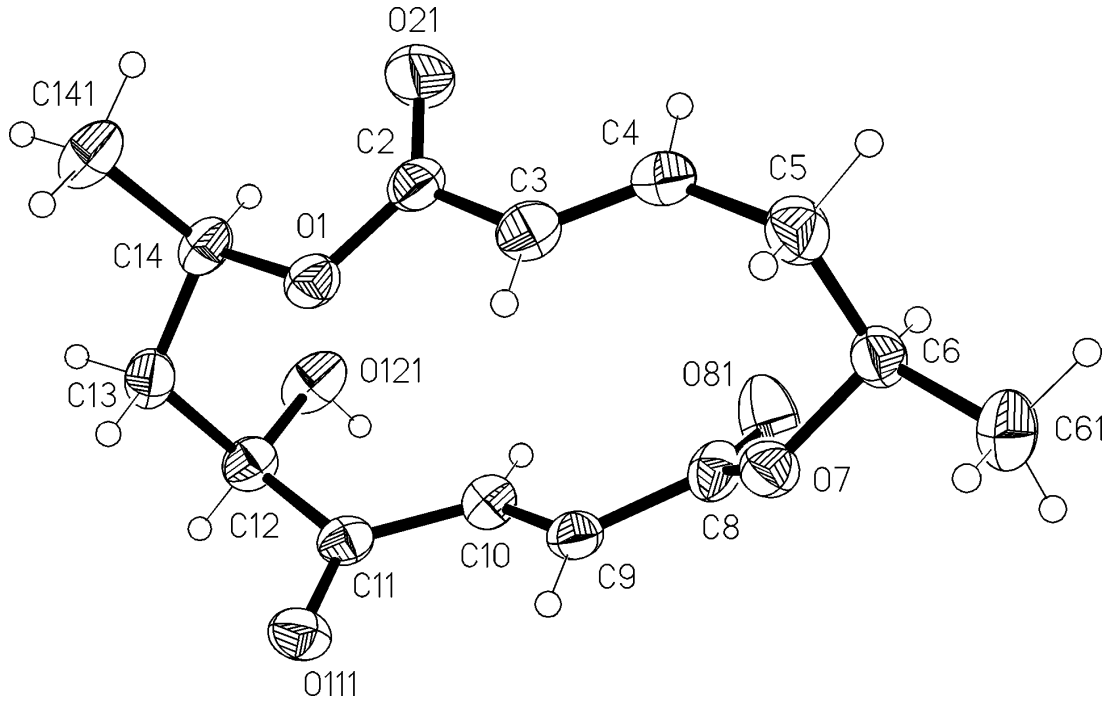
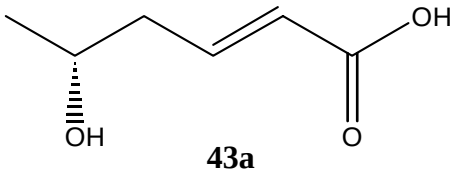


Figure 8: Result of the single crystal X-ray analysis of 43 (provided by Prof. Jones). Ellipsoids represent 50% probability levels. H atom radii are arbitrary.

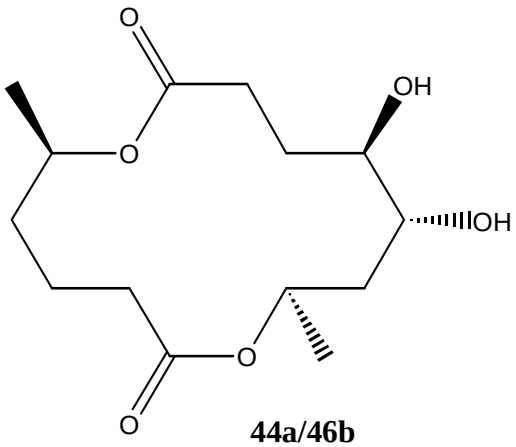
The absolute configuration at C-6 was determined as *R* by alkaline hydrolysis of **43** resulting in (-)-5-hydroxy-(2*E*)-hexenoic acid (**43a**) of known configuration as one product (MacMillan & Simpson, 1973). Thus, **43** could be shown to possess 6*R*,12*R*,14*R* configuration and is identical with colletoketol.



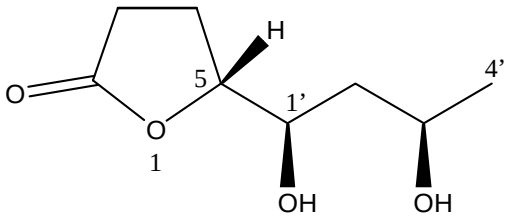
43a

Compound **46** appeared to be closely similar to **45** by comparing their EIMS, ^{1}H and ^{13}C NMR spectroscopic data. After assigning all hydrogens to their directly bonded carbons *via* a ^{1}H-^{13}C shift correlated NMR measurement (HMQC), **46** was shown to be a stereoisomer of **45**, differing in the configuration at one or more of the four chiral centres. The conformational flexibility of these 14-membered rings usually means the stereochemistry of each individual chiral centre has to be determined separately, e.g. by degradation studies. Attempts to produce crystals of **46** suitable for a X-ray

crystallographic analysis proved unsuccessful. Therefore, the 11,12-di-(*p*-bromobenzoate)-derivative of **46** (**46a**) was synthezised, unfortunately this compound was a clear oil. Its CD-spectrum showed for the two *p*-bromobenzoate units a negative ($\Delta\varepsilon_{255.2\ nm}$ -8.4), and a positive ($\Delta\varepsilon_{241.2\ nm}$ +7.8) cotton effect implying a negative chirality. MacMillan & Simpson (1973) calculated similar values for the two benzoate-units of the dibenzoate-derivative of **44**, and established the configuration at C-11 as *S* by degradation studies. This led in the initial determination of the absolute stereochemistry of colletodiol to the assignment of the 11*S*,12*R* configurations (MacMillan & Simpson, 1973). Although this assignment was later revised to 11*R*,12*R* after a X-ray crystallographic analysis (Amstutz, Hungerbühler & Seebach, 1981), both an 11*R*,12*R*- and an 11*S*,12*R*-configuration have to be in agreement with the observed cotton effect of the two benzoate units. This finding suggested one of these configurations also for **46**. As all other four isolated isolated (**42-45**) possessed the *R*-configuration at C-6 and C-14, **46** was likely also to have the 6*R*,14*R*-configuration. Taking this possibility into account, **46** was then possibly the 9,10-dihydro-derivative of (6*R*,11*R*,12*R*,14*R*)-colletodiol. Hydrogenation of **44** and **46** should then lead to identical products. The 3,4,9,10-tetrahydro-derivative of **44** (**44a**) was known and readily accessible by hydrogenation with H_2 over Pd/charcoal (MacMillan & Simpson, 1973).



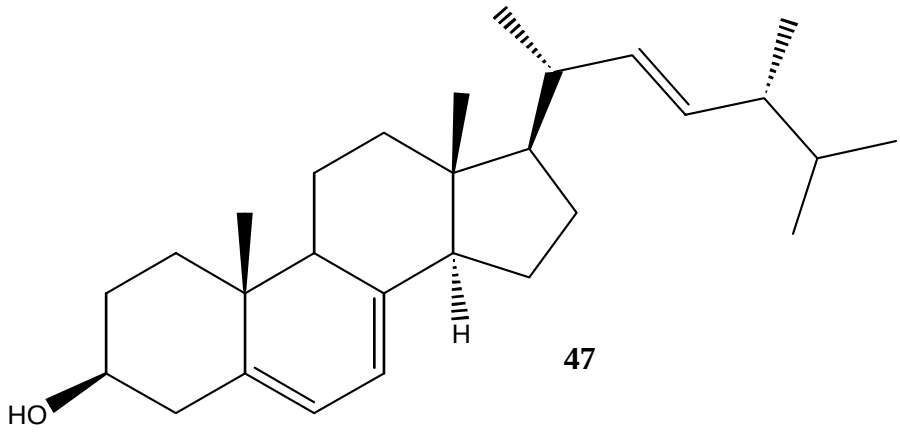
44a/46b



44b/46c

The 9,10-dihydro-derivative of **46** (**46b**) was prepared analogously. The hydrogenation products of both reactions proved to be unstable, and their spontanous decomposition/rearrangement started during the reaction and continued in the $CDCl_3$

solution during NMR measurements. Nevertheless, **44a** and **46b** could be shown to have identical ^{1}H NMR spectra, indicating the configurations of **46** at C-6, C-11, C-12 and C-14 to be either all *R* as in **44** or all *S*. The *R*-configuration, however, would be in accordance with the negative chirality observed for **46a**. The *R*-configurations could be further substantiated by a closer inspection of one major decomposition product of **44a**, which was shown to be (5*R*)-((1'*R*,3'*R*)-dihydroxybutyl)-dihydrofuran-2-one (**44b**), (MacMillan & Simpson, 1973). The identical compound (**46c**) was obtained from the decomposition of **46b**. Thus, **46** is 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol.



47

Additionally, the common fungal sterol ergosterol (**47**) was identified by ^{1}H NMR spectroscopy and MS (Shirane *et al.* 1996).

Grahamimycin A_1 (42): yellow solid (24 mg; 3.7 mg/L medium); mp. 68-70°C; $[\alpha]^{20}_{D}$ -5.5° (*c* 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (3H, d, *J* = 6.4 Hz, CH_3-14), 1.38 (3H, d, *J* = 6.4 Hz, CH_3-6), 2.30 (1H, dddd, *J* = 1.1, 7.2, 10.2, 13.6 Hz, H-5a), 2.42 (1H, dddd, *J* = 1.1, 3.8, 8.5, 13.6 Hz, H-5b), 2.46 (1H, ddd, *J* = 2.6, 10.2, 15.5 Hz, H-9a), 2.69 (1H, ddd, *J* = 3.0, 7.9, 15.5 Hz, H-9b), 2.73 (1H, dd, *J* = 6.0, 12.1 Hz, H-13a), 2.81 (1H, ddd, *J* = 3.0, 10.2, 19.6 Hz, H-10a), 3.29 (1H, dd, *J* = 5.3, 12.1 Hz, H-13b), 3.43 (1H, ddd, *J* = 2.6, 7.9, 19.6 Hz, H-10b), 4.95 (1H, ddq, *J* = 3.8, 10.2, 6.4 Hz, H-6), 5.26 (1H, ddq, *J* = 5.3, 6.0, 6.4 Hz, H-14), 5.68 (1H, dt, *J* = 15.8, 1.1 Hz, H-3), 6.58 (1H, ddd, *J* = 7.2, 8.5, 15.8 Hz, H-4); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 19.8 (q, CH_3-6), 20.6 (q, CH_3-14), 28.4 (t, C-9), 31.3 (t, C-10), 38.1 (t, C-5), 39.3 (t, C-13), 68.7 (d, C-14), 70.6 (d, C-6), 123.4 (d, C-3), 144.9 (d, C-4), 164.3 (s, C-2), 171.1 (s, C-8), 196.4 (s, C-11 or C-12), 197.3 (s, C-11 or C-12); EIMS *m/z* $[M]^+$ 282 (<1), 254 (15), 181 (15), 154 (32), 139 (26), 125 (13), 113 (100), 100 (22), 96 (40), 95 (51), 81 (31), 69 (78), 68 (76).

Colletoketol (43): light yellow crystals (EtOAc, 168 mg; 26 mg/L medium); mp 142-143°C; $[\alpha]^{D}_{20}$ -24.0° (*c* 0.68, $CHCl_3$); IR (film) ν_{max} 3460, 2980, 2935, 1725, 1715, 1705, 1650, 1630, 1445, 1320, 1290, 1240, 1175, 1120, 1050, 985 cm-1; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, d, *J* = 6.4 Hz, CH_3-14), 1.38 (3H, d, *J* = 6.4 Hz, CH_3-6), 1.85 (1H, ddd, *J* = 3.0, 7.2, 15.6 Hz, H-13), 2.07 (1H, ddd, *J* = 2.6, 5.7, 15.6 Hz, H-13), 2.27 (1H, ddd, *J* = 10.9, 11.5, 12.8 Hz, H-5), 2,49 (1H, dddd, *J* = 1.1, 3.0, 5.1, 12.8 Hz, H-5), 3.70 (1H, brs, O<u>H</u>-12), 4.46 (1H, dd, *J* = 3.0, 5.7 Hz, H-12), 5.09 (1H, ddq, *J* = 3.0, 11.5, 6.4 Hz, H-6), 5.31 (1H, ddq, *J* = 2.6, 7.2, 6.4 Hz, H-14), 5.76 (1H, brd, *J* = 15.8 Hz, H-3), 6.67 (1H, d, *J* = 15.8 Hz, H-9), 6.68 (1H, ddd, *J* = 5.1, 10.9, 15.8 Hz, H-4), 7.12 (1H, d, *J* = 15.8 Hz, H-10); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.8 (q, CH_3-14), 20.3 (q, CH_3-6), 40.2 (t, C-13), 40.3 (t, C-5), 66.6 (d, C-14), 70.6 (d, C-6), 73.0 (d, C-12), 126.8 (d, C-3), 132.2 (d, C-9), 134.2 (d, C-10), 143.7 (d, C-4), 165.4 and 165.8 (s, C-2/C-8), 201.3 (s, C-11); EIMS *m/z* $[M]^+$ 284 (2), 282 (2), 280 (3), 256 (16), 194 (18), 113 (100), 96 (56), 95 (86), 82 (78), 69 (62), 68 (84).

(5*R*)-Hydroxy-(2*E*)-hexenoic acid (43a): clear oil (10.5 mg); $[\alpha]^{20}_{D}$ -11.6° (*c* 1.05, EtOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (3H, d, *J* = 6.2 Hz, H-6), 2.40 (2H, ddt, *J* = 1.1, 6.2, 7.5 Hz, H-4), 3.99 (1H, tq, *J* = 6.2, 6.2 Hz, H-5), 5.91 (1H, dt, *J* = 15.7, 1.1 Hz, H-2), 5.51 (2H, brs, O<u>H</u>-5, COO<u>H</u>-1), 7.07 (1H, dt, *J* = 15.7, 7.5 Hz, H-3); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 23.2 (q, C-6), 41.8 (t, C-4), 66.8 (d, C-5), 123.2 (d, C-2), 147.7 (d, C-3), 170.8 (s, C-1); FAB-MS (glycerol, negative mode) *m/z* $[M - H]^-$ 129 (100).

(6*R*,11*R*,12*R*,14*R*)-Colletodiol (44): colourless crystals (680 mg; 105 mg/L); mp. 164°C; $[\alpha]^{20}_{D}$ +27.3° (*c* 0.41, $CHCl_3$); UV (EtOH) λ_{max} 214 nm (ε 1000); IR (film) ν_{max} 3440, 2980, 2935, 1720, 1655, 1450, 1350, 1315, 1265, 1230, 1175, 1110, 1055, 1025, 990 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (6H, d, *J* = 6.4 Hz, CH_3-14/CH_3-6), 1.49 (1H, ddd, *J* = 1.9, 6.0, 15.8 Hz, H-13a), 2.00 (1H, ddd, *J* = 1.1, 4.5, 15.8 Hz, H-13b), 2.16 (1H, ddd, *J* = 11.1, 11.1, 11.1 Hz, H-5a), 2.50 (1H, dddd, *J* = 1.1, 3.0, 4.9, 11.1 Hz, H-5b), 3.21 (2H, brs, OH-11/OH-12), 3.63 (1H, ddd, *J* = 1.1, 5.7, 8.3 Hz, H-12), 4.05 (1H, dd, *J* = 5.7, 9.0 Hz, H-11), 5.15 (1H, ddq, *J* = 1.9, 4.5, 6.4 Hz, H-14), 5.25 (1H, ddq, *J* = 3.0, 6.4, 11.1 Hz, H-6), 5.71 (1H, d, *J* = 15.8 Hz, H-3), 6.18 (1H, dd, *J* = 1.1, 15.8 Hz, H-9), 6.68 (1H, ddd, *J* = 4.9, 11.1, 16.0 Hz, H-4), 6.73 (1H, dd, *J* = 5.7, 15.8 Hz, H-10); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 18.1 (q, CH_3-14), 20.3 (q, CH_3-14), 36.2 (t, C-13), 41.1 (t, C-5), 68.1 (d, C-14), 68.7 (d, C-6), 71.8 (d, C-11), 73.8 (d, C-12), 123.7 (d, C-9), 125.6 (d, C-3), 144.2 (d, C-4), 146.6 (d, C-10), 165.2 (s, C-2), 166.7 (s, C-8); EIMS *m/z* $[M]^+$ 284 (<1), 280 (8), 113 (100).

3,4,9,10-Tetrahydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (44a/46b): white solid (8.0 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (3H, d, *J* = 6.4 Hz), 1.27 (3H, d, *J* = 6.4 Hz), 1.50-2.50 (12H, m), 3.58 (1H, ddd, *J* = 3.8, 4.9, 8.7 Hz), 3.72 (1H, ddd, *J* = 3.8, 3.8, 7.9 Hz), 5.04 (1H, ddq, *J* = 3.0, 12.4, 6.4 Hz), 5.15 (1H, ddq, *J* = 2.3, 13.2, 6.4 Hz).

(5*R*)-((1'*R*,3'*R*)-Dihydroxybutyl)-dihydrofuran-2-one (44b/46c): clear oil (8.9 mg); $[\alpha]^{20}_{D}$ -57.1° (*c* 0.45, $CHCl_3$); IR (film) ν_{max} 3440, 2965, 2920, 1765, 1645, 1455, 1420, 1375, 1195, 1135, 1035, 920 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (3H, d, *J* = 6.4 Hz, H-4'), 1.59 (1H, dt, *J* = 14.5, 2.8 Hz, H-2'), 1.72 (1H, dt, *J* = 14.5, 10.0 Hz, H-2'), 2.13 (1H, dddd, *J* = 6.6, 7.9, 10.2, 13.0 Hz, H-4), 2.25 (1H, dddd, *J* = 6.0, 7.5, 9.8, 13.0 Hz, H-4), 2.50 (1H, ddd, *J* = 7.9, 9.8, 17.7 Hz, H-3), 2.64 (1H, ddd, *J* = 6.0, 10.2, 17.7 Hz, H-3), 3.04 (2H, brs, OH), 3.89 (1H, ddd, *J* = 2.8, 4.3, 10.0 Hz, H-1'), 4.09 (1H, ddd, *J* = 2.8, 6.4, 10.0 Hz, H-3'), 4.42 (1H, ddd, *J* = 4.3, 6.6, 7.5 Hz, H-5); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 23.7 (t), 24.2 (q), 28.5 (t), 40.0 (t), 68.3 (d), 74.0 (d), 82.8 (d), 177.5 (s); FAB-MS (glycerol, negative mode) *m/z* 173 (100) $[M - H]^-$.

9,10-Dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (45): colourless crystals (23 mg; 3.5 mg/L medium); mp. 152-154°C; $[\alpha]^{20}_{D}$ -39.1° (*c* 0.46, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30 (3H, d, *J* = 6.1 Hz, CH_3-6), 1.33 (3H, d, *J* = 6.1 Hz, CH_3-14), 1.73 (1H, ddd, *J* = 7.1, 11.2, 14.8 Hz, H-13a), 1.80 (1H, dddd, *J* = 6.6, 14.2 Hz, H-10a), 1.90 (1H, dddd, *J* = 14.2 Hz, H-10b), 1.95 (1H, ddd, *J* = 2.5, 7.1, 14.8 Hz, H-13b), 2.30 (1H, ddd, *J* = 17.3 Hz, H-9a), 2.31 (1H, ddd, *J* = 3.1, 8.1, 12.9 Hz H-5a), 2.40 (1H, ddd, *J* = 8.1, 11.2, 12.9 Hz H-5b), 2.47 (1H, ddd, *J* = 2.0, 6.6, 17.3 Hz, H-9b), 3.30 (1H, brdd, *J* = 7.1, 7.1 Hz, H-12), 3.43 (1H, ddd, *J* = 1.0, 5.1, 7.1 Hz, H-11), 5.06 (1H, ddq, *J* = 2.5, 6.1, 11.2 Hz, H-14), 5.19 (1H, ddq, *J* = 3.1, 6.1, 11.2 Hz, H-6), 5.80 (1H, d, *J* = 16.0 Hz, H-3), 6.78 (1H, ddd, *J* = 8.1, 8.1, 16.0 Hz, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz), δ, 21.0 (q, 2C, CH_3-6/CH_3-14), 27.8 (t, C-10), 30.2 (t, C-9), 39.2 (t, C-5), 40.5 (t, C-13), 68.4 (d, C-11), 69.7 (d, C-14), 70.3 (d, C-6), 73.2 (d, C-12), 124.2 (d, C-3), 144.6 (d, C-4), 166.1 (s, C-2), 174.2 (d, C-8); EIMS *m/z* $[M]^+$ 286 (<1), 268 (3), 242 (5), 215 (12), 197 (7), 184 (9), 157 (23), 139 (25), 113 (100).

9,10-Dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (46): colourless crystals (12.7 mg; 2.0 mg/L medium); mp 146-147°C; $[\alpha]^{20}_{D}$ -5.0° (*c* 0.42, $CHCl_3$); IR (film) λ_{max} 3330, 2975, 2920, 1725, 1705, 1655, 1450, 1415, 1380, 1350, 1325, 1265, 1230, 1185, 1140, 1070, 1050, 1005 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (3H, d, *J* = 6.6 Hz, CH_3-14), 1.31 (3H, d, *J* = 6.1 Hz, CH_3-6), 1.61 (1H, dddd, *J* = 4.6, 4.6, 11.1, 13.7 Hz, H-10), 1.83 (1H, ddd, *J* = 3.1, 9.0, 15.3 Hz, H-13), 1.94 (1H, ddd, *J* = 2.1, 5.6, 15.3 Hz, H-13), 1.97 (1H, dddd, *J* = 4.8, 5.6, 7.4, 13.7 Hz, H-10), 2.28 (1H, dddd, *J* = 1.0, 8.1, 11.4, 13.2 Hz, H-5), 2.39 (1H, ddd, *J* = 4.6, 4.8, 13.2 Hz, H-9), 2.42 (1H, ddd, *J* = 3.0, 8.1, 13.2 Hz, H-5), 2.52 (1H, ddd, *J* = 5.6, 11.1, 13.2 Hz, H-9), 3.43 (1H, ddd, *J* = 4.6, 7.4, 10.7 Hz, H-11), 3.61 (1H, ddd, *J* = 3.1, 5.6, 10.7 Hz, H-12), 5.16 (1H, ddq, *J* = 3.0, 11.4, 6.1 Hz, H-6), 5.30 (1H, ddq, *J* = 2.1, 9.0, 6.6 Hz, H-14), 5.79 (1H, brd, *J* = 15.8 Hz, H-3), 6.73 (1H, ddd, *J* = 8.1, 8.1, 15.8 Hz, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.8 (q, CH_3-14), 21.1 (q, CH_3-6), 28.0 (t, C-10), 30.4 (t, C-9), 37.6 (t, C-13), 39.0 (t, C-5), 66.7 (d, C-14), 69.2 (d, C-6), 70.5 (d, C-11), 71.7 (d, C-12), 125.0 (d, C-3), 143.4 (d, C-4), 165.3 (s, C-2), 173.1 (s, C-8); EIMS *m/z* $[M]^+$ 286 (<1), 242 (5), 215 (10), 157 (15), 139 (20), 113 (100).

9,10-Dihydro-(11*R*,12*R*)-di-(*p*-bromobenzoate)-(6*R*,14*R*)-colletodiol (46a): colourless oil (8.8 mg); $[\alpha]^{20}_{D}$ +3.6° (*c* 0.29, $CHCl_3$); UV (EtOH) λ_{max} 245 (ε 28800), 280 (sh, ε 900) nm; CD (EtOH) $\Delta\varepsilon_{255.2\ nm}$ -8.4, $\Delta\varepsilon_{241.2\ nm}$ +7.8; IR (film) ν_{max} 2955, 2925, 1725, 1590, 1260, 1170, 1120, 1100, 1010, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (3H, d, *J* = 6.4 Hz, CH_3-14), 1.30 (3H, d, *J* = 6.0 Hz, CH_3-6), 1.99 (1H, m, H-13), 2.03 (2H, m, H-10), 2.14 (1H, m, H-13), 2.33 (1H, m, H-5), 2.44 (2H, m, H-9), 2.49 (1H, m, H-5), 5.12 (1H, m, H-14), 5.15 (1H, m, H-6), 5.33 (1H, ddd, *J* = 4.9, 7.2, 7.2 Hz, H-11), 5.56 (1H, ddd, *J* = 4.5, 4.5, 7.2 Hz, H-12), 5.93 (1H, d, *J* = 15.8 Hz, H-3), 6.93 (1H, ddd, *J* = 7.2, 8.7, 15.8 Hz, H-4), 7.54 (2H, m), 7.57 (2H, m), 7.86 (2H, m), 7.87 (2H, m); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.2 (q, CH_3- 14), 20.8 (q, CH_3-6), 25.7 (t, C-10), 29.5 (t, C-9), 35.9 (t, C-13), 39.3 (t, C-5), 67.2 (d, C-14), 69.5 (d, C-6), 70.7 (d, C-12), 71.6 (d, C-11), 124.4 (d, C-3), 128.2 (s), 128.4 (s), 128.6 (s), 128.7 (s), 131.1 (d, 2 × C), 131.2 (d, 2 × C), 131.7 (d, 2 × C), 131.9 (d, 2 × C), 144.8 (d, C-4), 164.5 (s), 165.0 (s), 165.2 (s), 171.1 (s, C-8); EIMS *m/z* $[M]^+$ 654 (1), 652 (3), 650 (1), 610 (<1), 608 (1), 606 (<1), 543 (1), 541 (2), 539 (1), 469 (5), 467 (5), 452 (3), 450 (3), 350 (4), 348 (4), 341 (12), 339 (12), 297 (3), 295 (3), 185 (98), 183 (100); HREIMS *m/z* 652.013 (calcd for $C_{28}H_{28}O_8{}^{79}Br^{81}Br$, 652.013).

Ergosterol (47): white solid (8 mg; 1.2 mg/L medium); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.63 (3H, s); 0.82 (3H, d, *J* = 6.7 Hz), 0.83 (3H, d, *J* = 6.7 Hz), 0.91 (3H, d, *J* = 6.9 Hz), 0.95 (3H, s), 1.03 (3H, d, *J* = 6.6 Hz), 1.24 - 2.10 (19H, m), 2.20 - 2.50 (2H, m), 3.64 (1H, m), 5.20 (2H, m), 5.37 (1H, m), 5.57 (1H, m); GC-EIMS *m/z* $[M]^+$ 396 (75), 363 (100), 337 (40).

4.5 Biological activity of pure compounds

Most secondary metabolites obtained in this study were evaluated for their antimicrobial activity in agar diffusion assays, the observed activities are summarised in Table 10. The same compounds, except **25** and **32 - 35**, were further investigated for inhibititory activity in ELISAs towards HIV-1 reverse transcriptase and tyrosine kinase at a test concentration of 0.2 µg/µL (TK) and 0.066 µg/µL (RT), respectively. Ulocladol (**39**) showed a complete inhibition of TK at this concentration. At a test concentration of 0.02 µg/µL a reduction of TK activity by 93% was observed. All other compounds did not significantly inhibit one of the enzymes tested.

Compounds **24**, **28**, **36**, **43** and **44** were further tested for their *in vitro* activity against three different *Plasmodium falciparum* clones; compounds **36** (pycnidione) and **43**

(colletoketol) proved to be active. The results of the investigations of the active compounds are given in Table 11.

Table 10: Antimicrobial activity of pure compounds in agar diffusion assays.[1]

Compound Nr.	Inhibition zone (cm) against test organism 50 µg compound / test disk						
	***E.c.*[2]**	***B.m.***	***M.v.***	***E.r.***	***F.o.***	***M.m.***	***C.f.***
9	n.a.[3]	0.4	0.1	n.a.	n.a.	0.1	n.a.
16	n.a.	n.a.	0.1	n.a.	n.a.	0.1	n.a.
18	n.a.	n.a.	n.a.	0.3	n.a.	n.a.	n.a.
19	n.a.	n.a.	0.2	0.1	n.a.	n.a.	0.1
20	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
21	n.a.	n.a.	0.2	0.2	n.a.	n.a.	n.a.
22	n.a.	n.a.	n.a.	0.2	n.a.	n.a.	n.a.
23	n.a.	0.3	n.a.	n.a.	n.a.	n.a.	n.a.
24	n.a.	n.a.	0.1	n.a.	n.a.	0.1	n.a.
25	n.a.	n.a.	0.2	0.7	n.a.	n.a.	n.a.
26	n.a.	n.a.	0.1	0.1	n.a.	n.a.	n.a.
27	n.a.	n.a.	n.a.	0.3	n.a.	n.a.	n.a.
28	n.a.	n.a.	0.1	n.a.	n.a.	n.a.	n.a.
29	n.a.	n.a.	0.1	0.1	n.a.	n.a.	n.a.
30	n.a.	n.a.	0.1	0.1	n.a.	n.a.	n.a.
32	n.a.	n.a.	0.3	0.3	n.a.	n.a.	0.4
33	n.a.	n.a.	0.2	n.a.	n.a.	n.a.	n.a.
34	n.a.	n.a.	0.5	0.3	n.a.	n.a.	n.a.
35	n.a.	n.a.	0.3	0.3	n.a.	n.a.	n.a.
36	n.a.	0.1	n.a.	n.a.	n.a.	n.a.	n.a.
37	n.a.	n.a.	1.0	2.5	n.a.	1.8	n.a.
39	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
41	n.a.	n.a.	0.2	0.2	n.a.	n.a.	n.a.
42	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
43	n.a.	n.a.	0.2	0.2	n.a.	n.a.	n.a.
44	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
45	n.a.	n.a.	n.a.	0.2	n.a.	n.a.	n.a.
46	n.a.	n.a.	n.a.	0.1	n.a.	n.a.	n.a.

[1] The following compounds were not tested: **1 - 8**, **10 -15**, **17**, **31**, **38**, **40**, **47**.
[2] *E.c. Escherichia coli*, *B.m. Bacillus megaterium*, *M.v. Microbotryum violaceum*, *E.r. Eurotium repens*, *F.o. Fusarium oxysporum*, *M.m. Mycotypha microspora*, *C.f. Chlorella fusca*.
[3] n.a. not active.

Table 11. Antimalarial activity (IC_{50}) of compounds 36 and 43 against *P. falciparum* strains FCR3F86, D6 and W2.

Inhibitor	***P. falciparum* strain tested**		
	FCR3F86	**W2**	**D6**
atovaquone (ng/mL)	0.4 ± 0.3 (n = 5)	0.8 (n = 2)	0.2 (n = 2)
36 (μg/mL)	0.3 (n = 2)	0.2 (n = 2)	0.4 (n = 2)
43 (μg/mL)	2.3 (n = 2)	3.1 (n = 1)	7.0 (n = 1)

5 Discussion

This study mainly aimed at the isolation of higher filamentous fungi from marine sponges and algae, investigation of their taxonomy, and evaluation of the biological activity of their extracts and secondary metabolite production. Both sponge- and algal-derived fungi only recently received broader interest in the natural products chemists community as producers of new and biologically active metabolites (cf. the reviews of, e.g., König & Wright, 1996; Pietra, 1997; Biabani & Laatsch, 1998). In this study, it could be shown that especially in sponges a great taxonomical variety of fungi is encountered, although it seemed doubtful that the majority of these isolates was actively growing in their hosts. Biological investigations of culture extracts of selected isolates suggested a high biosynthetic potential, especially for the production of antimicrobial metabolites. The corresponding chemistry turned out to be structurally diverse, and although obviously related to that of terrestrial fungi, several new natural products have been isolated. The interesting and biologically active metabolites already published from marine fungi (cf. 1.3.2 and reviews cited above) suggested them to be a prolific source for new compounds, which is clearly underlined by the present results.

5.1 Isolation and taxonomy of higher filamentous fungi from marine sponges

Two of the primary goals of the current study were to investigate the diversity of higher filamentous fungi associated with marine sponges, and to examine to what extent they produce biologically active secondary metabolites. It was therefore attempted to isolate species adapted to the marine environment, in particular to sponges, while trying to exclude ubiquitous, chemically well investigated, fast growing, and presumably terrestrial fungi. This was tried by either destroying fungal contaminants adherent to the surface of the sponges chemically or by inhibiting their growth by the use of selective isolation media. Literature data indicated, that to date no comparable studies about sponge-derived fungi were published.

The application of a surface sterilisation method using EtOH, analogously to methods established for terrestrial plants (Schulz *et al.*, 1993), was found to be not applicable to sponges. The EtOH could not be quickly and completely removed from the sponge

tissue, and thus damaged it already after a time insufficient to destroy most surface fungal spores (Schulz *et al.*, 1993). It therefore proved to be impossible to exclude fungi which were merely present by chance in sponge samples, such as inactive propagules filtered by the sponge from the water.

The exclusion of ubiquitous fungi was attempted further by the use of different isolation media. On nutrient rich agar media, fungi isolated were mostly members of genera well known from terrestrial habitats, e.g., *Acremonium*, *Fusarium* and *Penicillium*. Even though species of some of these genera have previously been observed to occur in sponges (Siepmann & Höhnk, 1962; Höhnk & Ulken, 1979; see also 1.3.3, Table 2), they were not desired in the present study due to the high possibility that these strains were terrestrial contaminants. Additionally, they were fast growing and quickly dominated on the agar plates, preventing the detection and isolation of slow growing species. The use of media supplemented with ASW did not significantly inhibit their growth. This finding is consistent with those of physiological studies (e.g., Jones & Byrne, 1976; Molitoris & Schaumann, 1986) which showed that the growth of many terrestrial fungi is not inhibited by salt concentrations equal to those found in the marine environment. Since a selective medium could not be obtained by adding salt, media poor in nutrients, and media supplemented with cyclosporine A (Dreyfuss, 1986) were used. These conditions hindered fast-growing isolates and led to the isolation of slow growing fungi otherwise not obtained.

5.1.1 Taxonomy of the fungal isolates

The majority of fungi isolated belonged to genera of mitosporic fungi. The number of strains identified as Ascomycetes was considerably lower, despite the fact that to date higher filamentous marine fungi have been reported primarily to be members of genera of the Ascomycetes (Kohlmeyer & Volkmann-Kohlmeyer, 1991). Although it can be assumed that most of the mitosporic fungi isolated are in effect Ascomycetes, the taxonomy of the isolates suggests that these are not obligate marine fungi. The only two obligate marine fungi, *Asteromyces cruciatus* and *Phialophorophoma litoralis*, obtained during this part of the current study were mitosporic fungi. The fact that not more obligate marine fungi have been obtained may be due to one or more of the

following reasons: 1) The obligate marine fungi described so far have been rarely isolated from the substrate sponge. 2) Marine Ascomycetes are best isolated using direct isolation methods, e.g., searching for ascomata which have developed on natural substrates, predominately wood (Kohlmeyer & Kohlmeyer, 1979). 3) In pure culture, as is the case for many terrestrial Ascomycetes, many marine Ascomycetes produce only their conidial state or no spores at all (Hyde Farrant & Jones, 1987), which means that their fruiting bodies will not be obtained and they thus can at best be identified as their anamorphs.

The 681 fungal strains isolated belonged to 13 genera of Ascomycetes, two genera of Zygomycetes and 38 genera of mitosporic fungi. The isolates of many genera obviously represented several different species. Except for members of the genera *Aspergillus* (Abrell, Borgeson & Crews, 1996a; Varoglu *et al.*, 1997), *Cladosporium* (Höhnk & Ulken, 1979), *Fusarium* (Siepmann & Höhnk, 1962; Höhnk & Ulken, 1979), *Microascus* (Yu *et al.*, 1996a), *Microsphaeropsis* (Keusgen *et al.*, 1996; Yu *et al.*, 1996b), *Penicillium* (Höhnk & Ulken, 1979) and *Trichoderma* (Kobayashi *et al.*, 1993; Amagata *et al.*, 1998), members of all other genera have so far not been reported from marine sponges. The great taxonomical variety of the fungal isolates obtained in this study raises the question of their "true" origin.

5.1.2 "True" origin of the fungal isolates

Dealing with the "true" origin of the fungi isolated, two major possibilities have to be considered: The fungus could have been growing in the sponge, e.g., as a symbiont, or it was inactive and present merely by chance, most probably filtered of the surrounding water by the sponge. This differentiation cannot be made unambiguously for each strain obtained, but some trends are obvious. Presuming the presence of symbiotic or associated fungi, it is likely that these species dominate by number in their hosts and, if at all capable to grow under the applied conditions *in vitro*, would have been obtained predominantly. Such a dominance of isolates of one or few species isolated only from a single sponge sample was not observed. The great variety of species, and the moderate difference between the spectra of isolates obtained from sponges of the same geographic origin, favours the possibility that the fungi have been filtered of the water

by the sponges. This might have occured in form of spores, which remained able to germinate and grow under laboratory conditions. The presence of such propagules in the sea water at different sites is well established (e.g., Roth, Orpurt & Ahearn, 1964; Schaumann, 1974; Miller & Whitney, 1981). In one of these studies (Schaumann, 1974), 495 isolates from sediment, water and air samples from the North Sea and North-East-Atlantic were reported, and "the majority of species is identical with well known and widespread terrestrial fungi". Members of the genera *Aspergillus*, *Cladosporium*, *Doratomyces*, *Fusarium*, *Penicillium* and *Verticillium* were reported to be among the most common isolates. These findings suggest the presence of similar fungi also for the sea around Helgoland and other oceans. If they were present in the water, it is likely that they were filtered of and accumulated by the sponges. In effect, members of all the genera mentioned above were also obtained in the current study from sponge samples from Helgoland; among them *Fusarium*, *Penicillium* and *Verticillium* ssp. were obtained in a great variety.

Not only land-born fungi, but also marine species are present in sea water and can therefore be filtered of by the sponges. In this respect, the unexpected isolation of eight strains of *Asteromyces cruciatus* from the sponge *Halichondria panicea* may also be the result of sea water containing conidia of this fungus. *A. cruciatus* is known as a saprobic marine fungus (Kohlmeyer & Kohlmeyer 1979) and has frequently been isolated from decaying algae.

There is some further evidence substantiating the proposition that most fungal strains isolated originated in fact from the seawater. Although the number of fungal isolates from each sponge sample is, due to modified experimental conditions, not strictly comparable in a quantitative sense, it is obvious that sponges from Australia and Tenerife yielded only few isolates compared to those from the other locations. This can be interpreted as the result of low concentrations of spores in the water at these collection sites. However, the possibility that antifungal metabolites produced by the sponges are involved must also be considered.

Finally, it is important to mention that none of the reports dealing with the chemistry of sponge-derived fungi contains direct evidence that the fungus in question had been

actively growing in the host sponge (cf. 1.3.3, literature cited in Table 2). Thus, the possibility that these fungal isolates were also filtered of the water by the sponges is likely.

Overall it is presumed that most fungi isolated during this study were inactive in the sponges and filtered of the water: They may be terrestrial species washed or blown into the sea, or they may represent facultative or even obligate marine species. To date, no experimental procedure is known to show if a selected strain is a terrestrial or facultative marine fungus, other than to observe *in vivo*, that is in the marine environment, its ability to grow. On the basis of *in vitro* studies, it can therefore only be suggested that, e.g., some *Phoma* isolates which were obtained during this study from sponge samples from Helgoland, and which were found to sporulate only on media supplemented with ASW, are facultative, or even obligate marine species. Thus, a clear distinction between terrestrial and facultative marine strains obtained in this study is not possible. It is most likely, however, that the majority of the obtained fungal isolates are terrestrial fungi, able to grow under the applied isolation conditions, and facultative marine fungi.

5.2 Isolation and taxonomy of fungal strains from algae

The usual method for the isolation of algae-inhabiting marine fungi is the microscopic examination of the algal thallus, sometimes after an incubation period in the laboratory, for fungal growth, and the separation and inoculation of single fungal spores (e.g., Kohlmeyer & Kohlmeyer, 1979; Nakagiri, 1993; Nakagiri & Ito, 1997). This method proves the isolate in question to be active *in vivo* in the alga, but implies that the fungus is sporulating and visible on its host. In contrast to this, in the current study it has been attempted to isolate endophytic fungi, causing no obvious symptoms on the algae.

As for marine sponges, surface sterilisation with EtOH (Schulz *et al.*, 1993) has been attempted. Algae investigated, which possessed thin thalli, proved to be very sensitive towards the sterilisation agent. It must therefore be presumed that their tissue, and therefore also the endophytic fungi, are destroyed much easier than fungal spores

present by chance on the surface of the algae. Thus, this method is applicable only to algae possessing a sturdy surface.

A further problem arose from the possibility, that fungi originally present only on the surface of the algae may have grown into the algal tissue during transport and storage. Such fungi cannot be differentiated from endophytes even if surface sterilisation was sufficient.

The isolated fungi represented 19 genera of mitosporic fungi, four genera of Ascomycetes, and one genus of Zygomycetes. Only one obligate marine (algicolous) fungus, *Varicosporina ramulosa*, was obtained.

Members of the genera *Acremonium* (Chen *et al.*, 1996), *Aspergillus* (Belofsky *et al.*, 1998), *Fusarium* (Chen *et al.*, 1996), *Leptosphaeria* (Takahashi *et al.*, 1994a and b, 1995) and *Penicillium* (Numata *et al.*, 1993, 1996; Takahashi *et al.*, 1996; Iwamoto *et al.*, 1998; Amagata, Minoura & Numata, 1998a) have previously been reported from algae in publications dealing with the chemistry of these isolates. Similar strains were also obtained in the current study. The results also agree with the spectrum of fungal genera obtained in a study of fungi from seaweeds off the Spanish coast (Genilloud *et al.*, 1994).

Additionally, some similarities were obvious concerning the spectrum of fungal genera isolated from sponge and algal samples of the same geographic origin. Representatives of the majority of all genera were obtained both from sponge and algal samples. These findings suggested that most fungi isolated originated from the sea water and were present only on the surface of the algae.

5.3 Biological activity of extracts from sponge-derived fungi

Due to the large number of isolates, only some strains could be tested for antimicrobial activity of their extracts, and a lower number for inhibition of HIV-1 RT and TK. The results are therefore, striktly spoken, not representative for all sponge-derived fungi obtained. It must be stressed, that members of ubiquitous genera, such as

Acremonium, *Aspergillus* and *Penicillium* which possess a well known secondary metabolite production, were not investigated in order to avoid the re-isolation of known metabolites. As these fungi are also known for the production of antimicrobial compounds, the percentage of extracts active in the agar diffusion assays might have been even higher. Nevertheless, the results obtained with the selected fungi demonstrated that a large number of strains, that is 81.5 %, produced antimicrobially active natural products. In comparison, 53 % of the phytopathogens, 70 % of the soil isolates and 91 % of the fungal endophytes that were tested using the same test organisms were found to have antimicrobial activity, (Schulz *et. al.*, 1999). The predominant antimicrobial activity of sponge-derived fungi was directed towards fungi and the alga *C. fusca*. In this respect it is interesting to note that the culture extracts of endophytes also predominately inhibited the alga and fungal test organisms (Schulz *et. al.*, 1999). The relatively high proportion of active culture extracts from sponge-derived isolates suggested that they are also creative producers of biologically active metabolites.

The percentage of *Coniothyrium* and *Phoma* strains whose extracts showed antimicrobial activity was very high, 100 % and 86 %, respectively (see 4.2.1.1, Table 5). TLC analysis of these extracts showed that the strains produced rather complex mixtures of substances. These results suggested that especially isolates of these genera are promising sources for biologically active secondary metabolites. Since the genus *Phoma* consists of about 2000 species or more (Sutton, 1980), a large array of secondary metabolites is likely. To date, according to the Chapman & Hall natural products data base, about 80 compounds from only 21 *Phoma* sp. have been described, eight of the producing strains have not been identified to the species level (Buckingham, 1996). From the genus *Coniothyrium*, 23 compounds have been described from only three strains (e.g., Krohn*et al.*, 1992; Krohn *et al.*, 1994).

The most active extracts in the agar diffusion assays, originating from 27 fungal strains, were further investigated for cytotoxicity towards KB cells, for inhibition of *Mycobacterium tuberculosis*, and in ELISAs for the inhibition of tyrosine kinase and HIV-1 reverse transcriptase. Whereas the assays for antimicrobial activity and cytotoxicity are whole cell test systems which do not allow the molecular targets

responsible for the observed inhibition to be directly determined, enzymatic assays can directly reveal specific activities.

Extracts of six strains exhibited significant activity when tested for cytotoxicity towards KB cells (IC_{50} < 20 µg/mL) and against *Mycobacterium tuberculosis* (inhibition > 90 % at 100 µg/mL), with two extracts being active in both assays. Together with the results obtained from the agar diffusion assays, this suggested that in some extracts metabolites with specific activity and no general toxicity were present.

HIV-1 reverse transcriptase and tyrosine kinase are important target enzymes for the treatment of AIDS and cancer, respectively (e.g., Chang & Geahlen, 1992; Mitsuya & Broder, 1987). Extracts of 37 % of the investigated fungal strains showed significant activity in these enzyme assays. In five cases both enzymes were inhibited. This may, of course, be interpreted as an unspecific activity. Specific activity was observed for two strains against tyrosine kinase and for three strains against HIV-1 reverse transcriptase. Although the number of tested strains was low and the concentration of the active compound(s) in the extracts is not known, the results certainly suggest that further investigations are warranted.

5.4 Biological activity of extracts from algal-derived fungi

The number (22) of algal-derived fungal strains tested for their production of biologically active metabolites was considerably lower than the number (92) of sponge-derived strains tested. From most genera only one species was tested, and only agar diffusion assays for antimicrobial activity were performed. A moderate overall activity of 63.6 % was observed. Nevertheless, extracts of four strains of Mycelia sterilia prominently and selectively inhibited *B. megaterium*. The potential of these algal-derived fungi to produce interesting secondary metabolites will have to be further evaluated by chemical investigations.

5.5 Metabolites from sponge- and algal-derived fungal strains and their biological activity

From extracts of 11 fungal strains, 39 pure compounds, including 30 secondary metabolites were isolated. Six compounds, (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde (**10**), (3*S*)-(3',5'-dihydroxyphenyl)butan-2-one (**16**), 2-((1'*E*)-propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol (**17**), microsphaeropsisin (**26**), ulocladol (**39**) and 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46)** proved to be new structures, and further three compounds, (3*S*,5*R*)-dimethyl-dihydrofuran-2-one (**11**), 5,7-dimethoxy-4,6-dimethylphthalide (**33**) and 9,10-dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**45**), were for the first time obtained as natural products. 22 secondary metabolites were obtained from sponge-derived fungal strains to a total of about 40 so far reported from such fungi (see 1.3.3, Table 2). Only *R*-mellein (**27**) has previously been reported from a sponge-derived fungus, *Aspergillus ?ochraceus* (Abrell, Borgeson & Crews, 1996a). The majority of all metabolites evaluated during this study exhibited biological, predominantly antimicrobial activity, thus explaining, or at least contributing to, the initially observed activity of the corresponding extracts. These results certainly justify further investigations of other fungal strains obtained.

5.5.1 Primary metabolites

Out of the 39 isolated compounds, 9 are supposed to be primary metabolites, or at least to be closely related to them. The most common metabolites derived from two obligate marine fungi: D-arabitol (**13**), D-mannitol (**14**), D-trehalose (**15**) from *Asteromyces cruciatus* and ergosterol (**47**) from *Varicosporina ramulosa*. Ergosterol is one of the main fungal sterols, and the other three metabolites are also frequently encountered in fungi. D-arabitol and D-mannitol have been reported to be accumulated in the mycelium of marine fungi in order to balance the osmotic pressure of the salt water (Clipson & Jennings 1992), while D-trehalose is a common fungal carbohydrate, important e.g. as a reserve compound (Pfyffer, 1998).

Tri-*O*-acetyl-glycerol (**12**) from *A. cruciatus*, 1-*O*-oleyl-glycerol (**34**) and 1-*O*-linoleyl-glycerol (**35**) from *Monochaetia* sp., and 2-phenylethanol (**21**) and

2-(*p*-hydroxyphenyl)ethanol (**22**) are closely related to common primary metabolites. According to literature data (Chapman & Hall and Beilstein data bases; Turner & Aldridge, 1983) out of the three glycerides, only 1-*O*-linoleyl-glycerol has previously been reported from a fungal source, namely as a sporogenic metabolite from *Sclerotina fructicola* (Katayama & Marumo, 1978). In the current study, compounds **34** and **35** have been shown to possess antimicrobial activity against *E. repens* and *M. violaceum* in agar diffusion assays, obviously contributing to the observed antimicrobial activity of the crude extract of the *Monochaetia* sp. Compounds **21** (2 phenylethanol) and **22** (2-(*p*-hydroxyphenyl)ethanol) both exhibiting antimicrobial activity, are presumably degradation products of phenylalanine and tyrosine. They have been previously reported from various fungi, e.g., Turner & Aldridge (1983).

5.5.2 Secondary metabolites

30 of the isolated compounds most probably represent secondary metabolites, and include polyketides, terpenes and metabolites of mixed biosynthetic origin.

5.5.2.1 Macrodiolides

Five structurally related macrodiolides (**42-46**) were obtained from culture extracts of the obligate marine fungus *Varicosporina ramulosa*. These metabolites formally consist of a triketide and a tetraketide unit. 9,10-Dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**45**) and 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46**) are new fungal metabolites, **45** being first reported as an intermediate in the total synthesis of **42** (Ohta *et al.*, 1993). The other three were already reported from terrestrial fungi, grahamimycin A_1 (**42**) from an isolate of the genus *Cytospora* (Gurusiddaiah & Ronald, 1981), and colletoketol (**43**; MacMillan & Simpson, 1973) and (6*R*,11*R*,12*R*,14*R*)-colletodiol (**44**; Grove, Speake & Ward, 1966) from the plant pathogen *Colletotrichum capsici*. In contrast to the prominent antimicrobial activity of **42** and **43** (as grahamimycin A, Gurusiddaiah & Ronald, 1981), no biological activities have been reported for colletodiol. Its abundant production of 105 mg/L medium suggests a biological function yet to be discovered.

5.5.2.2 Tetraketides

Three metabolites, aurantiogliocladin (**9**), 7-hydroxy-5-methoxy-4,6-dimethylphthalide (**32**) and 5,7-dimethoxy-4,6-dimethylphthalide (**33**) presumably are tetraketides.

Compound **9** was obtained in the current study from an *Acremonium* sp. It was first reported as an antibacterial agent from a *Gliocladium* sp. (Brian *et al.*, 1951). A similar activity against *B. megaterium* and additional antifungal activity against *M. mycotypha* and *M. violaceum* was observed in the current investigation.

The two phthalide-derivatives (**32** and **33**) were obtained from a *Monochaetia* sp. Both compounds showed antifungal activity in agar diffusion assays, contributing to the antifungal activity of the crude extract. Phthalide-derivatives are widespread fungal metabolites and have been reported with great structural diversity (e.g., Achenbach, Mühlenfeld & Brillinger 1985). Compound **32** was first described as an antifungal metabolite from a *Hansfordia* sp. (Schneider, Anke & Sterner, 1997), while compound **33** has been first obtained as a natural product in this study. So far it had been only described as the methylation product of both 5-hydroxy-7-methoxy-4,6-dimethylphthalide and 5,7-dihydroxy-4,6-dimethylphthalide, compounds isolated from *Aspergillus duricaulis* (Achenbach, Mühlenfeld & Brillinger 1985).

5.5.2.3 Pentaketides

A major group of common fungal metabolites contains mellein (**27**), and its derivatives and biosynthetic analogues. In this study a *Coniothyrium* sp. (**18**-**20**) and a *Microsphaeropsis* sp. (**27**-**31**) yielded such compounds.

Pentaketides of the mellein-type are frequently reported from various fungi, often with prominent biological, predominantly antimicrobial, activity. (-)-Mellein (**27**) was first described in 1933 from *Aspergillus melleus* (Nishikawa, 1933a and b). Since then both stereoisomers have been frequently reisolated (e.g., Krohn *et al.*, 1997; Turner & Aldridge, 1983), including also a report from a sponge-derived *A. ?ochraceus* strain (Abrell, Borgeson & Crews, 1996a). The mellein derivatives (3*R*)-6-methoxy-7-

chloromellein (**19**), (3*R*,4*S*)-hydroxymellein (**28**) and (3*R*,4*R*)-hydroxymellein (**29**) also represent typical fungal metabolites, first isolated from *Septoria nodorum* (Devys *et al.*, 1992), *Lasiodiplodia theobromae* (Aldridge *et al.*, 1971) and *Sporormia affinis* (McGahren & Mitcher, 1968). In contrast, (3*R*)-6-methoxymellein (**18**) was first reported from stored carrots (Sondheimer, 1957), and has since been described both as a fungal metabolite (e.g., McGahren & Mitcher, 1968), and as a phytoalexin produced by cultured carrot cells in response to fungal infection (Kurosaki & Nishi, 1983). Cryptosporiopsinol (**20**) is biosynthetically related to mellein and thus also belongs to this class of compounds (Giles & Turner, 1969; Holker & Young, 1975).

4,8-Dihydroxy-3,4-dihydro-2*H*-naphthalene-1-one (**30**) was first isolated from a fungus of the genus *Scytalidium* and named 4,8-dihydroxytetralone (Findlay & Kwan, 1973). Stereoisomers have since then been reported not only from other fungi, e.g., as (+)-isosclerone from *Sclerotinia sclerotiorum* (Morita & Aoki, 1974), but also from the stem bark of the walnut tree *Juglans regia* as (-)-regiolone (Talapatra *et al.*, 1988). The unidentified compound **31** is obviously structurally related to **30** and also presumed to belong to this group of metabolites.

Except for cryptosporiopsinol (**20**), all compounds tested, that is *R*-mellein (**27**), (3*R*)-6-methoxymellein (**18**), (3*R*)-6-methoxy-7-chloromellein (**19**), (3*R*,4*S*)-hydroxymellein (**28**), (3*R*,4*R*)-hydroxymellein (**29**) and 4,8-dihydroxy-3,4-dihydro-2*H*-naphthalene-1-one (**30**), showed antifungal activity in the agar diffusion assays. This finding in combination with the high content of *R*-mellein (**27**, 4.6 mg/L medium) and its two 4-hydroxyl-derivatives (**28**, 94 mg/L medium and **29**, 11 mg/L medium) in the crude extracts investigated explains the activity observed in the initial screening for antimicrobial activity. Their relatively high production, their antimicrobial activity, and the fact that these metabolites are particularly common to many fungi makes the selection of fungal strains for chemical investigations on the basis of the agar diffusion assays alone difficult. It seems therefore to be advisable to evaluate the presence of mellein-derivatives in extracts prior to further chemical investigations.

5.5.2.4 Heptaketides

Compounds 1233A (**37**), **38**, ulocladol (**39**), botrallin (**40**) and 1-hydroxy-6-methyl-8-hydroxymethylxanthone (**41**) are heptaketides.

From *Ulocladium botrytis* the new compound ulocladol (**39**) and a known xanthone-derivative (**41**) have been obtained. Metabolites with a basic structure similar to **39** have been reported from several fungi, e.g., botrallin (**40**), the presence of which in the current extract could only be tentatively determined, from *Botrytis allii* (Kameda *et al.*, 1974), and recently the 4-methoxyl-derivative of **39** from the lichen mycobiont of *Graphis scripta* var. *pulverulenta* (Tanahashi *et al.*, 1997). Xanthone-derivatives similar to compound **41** are mainly known from higher plants, but have also been reported from fungi with great structural variety (e.g., Turner & Aldridge, 1983). **41** was originally reported from *Cyathus intermedius*, a Basidiomycete (Ayer & Taylor, 1976).

The crude extract of *U. botrytis* showed the most prominent inhibition of all extracts tested for their tyrosine kinase inhibitory activity. By bio-assay guided fractionation it was possible to isolate the new metabolite ulocladol (**39**) and to show that it substantially contributed to this activity (93% inhibition of enzyme activity at 0.02 µg/µL).

Crude extracts of an isolate of *Scopulariopsis candida* were among the most active extracts in the agar diffusion assays for antifungal activity. Bioassay-guided work-up led to the isolation of 1233A (**37**), the by far most active antifungal metabolite obtained in this study. Additionally, the inactive derivative **38** was obtained. Compound **37** turned out to be originally reported from a *Cephalosporium* sp. (Aldridge, Giles & Turner, 1971), and has been particularly comprehensively studied. Its absolute configuration was determined by degradation studies and derivatisations (Chiang *et al.*, 1988). Additionally, a total synthesis (Chiang *et al.*, 1989) and the elucidation of its biosynthesis in a *Scopulariopsis* sp. (Kumagai, Tomoda & Omura, 1992) were reported. Investigations for biological activity revealed **37** to be the first

specific inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A synthase, and to be an inhibitor of cholesterol biosynthesis (Omura*et al.*, 1987; Greenspan *et al.*, 1987).

5.5.2.5 Terpenes

Only two terpenes, both being sesquiterpenes, have been obtained during this study, microsphaeropsisin (**26**) from a *Microsphaeropsis* sp. and dihydrobotrydial (**24**) from *Emericellopsis minima*.

Microsphaeropsisin is a new sesquiterpene of the eremophilane type. This structural class of sesquiterpenes has been reported mainly from higher plants (Buckingham, 1996), but is also encountered in fungi, e.g., in *Penicillium roqueforti* (Moreau *et al.*, 1976) and the marine mitosporic fungus *Dendryphiella salina* (Guerriero *et al.*, 1988, 1989, 1990). In most sesquiterpenes of the eremophilane type described to date CH_3-14 and CH_3-15 are cis, indicating microsphaeropsisin (**26**) to be a rare eremophilane derivative having these moieties trans configurated.

Dihydrobotrydial (**24**) was previously reported from *Botrytis cinerea* (Fehlhaber *et al.*, 1974), together with the similar botrydial, formally an oxidation product of **24**. Whereas for botrydial antimicrobial activity has been reported (Fehlhaber *et al.*, 1974), **24** was inactive in similar tests. This could explain the observed differences in antimicrobial activity of the extracts from *E. minima*. While the extract from the initial small scale culture probably contained botrydial, it showed antifungal activity; the extract from the up-scaled culture, which was investigated chemically, contained no botrydial but its hydrogenation product **24**, and was therefore inactive.

5.5.2.6 Compounds of mixed biosynthetic origin

The two metabolites fungerin (**25**) and pycnidione (**36**), obviously are of mixed biosynthetic origin. From the molecular structure of fungerin its biosynthesis may involve one isoprene unit and a histidine derivative, while pycnidione probably is derived from polyketide and terpenoid precursors.

Fungerin, from a *Microascus* sp. was the only nitrogen containing metabolite obtained during this study. Literature data about this compound is inconsistent and confusing. The metabolite seemed to be published twice with a different trivial name and structure as to the location of the N-methyl group, but with identical spectroscopic data: as visoltricin, isolated from a *Fusarium* sp., exhibiting toxicity against *Artemia salina* larvae, cytotoxicity against selected human tumour cell lines and showing an anticholinesterase activity (Visconti & Solfrizzo, 1994); and as fungerin isolated from a different *Fusarium* sp., exhibiting antifungal activity (Kato *et al.*, 1996). A recent total synthesis (Benhida, Lezama & Fourrey, 1998) proved the structure determined by Kato *et al.* to be correct. An antifungal activity, observed for the crude extract of the *Microascus* sp. was also exhibited by the pure compound. **25** turned out to be the second most active antifungal metabolite obtained in this study (Table 10). This finding stands in good agreement with the reported antifungal activity (Kato*et al.*, 1996).

Pycnidione (**36**) isolated in this study from the culture extract of a *Phoma* sp. derived from a sponge-sample from Helgoland, was first reported from a *Phoma* sp. originating from Micronesia (Harris *et al.*, 1993). The C-8 epimer eupenifeldin was described the same year by Mayerl *et al.* from *Eupenicillium brefeldianum*. For both compounds prominent biological activities were reported, mainly cytotoxicity for eupenifeldin and inhibition of stromelysin for pycnidione. Recently the re-isolation of pycnidione together with two new derivatives has been reported from an unidentified fungal strain originating from twigs out of a seasonal, deciduous alluvial forest from Brazil (Cai *et al.*, 1998). In the same study these compounds were shown to induce erythropoietin expression in human cells. In the current investigations, pycnidione showed prominent *in vitro* activity against different *Plasmodium falciparum* strains with an IC_{50} of 0.2 µg/mL against the chloroquine resistant W2 strain. In the agar diffusion assays only moderate activity against*B. megaterium* was observed.

5.5.2.7 Miscellaneous compounds

The remaining metabolites, (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde (**10**), (3*S*,5*R*)-dimethyl-dihydrofuran-2-one (**11**), (3*S*)-(3',5'-dihydroxyphenyl)butan-2-one

(**16**), 2-((1'*E*)-propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol (**17**) and 2-(hydroxymethyl)furan (**23**) are relatively small molecules, with **10**, **16** and **17** being new compounds, and **11** being first reported as a natural product. Only 2-(hydroxymethyl)furan (**23**) has so far been reported from fungi as a reduction product of furfural by yeasts (Windholz, 1983). A similar process may by involved in the current finding with furfural possibly originating from the decomposition of media ingredients during autoclaving.

5.6 Chemical investigations of the sponge samples

In the course of this study (since 1995), a growing number of reports about secondary metabolites from sponge-derived fungi has been published (see 1.3.3, Table 2). These compounds appeared to be typical fungal metabolites, deriving from metabolic pathways common to these organisms, and were clearly distinct from sponge metabolites. It seemed thus unlikely, that fungi are playing an important role in the production of metabolites found in sponges. Therefore only two sponge samples, parts of which were used for the isolation of fungi in this study, were thoroughly investigated chemically. This led to the isolation of eight pure compounds: taurine (**1**) from *Callyspongia* sp. cf. *C. flammea*, 22-*O*-methyl derivatives of five sesterterpene tetronic acids (**2**-**6**) from *Ircinia oros*, two of them, (8*Z*,13*Z*,18*S*,20*Z*)-strobilinin (**2**) and (7*E*,12*Z*,18*S*,20*Z*)-variabilin (**3**) being new metabolites, a clathridine Zn complex (**8**) and an as yet unelucidated metabolite (**7**) from *Leucosolenia challengeri*. Except for taurine, these compounds were not related to known fungal metabolites.

Sesterterpene tetronic acids are commonly encountered in sponges of the order Dictyoceratida (Demospongiae), mainly in the genera *Ircinia*, *Psammocinia* and *Sarcotragus*, where they are reported to be of chemotaxonomic significance (Perry *et al.*, 1987; Bergquist, 1978). Many similar metabolites have been reported, differing only in number, position and/or stereochemistry of the chain double bonds. Variabilin, the first representative of a group of secondary metabolites termed variabilins, sesterterpene tetronic acids with a $\Delta^{7,8}$ double-bond, was first reported from the sponge *Ircinia variabilis* as an antimicrobial agent (Faulkner, 1973). Sesterterpene tetronic acids of this type with a $\Delta^{8,10}$ double-bond are referred to as strobilinins; the

basic structure of strobilinin without stereochemistry and double-bond geometry was first described from *Ircinia strobilina* (Rothberg & Shubiak, 1975). In 1994 Davis & Capon showed that the originally described strobilinin was in fact a mixture of two isomers, namely (8*E*,13*Z*,20*Z*)- and (8*Z*,13*E*,20*Z*)-strobilinin. Several stereo-, geometric and regioisomers of variabilin and strobilinin and other related types have been described to date (Martinez *et al.*, 1997; Gonzáles, Rodríguez & Barrientos, 1983; Shubiak, 1976; Barrow *et al.*, 1988) and shown to possess antiviral and cytotoxic activity (Barrow *et al.*, 1988; Jacobs, White & Wilson, 1981).

6 Summary

Higher filamentous fungi were isolated from marine sponges and algae. Their taxonomy, the biological activity of their extracts, their secondary metabolite production, and the biological activity of the obtained pure compounds were investigated. Additionally, selected sponge samples were investigated chemically.

Out of 16 sponge samples from six different locations, Australia (Bear Island and The Great Barrier Reef), Dominica (Caribbean), Helgoland (North Sea, Germany), Malta and Tenerife (Spain), 681 fungal strains, representing 53 genera (38 genera of mitosporic fungi, including two clearly obligate marine fungi, 13 genera of Ascomycetes, two genera of Zygomycetes), and 37 strains of Mycelia sterilia, were isolated. Representatives of most of these fungal genera have thus far not been reported from marine sponges. The taxonomy and diversity of the isolated fungal strains suggested, however, that the majority of them were not associated with the host-sponge, but had been simply filtered from the sea water. These isolates were most likely to be mainly terrestrial and facultative marine species.

In order to enable preliminary investigations of their biologically active secondary metabolite production, 92 sponge-derived fungal strains were chosen for small scale cultivation on different media. EtOAc extracts of 75 strains (81.5 %) showed antimicrobial activity in agar diffusion assays for antibacterial, antifungal and antialgal activity. Extracts of 27 strains were further tested in different assays: Extracts of six strains showed significant cytotoxicity towards KB cells, and further six exhibited prominent *Mycobacterium tuberculosis* inhibition. HIV-1 reverse transcriptase was inhibited by extracts of eight strains, and tyrosine kinase by extracts of seven strains.

Out of 10 algal samples from Tenerife (Spain) and Helgoland (Germany) 105 fungal strains, representing 25 genera (four genera of Ascomycetes, one genus of Zygomycetes, 19 genera of mitosporic fungi, including one obligate marine fungus) and seven strains of Mycelia sterilia, were isolated. Agar diffusion assays showed extracts of 14 (63.6 %) out of the 22 algal-derived strains investigated to have antimicrobial activity.

Extracts of 11 fungal strains, including 10 sponge-derived and one algal-derived strain, were investigated chemically. This investigation resulted in the isolation and structure elucidation of 39 pure compounds, including 30 secondary and 9 primary metabolites. Six compounds, (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde (**10**), (3*S*)-(3',5'-dihydroxyphenyl)butan-2-one (**16**), 2-((1'*E*)-propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol (**17**), microsphaeropsisin (**26**), ulocladol (**39**) and 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46**) proved to be new compounds, and a further three compounds were obtained for the first time as natural products: (3*S*,5*R*)-dimethyl-dihydrofuran-2-one (**11**), 5,7-dimethoxy-4,6-dimethylphthalide (**33**) and 9,10-dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**45**).

From the three sponge species investigated eight pure compounds were isolated, including 22-*O*-methyl derivatives of the two new sesterterpene tetronic acids (8*Z*,13*Z*,18*S*,20*Z*)-strobilinin (**2**) and (7*E*,12*Z*,18*S*,20*Z*)-variabilin (**3**).

The antimicrobial activity of 28 compounds was investigated in agar diffusion assays: 22 were found to possess antifungal, three antibacterial, and two antialgal activity. These findings explained in many cases the previously observed activity of the corresponding fungal crude extracts. Two of the isolated metabolites exhibited additional prominent biological activities: the new compound ulocladol (**39**) inhibited tyrosine kinase (93% inhibition of enzyme activity at 0.02 µg/µL), and pycnidione (**36**) showed an *in vitro* antimalarial activity against the *Plasmodium falciparum* strains FCR3F86, W2 and D6 with IC_{50} values of 0.3, 0.2 and 0.4 µg/mL, respectively.

7 References

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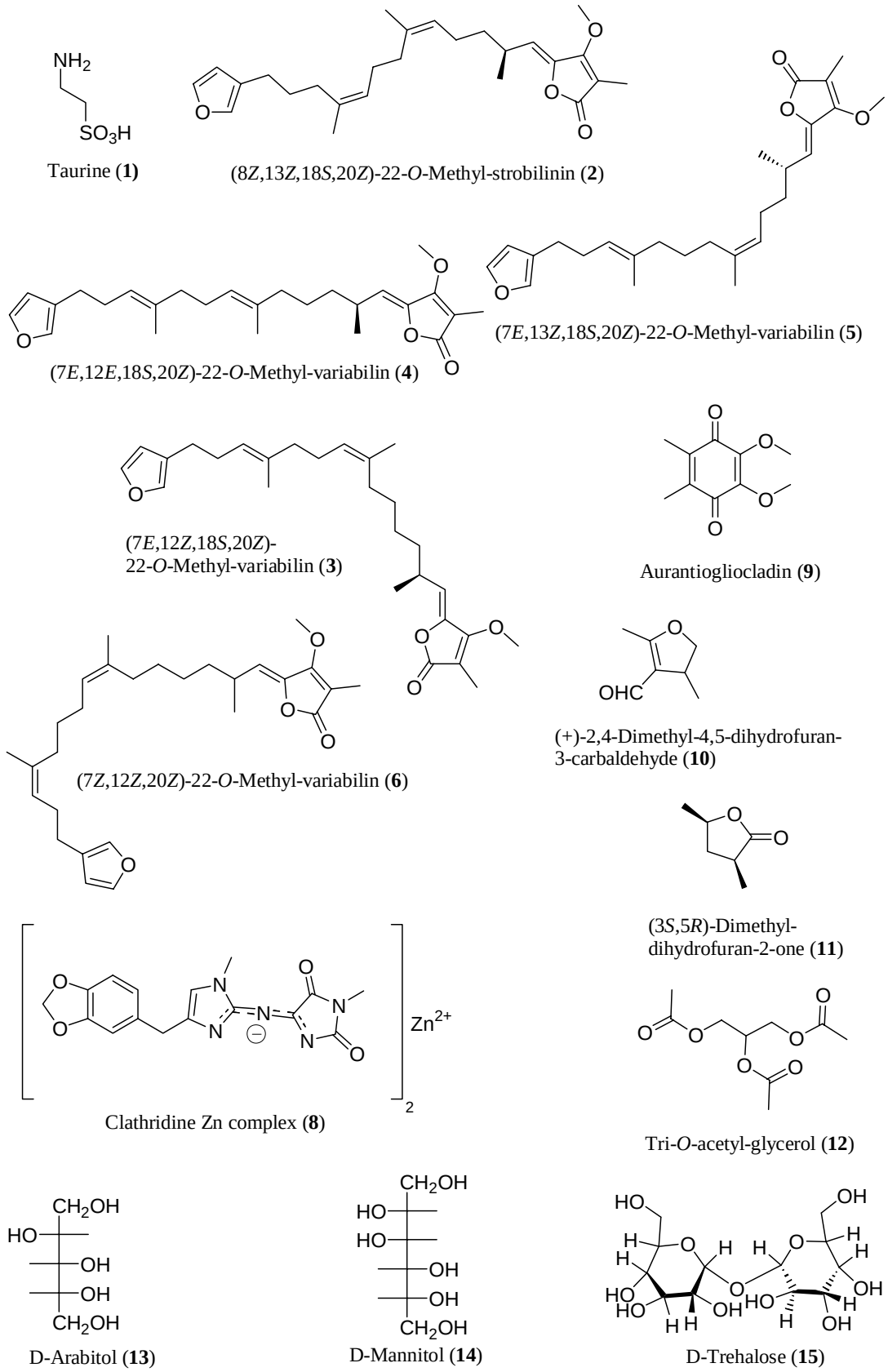
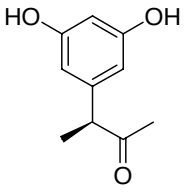
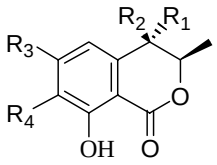


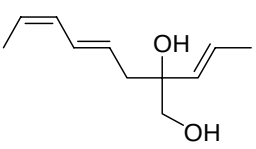
Figure 9: Metabolites 1-6 and 8-15 isolated during this study.



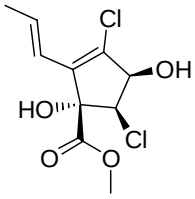
(3*S*)-(3',5'-Dihydroxyphenyl)-butan-2-one (**16**)



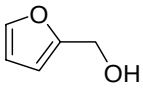
R_1	R_2	R_3	R_4	
H	H	H	H	*R*-Mellein (**27**)
OH	H	H	H	(3*R*,4*S*)-Hydroxymellein (**28**)
H	OH	H	H	(3*R*,4*R*)-Hydroxymellein (**29**)
H	H	OMe	H	(3*R*)-6-Methoxymellein (**18**)
H	H	OMe	Cl	(3*R*)-6-Methoxy-7-chloro-mellein (**19**)



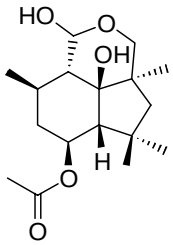
2-((1'*E*)-Propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol (**17**)



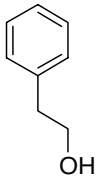
Cryptosporiopsinol (**20**)



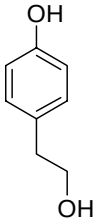
2-(Hydroxymethyl)furan (**23**)



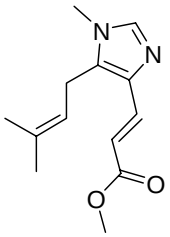
Dihydrobotrydial (**24**)



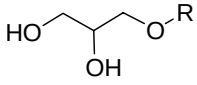
2-Phenylethanol (**21**)



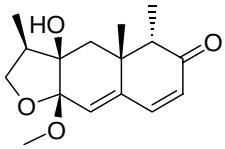
2-(*p*-Hydroxyphenyl)-ethanol (**22**)



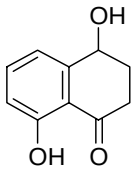
Fungerin (**25**)



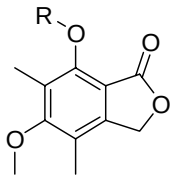
1-*O*-Oleyl-glycerol (**34**) R = oleyl
1-*O*-Linoleyl-glycerol (**35**) R = linoleyl



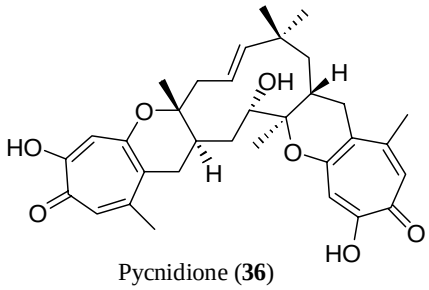
Microsphaeropsisin (**26**)



4,8-Dihydroxy-3,4-dihydro-2*H*-naphthalene-1-one (**30**)

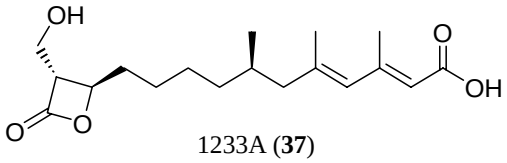


7-Hydroxy-5-methoxy-4,6-dimethylphthalide (**32**) R = H
5,7-Dimethoxy-4,6-dimethylphthalide (**33**) R = CH_3

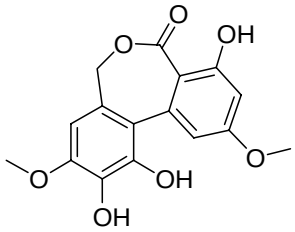


Pycnidione (**36**)

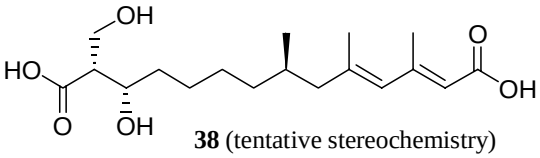
Figure 10: Metabolites 16-30 and 32-36 isolated during this study.



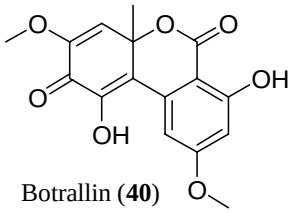
1233A (**37**)



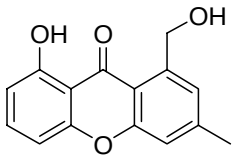
Ulocladol (**39**)



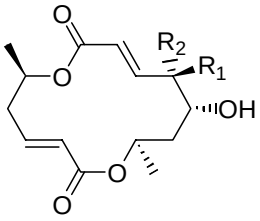
38 (tentative stereochemistry)



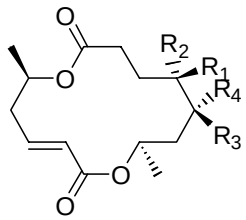
Botrallin (**40**)



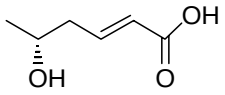
1-Hydroxy-6-methyl-8-hydroxy-methylxanthone (**41**)



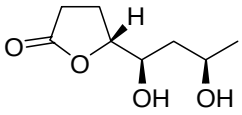
Colletoketol (**43**) R_1, R_2 = O
(6*R*,11*R*,12*R*,14*R*)-Colletodiol (**44**) R_1 = OH; R_2 = H



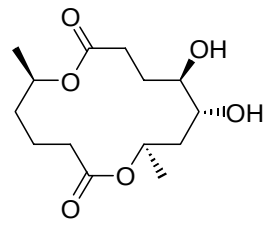
Grahamimycin A_1 (**42**) R_1,R_2=O; R_3,R_4=O
9,10-Dihydro-(6*R*,11*S*,12*S*14*R*)-colletodiol (**45**)
R_1 = R_4 = H; R_2 = R_3 = OH
9,10-Dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46**)
R_1 = R_4 = OH; R_2 = R_3 = H
9,10-Dihydro-(11*R*,12*R*)-di-(*p*-bromobenzoate)-(6*R*,14*R)*-colletodiol (**46a**)
R_1 = R_4 = *p*-bromobenzoate; R_2 = R_3 = H



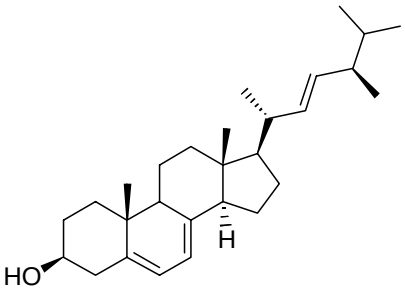
(5*R*)-Hydroxy-(2*E*)-hexenoic acid (**43a**)



(5*R*)-((1′*R*,3′*R*)-Dihydroxybutyl)-dihydrofuran-2-one (**44b**/**46c**)



3,4,9,10-Tetrahydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**44a**/**46b**)



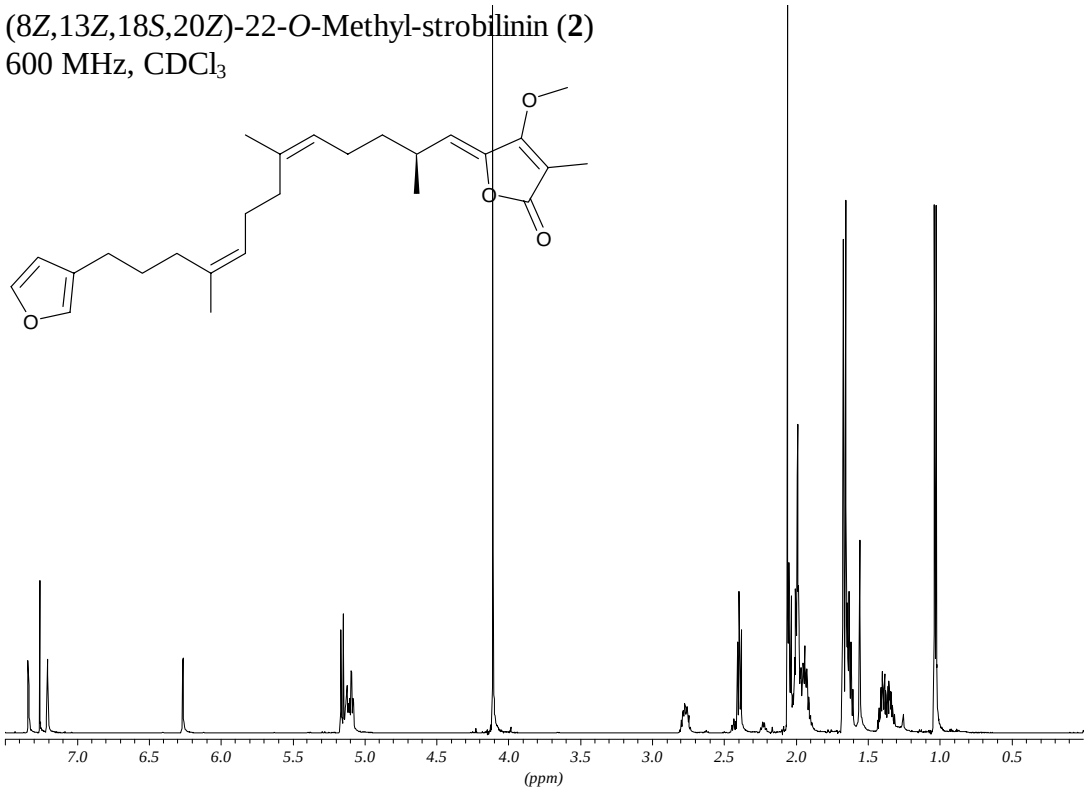
Ergosterol (**47**)

Figure 11: Metabolites 37-47 isolated during this study.

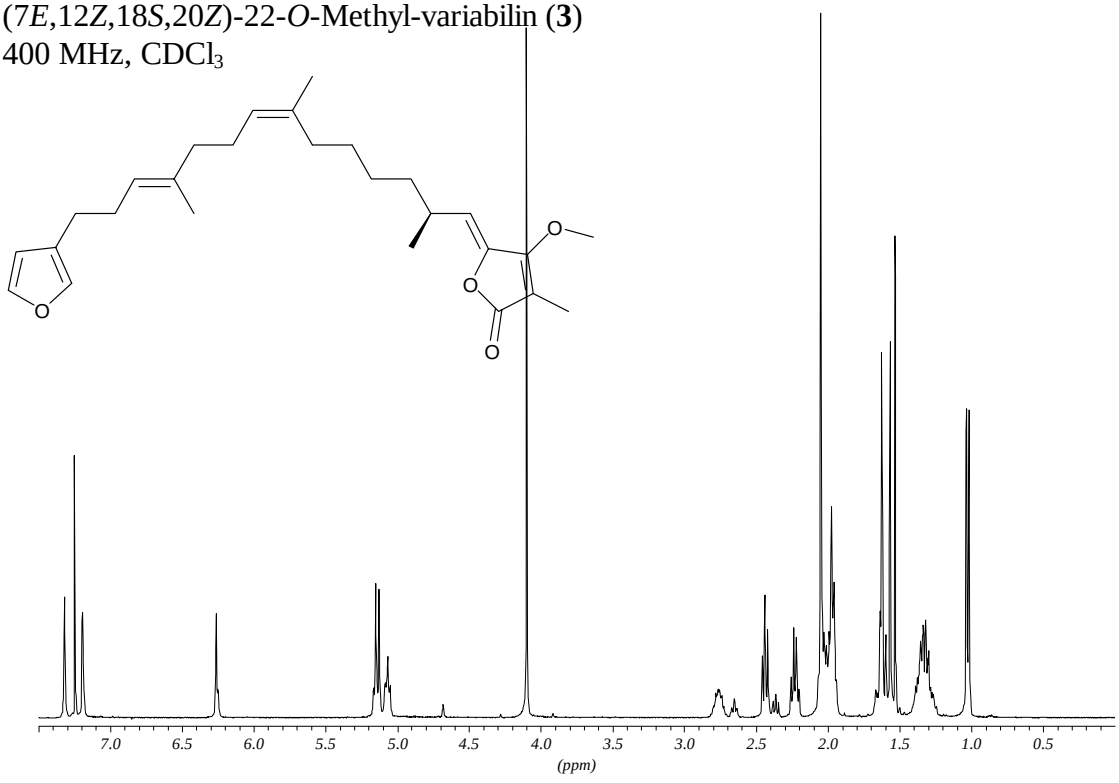
8.2 [1]H NMR spectra of selected compounds

A "X" marks resonances due to an impurity.

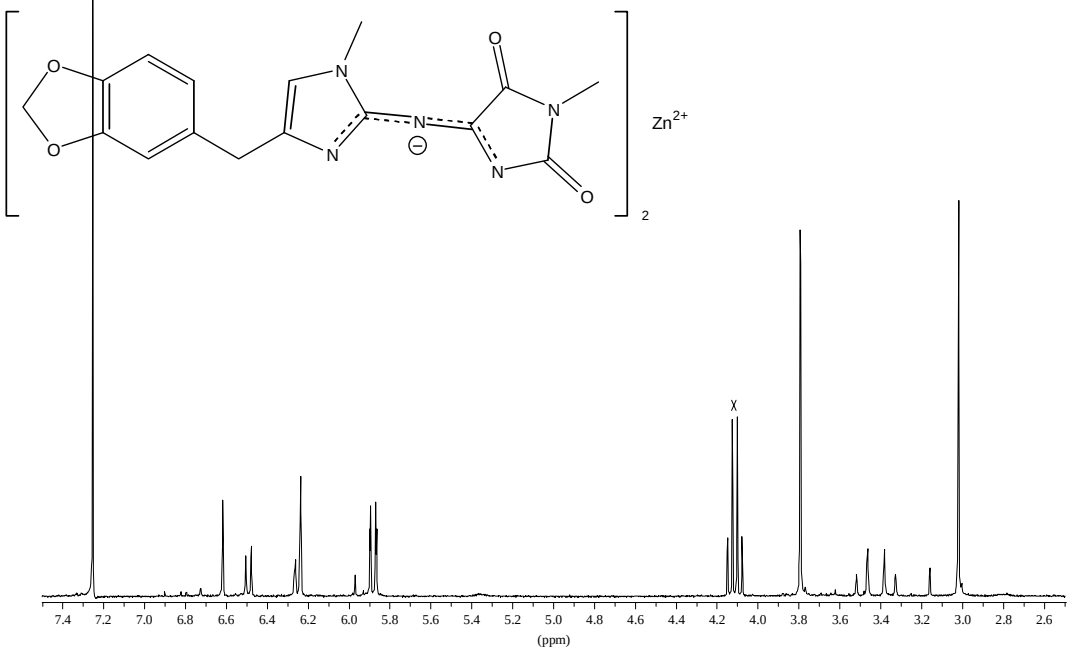
(8*Z*,13*Z*,18*S*,20*Z*)-22-*O*-Methyl-strobilinin (**2**)
600 MHz, $CDCl_3$



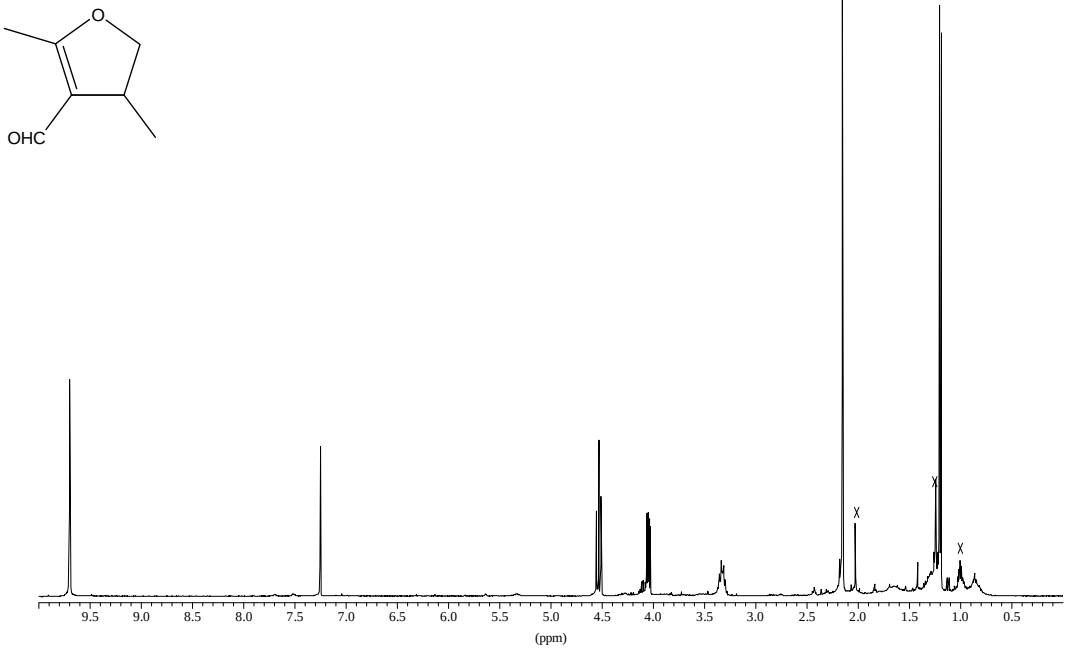
(7*E*,12*Z*,18*S*,20*Z*)-22-*O*-Methyl-variabilin (**3**)
400 MHz, $CDCl_3$



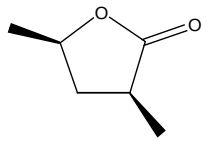
Clathridine Zn complex (**8**)
300 MHz, $CDCl_3$



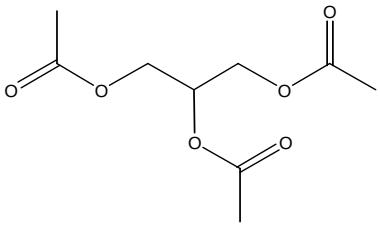
(+)-2,4-Dimethyl-4,5-dihydrofuran-3-carbaldehyde (**10**)
400 MHz, $CDCl_3$



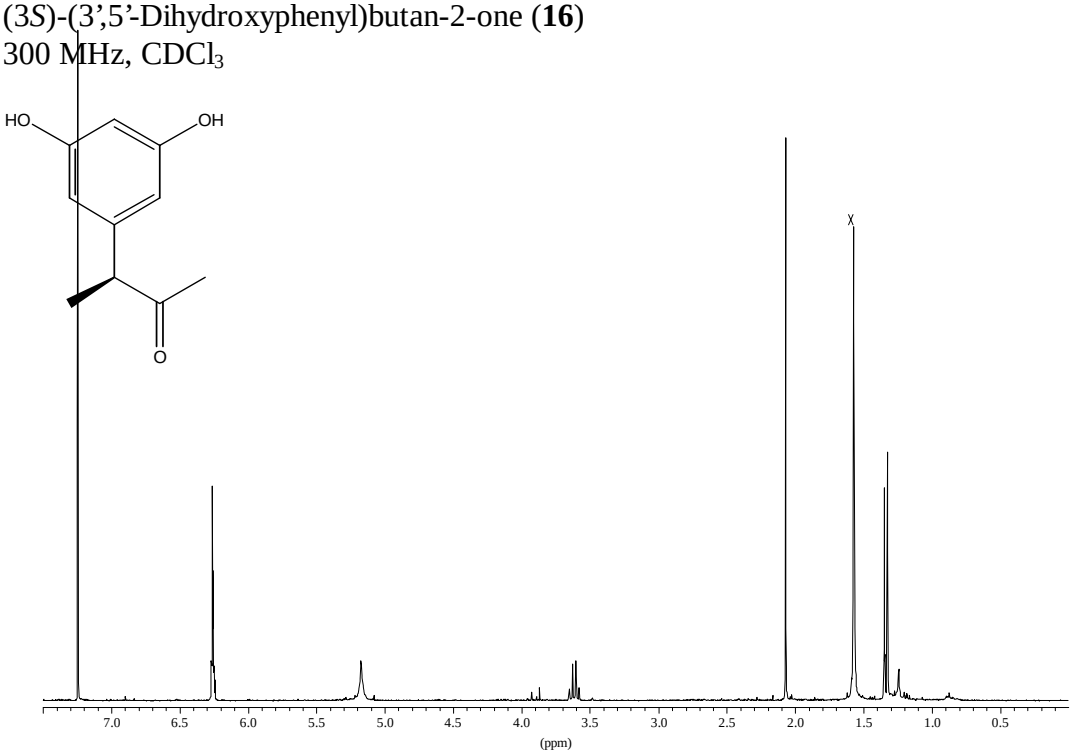
(3*S*,5*R*)-Dimethyl-dihydrofuran-2-one (**11**)
400 MHz, $CDCl_3$



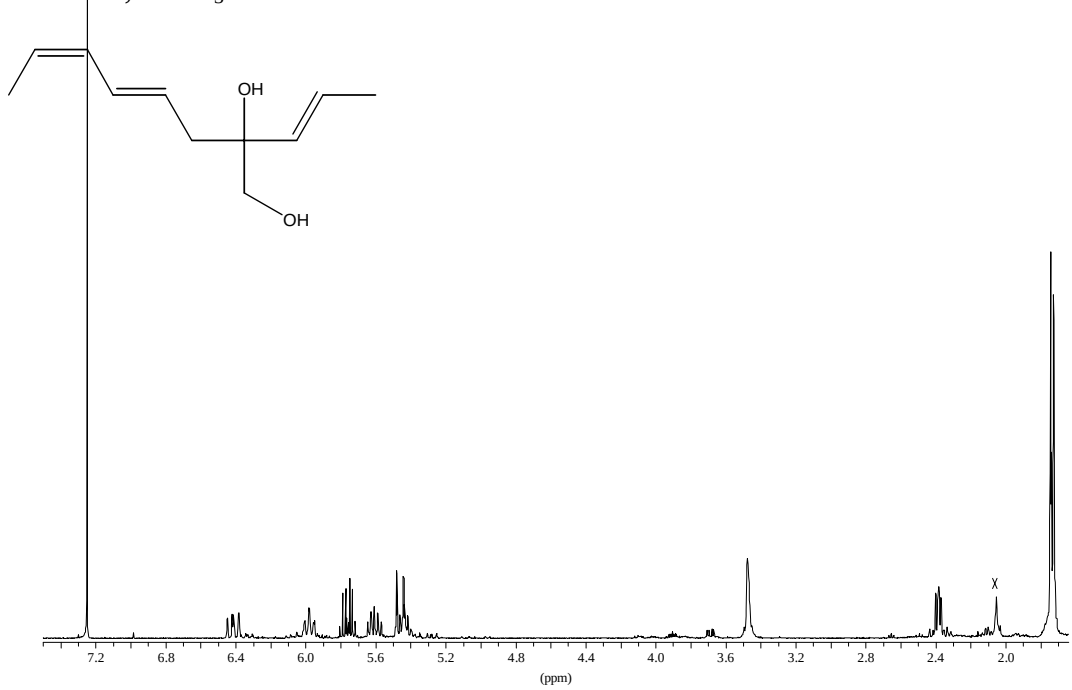
Tri-*O*-acetyl-glycerol (**12**)
300 MHz, $CDCl_3$



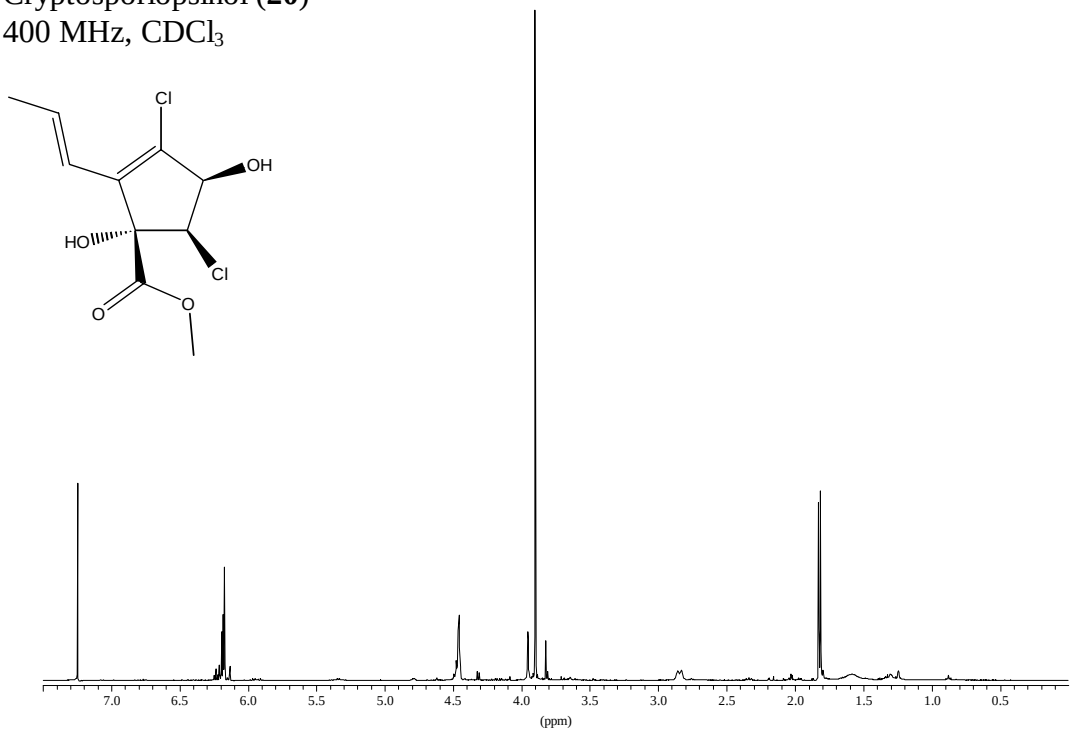
(3*S*)-(3',5'-Dihydroxyphenyl)butan-2-one (**16**)
300 MHz, $CDCl_3$



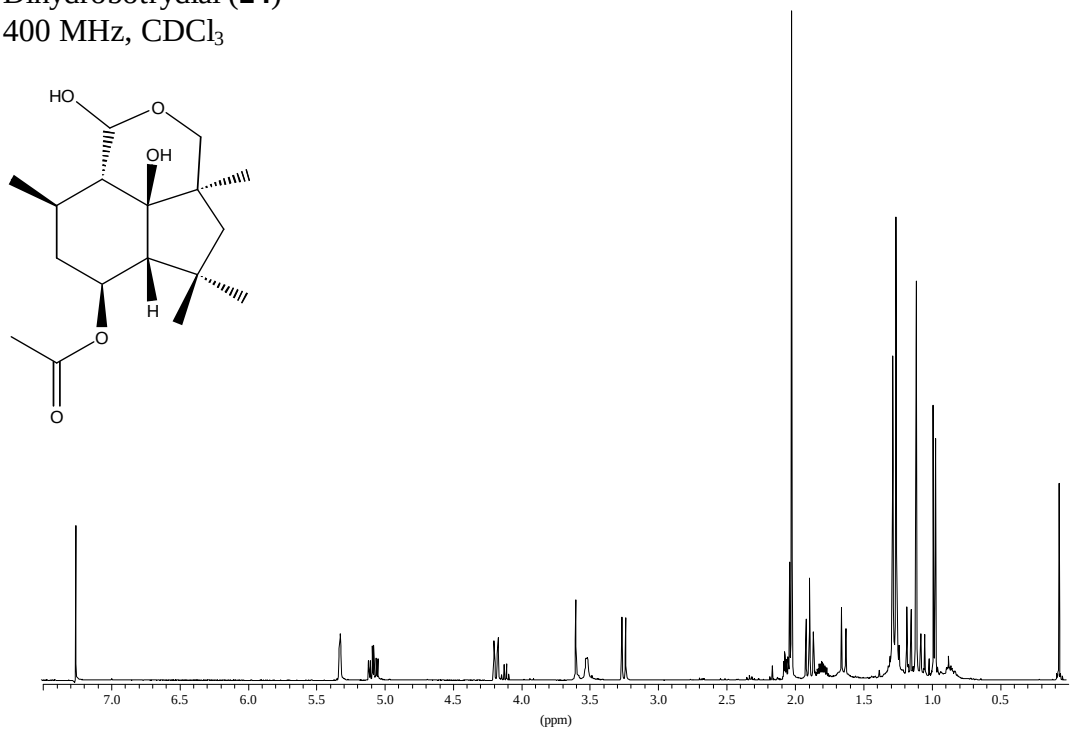
2-((1'*E*)-Propenyl)-octa-(4*E*,6*Z*)-diene-1,2-diol (**17**)
300 MHz, $CDCl_3$



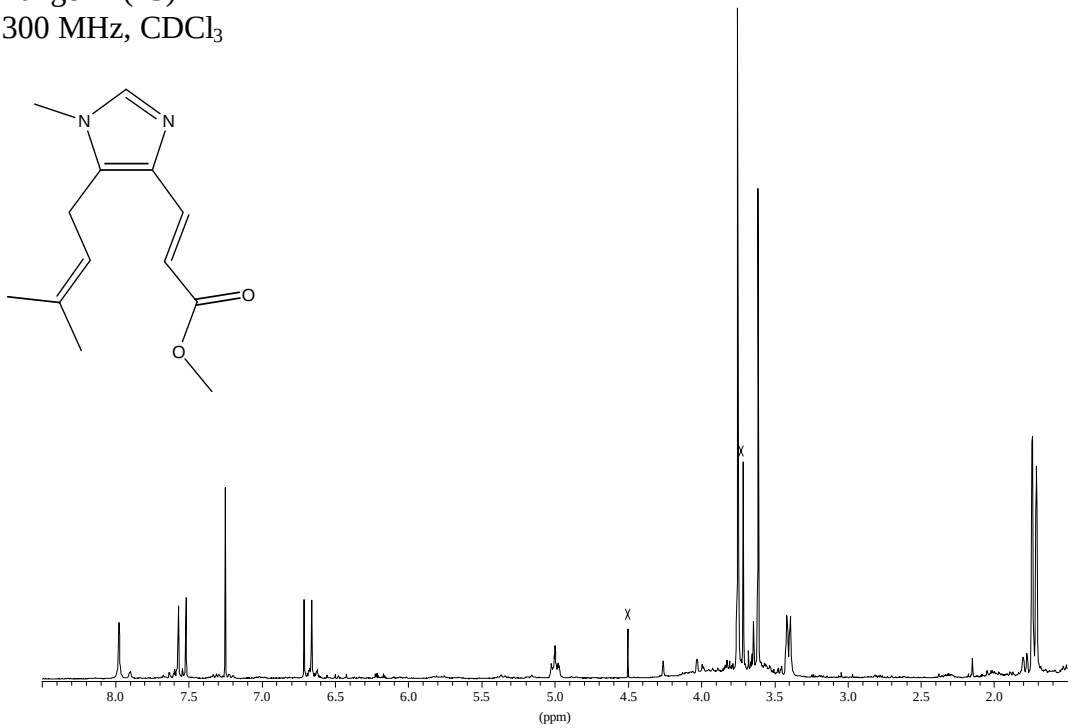
Cryptosporiopsinol (**20**)
400 MHz, $CDCl_3$



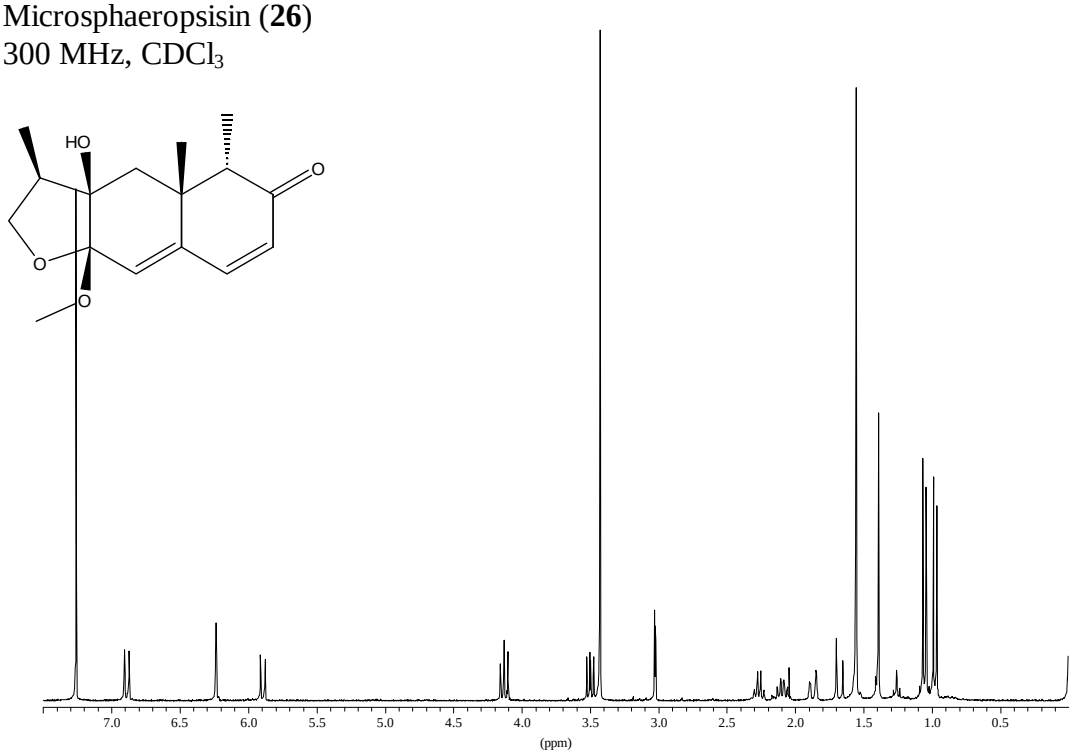
Dihydrobotrydial (**24**)
400 MHz, $CDCl_3$



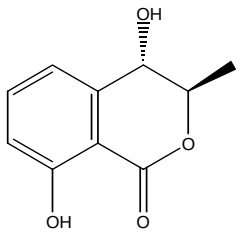
Fungerin (**25**)
300 MHz, $CDCl_3$



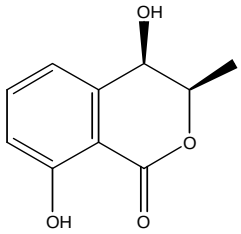
Microsphaeropsisin (**26**)
300 MHz, $CDCl_3$



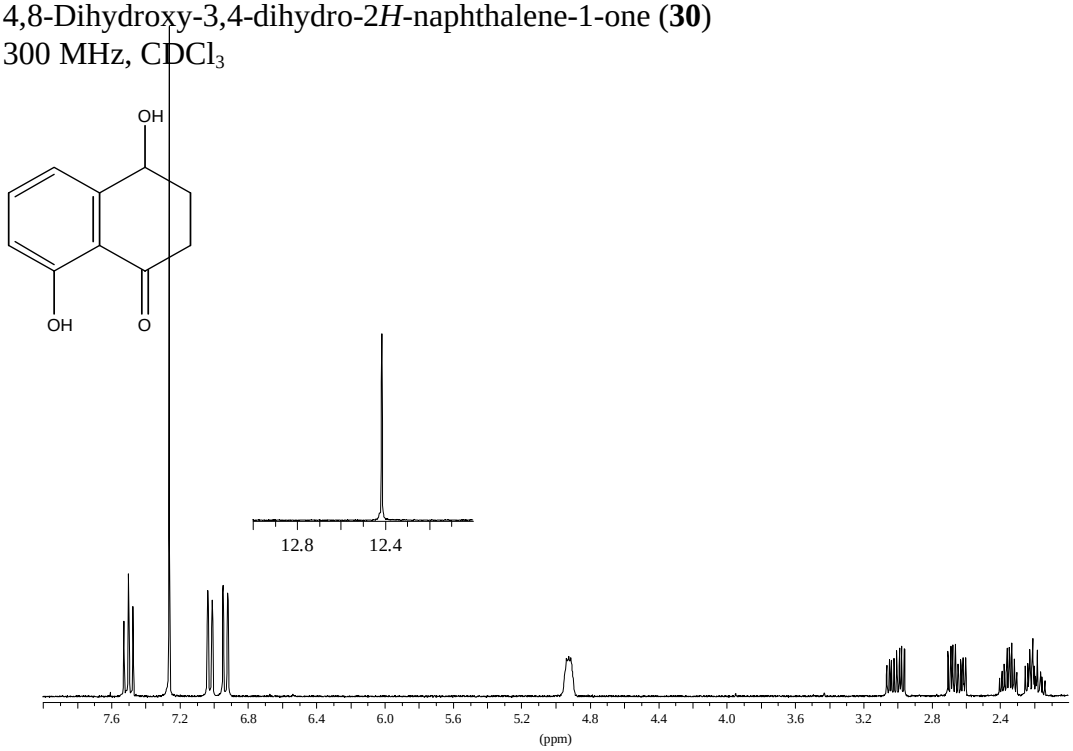
(3*R*,4*S*)-Hydroxymellein (**28**)
300 MHz, $CDCl_3$



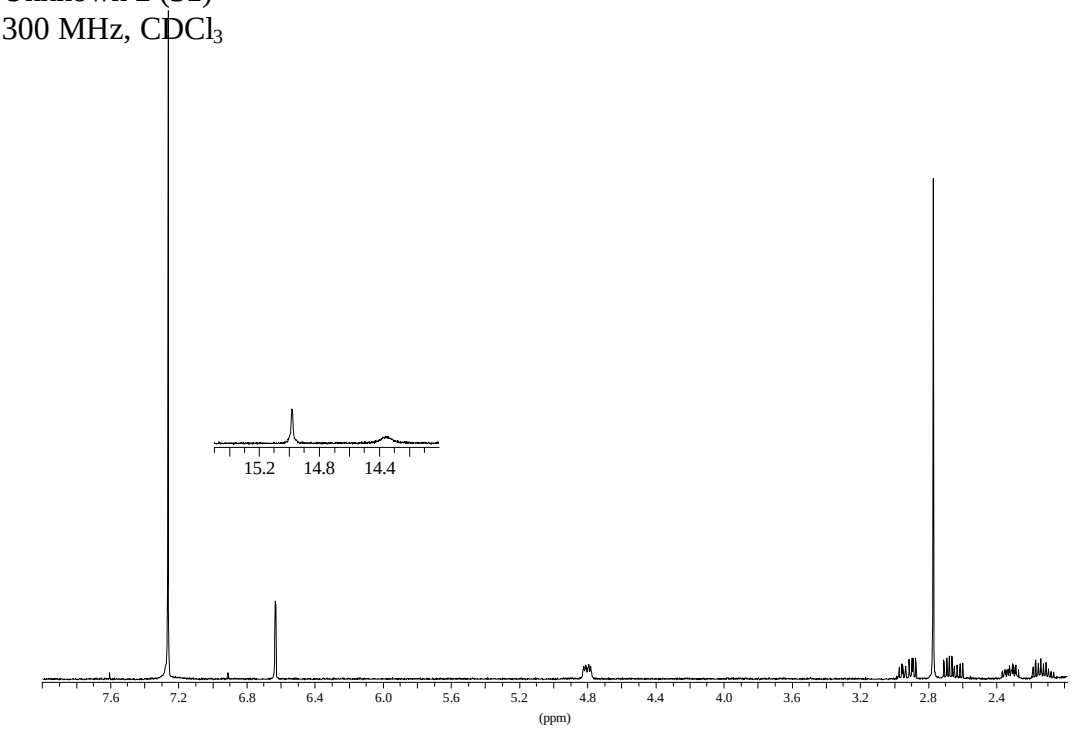
(3*R*,4*R*)-Hydroxymellein (**29**)
300 MHz, $CDCl_3$



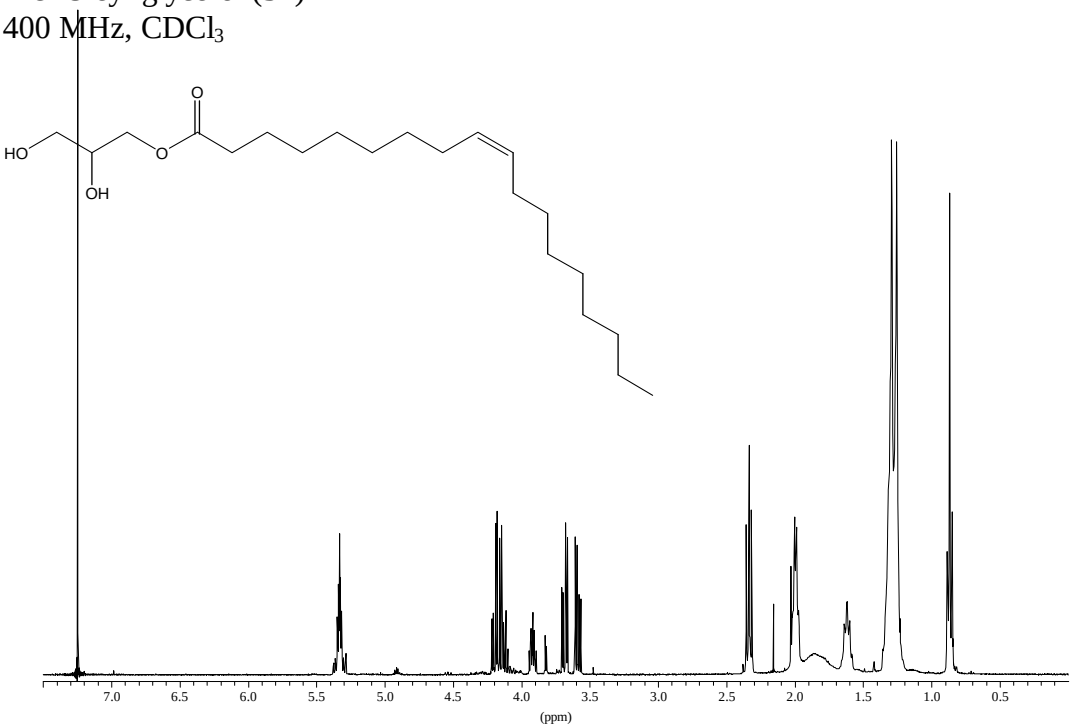
4,8-Dihydroxy-3,4-dihydro-2*H*-naphthalene-1-one (**30**)
300 MHz, $CDCl_3$



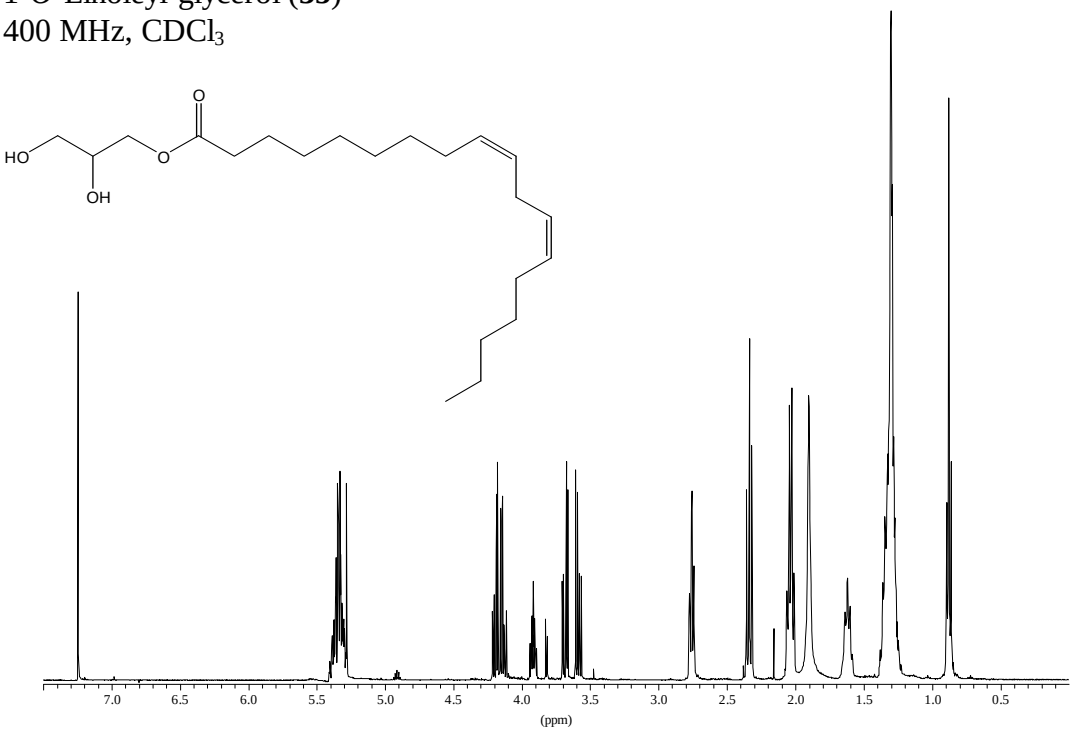
Unknown 2 (**31**)
300 MHz, $CDCl_3$



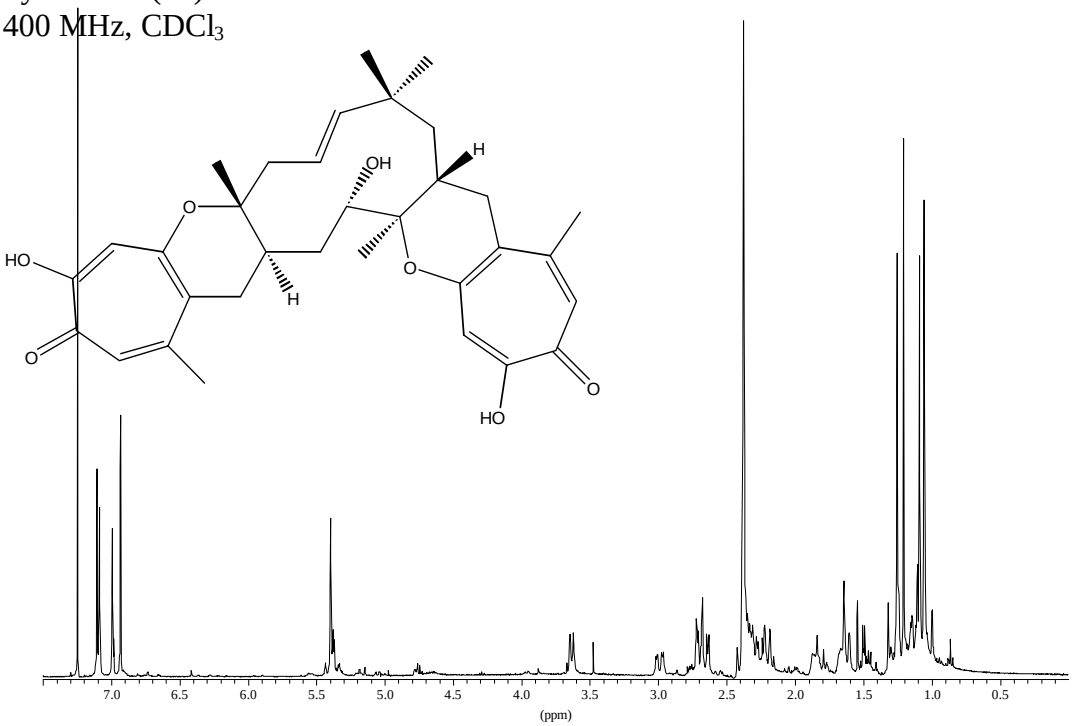
1-*O*-Oleyl-glycerol (**34**)
400 MHz, $CDCl_3$



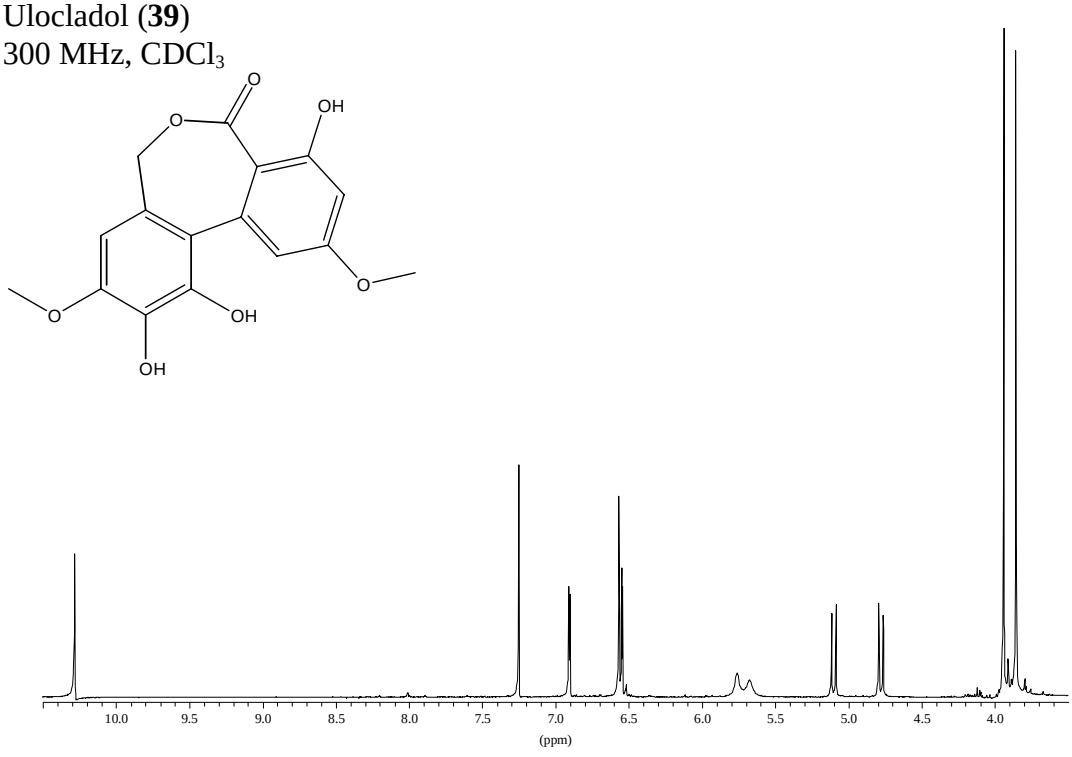
1-*O*-Linoleyl-glycerol (**35**)
400 MHz, $CDCl_3$



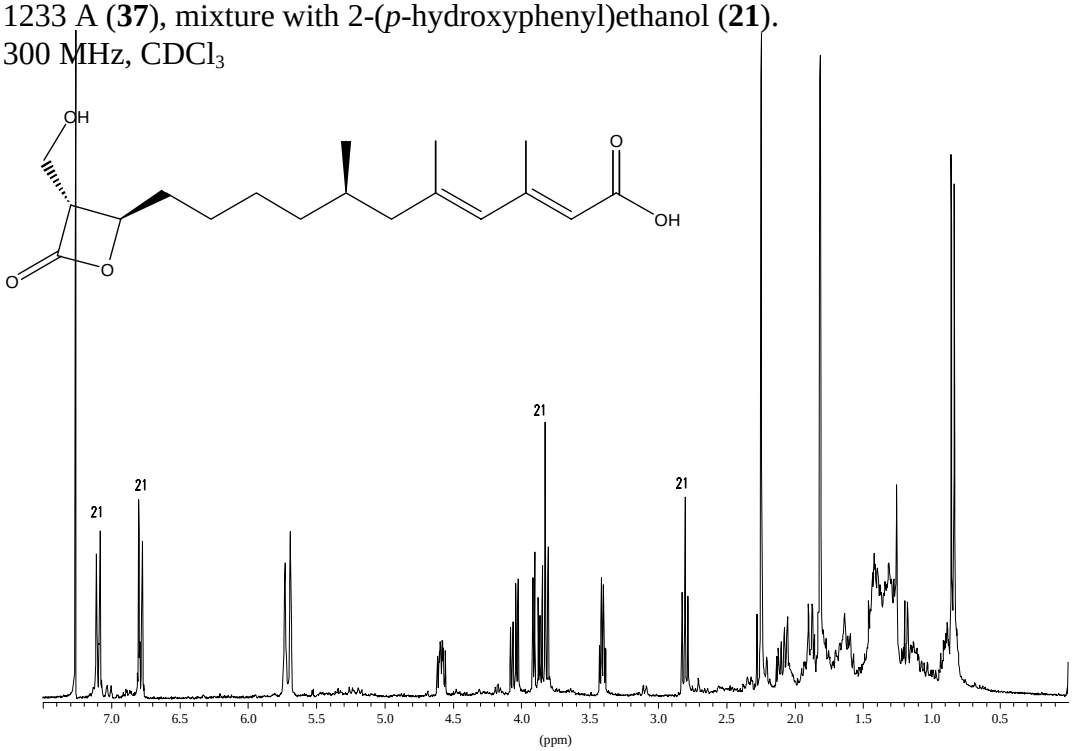
Pycnidione (**36**)
400 MHz, $CDCl_3$



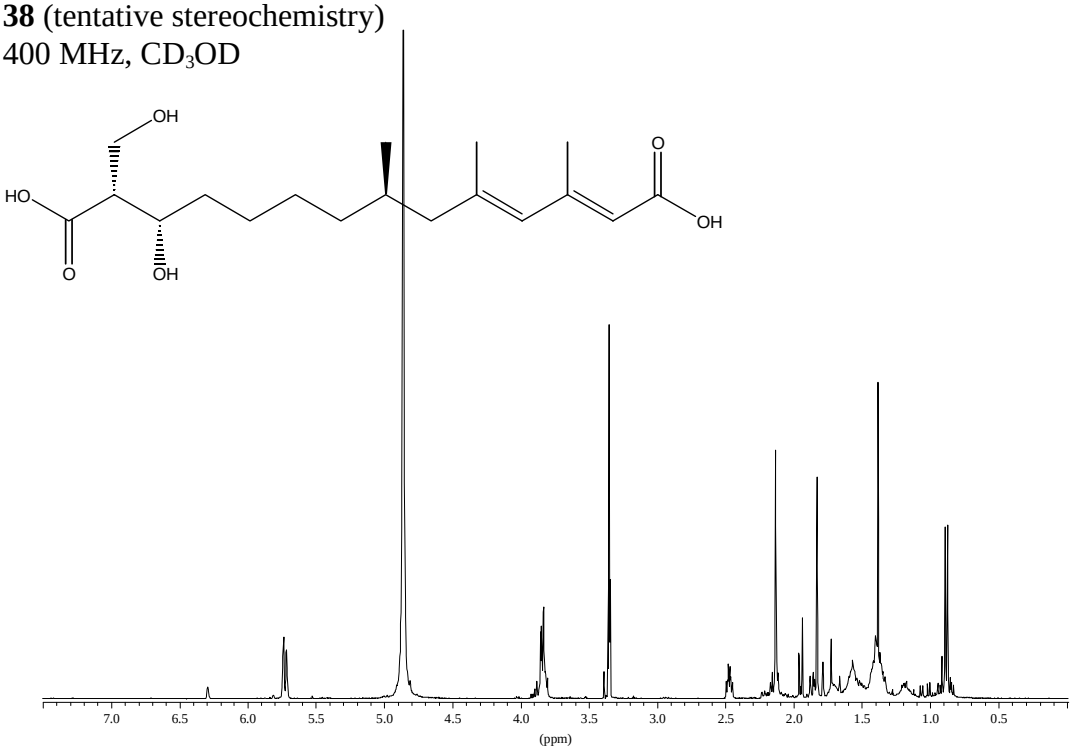
Ulocladol (**39**)
300 MHz, $CDCl_3$



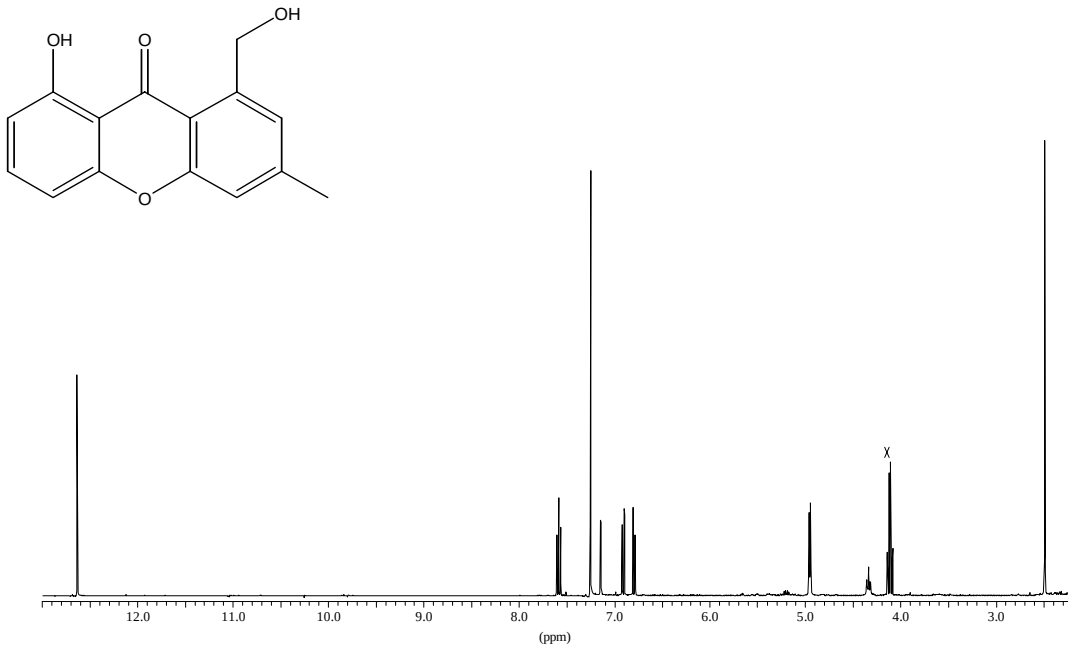
1233 A (**37**), mixture with 2-(*p*-hydroxyphenyl)ethanol (**21**).
300 MHz, $CDCl_3$



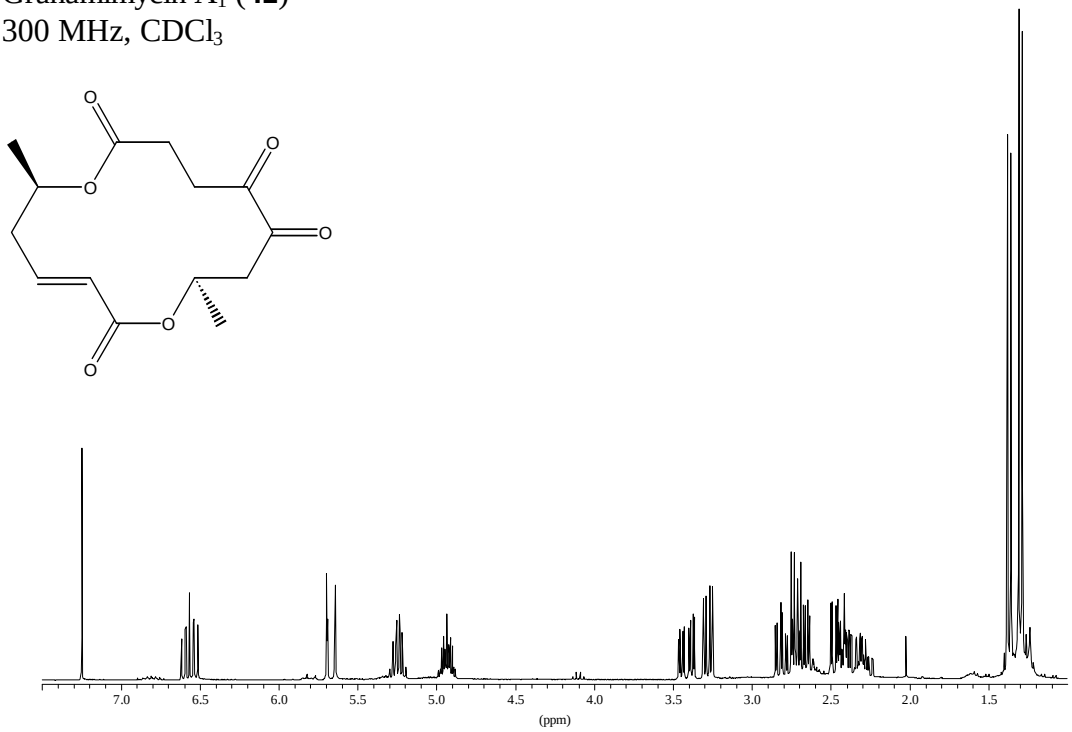
38 (tentative stereochemistry)
400 MHz, CD_3OD



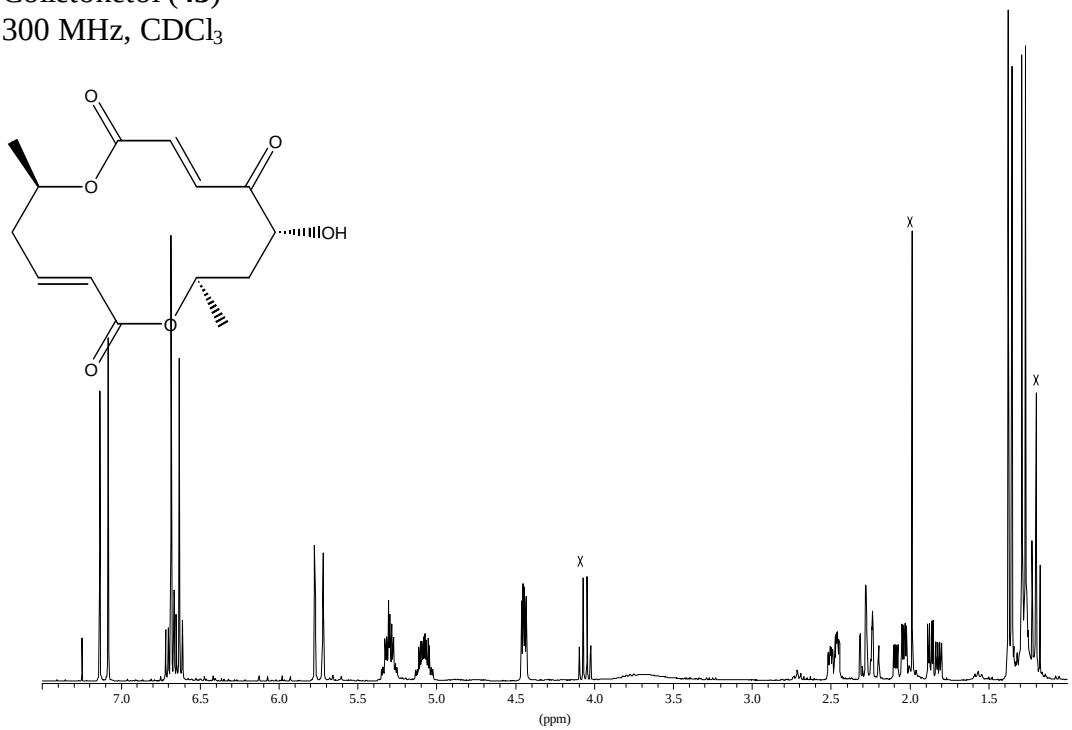
1-Hydroxy-6-methyl-8-hydroxymethylxanthone (**41**)
400 MHz, $CDCl_3$



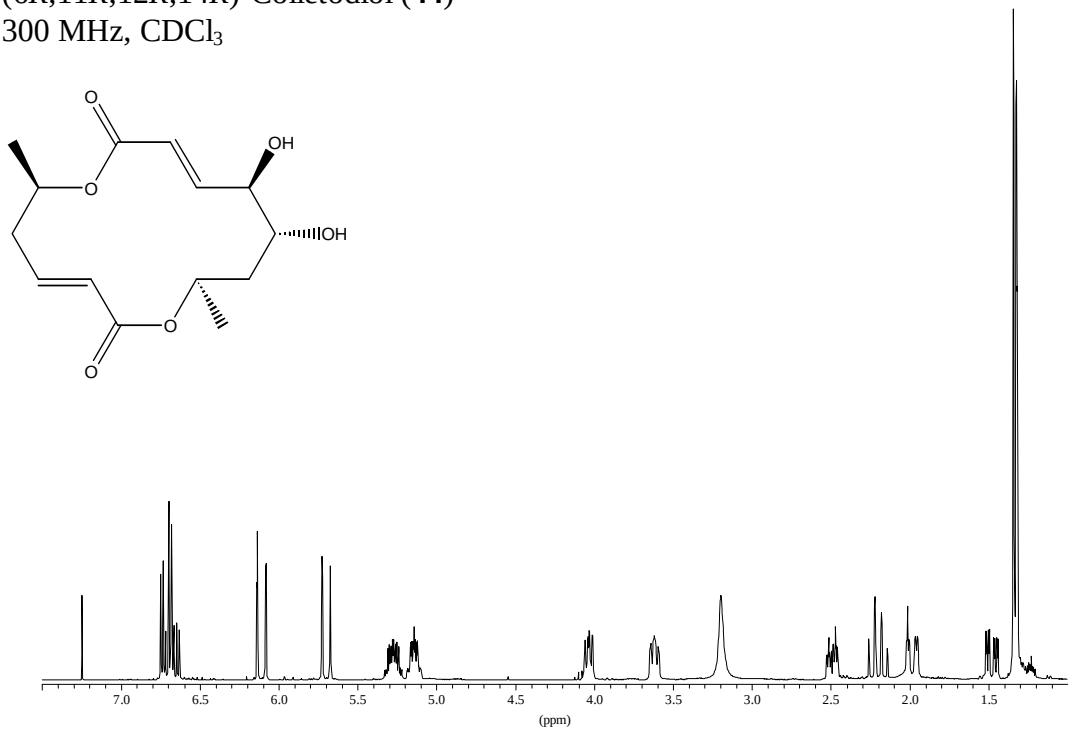
Grahamimycin A_1 (**42**)
300 MHz, $CDCl_3$



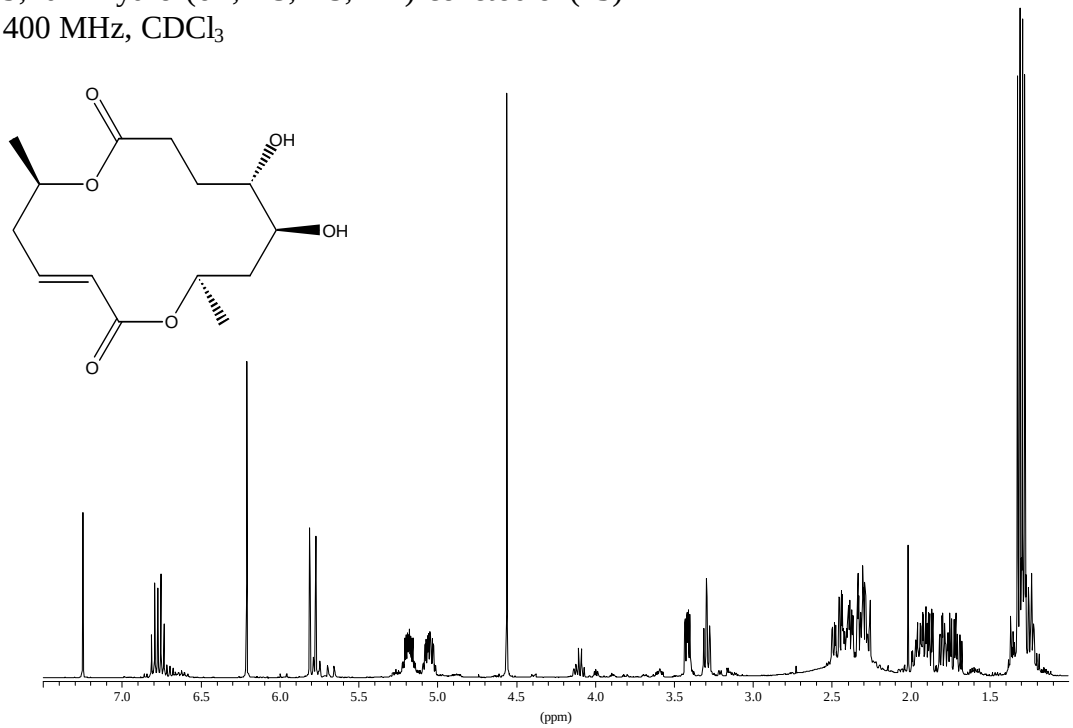
Colletoketol (**43**)
300 MHz, $CDCl_3$



(6*R*,11*R*,12*R*,14*R*)-Colletodiol (**44**)
300 MHz, $CDCl_3$



9,10-Dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**45**)
400 MHz, $CDCl_3$



9,10-Dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**46**)
400 MHz, $CDCl_3$

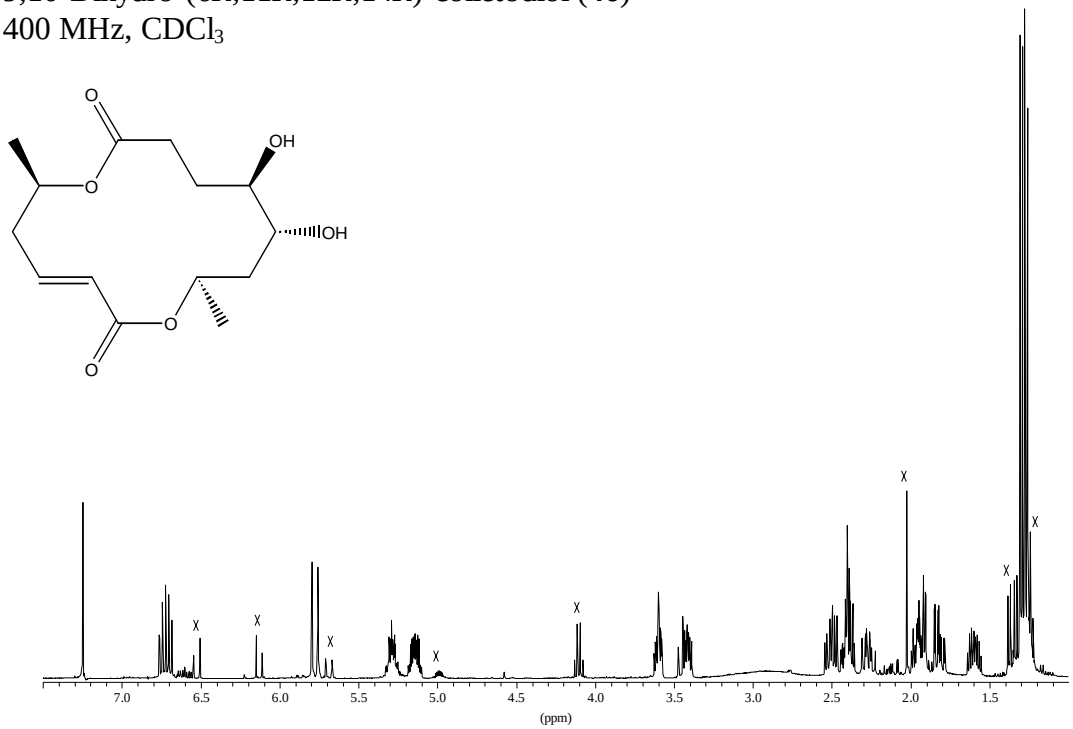


Table 12: Results of the agar diffusion assays with extracts of fungal cultures;
for abbreviations and details see p. 151

Fungal strain	Me-di-um	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
Aspergillus Ja23	B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bs	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bfl	-	-	0.2	-	-	-	-
Myxotrichum deflexa Ka12	B	-	0.3	0.2	-	0.2	-	0.5
	Bs	-	0.3	0.2	-	0.3	-	0.3
	Bfl*	-	0.1	-	0.4	0.2	0.3	P
Monascus K12	B	-	-	0.3	0.5	-	-	0.5
	Bs	-	-	0.5	1.0 T	-	0.2	0.3
	Bfl	-	-	1.0	1.0 T	-	-	0.5
Microascus K14	B*	-	0.1 T	0.2	1.3 T	-	-	-
	Bfl	0.2	-	0.3	-	-	-	-
	MS*	-	-	-	1.5 T	-	-	-
Ulocladium botrytis 193A4	B*	-	0.4 T	0.4 T	-	-	0.2 T	-
	MS	-	-	0.1	-	-	-	-
	Bfl	-	0.2	-	-	-	-	-
Monochaetia 193A6	B	-	-	-	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-
Monochaetia 193A18	B	-	0.2	-	-	-	-	-
	MS	-	-	0.1	-	-	-	-
	Bfl	-	0.1	0.1	-	-	-	-
Monochaetia 193A20	B	-	-	0.5 T	1.0 T	0.3	-	-
	MS*	-	0.3	0.8 T	1.4 T	0.4	0.4 T	-
	Bfl	-	-	0.3	0.5	-	0.2	-
Leptosphaeria 193A21	B	-	-	-	-	-	0.2 T	-
	MS	-	0.3 T	0.2 T	-	-	0.3	-
	Bfl	-	-	-	-	-	-	-
M. s. 193A23	B	-	-	-	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	-	0.1	-	-	-	-	-
Myrothecium rorideum 193A25	B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	MS	-	0.8 T	-	-	-	-	-
	Bfl	-	0.2	1.2 T	2.5 T	-	0.5	3.0 T
Phoma 193A26	B	-	0.4 T	0.2 T	0.1	0.1	0.4 T	-
	MS	-	-	-	-	-	-	-
	Bfl	-	0.1	0.2 T	0.1	0.1	0.4 T	-
M. s. 193A27	B*	-	0.8 T	0.7 T	1.1 T	0.4 T	0.6 T	0.1
	MS	-	0.5 T	0.5 T	0.4 T	0.1	0.3 T	-
	Bfl	-	0.2	-	-	-	-	-
Myrothecium gramineum 193A34	B	-	-	-	-	-	-	-
	MS	-	0.1	-	-	-	-	-
	Bfl	-	0.1 T	-	-	-	-	-

Table 12 continued; for abbreviations and details see p. 151

Fungal strain	Me-di-um	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
Phoma 193A35	B	-	0.1	0.2 T	-	-	0.1 T	-
	MS	-	-	0.1	-	-	-	-
	Bfl	-	-	-	-	-	-	-
Myrothecium verrucaria 193A45	B	-	-	0.1 T		-	-	-
	MS	0.1	0.9 T	0.6 T	0.1	0.1	0.2 T	-
	Bfl	-	0.3	-	-	-	-	-
M. s. 193A46	B	-	-	-	-	-	-	-
	MS	-	0.1	0.1	-	-	-	-
	Bfl	-	-	-	-	-	-	-
Aplosporella 193H1	B	0.2	-	2.0	0.3	-	0.5	0.3
	Bs	-	-	0.3	0.5	-	-	0.3
	Bfl	-	-	0.3	0.5	-	-	0.5
Coniothyrium 193H3	B	-	-	-	0.2	-	-	0.3
	Bs*	-	-	-	0.3	-	-	0.4
	Bfl	-	-	-	0.2	-	-	0.1
M. s. 193H5	B	-	0.2 T	0.2 T	-	-	-	0.5 T
	Bfl	-	0.2	0.1	-	-	-	-
M. s. 193H6	B	-	-	0.3	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	-	-	0.3	-	-	-	-
M. s. 193H7	B	-	0.1	0.4 T	-	-	0.2	0.1
	Bfl	-	-	0.3 T	0.1	-	-	-
M. s. 193H8	B	-	0.2 T	-	-	0.3	0.4	0.3
	Bfl	-	-	0.1	0.3	-	-	-
Gliocladium 193H15	B	-	-	0.2	0.3	0.2	0.3	0.2
	Bs*	0.4	0.3	1.5	0.9 T	0.5	0.7 T	0.1
	Bfl	-	-	0.2	-	-	-	0.5
Phoma 193H16	B	-	-	-	0.4	-	0.1	0.1
	Bs	-	-	0.2 T	0.5	-	0.1	0.1
	Bfl	-	0.2	0.2	0.4	-	0.1	0.1
Cladosporium 193H17	B	-	-	-	-	-	-	-
	Bfl	-	-	0.1	0.1	-	-	-
Phoma 193H19	B	-	-	-	0.2	-	-	-
	MS	-	-	-	-	-	-	0.1 T
	Bfl	-	-	-	0.3	-	-	-
M. s. 193H28	B	-	0.1	0.3	0.3	0.1	-	0.1
	Bfl	-	0.1	0.4	0.1	-	-	0.1
Sporothrix 193H29	B	-	-	-	0.3	-	-	-
	Bs	0.2	0.3	-	-	-	-	-
	Bfl	-	0.5	0.3 T	0.3	-	-	-
M. s. 193H31	B	-	-	-	0.2	-	0.2	-
	Bfl	-	-	0.1	-	-	-	-

Table 12 continued; for abbreviations and details see p. 151

Fungal strain	Me-di-um	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
Coniothyrium 193H34	B	0.4	0.4 T	0.5 T	0.5	-	0.1	0.3 T
	Bs	0.2	-	0.3 T	0.5	-	0.1	0.2 T
	Bfl	0.5	0.4 T	0.5 T	0.5	-	0.1	0.1 T
Coniothyrium 193H36	B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bs	-	-	-	0.3	-	-	-
	Bfl	-	-	-	0.3	-	-	-
Aplosporella 193H38	B	-	-	-	-	-	-	-
	Bs	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bfl	-	0.1 T	-	0.5	-	-	-
Coniothyrium 193H39	B*	-	-	1.0 T	0.5	0.8	1.0 T	1.0 T
	MS	-	-	0.3	0.3	-	0.3	-
	Bfl	-	-	0.2	-	-	-	P
Phoma 193H41	B	-	-	0.2	0.2	-	-	0.5
	Bs	-	-	-	0.2	-	-	0.5
	Bfl	-	-	-	0.2	-	-	0.5
Beauveria 193H43	B	0.2	0.2	-	-	-	-	-
	Bs	-	-	-	-	-	-	-
	Bfl	0.2	0.2	-	-	-	-	-
Coniothyrium 193H44	B*	-	0.1	0.3 T	0.5	0.5	-	0.2
	Bs	-	-	-	0.3	0.5	-	0.6
	Bfl	-	-	0.5 T	0.2	0.3	-	0.7
Phoma 193H45	B	-	-	-	-	-	-	-
	MS	-	0.1	0.2	-	-	-	0.2
	Bfl	-	0.1	0.3	-	-	-	0.2
Phoma 193H48	B	-	-	-	-	-	0.2	-
	MS	-	-	-	-	-	0.2	-
	Bfl	-	-	-	-	-	0.2	-
Coniothyrium 193H49	B	-	0.3 T	0.3	0.5	-	-	0.2
	Bs	-	0.2	0.2	0.5	-	0.2	0.5
	Bfl	-	-	0.2	0.3	-	-	0.3
Coniothyrium 193H52	B	-	-	0.3	0.2	-	-	-
	Bs	-	-	0.2	0.2	-	-	-
	Bfl	-	-	0.2	0.3	-	-	-
Phoma 193H57	B	-	-	0.3	-	-	-	-
	MS	-	-	0.3	0.5	-	-	-
	Bfl	-	-	0.2	0.2	-	-	-
M. s. 193H60	B*	-	0.1	0.2	0.5	-	0.3	0.5 T
	Bfl	-	-	-	-	-	-	-
M. s. 193H64	B	-	-	-	-	-	0.2	-
	Bfl	-	-	0.2	0.2	-	-	0.3
M. s. 193H75	B	-	-	-	-	-	0.3	-
	Bfl	-	-	0.2	0.2	0.1	-	P
Phoma 193H76	B	-	-	-	-	-	-	-
	MS	-	-	-	-	-	-	-

Table 12 continued; for abbreviations and details see p. 151

Fungal strain	Medium	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
Coniothyrium 193H77	B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bs	-	-	0.2 T	0.7	-	0.1	0.7 T
	Bfl	-	0.2 T	1.0 T	1.0	-	0.5	0.8 T
M. s. 193H79	B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bfl	-	-	0.2	0.2	0.1	-	-
M. s. 193H82	B	-	-	0.5	-	-	-	-
	Bfl	-	-	0.1	0.1	-	-	-
Ulocladium 193I2	B	-	0.6 T	-	-	-	-	-
	MS	-	0.4	-	-	-	-	-
	Bfl	-	0.2	-	-	-	-	-
M. s. 193I10	B	-	0.2	-	-	-	-	-
	MS	0.6 T	-	-	-	-	-	-
	Bfl	-	0.1	-	-	-	-	-
Phoma 193I27	B	-	0.2	-	0.4	-	-	0.1
	MS	-	-	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-
M. s. 193I28	B	0.5	-	-	-	-	-	-
	MS	-	0.2	0.2	-	-	-	-
	Bfl	-	-	-	-	-	-	-
Phoma 193I31	B	-	0.2	-	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	0.1	-	-	-	-	-	-
M. s. 193I35	B	-	0.2	-	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	-	0.4 T	-	-	-	-	-
M. s. 195I36	B*	-	0.3	0.4 T	1.2 T	-	0.3 T	0.1
	MS	-	-	-	-	-	-	-
	Bfl	-	0.3	-	-	-	-	-
Stachylidium 293K4	B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bs	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bfl	-	-	0.2	0.5	-	-	-
Sporormiella 293K5	B	-	-	-	0.2	-	-	0.2
	Bs	-	-	-	0.2	-	-	0.5
	Bfl	-	-	-	0.2	-	-	0.5
M. s. 293K6	B	-	-	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-
M. s. 293K7	B	-	0.1	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-
M. s. 293K8	B	-	-	-	0.3	-	-	-
	Bfl	-	0.2	0.3	0.3	0.3	-	-
M. s. 293K9	B*	0.5 T	2.8 T	3.0 T	1.9 T	0.1 T	0.9 T	0.1
	Bfl	0.3 T	1.4 T	0.4 T	0.5 T	-	0.2 T	-

Table 12 continued; for abbreviations and details see p. 151

Fungal strain	Me-di-um	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
M. s.	B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
H1-1	Bfl	-	-	0.3	0.1 T	-	-	0.2
M. s.	GPY	-	-	0.2	-	-	-	-
H-2	MS	-	-	0.2	-	-	-	-
Westerdykella	B	-	0.3 T	-	-	-	-	-
H3-1	MS	-	-	-	-	-	-	-
	Bfl	-	-	0.2	-	-	-	-
Gymnoascus	B	-	0.1	-	-	-	0.2 T	-
H3-4	MS	-	0.2 T	1.0	-	-	-	0.5
	Bfl	-	-	0.2	-	-	1.4	0.2
Phoma	B	-	-	0.2	0.3	-	-	0.3
H3-13	Bs	-	-	0.2	0.3	-	0.1	0.7
	Bfl	-	-	0.2	0.2	-	-	0.3
Phoma	B	-	-	-	-	-	-	-
H3-23	MS	-	-	0.3	-	-	-	-
	Bfl	-	0.1	0.2	-	-	-	-
Phoma	B	-	-	0.3	0.2	-	-	-
H4-12	Bs	-	-	-	0.5	-	-	-
	Bfl	-	-	-	0.2	-	-	-
Coniothyrium	B/Bs	-	-	0.2	-	-	-	-
H4-52	Bfl	-	-	0.2 T	0.1	-	-	-
Phoma	B	-	0.3 T	0.3	-	-	-	0.5
H4-71	MS	-	0.5 T	0.3	0.5	-	-	0.2
	Bfl	-	-	0.2	-	-	-	-
Phoma	B	-	0.2	-	0.2	-	-	0.5
H4-77	Bs*	-	0.3 T	-	0.4	-	-	0.6
	Bfl	-	0.2 T	-	0.2	-	-	0.3
Coniothyrium	B	-	0.2	-	-	-	0.2	-
H4-83	MS	-	-	0.3	-	-	-	-
	Bfl	-	0.2	0.1 T	0.4	-	0.2	-
Coniothyrium	B	0.3 T	-	-	-	-	0.5	0.2
H4-87	MS	-	-	0.2	-	-	-	-
	Bfl	-	-	0.2 T	0.2	-	-	0.1
Beauveria	B	-	-	-	-	-	-	-
H5-6	Bs	-	0.5 T	-	-	-	-	0.2
	Bfl	-	0.5 T	-	-	-	-	0.2
Drechslera	B*	-	0.1	2.0	1.0	0.3	0.5 T	0.1
H5-28	Bs	0.2	0.1	1.5	0.8	0.2	0.3 T	0.3
	Bfl	-	0.1	0.8	0.8	0.2	0.5 T	0.3

Table 12 continued; for abbreviations and details see p. 151

Fungal strain	Me-di-um	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
Micro-	B*	-	0.3 T	1.0	0.5	-	-	0.3
sphaeropsis	Bs	-	-	-	0.3	-	-	0.3
H5-50	Bfl	-	-	-	0.3	-	-	0.6
Phoma	B	-	-	1.0 T	-	-	-	-
H5-58	MS	-	0.3 T	0.3 T	-	-	-	0.2
	Bfl	-	-	0.2	-	-	-	0.3
Coniothyrium	B	-	0.2	-	-	-	0.2	-
H5-63	MS	-	-	0.5 T	-	-	-	0.2
	Bfl	-	0.5	-	-	-	0.2	-
Preussia	B*	-	0.6 T	0.5 T	0.5	-	-	P
H5-73	MS	-	0.5 T	0.3	0.2	-	-	-
	Bfl	-	-	0.3	0.3	-	-	-
Phoma	B	-	0.2	-	-	-	0.3	-
H5-75	MS	0.3	-	-	0.3	-	-	-
	Bfl	-	0.2	-	-	-	0.3	-
Phoma	B	0.1	-	-	0.3	-	-	-
H5-76	MS	0.3	-	0.2	0.3	-	-	-
	Bfl	-	-	0.1	-	-	-	0.1
Coniothyrium	B	-	-	0.3	-	-	0.2	-
H5-78	MS	-	-	-	-	-	0.2	0.5 T
	Bfl	-	-	-	-	-	0.2	-
Coniothyrium	B*	0.2	-	0.4 T	0.5	-	-	0.5 T
H5-80	Bs	-	-	0.2	0.4	-	-	0.3
	Bfl	-	-	-	0.5	-	-	0.1
Asteromyces	B	-	-	-	-	-	-	0.2
cruciatus	Bs	-	-	-	-	-	-	0.3
H5-81	Bfl*	0.2	0.2	0.2	-	-	-	0.5
Phoma	B	-	-	0.2	0.2	-	-	0.2
H5-83	Bs	-	-	0.3	0.5	-	-	0.4
	Bfl	-	-	0.2	0.2	-	-	0.2
Phoma	B	-	-	0.2 T	0.2	-	-	0.7
H5-85	Bs	-	-	0.3 T	0.3	-	-	0.5
	Bfl	-	-	-	0.5	-	-	0.5
Niessla/	B*	-	0.2 T	0.3	2.0	-	-	-
Monocillium	MS*	-	-	-	1.5	-	-	-
H6-9	Bfl	-	-	-	1.0	-	-	0.2
Gonatobotrys	B	0.1	0.1	-	-	-	-	-
H6-15	Bs	0.1	-	-	-	-	-	-
	Bfl	0.1	0.2	-	-	-	-	-
Phoma	B*	-	-	0.8	-	-	0.2 T	1.2
H6-51	MS	-	-	0.3	-	-	0.3	0.3
	Bfl	-	-	-	-	-	-	-

Table 12 continued; for abbreviations and details see p. 151

Fungal strain	Me-di-um	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
Microascus H6-57	B	-	0.2	2.5 T	1.7 T	-	1.0 T	-
	MS	-	0.2	0.5	-	-	-	-
	Bfl	-	0.2	-	-	-	-	-
Scopulariopsis candida H7-19	B*	-	-	2.5 T	2.0 T	-	1.3 T	-
	Bs	0.2	0.3 T	0.7 T	0.5 T	-	-	-
	Bfl	-	-	2.0 T	1.5 T	-	0.7 T	-
Phoma H7-58	B	-	-	-	0.2	-	-	0.3
	MS	-	-	-	0.5	-	-	-
	Bfl	-	-	-	0.3	-	-	0.2
Emericellopsis minima H7-65	B*	-	0.6 T	1.5 T	0.8 T	0.2	-	0.5
	Bs	-	0.3 T	1.2 T	0.5 T	0.2	-	0.3
	Bfl	-	0.3	0.5	-	0.2	-	0.1
Drechslera H8-31	B	0.1	-	0.8	0.3	-	-	0.3
	Bs	0.1	-	1.0	-	-	-	0.2
	Bfl	0.1	-	1.3	-	-	0.2	0.3
Coniothyrium H8-42	B*	-	-	0.3	0.3	-	-	0.8 T
	Bs	-	-	0.2	0.3	-	-	0.3
	Bfl	-	-	0.3	0.5	-	-	0.5
Beauveria H8-49	B	0.1	-	-	-	-	-	-
	Bs	0.2	-	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-
Coniothyrium H8-53	B	-	0.2	0.2	-	-	0.2	0.5
	MS	-	-	-	-	-	0.2	-
	Bfl	-	-	-	-	-	-	-
Phoma H8-54	B	-	-	0.5	0.7 T	-	-	0.3
	Bs*	-	-	1.5	0.2	-	0.2	0.3
	Bfl	-	-	-	0.2	-	-	0.5
Coniothyrium H8-57	B*	-	0.3 T	1.0	0.5	-	-	0.3
	Bs	-	0.3 T	0.5	0.3	-	-	1.0
	Bfl	-	0.1	0.7	0.3	-	-	0.7
M. s. 195-2 (I)	B	0.9 T	0.6 T	-	1.0 T	-	1.0 T	-
	MS	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bfl	0.3	0.3 T	-	0.6 T	-	0.4 T	-
M. s. 195-8 (Y)	B	0.1	0.2	0.1	-	-	-	0.1
	MS	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	Bfl	-	-	-	-	-	-	-
Curvularia 195-9 (Y)	Bs	-	0.1	-	-	-	-	-
	MS	-	0.1	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-
Phoma 195-10 (Y)	B	-	-	0.2	-	-	-	-
	MS	-	-	0.3	-	-	-	-
	Bfl	-	-	-	-	-	-	-

Table 12 continued; for abbreviations and details see p. 151

Fungal strain	Medium	Inhibition (cm) against test organism						
		E. c.	***B. m.***	***M. v.***	***E. r.***	***F. o.***	***M. m.***	***C. f.***
Alternaria 195-15 (I)	B	-	-	-	-	-	-	-
	MS	0.2	-	0.5	0.5	-	-	-
	Bfl	0.2	-	-	-	-	-	-
Ulocladium 195-18 (Y)	B	-	-	-	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-
Scoleco-basidium constrictum 195-19 (E)	Bs	-	-	-	-	-	-	-
	MS	-	-	-	-	-	-	0.1
	Bfl	-	-	-	-	-	-	-
Phoma 195-22 (I)	B	-	-	-	-	-	-	-
	MS	-	-	0.2	-	-	-	-
	Bfl	-	0.3	-	-	-	-	-
Varicosporina ramulosa 195-31 (L)	B	-	-	-	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	-	-	-	0.2 T	-	-	-
M. s. 195-32 (I)	Bs	-	1.0 T	-	-	-	-	-
	MS	-	1.1 T	-	-	-	-	-
	Bfl	-	0.3 T	-	-	-	-	-
Chryso-sporium 195-38 (L)	B	-	-	0.2	-	-	-	-
	MS	-	-	0.2	-	-	-	-
	Bfl	-	-	-	-	-	-	-
M. s. 195-39 (I)	Bs	-	1.4 T	-	-	-	-	-
	MS	-	1.0 T	-	-	-	-	-
	Bfl	-	1.1 T	-	-	-	-	-
Phialophoro-phoma litoralis 195-40 (T)	B*	-	-	-	-	-	-	-
	MS*	-	0.4 T	-	-	-	-	0.3
	Bfl	-	-	0.2	-	-	-	-
Drechslera 195-42 (Y)	B	-	-	-	-	-	-	-
	MS	-	-	-	0.3	-	0.9 T	-
	Bfl	0.2	-	-	-	-	-	-
Leptosphaeria 195-43 (Y)	B							
	MS*	-	-	1.4 T	1.5 T	-	0.5 T	0.5
	Bfl	-	-	0.2	-	-	-	-
M. s. 195-51 (X)	B	-	0.5 T	-	-	-	-	-
	MS	-	0.5 T	-	-	-	0.1	-
	Bfl	-	0.3 T	-	-	-	-	-
Scoleco-basidium variabile 195-54 (Y)	B	-	-	-	-	-	-	-
	MS	-	-	-	-	-	-	-
	Bfl	-	-	-	-	-	-	-

Table 12: Results of the agar diffusion assays with extracts of fungal cultures: abbreviations and details.

Extracts were prepared, and agar diffusion assays were performed as described in the materials and methods section. Applied amounts were ~250 µg extract per test disk. Inhibition zones (cm) were measured from the edge of the filter disks.

Fungal strain:
Genus, and if determined species name of fungal strain tested, M. s. = Mycelia sterilia. The code marks the sponge or algal sample from which the fungal isolate was obtained, and the no. of the strain; e.g. 193H77 = fungal isolate no. 77 from the sponge sample CT 193 H; H5-50 = fungal isolate no. 50 from the sponge sample H-5. Strain Ja23 was isolated from sample CT 912 J, strains Ka12 and K14 were isolated from sample CT 912 K. For strains from Tenerife, the origin is indicated in brackets; e.g. 195-31 (L) = fungal isolate no. 31 from sample CT 195 L, for origin of samples see 3.1.

Medium: B = solid biomalt medium; Bs = solid biomalt medium with ASW; MS = solid malt extract soymeal medium with ASW; Bfl = liquid biomalt medium with ASW; GPY = glucose peptone yeast extract medium; for media compositions see materials and methods section.

Some fungal cultures were additionally extracted with *n*-BuOH. Both the EtOAc and the *n*-BuOH extract were also tested for inhibition of tyrosine kinase and HIV-1 reverse transcriptase, for cytotoxicity towards KB cells and inhibition of *Mycobacterium tuberculosis*. They are marked with an asterics (*) after the abbreviation of the medium. If two media are marked, a 1:1 mixture of both resulting extracts was tested. For results of the active extracts see 4.2.1.2.

Test organisms: *E. c. Escherichia coli, B. m. Bacillus megaterium, M. v. Microbotryum violaceum, E. r. Eurotium repens, F. o. Fusarium oxysporum, M. m. Mycotypha microspora, C. f. Chlorella fusca*.

T: complete inhibition, no growth in the indicated zone.
P: inhibition only on the test disk itself
- no inhibition observed
n.t. not tested