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GRISEOFULVIN AND OTHER BIOLOGICALLY ACTIVE, HALOGEN CONTAINING COMPOUNDS FROM FUNGI

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ABSTRACT: Fermentation of produced strains, purification, isolation and biosynthesis of griseofulvin are described. Metabolites similar to griseofulvin (precursors, side products of biosynthesis, *etc.*) are also discussed. Most important fungal metabolites containing halogen atoms are included in the second part of the review. In brief, metabolites are included that were discovered and isolated in 1995 and later. Pharmacological properties are only described when referred to in the original papers.

INTRODUCTION

Chlorine and bromine forming various compounds are important components of the environment and are among the most common biogenic elements on the Earth. They are thus present in all living organisms, as well as in both inorganic and organic compounds. Compounds containing organic chlorine containing and to a lesser extent bromine are also synthesized by fungi. These often include unusual compounds with fascinating properties including biologically active compounds, antibiotics in the first place. Griseofulvin, a chlorine-containing antibiotic produced commercially, is one of the most important examples.

First Part - Griseofulvin

In 1939, the isolation of griseofulvin from mycelia of *Penicillium griseofulvum* was reported [1]. This compound has the empirical formula $C_{17}H_{17}ClO_6$ and Grove *et al.* [2] determined its structure **1** in 1952. Fermentation conditions for griseofulvin production have appeared infrequently in the scientific literature, because this substance is of great economic importance. It is one of the few antifungal compounds that are

synthesized by fungi themselves and are produced commercially. Table **1** lists numerous organisms that have been shown to produce griseofulvin. The fermentation media and culture conditions for most of the producing organisms appear to be quite similar.

Chemical synthesis of griseofulvin is economically not feasible, since a number of intermediate steps are involved in the final product formation.

Although a number of synthetic routes to the spiro system have been reported, the methods generally fall into two broad categories, *viz.*, the building up of a spiro ring C onto the A-B ring system and/or the forging together of ring A and C components to develop the oxygen containing ring B. The latter approach is exemplified in the elegant synthesis of griseofulvin developed [3,4]. This synthesis is based on the double Michael addition of vinyl ethynyl ketones to active methylene compounds [5].

The synthesis of griseofulvin by the Merck group represents the second approach to the antifungal antibiotic [6].

Table 1. List of griseofulvin producing microorganisms.

Name of microorganism	Literature
Penicillium griseofulvum	[1]
Penicillium janczewskii	[7]
Penicillium nigricans	[8]
Penicillium urticae	[8]
Penicillium raistrickii	[8]
Penicillium albidum	[9]
Penicillium raciborskii	[9]
Penicillium melinii	[9]
Penicillium patulum	[9]
Aspergillus versicolor	[10]
Carpenteles brefeldianum	[11]
Khauskia oryzae	[12]
Nigrospora musae	[13]
Nigrospora splaerica	[13]
Streptomyces albolongus	[14]

Fermentation

Typically, high yield industrial fermentations in submerged cultures have employed complex media with sugar, inorganic salts and corn steep liquor as the nitrogen source.

The optimum chloride concentration for optimal griseofulvin production was found to be 0.001-0.05 % KCl [15]. Fe^{2+} shows a specific effect on the formation of mycelianamide, a second compound, which is considered as interfering substance. By a proper strain selection and by keeping $FeSO_4$ content in the media at 0.1% the formation of mycelianamide was eliminated [16].

When the nitrogen concentration was less than 0.04 % or more than 0.4 %, production of griseofulvin was inhibited. The retarding effect of the high nitrogen concentration levels was explained by a specific inhibition of NH_4^+ on oxaloacetate formation [17].

Large-scale production of griseofulvin was carried out using 30000 l of culture medium containing (g/l) corn seed (5-15), corn extract (5-8), lactose (10-14), glucose (7-15), $CaCO_3$ (10-14), KCl (1-2), KH_2PO_4 (6-10), $MgSO_4$ (0.05-0.1), urea (1-2), and hydrogenated sunflower oil (2-10). The medium was inoculated with 5-10 % precultivated inoculum of *Penicillium* and aerobically fermented for 300-350 hours at pH 5.8-7.0 and 26-30 °C. The maximum production of griseofulvin achieved was 1200 μg/ml without the formation of intermediates [18].

In 450 liter batch fermentation medium [9] containing (in g/l) corn steep liquor (2), lactose (70), $CaCO_3$ (8), K_2HPO_4 (4) and KCl (1), the maximum production by the *P. patulum* mutant was about 1500 μg/ml. A significant improvement in the fermentation technology of griseofulvin production was realized with the introduction of the fed batch process described by Hockenhull [19]. A yield of 6000 μg/ml of griseofulvin was obtained after a 220-h cultivation.

When the corn steep liquor was increased to ~5 g/l, the yield of griseofulvin after a 260-hour cultivation increased to 11000 μg/ml [20].

Partial replacement of corn steep liquor with $(NH_4)_2SO_4$ yielded 14000 μg/ml in 3,786 liters fermentor [21]. The precise conditions of the cultivation are described bellow (g/l): corn steep liquor (3.5), $(NH_4)_2SO_4$ (0.5), KH_2PO_4 (4), KCl (1), $CaCO_3$ (4), H_2SO_4 (0.125), Mobilpar S (0.275, white mineral oil (0.275). Further, the inoculation was performed

with 10 % of inoculum. The cultivation temperature was 25 °C and the rotation impeller speed was about 3.33 Hz. Between 0-5 hours of cultivation, the feeding rate was kept at 2.26 m^3/min. In the interval of 5 to 10 hour, the feeding rate was maintained at 4.8 m^3/min and this value could be conserved after 10 hours. The additional feeding of a solution containing 50 % of glucose was used to maintain pH in the range 6.8-7.2.

The production of griseofulvin was improved by a careful control of the growth conditions of *P. urticae*. The medium containing (g/l) glucose (60), corn steep liquor (1.5), KH_2PO_4 (4), KCl (1) and $CaCO_3$ (8) was incubated for 13 days and production was up to 14000 μg/ml [22].

The use of hydrocarbons as carbon and energy sources for fermentation production of griseofulvin was described in [23] and the yield of 2550 μg/ml after 96 hours was obtained.

Further, the production of griseofulvin by a 23-day surface cultivation of *P. urticae* yielded about 9000 μg/ml [24].

Similarly to the biosynthesis of tetracyclines, even here the bromo analog was produced by simply substituting KBr for KCl in the medium [25]. Deuterated griseofulvin has been obtained by cultivation of *P. janczewskii* in culture medium with D_2O.

Methods to improve the production are kept secret and those described in respective patents (see below) are also quite successfully camouflaged. In the fifties and sixties, classical methods based on mutagenesis with UV light or chemical mutagens such as ethyl methane sulfonate (with radioactive sulfur isotopes) and also N-nitrosomethylurea and N-nitroso-N-methylbiuret were mainly used. For example, the sulfur isotope procedure on the 18th day provided a culture that was reported to yield ~146 % more than its parent strain [26]. Kiuchi *et al.* [27] have reported the transformation of *Penicillium urticae* with plasmids containing the hygromycin-B resistance. The transformation system used plasmid pDH25MC and its derivatives containing fragments of the *Penicillium urticae* genome. Tandem repeated integration and random integration of vector DNA were observed. Although *Penicillium urticae* was able to grow in the presence of 0.2 kg/m^3 hygromycin-B, transformants were resistant to more than 5.0 kg/m^3 hygromycin-B.

The strain of *P. patulum* was induced by exposure to $Na_2{}^{35}SO_4$ (~1 mg/l) and after 13 days the best strain produced 2964 μg/ml of

griseofulvin, compared to the production of 1640 μg/ml of the parent strain [26]. Another strain of *P. patulum* showed a high tolerance to Cl^- anions and increased production of griseofulvin with increasing Cl^- concentration that reached a maximum at 3 % of Cl^- in the cultivation broth [28].

Purification and/or Isolation

The mycelium of different strains was extracted three times by homogenization in the presence of CH_2Cl_2. The solvent extracts were concentrated to 10 % of the original volume and cooled to 4 °C to remove some impurities. After decolorization with charcoal, the crystallization was accomplished by evaporation at 50 °C to 6.6 % of the original volume and cooling to 0 °C. The recovery was 95 % pure griseofulvin [29].

Biosynthesis

The carbon skeleton of the griseofulvin has been shown by Birch [30,31] to arise from 7 acetates. Radioactive (1-^{14}C)-acetates were incorporated into griseofulvin by *P. griseofulvum*. The incorporation of 2-^{13}C acetate by *P. urticae* was proved by ^{13}C NMR and so identical carbon sites were reported [32]. Simpson and Holker [33] have similarly shown alternating site incorporation of 1-^{13}C-acetates and/or 2-^{13}C acetates into griseofulvin by *P. patulum*. Incorporation of doubly labeled acetate ($^{13}CH_3C^{18}O_2H$) into griseofulvin by *P. griseofulvum* revealed that all of its oxygen is derived from the acetate. The location of labeling was determined by pulse sequence in ^{13}C NMR to detect ^{18}O induced isotope shifts [34]. Studies of Sato *et al.*, [35,36] using 2-^{2}H- and 2-^{3}H-acetates incorporation by *P. urticae,* fully validate the earlier experiments with acetates labeled by isotopes ^{13}C and ^{14}C.

Intermediates isolated from *P. patulum* were reported by Rhodes *et al.* [37] and a biosynthetic scheme for griseophenones (**2, 3**) was postulated. Many additional papers in which speculative biosynthetic pathways were proposed were published [11,30,33,38-40]. Final biosynthetic steps including methylation or rather a series of methylations [32,33,40,41] and reductions [31,42] were also investigated.

The biosynthetic study of griseofulvin by *Penicillium urticae* and microbial transformation of (-)- and (+)-dehydrogriseofulvin (**4**) and their derivatives by *Streptomyces cinereocrocatus* followed by NMR

spectroscopy showed that in the reduction of (-)-dehydrogriseofulvin into (+)-griseofulvin by a partially purified enzyme system of *S. cinereocrocatus*, the origin of the 6'-α-hydrogen of (+)-griseofulvin was a hydride ion donated by pro-4*R*-hydrogen of NADPH [43].

Determination

In a recent review, Dasu *et al.* [44] described determination of griseofulvin by means of different analytical methods. Although published as recently as in 2000, the review unfortunately includes references most typically from the fifties and sixties of the last century. Spectrophotometric methods, (*e.g.* UV determination [45]), spectrofluorimetric methods and microbiological assay using *Microsporum gypseum* [46] were developed. A major part of the paper is devoted to chromatographic methods, both to paper and thin-layer chromatography, and to more modern gas chromatography and HPLC. We concluded that just the latter method, *i.e.* HPLC, is of a real practical significance. For the identification of griseofulvin, standard RP-HPLC with acetonitrile-water as mobile phase with a UV detector or fluorescence detector seems to be the most useful. In addition to the above-mentioned RP-HPLC, other examples of analytical methods, such as determination of griseofulvin by LC-MS, will be described here.

The non-volatile metabolites from several different molds were separated by HPLC, and then derivatized and analyzed by GC-MS [47].

New LC-MS and LC-MS-MS methods for the simultaneous determination of mycophenolic acid, griseofulvin, roquefortine C, chaetoglobosin B, verruculogen and penitrem A, and other *Penicillium* derived mycotoxins in food and feed samples were described. The methodologies involve sample extraction with acetonitrile-water, defatting with hexane and quantification using LC-MS with atmospheric pressure chemical ionization or LC-MS-MS. Detector responses for all mycotoxins were found to be linear over the range 10-1000 ng of mycotoxin/g of extracted food mixture material. The limits of detection for the mycotoxins using MS and MS-MS were 70 and 10 ng/g for griseofulvin, respectively [48].

Biological Activity

A review covering most aspects of the mechanism of action of griseofulvin was published in 1974 [49]. Gull and Trinci [50] reported

that griseofulvin produced multinucleation. This drug is fungistatic *in vitro* for various species of dermatophytes, for example *Microsporum* (see above), *Epidermophyton* or *Trichophyton*. The drug has no effect on other fungi including yeast, and actinomycetes or *Nocardia*. It kills young and actively metabolizing cells and inhibits the growth of older and dormant cells.

Griseofulvin was the first available oral agent for the treatment of dermatophytoses and has now been used for more than forty years [51].

Griseofulvin is fungistatic, the exact mechanism by which it inhibits the growth of dermatophytes being still doubtful. However, several mechanisms have been proposed: inhibition of fungal cell mitosis and nuclear acid synthesis and probable interference with the function of microtubules. Griseofulvin has also anti-inflammatory properties and some direct vasodilatory effects when used in high doses. Griseofulvin is poorly absorbed from the gastrointestinal tract but absorption is enhanced by administration with fatty meal, and peak plasma concentration occurs four hours after oral administration.

Griseofulvin is detected in the outer layer of the stratum corneum soon after ingestion; it is diffused from the extracellular fluid and sweat. There is no information regarding the mechanism by which the drug is delivered to nails and hair. Deposition in the newly formed cells could be the major factor. It is metabolized by the liver microsomal enzyme system and excreted in the urine. Its half-life is 9 to 21 hours.

Griseofulvin has been used in the therapy of dermatophyte onychomycosis; however, treatment periods from 6 to 18 months were necessarily with disappointing results and numerous relapses. Therefore, the newer oral antifungal agents itraconazole, terbinafine and fluconazole have superseded griseofulvin as agents of choice for onychomycosis. Unlike griseofulvin, the new agents have a broad spectrum of action that includes dermatophytes, *Candida* species and nondermatophyte moulds. Each of the new antifungal agents is more cost-effective than griseofulvin and is associated with high compliance, in part because of the shorter duration of therapy.

The main use of griseofulvin currently is to treat *tinea capitis*, which is the most common dermatophyte infection during childhood [52]. The treatment of *tinea capitis* requires an oral antifungal agent [53], and griseofulvin is well tolerated particularly in children. However, some

clinical studies over the past decade that have investigated the response of *tinea capitis* to griseofulvin suggest a decrease in sensitivity to this pharmacologic agent [54]. Systemic therapy of scalp ringworm with itraconazole and terbinafine, as well as perhaps fluconazole, seems to be an equivalent or a superior therapeutic approach as compared to the use of griseofulvin. In addition, the data from the use of itraconazole, terbinafine, and fluconazole suggest that they are safe in children [55]and shorten the duration of therapy.

Doses of griseofulvin are 15-20 mg/kg/d for 6 to 8 weeks in children with the microsized form. More frequent side effects are minor: headaches, gastrointestinal reactions and cutaneous eruptions. The major drug interactions have been noted with phenobarbital, anticoagulants and oral contraceptives.

The absorption of griseofulvin with normal particle size in humans is minimal and unpredictable. However, the absorption can be increased by the costly process of size reduction to microsize or ultramicrosize [56].

A number of griseofulvin derivatives were synthesized, of which some exhibited a significant biological activity. The hitherto data on structure-function relationships can be summarized as follows:

1. The spatial arrangement of griseofulvin plays a determining role. Only the natural griseofulvin exhibits the fungistatic activity, the 3 remaining isomers are inactive.

2. Chlorine in position 7 of the A ring is not essential for the biological activity. Its elimination or eventually the substitution by fluorine or bromine yields highly active compounds. For a high biological activity, it is required that position 6 of the aromatic ring remains free. Elimination of methoxy groups also does not yield useful compounds.

3. Substitution of oxygen in position 1 with sulfur or methylimino group does not produce compounds with an interesting biological activity as compared to griseofulvin itself.

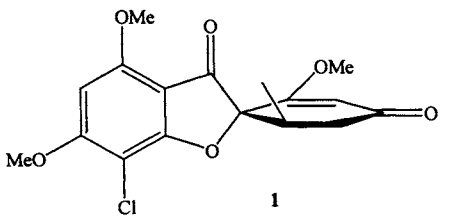
4. Substitution of the whole ring C with 2,2-dimethyl group results in a significant decrease of the biological activity.

5. A number of modifications of the C ring can be made while preserving a high biological activity. Thus substitution of methoxy group in position with 2′ propoxy- or butoxy- group yields compounds 20-50-

times more active *in vitro* than griseofulvin. Substitution of methoxy group with methyl group decreases the activity, elimination of the whole amino group results even in a complete activity loss. Substitution of keto group with CH_2 group yields an inactive compound, however, the activity is preserved when carbonyl group is in position 2′. However, two carbonyl groups, in positions 2′ and 4′, lead to the loss of the activity. Elimination of the 6′-asymmetric center decreases the antifungal activity, whereas a change of the configuration results in a complete activity loss.

Similar Metabolites

Metabolites similar to griseofulvin, *i.e.* geodin (**2**) and erdin (**3**) were isolated from *A. terreus* and later also from *Penicillium* sp. [57-59]. Structurally similar compounds including *e.g.* dehydrogriseofulvin (**4**) from *P. patulum* [60] and *P. martinsii* (Kamal *et al.*, 1970) and dihydrogriseofulvin (**5**) from *P. martinsii* were also isolated [61]. Further, structurally similar metabolites as geodoxin (**6**) [62] and gillusdin (**7**) [63] have been discovered from *A. terreus*. These fungi also produce the presumed biosynthetic precursors, as mentioned above, griseophenones A, B (**8, 9**); dihydrogeodin (**10**) and its structural analogs **11** and **12** [37,64]. The marine annelid, *Thelepus setosus* produces thelepin (**13**), a novel brominated spiro compound, which has antifungal activity comparable with griseofulvin [65,66].



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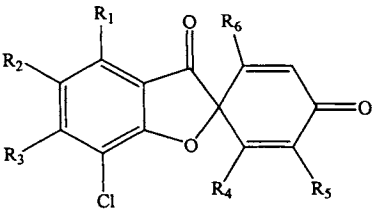
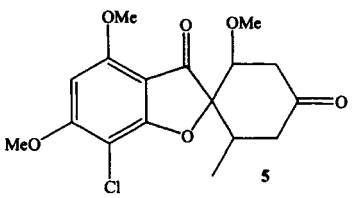
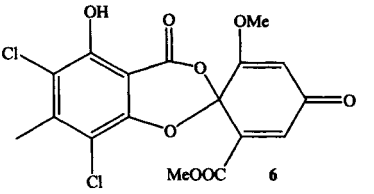
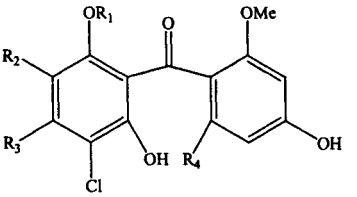


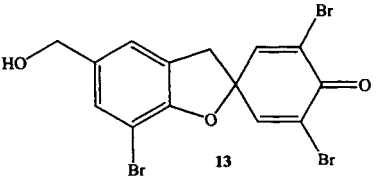
	R_1	R_2	R_3	R_4	R_5	R_6
2	OH	Cl	Me	CO_2Me	H	OMe
3	OH	Cl	Me	CO_2H	H	OMe
4	OMe	H	OMe	Me	H	OMe
7	Me	Cl	OH	OMe	OH	CO_2Me







	R_1	R_2	R_3	R_4
8	Me	H	OMe	Me
9	H	H	OMe	Me
10	H	Cl	Me	CO_2Me
11	H	H	Me	CO_2Me
12	Me	H	Me	Me



Second Part - Other Halogen Containing Compounds from Fungi

In the second part of the review, we would like to concentrate primarily on pharmacologically significant compounds containing halogen atom(s) in their molecules and are isolated from fungi. This part aims to review papers published after 1996, since earlier studies had been previously reviewed by Gribble [67,68]. Additional papers published by the same author [69-72], although highly interesting, are not extensive enough, and none of them deals in depth with natural compounds isolated from fungi and containing halogen atom(s) in their molecules.

Most organohalogens produced by fungi have an aromatic structure; important groups include the chlorinated anisyl metabolites, drosophilins, and other chlorinated hydroquinone methyl ethers, chlorinated sesquiterpenens, chlorinated anthraquinones and strobulirins [72].

Many different reviews have been devoted to the toxicity of mycotoxins [73-76] or their analysis in the natural material [77].

Azaphilones

Azaphilones are a large group of pyrano-quinone structures with a high electron acceptor tension determining sensitivity of oxygen in the primary ring yielding γ-pyridones, which exhibit chromophore properties. The color of azaphilone pigments depends on their chemical structure. Their name stems from their reaction with ammonia resulting in γ-pyridone derivatives. Metabolites in the medium readily react with compounds containing amino groups such as proteins, amino acids, or nucleic acids resulting in water-soluble colored products.

Most of them have their absorption maximum within the visible range of the spectrum (400 and 500 nm for yellow and red pigments, respectively). In general, yellow structures are more hydrogenated than orange and red, and amino forms usually have their maximum at higher wavelengths of the absorption spectrum.

They were identified in different filamentous fungi indicating that these microorganisms could serve as a useful source of a new group of pigments of natural origin applicable in food industry. Microorganisms tested include the genera *Monascus*, *Penicillium,* and *Chaetomium*, all well-known producers of pigments with the azaphilone basic structure. Some compounds of this type were also identified in *Aspergillus ustus*, *Cochliobolus lunata*, *Talaromyces* sp. and *Emericella falconensis.* The natural origin of the azaphilone pigments and their easy derivatization, together with increased thermostability in comparison to other natural dyes, open new possibilities of their applications in food industry and cosmetics. Chemical structures of azaphilones isolated from the mycelium and cultivation broth are summarized below.

Data concerning their biological activity are contradictory, namely with respect to their antimicrobial, *viz.* antibacterial and antifungal activities. However, it is generally accepted that they exhibit a significant inhibitory effect on the activity of acyl-CoA:cholesterol acyltransferase (ACAT) and diacylglycerol acyltransferase (DGAT). The azaphilone skeleton is essential for certain biological activities of these metabolites and differences in their activity can be ascribed to differences in their reactivity with amines.

Production of azaphilone metabolites (including also non-halogen compounds) by filamentous fungi is summarized in Table 2.

Table 2. Production of azaphilone metabolites by filamentous fungi.

Metabolite	Microorganism
monascin	*Monascus purpureus*
	Monascus rubiginosus
	Monascus rubropunctatus
rubropunctatin	*Monascus rubropunctatus*
rubropunctatamine	
ankaflavin	
monascorubrin	*Monascus purpureus*
monascorubramine	
N-glutarylrubropunctatamine	*Monascus ruber*
N-glutarylmonasconibramine	*Monascus ruber*
	Penicillium rubrum
mitorubrin	*Talaromyces udagawae*
mitorubrinol	*Penicillium rubrum*
	Penicillium wortmannii
	Penicillium vermiculatum
	Penicillium funiculosum
mitorubrinic acid	*Penicillium vermiculatum*
monomethyl-(+)-mitorubrin	*Talaromyces tardifaciens*
wortmin	*Penicillium wortmannii*
austdiol	*Aspergillus ustus*
chaetoviridin A-D	*Chaetomium globosum* var. *flavoviride*
lunatic acid	*Cochhobolus lunata*
falconensin A-D,H	*Emericella falconensis*
rubrorotiorin	*Penicillium hirayamae*
	Talaromyces luteus
luteusin A-E	*Penicillium vonarxii*
isochromophilone I-VI	*Penicillium multicolor*
isochromophilone VII,VIII	*Penicillium* sp.

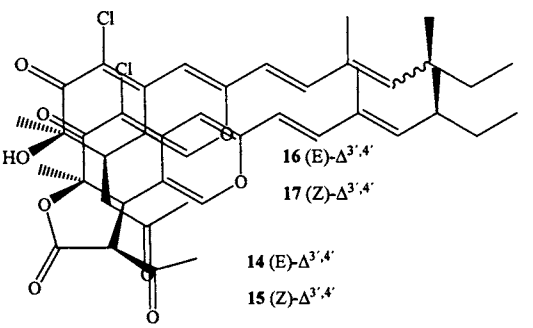
Isochromophilones I (**14**, **15**), the new gp 120-CD4 binding inhibitors, were isolated from a culture broth of *Penicillium multicolor* FO-2338 grown on a medium with carbon and nitrogen sources. Their amounts in the medium were ~2 mg/l and their chemical structures were elucidated

by NMR experiments. Both compounds have an azaphilone skeleton substituted by a chlorine atom at C-5 and a side chain, 3,5-dimethyl-1,3-heptadien at C-3. Additionally, they contain a γ-lactone ring [78].

These metabolites are the first non-peptide compounds, inhibiting the specific binding of the HIVgp120 protein with the CD4 molecule on the surface of sensitive cells of the organism that is attacked by the virus and thus prevent the HIV virus from entering the cells.

Isochromophilones I (**14** and **15**) inhibited gp120-CD4 binding with IC_{50} values of 6.6 and 3.9 μM, respectively, by ELISA method. Isochromophilone (**15**) exhibited anti-HIV activity at 25 μM, but did not affect cell proliferation in lymphocytes at the same concentration although it inhibited somewhat the cell proliferation at 250 μM.

Isochromophilones II (**16** and **17**) were inactive against bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* and *Staphylococcus aureus*) and fungi (*Candida albicans*, *Aspergillus niger* and *Piricularia oryzae*) at 1.0 mg/ml on paper disc method.

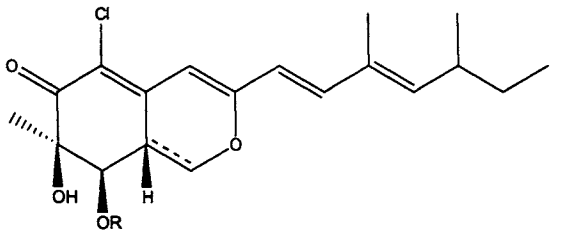


New azaphilones named isochromophilones III-VI (**18-21**) were isolated from the culture broth of *Penicillium multicolor* FO-3216 as inhibitors of ACAT. Their structures were elucidated by NMR and other spectroscopic analyses. The IC_{50} values of isochromophilones (**17-20**) (ACAT) activity in an enzyme assay using rat liver microsomes were calculated to be 110, 50, 50 and 120 μM, respectively [79].

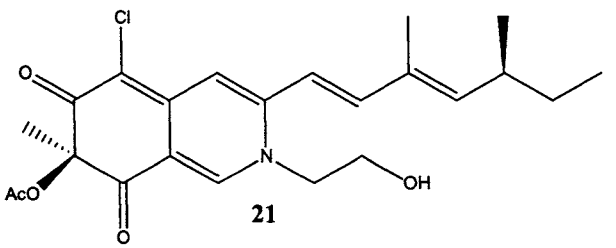
The compound **19** also inhibited the activity of cholesteryl ester transfer protein (CETP) with an IC_{50} value of 98 μM. The compounds **18**, **20** and **21** weakly inhibited the activity of CETP in 300 μM.

Antimicrobial and cytotoxic activities of **18**, **20** and **21** were tested. They inhibited the growth of *Staphylococcus aureus*, *Bacteroides fragillis*, and *Pyricularia oryzae* at 50 μg/disk. However, they did not

inhibit the growth of *Bacillus subtilis*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Xanthomonas oryzae*, *Acholeplasma laidlawii*, *Candida albicans*, *Saccharomyces sake*, *Aspergillus niger* and *Mucor racemosus* in the same concentration. The IC_{50} values of **18**, **20** and **21** against the growth of B-16 melanoma cells *in vitro* were 33, 36 and 30 μM, respectively.



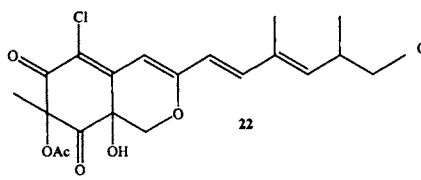
	R	Δ
18	H	No
19	Ac	No
20	H	Yes



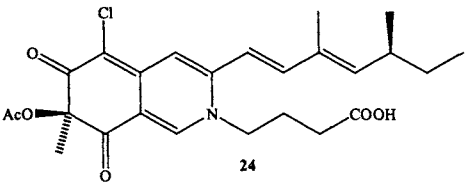
21

New isochromophilones VII-VIII (**22**) and (**23**) were isolated from the culture broth of *Penicillium* sp. FO-4164. Both isochromophilones inhibited DGAT activity (assayed *in vitro* using rat liver microsomes) with IC_{50} values of 20.0 and 127 μM and ACAT activity with IC_{50} values of 24.5 and 47.0 μM, respectively [80].

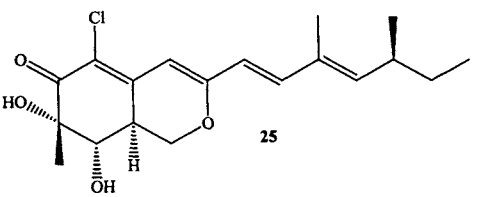
Antimicrobial activity was tested at a concentration of 10 μg/paper disk. Both isochromophilones showed antimicrobial activity against *Bacillus subtilis*, *Mycobacterium smegmatis*, *Micrococcus luteus* and *Pyricularia oryzae*. But no antimicrobial activity was observed against the following microorganisms: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Saccharomyces sake*, *Mucor racemosus* and *Aspergillus niger*.



Isochromophilone IX (**24**), a novel GABA-containing metabolite, was isolated from a cultured fungus, *Penicillium* sp. [81].



The azaphilone (**25**) produced by *Penicillium sclerotiorum* active in assays for the detection of antagonists of the endothelin-A (ET(A)) and endothelin-B (ET(B)) receptors has been identified. Data for the inhibition of endothelin-1 (ET-1) and endothelin-3 (ET-3) binding in the ET(A) and ET(B) receptor assays, respectively, have been reported for this series. Compound **25** was more selective for the rabbit ET(A) receptor than for the rat ET(B) receptor. The IC_{50} value for (**25**) was 9 μM in an assay based on binding of ET-1 to rabbit ET(A) receptors. In an assay based on the binding of ET-3 to the rat ET(B) receptor compound **25** exhibited IC_{50} of 77 μM. This compound demonstrated an antagonistic behavior in a secondary assay based on blockade of ET-1 stimulated arachidonic acid release from rabbit renal artery smooth muscle cells, when present at concentrations greater than or equal to 30 μM [82].



A novel brominated azaphilone derivative, 5-bromoochrephilone (**26**) and known derivatives, were isolated from the culture broth of a producing organism *Penicillium multicolor*, fermented in a medium

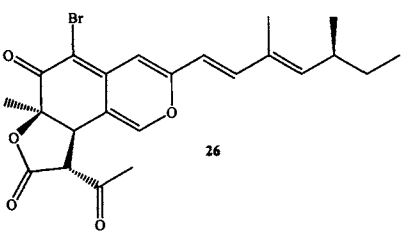
containing potassium bromide. Nineteen azaphilone-related compounds isolated from the above strain and from other fungi were tested for the inhibition of gp120-CD4 binding, and the structure-activity relationship was discussed. 5-Bromoochrephilone was found to be the strongest inhibitor (IC_{50}, 2.5 μM). A halogen atom at C-5, a proton at C-8 and a diene structure in C-2 side chain of 6-oxoisochromane ring are necessary for gp120-CD4 binding [83].

The results in Table **3** indicate that the halogen atom at C-5 and the orientation from C-8 to C-10 in the isochromane ring of azaphilones, in addition to the diene structure in C-2 side chain, are very important for the inhibition of gp120-CD4 binding.

Table 3. Inhibitory activities of azaphilones on gp120-CD4 binding.

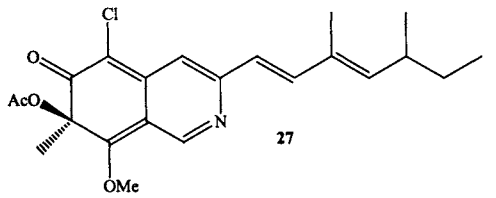
Inhibitor	IC_{50} (μM)
isochromophilone I	6.6
isochromophilone II	3.9
isochromophilone III	48
isochromophilone IV	96
isochromophilone V	14.6
tetrahydroisochromophilone I	>260
sclerotiorin	>250
rubrorotiorin	>240
5-bromoochrephilone	2.5
rotiorin	>240
luteusin A	9.4
chaetoviridin A	>230
chaetoviridin B	140

The effect of thirteen different fungal azaphilones on cholesteryl ester transfer protein activity was tested [84].



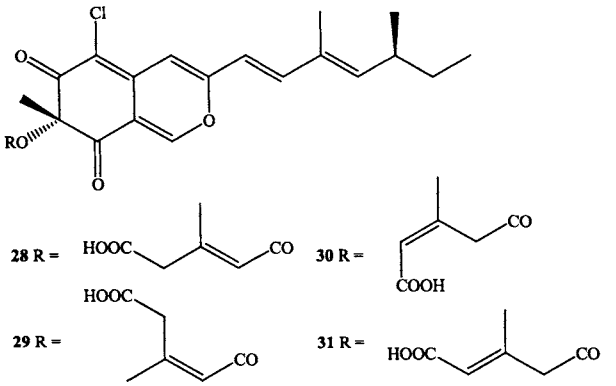
26

A new secondary metabolite, 8-O-methylsclerotiorinamine (**27**), was isolated from a strain of *Penicillium multicolor*, and its structure was established using NMR spectroscopy and chemical evidence. The metabolite significantly inhibited the binding between the Grb2-SH2 domain and the phosphopeptide derived from the Shc protein and also blocked the protein-protein interactions of Grb2-Shc in cell-based experiments, with IC_{50} values of 5.3 and 50 μM, respectively [85].



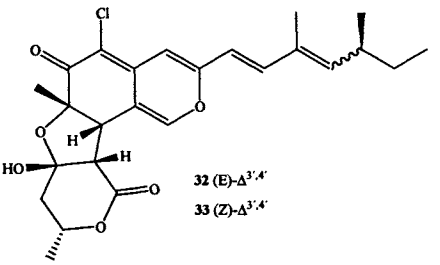
27

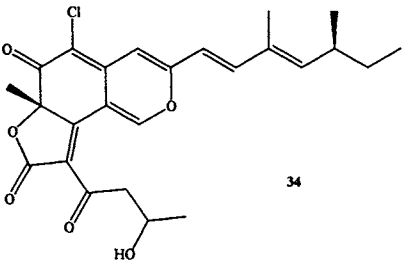
Four new azaphilones, named helicusins A, B, C and D (**28-31**), were isolated from *Talaromyces helicus*. These four new azaphilones showed weak monoamine oxidase-inhibitory effects [86].



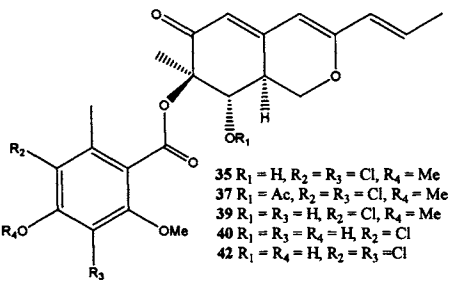
Three new azaphilones (**32-34**), named luteusins C, D and E, were isolated after a 21-h surface cultivation of the ascomycete *Talaromyces luteus* (anamorph of *Penicillium vonarxii*) on rice, together with luteusins A and B, previously known as inhibitors of monoaminooxidase [87]. The new compounds had no MAO-inhibitory activity [88].

The potential to inhibit MAO is lost after conversion of hydroxyl group in position C-8 to oxo-group and hydrogenation of the side chain double bonds.

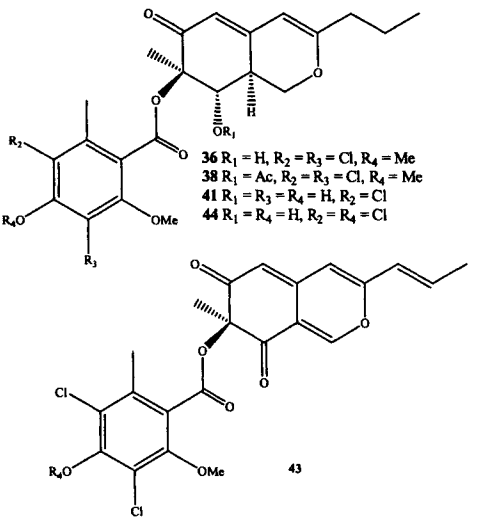




Falconensins, *i.e.* azaphilones esterified by chloroorselinic acid, A (**35**), B (**36**) and C (**37**), were isolated from mycelial extracts of the Venezuelan soil fungus ascomycete *Emericella falconensis* [89]. Itabashi *et al.* [90] later isolated falconensone H (**43**) together with falconensins A, B, C and D (**38**) from the same culture. Their chemical structures are shown below.



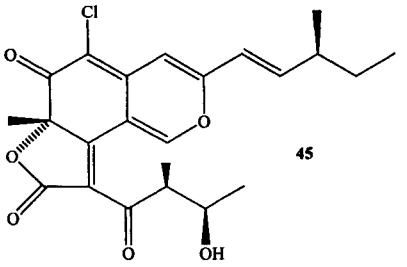
Further falconensins, *i.e.* falconensins E (**39**), K (**40**), L (**41**), M (**42**) and N (**44**), were isolated also from *Emericella falconensis.* Three new azaphilone derivatives designated falconensins E, F and G were isolated from the mycelium of *Emericella falconensis*, along with falconensins A-D and H. The structures of new falconensins were established by spectroscopic investigation and chemical correlations. The absolute stereochemistry of falconensins A-G was also established [91].



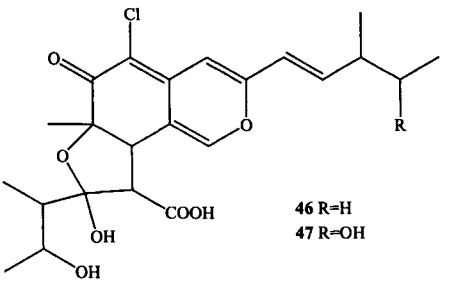
Six new hydrogenated azaphilones designated falconensins I-N were isolated as minor components from mycelia of *Emericella falconensis* and/or *E. fruticulosa* along with nine azaphilone derivatives, falconensins A-H. The structures of falconensins I-N were determined by spectroscopic investigation and chemical correlation [92].

Chaetoviridines A (**45**), B (**46**), C (**47**) and D (**48**), also belonging to the azaphilone group, can be isolated from the fungus *Chaetomium globosum* var. *flavoviride* grown on wheat. Spectral data show that chlorine-containing azaphilones are involved with a conjugated γ-lactone connected angularly with the azaphilone unit.

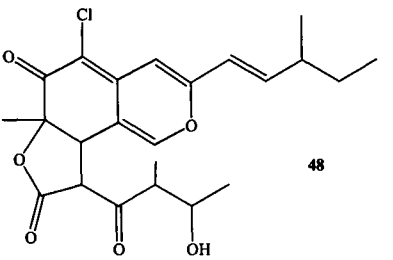
Chemical structure of chaetoviridin A was determined by NMR, and so the absolute configuration has been established [93]. The red pigment chaetoviridin A contains an unsaturated γ-lactone ring, whereas the yellow chaetoviridins B, C and D contain a saturated γ-lactone ring as shown. Chaetoviridin A was shown to inhibit monoaminooxidase and growth of *P. oryzae* at 2.5 μg/ml.



45

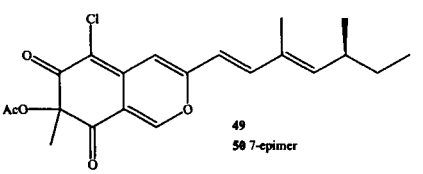


46 R=H
47 R=OH

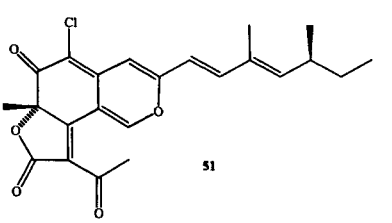


48

The fungal metabolite, sclerotiorin (**49**) was first isolated independently by two groups from *Penicillium sclerotiorum* [94,95] and later from *P. multicolor* [95]. The 7-epimer (**50**) is also a known metabolite of *P. hirayamae* [96]. Sclerotiorin was isolated for the first time from lichen mycobiont *Pyrenula japonica* [97]. The related rubrorotiorin (**51**) was also found in *P. hirayamae* [98].



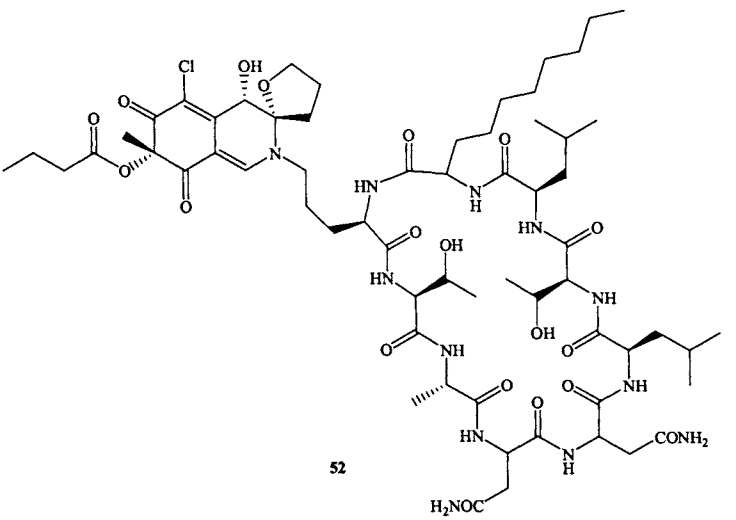
49
50 7-epimer



51

Chlorofusin (**52**) was one of the major components produced by fermentation of *Fusarium* sp. 22026. The DELFIA-modified ELISA was used to guide to the purification of inhibitors of the p53/MDM2 interaction from these fermentation extracts. Chlorofusin was the most abundant inhibitory compound. Titration of purified chlorofusin in the

DELFIA-modified ELISA gave an IC_{50} of 4.6 μM. In simultaneous cross-screen testing, chlorofusin was inactive at a concentration of 4 μM in the TNFα:TNFα receptor protein-protein interaction, which was configured in the same format as the primary assay. The compound showed no cytotoxic effects against Hep G2 cells at a concentration of 4 μM. At a test concentration of 7.3 μM, chlorofusin did not exhibit any antimicrobial activity against the following test strains: *Escherichia coli*, *Staphylococcus aureus*, *Serratia marcescens*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus niger* [99].



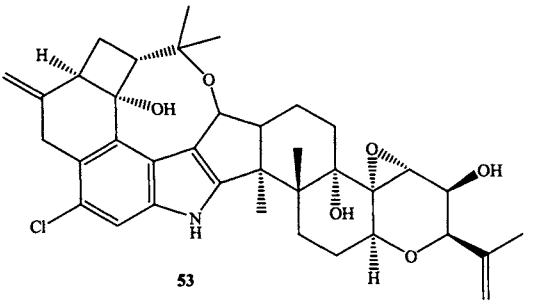
52

Indoles

Indole-alkaloid isoprenoid was isolated from extracts of *Penicillium crustosum* grown on rice. This compound, designated thomitrem (**53**), contains a 18(19)-double bond and lacks the characteristic penitrem 17(18)-ether linkage [100].

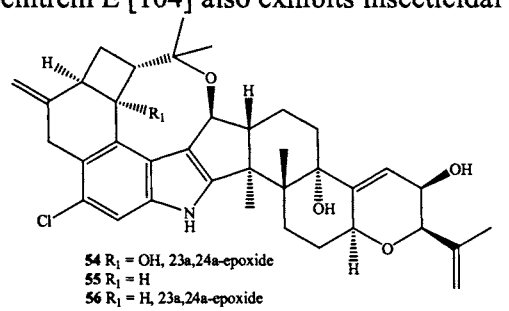
Penitrems are a group of tremorgenic mycotoxins produced by a variety of *Penicillium* and *Aspergillus* species, amongst which *Penicillium crustosum* is generally regarded as the most important producer of this group of mycotoxins. They have been reported to intoxicate animals. Generally, penitrem A is considered to be the most significant of the series of *P. crustosum* mycotoxins, which includes

penitrems B, C, D, E and F, and other related metabolites such as PC-M4, PC-M5, PC-M5' and PC-M6.



53

A fungus, *Penicillium crustosum,* is known to produce the tremorgenic mycotoxins penitrems A, C, F (**54-56**), when grown in surface culture [101-103]. All of these tremorgens have a common core structure composed of an indole moiety, biosynthetically derived from tryptophan, and a diterpenoid unit from four mevalonate-derived isoprenes. Penitrems are also produced by other *Penicillium* species and by *Aspergillus sulphureus*. This group of metabolites is capable of eliciting tremors in vertebrates, and some specific members have also shown insecticidal activity. For example, compound **54** shows convulsive and insecticidal activities against *Bombyx mori*, *Spodoptera frugiperda*, and *Heliothis zea*, and its use as an insecticide was patented in 1990. Natural penitrem analog 6-bromopenitrem E [104] also exhibits insecticidal activity.

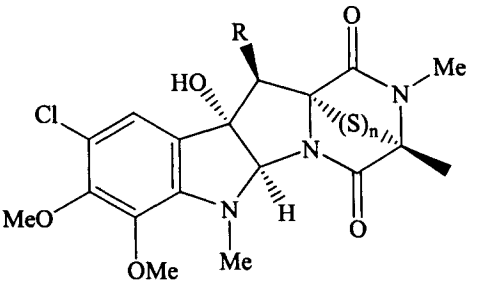


54 R_1 = OH, 23a,24a-epoxide
55 R_1 = H
56 R_1 = H, 23a,24a-epoxide

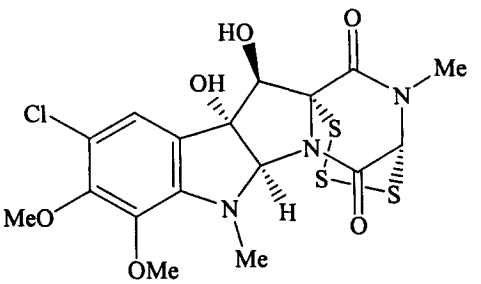
Twenty-five *Penicillium* species were isolated and mycotoxins produced by several of these species, including penitrems A-F, were detected. The levels of penitrem A in these samples were in the range 35-7500 μg/kg [105]. HPLC and diode array detection were used to confirm

the chemical structure of the mycotoxins, *e.g.* penitrem A and ochratoxin A in extracts from three mycotoxigenic fungi (*Penicillium crustosum*, *Penicillium glabrum/spinulosum*, and *Penicillium discolor*) that dominated on *Castanea sativa* nuts sold in Canadian grocery stores [106]. Another paper includes data for detection and dereplication of >400 fungal metabolites using MS/ESI$^+$ methods [107].

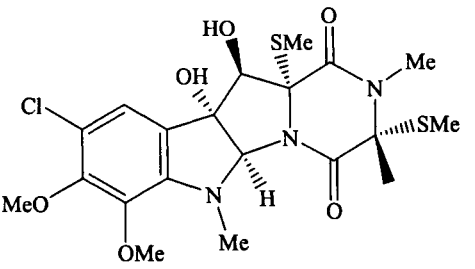
Sporidesmins, the series of sulfur-containing physostigmine-like metabolites, were isolated from *Sporidesmium bakeri* A (**57**), B (**58**) and C (**59**), a fungus that causes facial eczema and liver damage in farm animals, *e.g.* New Zealand sheeps [108-110]. Later studies identified sporidesmins D (**60**) [111], E (**61**) [112], F (**62**) [111], G (**63**) [113,114], H (**64**) [115]) and J **65**) [115] from *P. chartarum*. Protonated $[M+H]^+$ and deprotonated $[M-H]^-$ ions were observed in positive and negative ion ESI modes, respectively [116]. In a further paper, complexation of sporidesmin A, with metals, was used for its analysis [117].



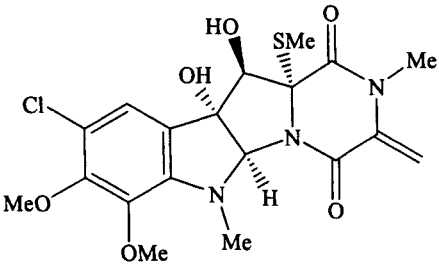
57 R = OH, n= 2
58 R = H, n= 2
61 R = OH, n= 3
63 R = OH, n= 4



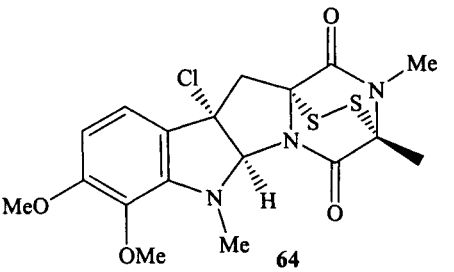
59



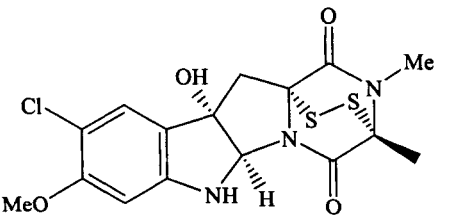
60



62

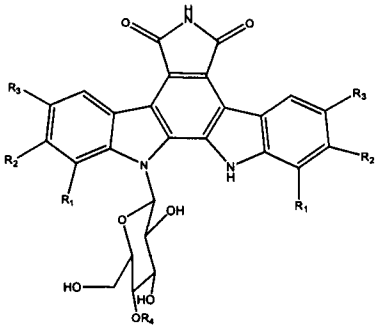


64



65

Saccharothrix aerocolonigenes produces an indolocarbazole antitumor agent rebeccamycin (**66**) under submerged fermentation conditions. Adding D,L-6-fluorotryptophan to culture of *S. aerocolonigenes* induces the formation of two novel indolocarbazoles, fluoroindolocarbazoles A (**67**) and B (**68**). Feeding *D,L*-5-fluorotryptophan to culture of *S. aerocolonigenes* induces the production of a novel indolocarbazole, fluoroindolocarbazole C (**69**). These fluoroindolocarbazoles have been isolated from culture broth and purified to homogeneity by vacuum liquid chromatography and column chromatography. All three fluoroindolocarbazoles are more potent than rebeccamycin against P388 leukemia via ip route in murine model [118].

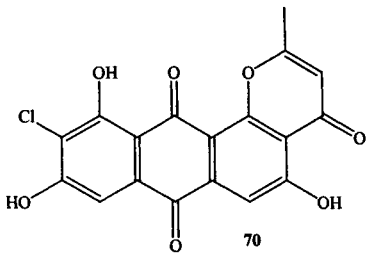


	R_1	R_2	R_3	R_4
66	Cl	H	H	Me
67	H	F	H	Me
68	H	F	H	H
69	H	H	F	H

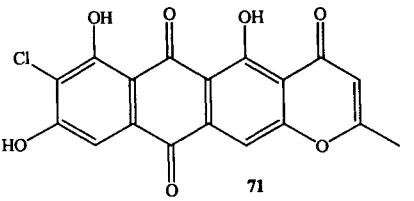
Antraquinones

Like the fungal and lichen xanthones, anthraquinones, which are also produced both by lichens and fungi, are derived from extended polyketides by cyclization. Several chlorinated compounds were described.

The structures of novel topoisomerase I inhibitors, topopyrones A and B (**70** and **71**), were elucidated by spectral analysis of the chemical derivatives. It was suggested that topopyrone B is converted from topopyrone A [119]. Topopyrones A and B selectively inhibited recombinant yeast growth dependent on the expression of human topoisomerase I with IC_{50} values of 1.22 and 0.15 ng/ml, respectively. The activity and selectivity of **71** were comparable to those of camptothecin. The relaxation of supercoiled pBR322 DNA by human DNA topoisomerase I was inhibited by these compounds, however, they did not inhibit human DNA topoisomerase II. Both topopyrones were cytotoxic to all tumor cell lines when tested *in vitro*. Topopyrone B has potent inhibitory activity against herpesvirus, especially varicella tester virus (VZV). It inhibited VZV growth with EC_{50} value of 0.038 μg/ml, which is 24 fold stronger than that of acyclovir (0.9 μg/ml). Both topopyrones were inhibitory against Gram-positive bacteria.

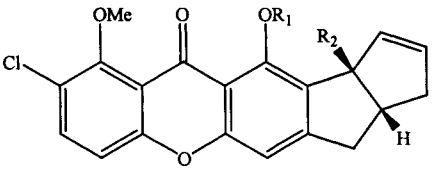


70



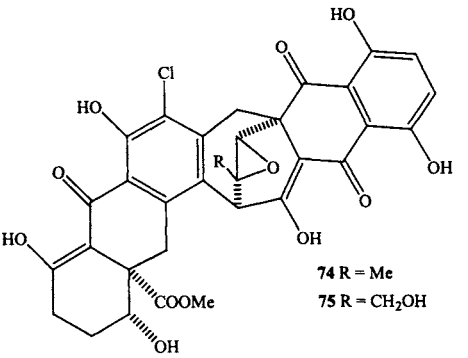
71

Aspergillus ustus produces several novel pentacyclic metabolites, the austocystins, two of which, i.e. A (**72**) and C (**73**), contain chlorine [120].

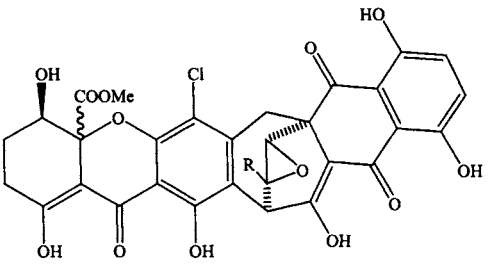


72 R_1 = Me, R_2 = H
73 R_1 = H, R_2 = OH

The fungus *Cercospora beticola,* which is a highly destructive disease of sugar beets worldwide, has been shown by several groups to produce a series of highly intricate metabolites, beticolins 1 (**74**) (= cebetin A), 2 (**75**), 3 (**76**), 4 (**77**), 6 (**78**), 8 (**79**) and cebetin B (not shown), the latter of which is *bis*-Mg complex of cebetin A [121-126]. Following some initial confusion regarding the complexity of structures and the fact, that beticolin 2 and cebetin A are in equilibrium, the situation now appears to be in order [125,126].

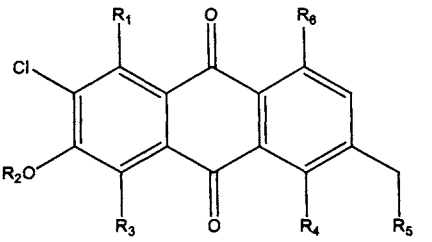


74 R = Me
75 R = CH_2OH



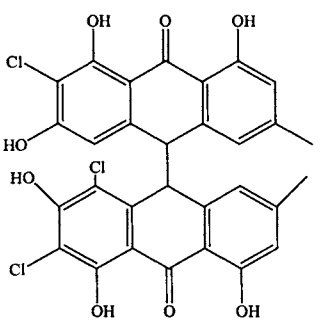
76 R = Me; α-COOMe
77 R = CH_2OH; α-COOMe
78 R = Me; β-COOMe
79 R = CH_2OH; β-COOMe

The cheese mold *Penicillium nalgiovensis* produces nalgiolaxin (**80**), which appears to be the first natural chlorinated anthraquinone isolated [127,128], although fragilin (**81**) was the first fully characterized [129]. A summary of the known chlorinated anthraquinones (**80-88**) is given below.

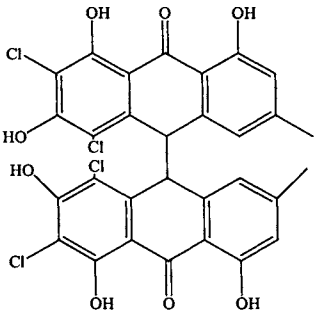


No.	R_1	R_2	R_3	R_4	R_5	R_6
80	Me	Me	-	-	MeCHOH	OH
81	OH	Me	-	-	H	OH
82	OH	H	-	-	H	OH
83	OH	H	-	-	H	OH
84	OH	Me	-	-	H	OMe
85	OH	Me	-	-	H	OMe
86	OH	H	Cl	-	H	OMe
87	OH	H	OH	-	H	OH
88	OH	H	-	OH	H	OH

Several chlorinated metabolites closely related to anthraquinones are also known. For example, the aspen tree fungus *Phialophora alba* which protects the tree against attack by the decaycausing fungus *Phellinus tremulae* produces anthrone, in addition to other derivatives [130]. Anthrone was found in cultures of *Aspergillus fumigatus* [131], and the corresponding bromo compound was formed in the presence of bromide [131]. The novel bis-anthrones, flavoobscurin A (**89**), B (**90**) and B_2 (the latter two are rotational isomers) were produced by *Anaptychia obscurata* [132].



89

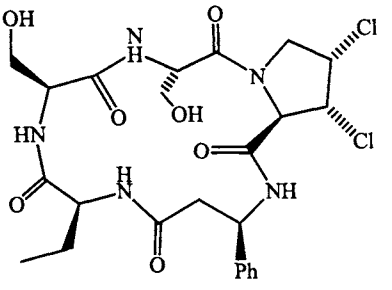


90

Macrocycles

Several relatively large cyclic peptides have been found to contain halogen. Islanditoxin (**91**) which was isolated in 1955 from a culture of

Penicillium islandicum is a chlorine-containing peptide whose structure was determined later. This organism also produced cyclochlorotine (**92**), which is an infectant of yellowed rice [133]. The fungus *Metarhizium anisopliae* produces the chlorohydrin cyclic peptide (**93**) [134]. Cyclochlorotine, a hepatotoxic mycotoxin, was also isolated from *Penicillium islandicum*.



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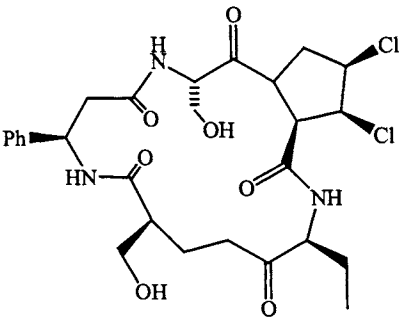
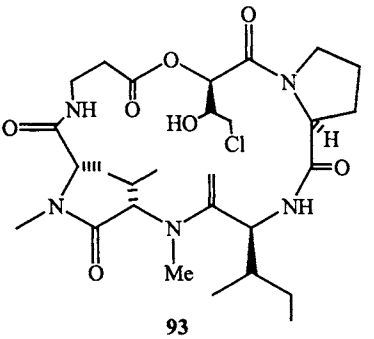


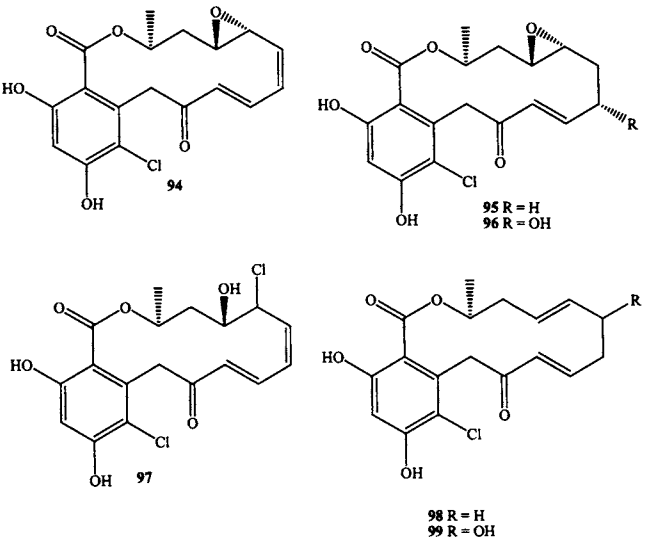
92



93

Monorden (**94**) and the novel resorcylic acid lactones pochonins A (**95**), B (**96**), C (**97**), D (**98**) and E (**99**) were isolated from cultures of the clavicipitaceous hyphomycete *Pochonia chlamydosporia* var. *catenulata* strain P 0297. Fermentation of P 0297 in bromide-containing culture media led to a shift in secondary metabolite production and yielded monocillins (compounds without bromine in the molecule) as major metabolites besides monorden (**94**) as well as the novel compounds pochonin F and a monocillin II glycoside as minor metabolites, Fig. (**1**). Most of these compounds showed moderate activities in a cellular replication assay against herpes simplex virus and against the parasitic protozoan *Eimeria tenella*. In contrast to the structurally related zearalenone derivatives, none of the metabolites of strain P 0297 was

found to be active in a fluorescence polarization assay for the determination of modulatory activities on the human estrogenic receptor ERbeta [135].



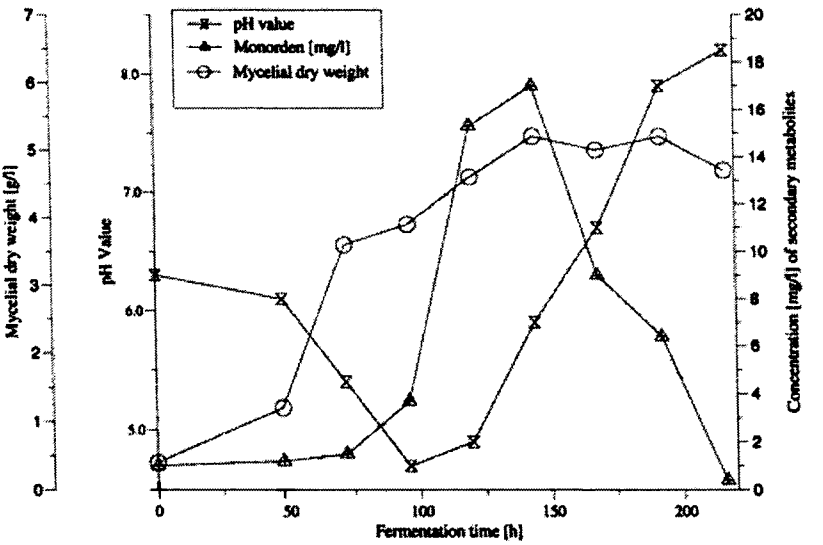
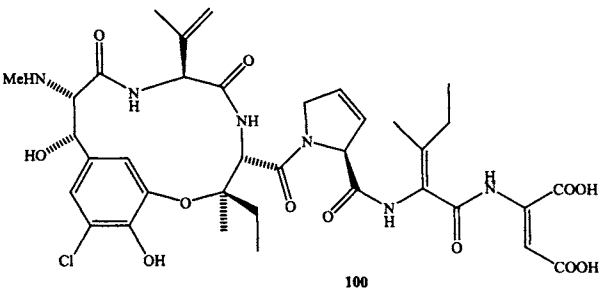


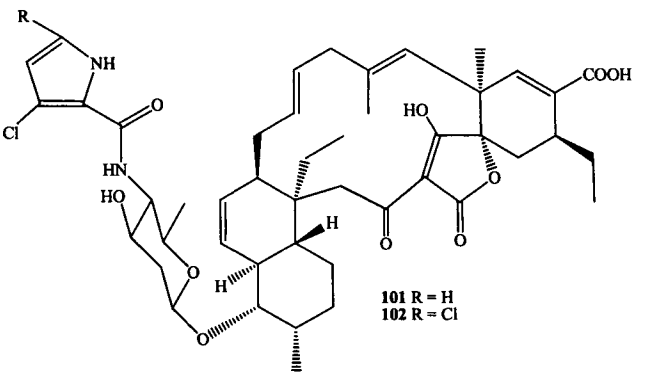
Fig. (1). Time course of production of monorden (94) in Q6/2 medium (150 ml shake flasks) by strain P 0297.

Phomopsin A (**100**), the main mycotoxin isolated from cultures of *Phomopsis leptostromiformis* and the cause of lupinosis disease, is the hexapeptide containing unusual amino acid [136]. The *L*-configuration of the indicated amino acids was established by comparison of the *N*-trifluoroacetyl n-butylester derivatives of the acid hydrolysis products of phomopsin A with samples prepared from authentic amino acids, using capillary gas chromatography on a chiral stationary phase. The *E* configuration of the two 2,3-didehydro amino acids is based on the products obtained by catalytic hydrogenation and sodium borohydride reduction of phomopsin A followed by acid hydrolysis (for 2,3-didehydroisoleucine) or by analysis of the coupled ^{13}C NMR spectrum of phomopsin A (for 2,3-didehydroaspartic acid). Evidence was presented, which shows that the glycine formed during the acid hydrolysis of phomopsin A is derived from the 3,4-didehydrovaline moiety. The sequence of the amino acids was established by heteronuclear ^{13}C ^{1}H selective population inversion experiments and by fast atom bombardment mass spectrometry of phomopsin A and its derivatives. An X-ray crystallographic study of phomopsin A confirmed the amino acid sequence and showed that the hexapeptide is modified by an ether bridge in place of the 5-hydroxy group of the *N*-methyl-3-(3-chloro-4,5-dihydroxyphenyl)serine and the hydroxy group of the 3-hydroxyisoleucine units. In addition, the X-ray study specified the absolute configuration of phomopsin A as 22*E*, 25*E*, 3*R*, 4*S*, 7*S*, 10*S*, 11*S*, 19*S*. The consumption of lupins or post-harvest lupin roughage infected with the fungus *Phomopsis leptostromiformis* has been identified as the cause of lupinosis, mycotoxicosis of sheep, cattle, and horses. The condition, characterized by severe liver damage, is of considerable economic importance in Australia, and field cases have also been reported in South and New Zealand [137].



100

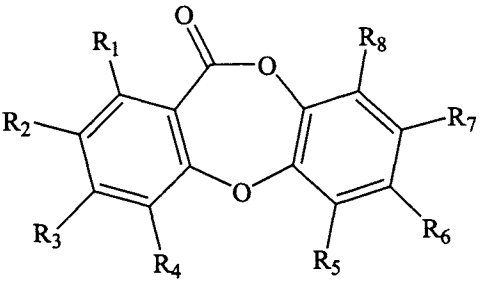
The structures of decatromicins A and B (**101** and **102**) that strongly inhibit the growth of methicillin-resistant staphylococci MRSA were elucidated. Decatromicins A and B inhibited the growth of Gram-positive bacteria including multi-drug resistant strains such as *Staphylococcus aureus* and methicilin resistant *S. aureus*. The antimicrobial activity of decatromicin B was stronger than that of decatromicin A. This observation suggests that the antibacterial activity against Gram-positive bacteria of decatromicins might increase with increase in the number of chlorine atoms attached to the pyrrole ring. However, these antibiotics did not inhibit growth of Gram-negative bacteria and yeast at 100 μg/ml. The acute toxicities (LD_{50}) of decatromicins A and B in mice (*ip*) were estimated to be more than 100 mg/kg [138].

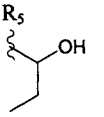
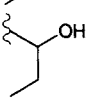
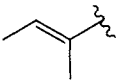
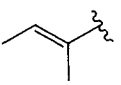
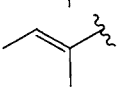
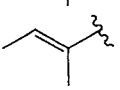
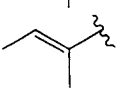
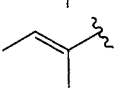
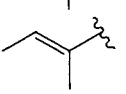
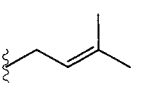
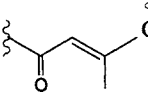


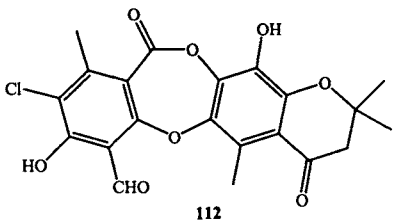
Depsides and Depsidones

Similarly, to lichens, depsidones were also detected in fungi. The fungus *Aspergillus unguis* contains haiderin (**103**), rubinin (**104**), shirin (**105**) and nasrin (**106**) [139]. The 2-chlorounguinol and emeguisins A-C (**107-109**) were found in *Emericella unguis* [140,141]. A marine isolate of the fungus *Emericella unguis* gave a new antibacterial depside, guisinol. The structure determination was based on mass spectrometry and NMR spectroscopical studies. The fungus, *Chaetomium mollicellum* produces several new depsidones three of which are chlorinated, mollicellins D (**110**), E (**111**) and F (**112**). The lactones canesolide (**113**) and buellolide (**114**), which may arise from depsidones by catabolism, were also found in *Buellia canescens*. Structurally, guisinol (**115**) resembles the depsidones emeguisin A-C previously isolated from another strain of the same species. Five other isolates of *E. unguis* also produced the same

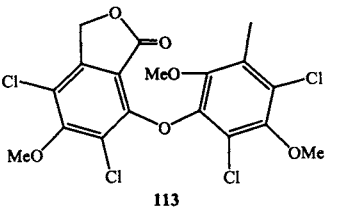
qualitative profile of secondary metabolites including guisinol and dechloronidulin. Other species of *Emericella* did not produce any of these compounds that are thus of chemotaxonomic significance [142].



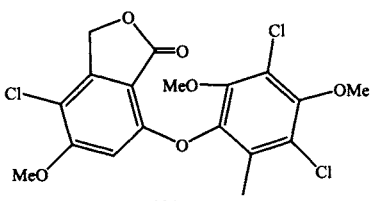
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
103	Me	H	OH	Cl		H	OH	Me
104	Me	H	OH	Cl		H	OMe	Me
105	Me	Cl	OH	Cl	i-Bu	Cl	OH	Me
106	Me	Cl	OH	Cl		Cl	OH	Bu
107		H	OH	Me		Cl	OH	Me
108		H	OMe	Me		Cl	OH	Me
109		Cl	OH	Me		Cl	OMe	Me
110	Me	Cl	OH	CH_2O H	H	OH		Me
111	Me	Cl	OH	CHO	Me		OMe	OH



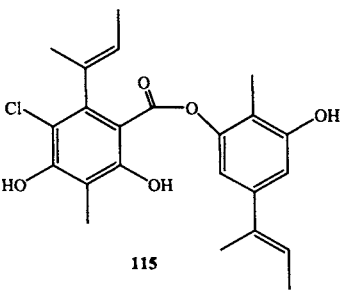
112



113

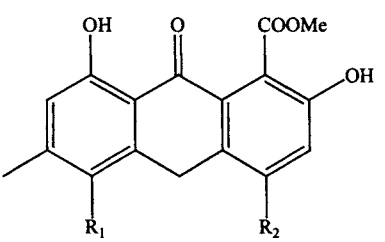


114

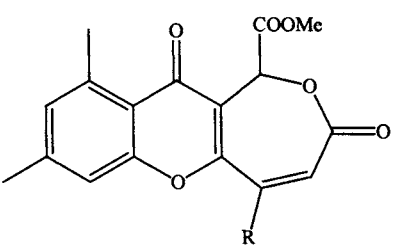


115

The cherry rot fungus, *Monilinia fructicola,* which was earlier shown to produce chloromonilinic acids A and B, also contains 4-chloropinselin (**116**) [143] and its biosynthetic product chloromonilicin (**117**) [144-146]. In the paper [143], the structural elucidation of a new antifungal metabolite, chloromonilicin (**117**), isolated as a growth self-inhibitor of phytopathogenic fungus *Monilinia fructicola,* was reported. It contained a novel, seven-membered lactone ring presumably formed by oxidative cleavage of a benzene nucleus in a xanthone system. Subsequently was isolated a new chlorine containing metabolite, 4-chloropinselin (**116**), a probable precursor of (**117**). In addition, bromomonilicin (**118**) and bromopinselin (**119**) were prepared for activity tests and biosynthetic studies. Biosynthetic incorporation of (**119**) into (**118**) was accomplished using *M. fructicola* culture.



116 R_1 = H, R_2 = Cl
119 R_1 = H, R_2 = Br

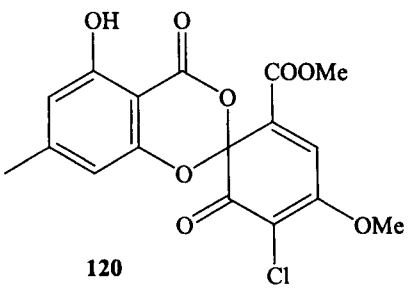


117 R = Cl
118 R = Br

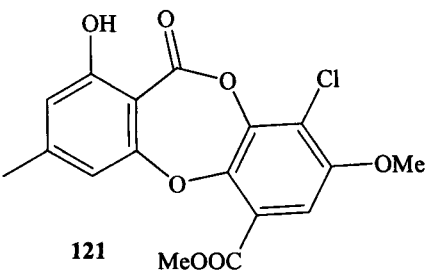
Table 4. Antimicrobial spectrum of bromomonilicin (118), by agar dilution method on glucose nutrient agar.

Test organism	MIC (μg/ml)
Staphylococcus aureus	>50
Escherichia coli	>50
Shigella flexneri	>50
Pseudomonas aeruginosa	>50
Candida albicans	25
Trichophyton asteroides	6.2
T. interdigitale	6.2
T. rubrum	6.2

A new chlorinated depsidone (maldoxone **120**) and a new spirocyclohexadienone (maldoxin **121**) have been isolated from the culture medium of an as yet unidentified *Xylaria* species. Their role in the grisan-depsidone biosynthetic pathway was discussed [147].



120

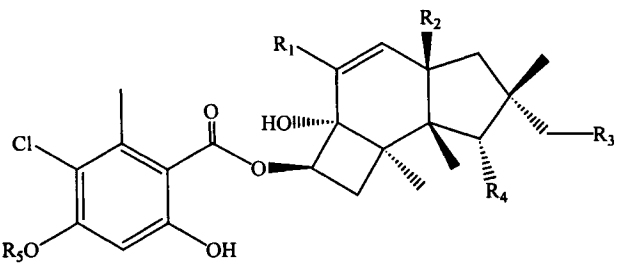


121

Terpenes

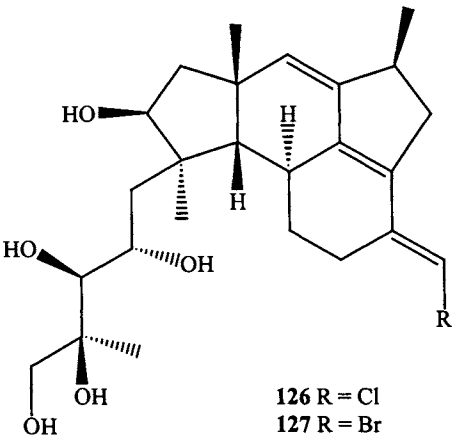
Over 30 sesquiterpene aryl esters have been isolated from *Armillaria* spp.; of these, eight are esterified with chlorinated orsellinate. The honey mushroom, *A. mellea,* produces 70 mg of the chlorinated compound armillaridin (**122**) per kg dry mycelium [148], while liquid cultures of *A. ostoyae* contain the chlorinated compounds, melledonal C (**123**) (50 mg/l) and melleolide D (35 mg/l) (**124**) [149]. *Clitocybe elegans* is the only other genus for which chlorinated sesquiterpene aryl esters have been reported [150], including melledonal D (**125**), which has not yet been detected in *Armillaria* spp. The pathogenic basidiomycete, *Armillaria* causes root disease in both coniferous and deciduous trees. *Armillaria*

spp. were the most frequently isolated fungi associated with root in living spruce and balsam fir trees in Ontario, Canada [151]. It is not known yet what makes some of the *Armillaria* spp. such virulent parasites, but secondary metabolites are thought to be the major cause. In a test for fungi or phytotoxicity of fourteen *Armillaria* metabolites, four of them chlorinated, the toxicity decreased with increasing hydrophobicity, *e.g.* methylating the hydroxy group of the orsellinate and/or adding a chlorine atom [152]. Sonnenbichler *et al.* [149] found that increased amounts of melledonal C (**123**) and other nonchlorinated sesquiterpene aryl esters were produced in cultures of *A. ostoye* growing in the presence of an antagonistic fungus or host plant cells [149,152]. The biosynthesis of the chlorinated metabolite was enhanced up to 5-fold upon antagonization [149], indicating that the physiological purpose of the sesquiterpene aryl esters is their antibiotic activity.



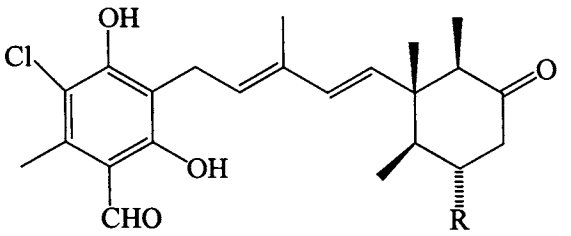
	R_1	R_2	R_3	R_4	R_5
122	CHO	H	H	H	Me
123	CHO	OH	H	OH	Me
124	CHO	OH	OH	OH	Me
125	CH_2OH	OH	H	OH	Me

Two novel sesterterpenes, neomangicols A (**126**) and B (**127**), were isolated from the mycelial extract of a marine fungus belonging to the genus *Fusarium*. The carbon skeleton of the neomangicols is undescribed and constitutes a new class of C25 rearranged sesterterpenes. Neomangicol A was most active against MCF-7 (human breast carcinoma) and CACO-2 (human colon carcinoma) cell lines, displaying IC_{50} values of 4.9 and 5.7 μM, respectively. Neomangicol B was less active, having a mean IC_{50} value of 27 μM across the entire cell panel (versus 10 μM for neomangicol A), while neomangicol B displayed antibacterial activity similar to that of the known antibiotic, gentamycin, against the Gram-positive bacterium *Bacillus subtilis* [153].



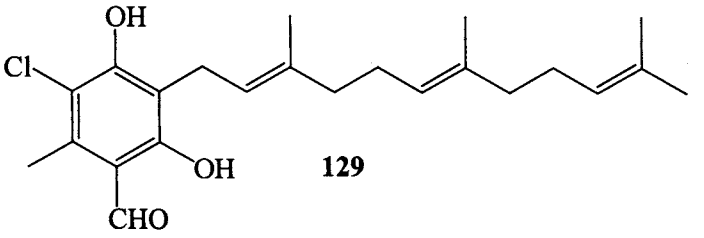
126 R = Cl
127 R = Br

The fungal metabolite ascochlorin (**128**), which is produced by *Ascochyta viciae* [154-156], was simultaneously isolated as "LL-Z1272*y*" from *Fusarium* sp. [157] and as "ilicicolin D" from *Cylindrocladium ilicicola*, a fungus of dead beech leaves [158,159]. Other metabolites from these fungi include: LL-Z1272α (**129**), LL-Z1272λ (**130**) [157,158], and cylindrochlorin (**131**) [160]. The fungus *Nectria coccinea* also produces several of these metabolites, including the new chloronectrin (**132**) [161]. The hydroxy analog (**133**) was produced by *Ascochyta viciae* [162]. The tobacco pathogen *Colletotrichum nicotianae* produces colletochlorin B (**134**), C (**135**), and D (**136**) [163-165]. The hypolipidemic active metabolites, ascofuranone (**137**) and ascofuranol (**138**), have been isolated from *Ascochyta viciae* [166]. The fungus *Acremonium luzulae* also produces ascochlorin (**138**) [167], while *Strobilurus tenacellus* and *Mycena* spp. contain the new strobilurin B (**139**) [168].

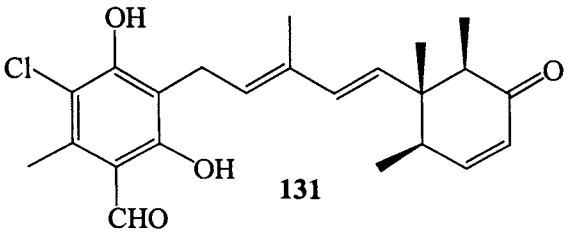


128 R = H

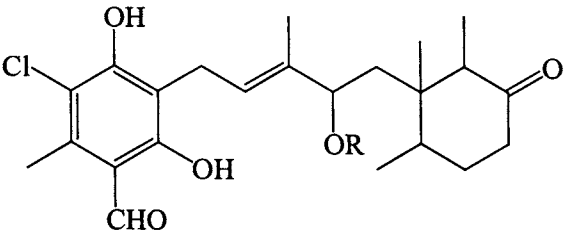
130 R = OAc



129

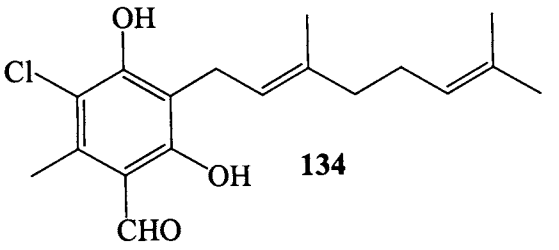


131

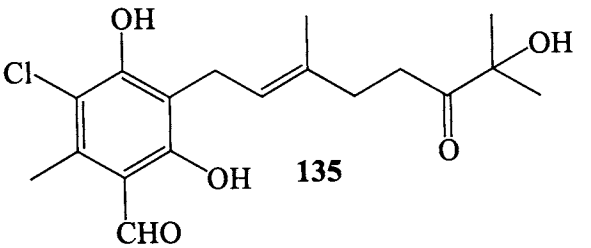


132 R = Ac

133 R = H



134



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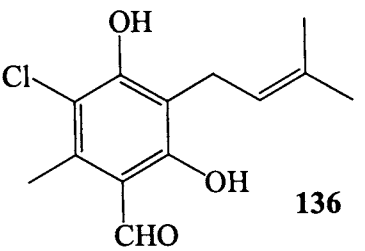

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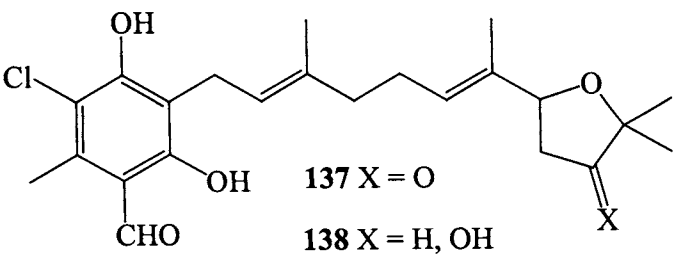
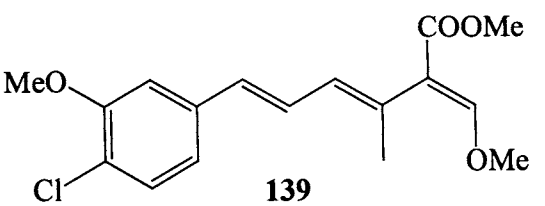


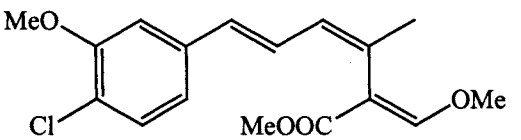
137 X = O

138 X = H, OH



139

The strobilurins were first isolated from *Strobilurus tenacellus* [169]. The chlorinated strobilurin B (**140**) has been isolated from three genera and seven species, *e.g. Mycena alkalina, M. avenacea, M. crocata, M. vitilis, Xerula longipes, X. melanotricha,* and *S. tenacellus.* Chlorinated oudemansin B (**141**) is produced by *X. melanotricha* [170]. The chlorinated and nonchlorinated strobilurins and the closely related oudemansins are new respiration inhibitors, binding to cytochrome b. Thirteen strobilurins and three oudemansins have been isolated so far. Their high antifungal activity against phytopathogenic fungi and insects and their low toxicity toward mammals and bacteria make them attractive lead compounds for the synthesis of agricultural fungicides [171]. Most fungi that produce strobilurins and oudemansins grow on wood. *Oudenmansiella mucida* also produces a nonchlorinated strobilurin on sterilized beech wood. Therefore, it appears that the strobilurins play a role in securing nutrient resources for the producers from competing fungi [171]. The aromatic ring and the benzylic carbon atom in the strobilurins and oudemansins are derived from the shikimate pathway, whereas the side chain is built up from acetate units [171].



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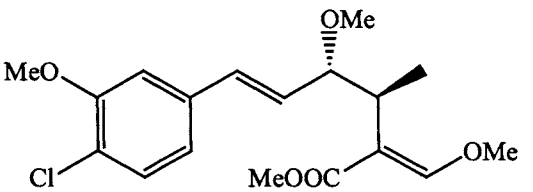
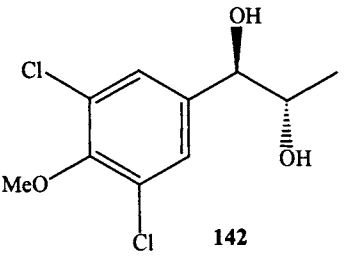


141

Aromatic Compounds

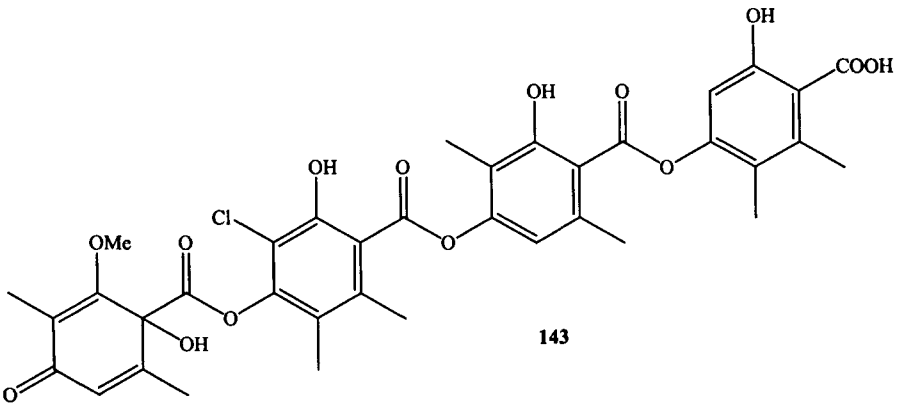
Two strains of the basidiomycete, *Bjerkandera adusta* produces in static liquid culture, phenyl, veratryl, anisyl, and chloroanisyl metabolites as well as a series of compounds not previously known to be produced by *Bjerkandera* species. A new metabolite, for which the name bjerkanderol B (**142**) was given, has been proposed. Experiments with static liquid cultures supplied with $^{13}C_6$- and $^{13}C_9$-*L*-phenylalanine showed that all

identified aromatic compounds (with the exception of phenol) could be derived from L-phenylalanine. For the aryl propane diols, the ^{13}C label appeared only in the phenyl ring and the benzylic carbon, suggesting a stereoselective resynthesis from a C_7 and a C_2-unit, likely aromatic aldehyde and decarboxylated pyruvate, respectively. For both strains, cultures supplied with $Na^{37}Cl$ showed incorporation of ^{37}Cl in all identified chlorometabolites. The compounds have been reported to exhibit important physiological functions in this white rot fungus. Possible mechanisms for their formation through the newly discovered compounds have been discussed [172].



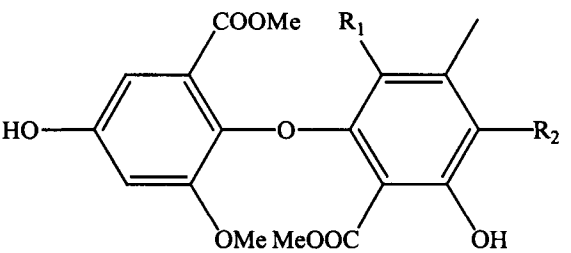
142

A new D-glucose-6-phosphate phosphohydrolase (G6Pase) inhibitor (**143**), CJ-21,164 was isolated from the fermentation broth of the fungus *Chloridium* sp. The structure was elucidated to be a novel tetramer of the salicylic acid derivatives by spectroscopic analyses. The compound inhibited G6Pase in rat liver microsomes with an IC_{50} of 1.6 μM. Glucose output from hepatocytes isolated from rat liver was inhibited when it was present in the incubation medium, consistent with the role of this compound as a G6Pase inhibitor. It dose-dependently inhibited G6Pase, with an IC_{50} value of 1.6 μM. At a concentration of 133 μM, this compound inhibited the rate of glucose output stimulated with 25 nM glucagon by 81 %. Hepatocytes incubated with this compound did not show significant cytotoxicity at these concentrations, suggesting that the reduction in glucose output might not be a consequence of cytotoxicity [173].



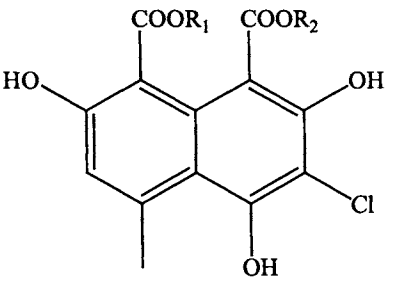
143

In the search for new, naturally occurring, anti-angiogenic compounds, it was found that a culture broth of an unidentified fungal strain B90911 exerted inhibitory activity on capillary-like tube formation of human umbilical vein endothelial cells (HUVEC) *in vitro*. Active compounds were isolated by bioassay-guided separation and their structures were identified to be two new asterric acid derivatives, i.e. 3-chloroasterric acid (**144**) and 3,5-dichloroasterric acid (**145**), by spectroscopic analyses. These compounds significantly inhibited (10 μg/ml) the VEGF-induced tube formation of HUVEC, suggesting that asterric derivatives could be useful for further study as anti-angiogenic agents [174].



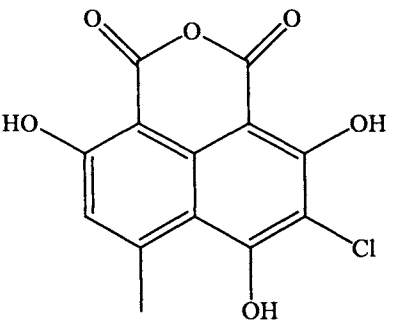
144 $R_1 = H, R_2 = Cl$
145 $R_1 = R_2 = Cl$

Simple naphthalene metabolites (**146** and **147**) were produced by *Verticillium lamellicola* [175] and (**148**) found in the fungus *Scolecobasidiella avellanea.*



146 $R_1 = H, R_2 = Me$

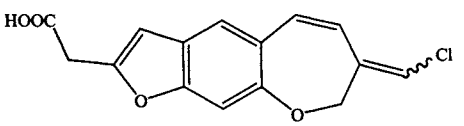
147 $R_1 = Me, R_2 = H$



148

The structures of two novel fungal antibiotics, isolated from a *Pterula* species that interfere with the NADH: ubiquinone oxidoreductase and inhibit the respiration of eucaryotes, were determined by spectroscopic techniques. The compounds, pterulinic acid (**149**) and pterulone (**150**), contain a 1-benzoxepin ring system and are chlorinated [176]. In the serial dilution assay, both compounds showed antibacterial activities at concentrations up to 100 μg/ml (*Acinetobacter calcoaceticus*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus brevis*, *Bacillus subtilis*, *Micrococcus luteus*, *etc.*). They exhibited weak cytotoxicity activities toward L1210 and BHK cells but showed moderate activity toward HL60 and HeLa cells. In contrast, **150** was not cytotoxic against L1210, HL60, BHK, and HeLa cells at concentrations up to 100 μg/ml and has phytotoxic activities. The germination of *Lepidium sativum* and *Setaria italica* was inhibited at concentrations 10-50 μg/ml.

Both compounds neither showed nematocidal activity against *Meloidogyne incognita* and *Caenorhabditis elegans*, nor hemolytical effects at concentration up to 100 μg/ml.



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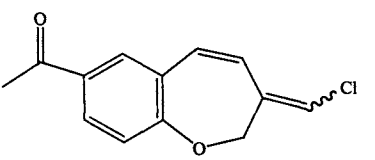
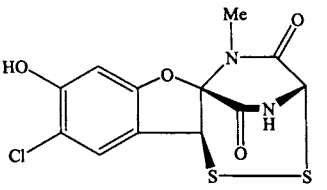


150

Antibiotic aspirochlorine was originally isolated from *Aspergillus tamarii* [177], later from *A. flavus* [178] and *A. oryzae* [179], and shown

to have the novel structure (**151**) by X-ray crystallography and total synthesis [180]. Armillaridin (**152**) is a novel phenolic sesquiterpene containing a cyclobutane ring that is produced by *Armillaria mellea* [181]. Later work with this organism revealed the related metabolites: melleolide D (**153**) [182], melledonals B (**154**), C (**155**) [183] and armillaricin (**156**) [184].



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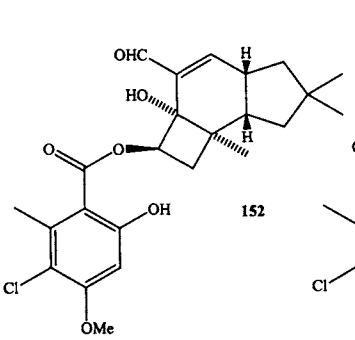
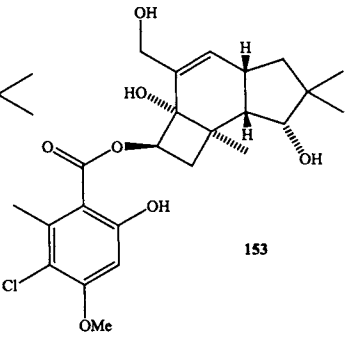
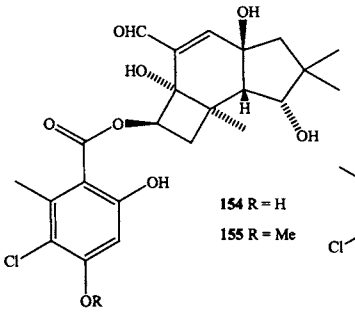


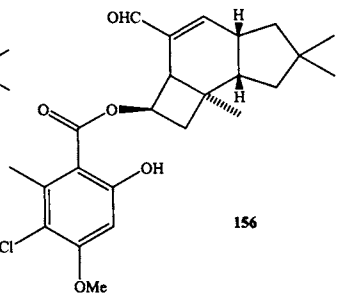
152



153

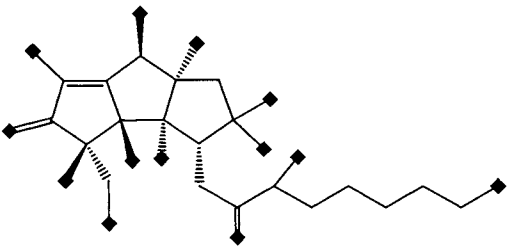


154 R = H
155 R = Me



156

In one of the few studies of marine fungi, the novel chloriolins A-C (*e.g.* the structure of chloriolin B, **157**) have been reported from a cultured, unidentified fungus associated with the sponge *Jaspis johnstoni* [185].



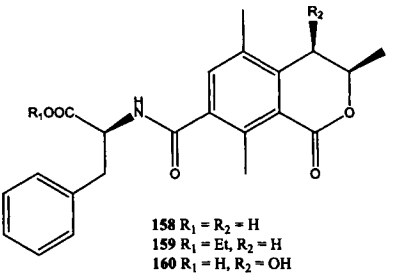
157

Coumarins and Isocoumarins

One of the most ubiquitous of all fungal metabolites is the isocoumarin derivative ochratoxin A (**158**), which was first isolated from *Aspergillus ochraceus* [186,187] and later from *Penicillium viridicatum* [188], *P. cyclopium*, *P. commune*, *P. variabile*, *P. purpurescens* [189], *Aspergillus melleus* and *A. sulphureus* [190]. The related ochratoxin C (**159**) is produced by *Aspergillus ochraceus* [191], and 4-hydroxyochratoxin A (**160**) was isolated from cultures of *Penicillium viridicatum* [192]. When ochratoxin A was administered in the diet, hepatocellular tumors, renal-cell tumors, hepatomas and hyperplastic hepatic nodules were observed in male mice. Incidence of and mortality from urothelial urinary tract tumors have been correlated with the geographical distribution of Balkan endemic nephropathyin. Ochratoxin A has been detected in moldy cereals including wheat, maize, rye, barley and oats, peanuts, coffee beans, bread, flour, rice, peas and beans from 0.03 to 27.5 ppm. Residues of ochratoxin A have been detected in samples of meat from animals slaughtered immediately after consuming contaminated feed and were detected at levels of 10 to 920 μg/kg in sausage, ham and bacon samples.

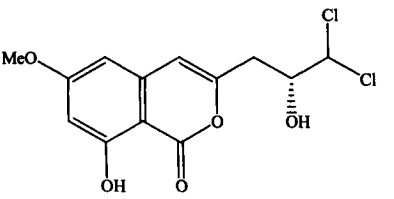
The quantification of ochratoxin A, at levels within the range 0.25-10 ng/ml from wine by HPLC-fluorescence detection, was described [193]. RP-HPLC - fluorescence method for the detection of ochratoxin A in wine with a detection limit of 0.05 ng/ml was also published [194]. A stable isotope dilution assay by LC-MS/MS was developed for quantification of the ochratoxin A by using $[D_5]$-ochratoxin A as internal standard with a low detection and quantification limits of 0.5 and 1.4 μg/kg, respectively [195]. The LC-MS/MS method (ESI and APCI) was also applied to the analysis of contaminated coffee samples by ochratoxins A and B with absolute minimum detection limit around 10-20 pg per injection. Fragment ions from the $[M+H]^+$ and $[M+Na]^+$ ions of

both ochratoxins were monitored in the multiple reaction monitoring mode [196].



158 $R_1 = R_2 = H$
159 $R_1 = Et$, $R_2 = H$
160 $R_1 = H$, $R_2 = OH$

The metabolite profiles of three different isolates of *Penicillium nalgiovense* from cheese were analyzed. The novel isocoumarin metabolite, dichlorodiaportin (**161**) was isolated from the cultures of *P. nalgiovense* along with further metabolites. The reason isocoumarins occur naturally and the possible toxicity or health benefits of these compounds in cheese and other relevant food products should be investigated [197].

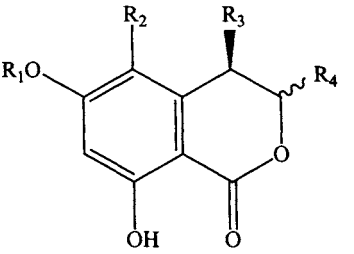


161

The structures of four new biologically active nematocidal halogenated dihydroisocoumarins (**162-165**) isolated from submerged cultures of the wood-inhabiting ascomycete *Lachnum papyraceum* have been elucidated by spectroscopic methods. The compounds are structurally related to lachnumon and mycorrhizin A, which are also produced by the fungus [198].

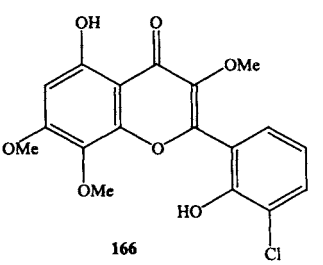
The brominated metabolites isolated from *L. papyraceum* in this investigation are further examples of how bromine can be introduced into normally chlorinated fungal metabolites when bromide salts are added to the culture medium. Besides obtaining derivatives that are useful for QSAR studies and for assessing the importance of the halogen atom for the biological activity of the metabolites, the shifts in the secondary metabolism of the fungus induced by the addition of bromide to the

culture medium may also be helpful during studies of the biosynthesis of mycorrhizins.

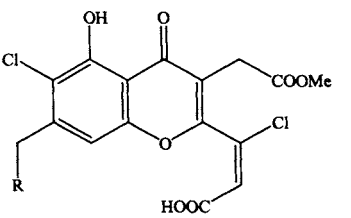


	R_1	R_2	R_3	R_4
162	H	H	Br	(R)-Me
163	H	OH	Cl	(S)-Me
164	H	OH	Cl	(R)-Me
165	Me	H	Cl	(R)-Me

Chlorflavonin (**166**) was discovered in the culture broth of *Aspergillus candidus* [199-201]. A biosynthetic study of this fungal metabolite indicates that it is a true metabolite and is synthesized *de novo* by this microbe [202]. It was also detected in *Aspergillus candidus* and *A. campestris* [203]. The fungus *Monilinia fructicola* produces chloromonilinic acids A (**167**) and B (**168**) derived from chloromonilicin [204], to be discussed later.

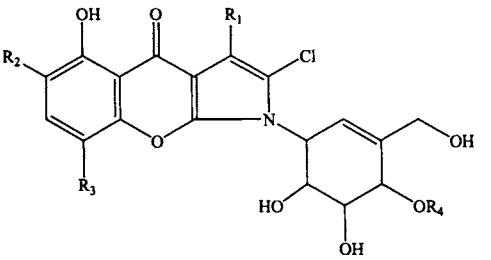


166

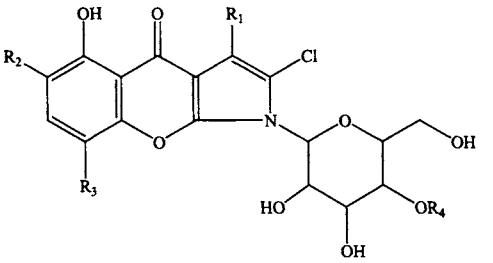


167 R = OH
168 R = H

The strain of *Actinamadura spiralis* isolated from a soil sample produced new antibiotics, pyralomicins (**169-175**). Pyralomicins inhibited the growth of *Micrococcus luteus* and *Escherichia coli* at the concentration of 0.2-25 μg/ml by agar dilution method. Pyralomicin la (**169**) and pyralomicin 2a (**173**) did not show any acute toxicity in mice at 100 mg/kg ip [205].



	R_1	R_2	R_3	R_4
169	H	Cl	Me	Me
170	H	Me	Cl	Me
171	H	Cl	Me	H
172	Cl	Cl	Me	H

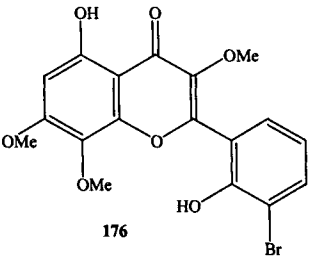


	R_1	R_2	R_3	R_4
173	H	Cl	Me	Me
174	H	Me	Cl	Me
175	H	Cl	Me	H

A new antifungal agent, CJ-19,784 (**176**) was isolated from the fermentation broth of a fungus, *Acanthostigmella* sp. This compound inhibits the growth of pathogenic fungi, *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* with IC_{50} values of 0.11, 20 and 0.54 μg/ml, respectively. Compound **176** and 3′-Cl analog did not exhibit significant activity against HeLa cells.

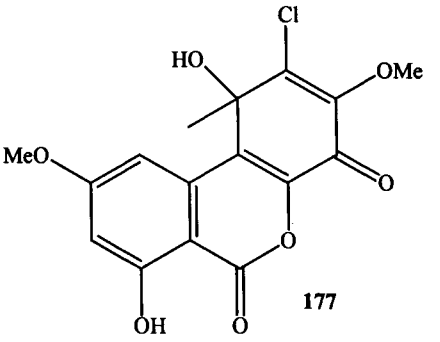
It is well known that the production of halogenated microbial metabolites depends on the presence of halogen atoms in the fermentation medium. For example, chlortetracyclin and chloromonilicin are produced well under the fermentation in chlorine-containing media, and the chlorine atom is easily exchanged for a bromine atom by replacing the media with bromine-containing media. It is very interesting that

compound **176** but not 3′-Cl analog was produced as a major product under the fermentation in chlorine-containing medium (0.2 % sodium chloride). This suggests that the fungus *Acanthostigmella* sp. would have unknown biosynthetic mechanisms enabling prior use of the bromine atom [206].



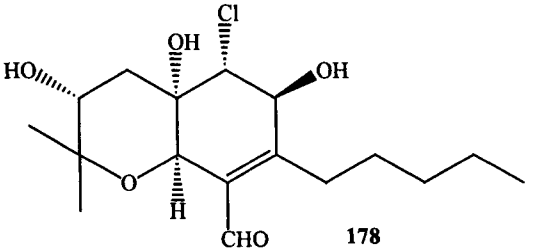
176

A novel inhibitor of STAT6 activation, named as TMC-264 (**177**), was discovered from the fermentation broth of *Phoma* sp. TC 1674. TMC-264 suppressed expression of IL-4 driven luciferase and germline C epsilon mRNA with IC_{50} values of 0.3 μM and 0.4 μM, respectively. It exhibited a potent inhibitory activity against tyrosine phosphorylation of STAT6 with an IC_{50} value of 1.6 μM, whereas weakly inhibited tyrosine phosphorylation of STAT5 with an IC_{50} value of 16 μM, but did not inhibit the phosphorylation of STAT1 up to 40 μM. TMC-264 blocked formation of the complexes between phosphorylated STAT6 and STAT6 oligonucleotides in a dose-dependent manner, while it did not affect the formation of phosphorylated STAT1/STAT1 oligonucleotides complexes. These results suggested that TMC-264 selectively inhibited IL-4 signaling by interfering both with phosphorylation of STAT6 and binding of the phosphorylated STAT6 to the recognition sequence [207]. The structure was elucidated on the basis of NMR analyses of normal abundance and biosynthetically ^{13}C enriched TMC-264 (**177**) [208].



177

In the process of screening microbial extracts from endophytic *Cytospora* sp., three novel compounds cytosporins A, B and C (**178**) were found as specific inhibitors of angiotensin II binding to receptors of AT_2. The IC_{50} of component A in the biochemical assay was found to be approximately 25-30 μM in AT_1 and 1.5-3 μM in AT_2. These results were obtained by blocking one of the two angiotensin II binding sites in rat adrenal glands. Further analysis suggested reversible inhibition by this compound, and thus it appears to be a competitive inhibitor [209].



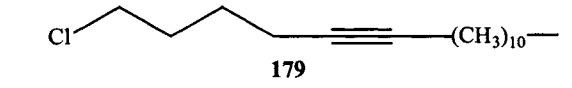
178

Miscellaneous Compounds

In addition to the numerous fungal and lichen metabolites discussed in the preceding sections, there are several others that do not easily fit into the well-defined structural categories.

Several other chlorinated aliphatic metabolites are produced; a volatile organohalogen, 1-chloro-5-heptadecyne (**179**) was detected in an edible wild milk cap *Lactarius* spp. [210]. Lepiochlorin (**180**), an antibacterial lactol, was isolated from liquid cultures of a *Lepiota* sp., a fungus cultivated by gardening ants [211]. Another aliphatic halogenated compound with an interesting trichloromethyl group was isolated from the mycelium of the fungus *Resinicium pinicola* in 120 mg/kg yield [212].

The compound, pinicoloform (**181**), showed antibiotic and cytotoxic activities.



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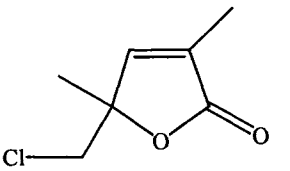

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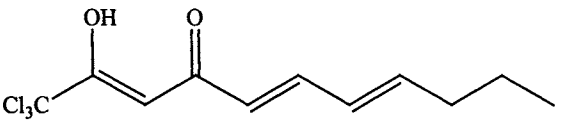
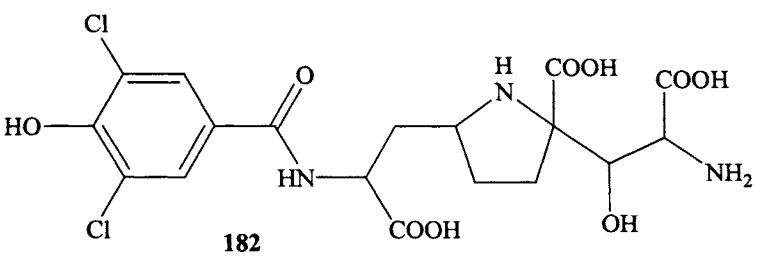


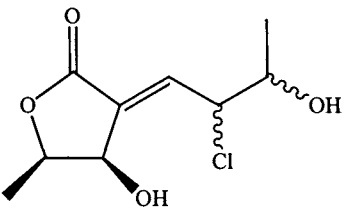
181

Kaitocephalin (**182**) was isolated from *Eupenicillium shearii* and protected chick primary telencephalic and rat hippocampal neurons from kainate toxicity at 500 μM with EC_{50} values 0.68 μM and 2.4 μM, respectively, without showing any cytotoxic effect. Although a well-known AMPA/KA antagonist CNQX with a quinoxalinedione skeleton effectively protected chick primary telencephalic neurons from kainate toxicity with EC_{50} value 0.53 μM, it exhibited cytotoxicity against chick primary telencephallic and rat hippocampal neurons at the concentrations of 20 μM and 2 μM, respectively. Kaitocephalin also protected chick primary telencephalic and rat hippocampal neurons from AMPA/cyclothiazide (500 μM/50 μM) toxicity with EC_{50} values 0.6 and 0.4 μM, respectively. Kaitocephalin is the first AMPA/KA antagonist from nature, consisting of a quite different skeleton from other known AMPA/KA antagonists [213].



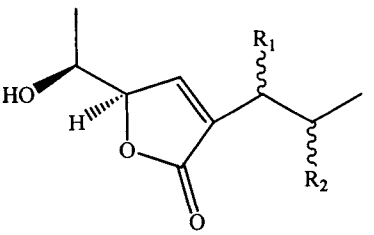
182

The salt-water culture of *Aspergillus ochraceus* separated from the Indo-Pacific sponge *Jaspis coriacea* has yielded two new chlorine containing polyketides, chlorocarolide A (**183**) and B (**184**). These compounds have an overall structural analogy to penicilic acid whose biosynthesis has been intensely studied. The structures and stereochemical features of the chlorocarolides were reported [214].

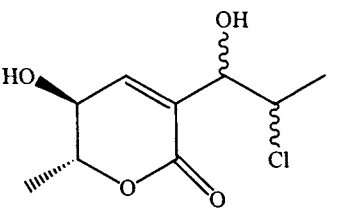


183 7R* 8R*
184 7S* 8S*

Three new chlorine-containing compounds (**185-187**) together with penicillic acid were obtained from a marine-derived fungus *Aspergillus ostianus* isolated from a marine sponge at Pohnpei. Compound **185** inhibited the growth of *R. atlantica* at 5 μg/disc (inhibition zone 12.7 mm), while **186** and **187** were active at 25 μg/disc (10.1 and 10.5 mm, respectively). The growth of *E. coli* and *S. aureus* was also inhibited by these compounds. Compounds (**185-187**) did not inhibit the growth of *S. cerevisiae* and *M. hiemalis* even at 100 μg/disc. Compound **185** was the most potent among the three new components. Thus, the position of Cl affects the activity of these compounds [215].



185 R_1 = Cl, R_2 = OH
186 R_1 = OH, R_2 = Cl



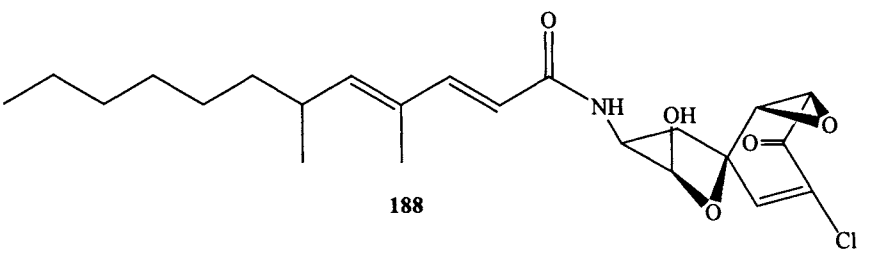
187

Two new compounds named aranochlors A (**188**) and B (**189**) were detected as minor components from the fermented broth of the fungal strain *Pseudoarachniotus roseus* [216]. Fermentations were carried out in shake flasks as well as in laboratory fermenters. For the isolation of **188** and **189** six batches of each 100 liters were processed. Both compounds were present both in the culture filtrate and the mycelium. Both aranochlors exhibited antibacterial and antifungal activities. The

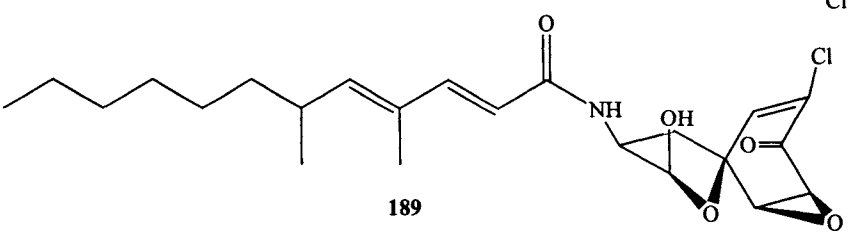
minimum inhibitory concentrations (MIC) of **188** and **189** required to inhibit a variety of bacterial and fungal strains are listed in Table 5.

Table 5. Inhibitory activities of aranochlors against different microorganisms.

Test Organism	MIC (μg/ml)	
	Aranochlor A	Aranochlor B
Staphylococcus aureus	3.12	1.56
Bacillus subtilis	3.12	3.12
Micrococcus luteus	0.39	0.39
Escherichia coli	50	25
Pseudomonas aeruginosa	>200	>200
Candida albicans	>200	>200
Saccharomyces cerevisiae	1.56	6.25
Aspergillus niger	>200	>200

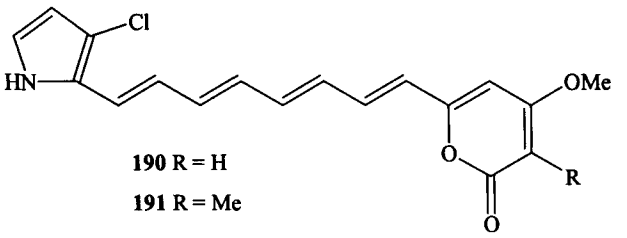


188



189

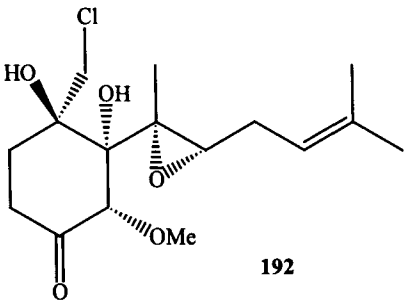
The red pigments, auxarconjugatins A and B (**190,191**) were isolated from mycelia of *Auxarthron conjugatum*, an ascomycetous fungus belonging to the Onygenaceae, in which the causative fungi of severe mycoses are also found [217].



190 R = H

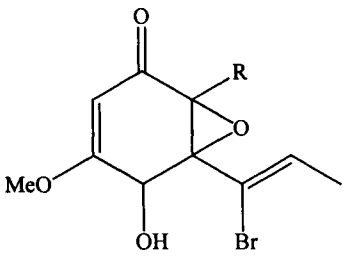
191 R = Me

Selective growth inhibition of IL-6 dependent cells was detected in fermentation extracts of a fungal strain, which was characterized as *Sporothrix* species. An active metabolite, **192** termed chlovalicin was isolated. Chlovalicin dose-dependently inhibited the growth of IL-6 dependent MH60 cells (IC_{50}, 7.5 μM) in the presence of 0.2 U/ml IL-6 and, to a lesser extent, the growth of B16 melanoma cells (IC_{50}, 38 μM). This compound did not show any antimicrobial activity at a concentration of 1 mg/ml [218].



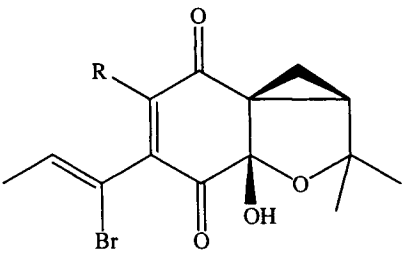
192

The structure determination of lachnumon (**193**), brominated derivatives of lachnumon (**194**) and mycorrhizin (**195**) and brominated derivatives of mycorrhizin A (**196**) was described. The compounds, which exhibit similar antimicrobial and nematocidal activities as their chlorinated analogues, were isolated from extracts of cultures of the ascomycete *Lachnum papyraceum* to which $CaBr_2$ had been added [219].



193 R = H

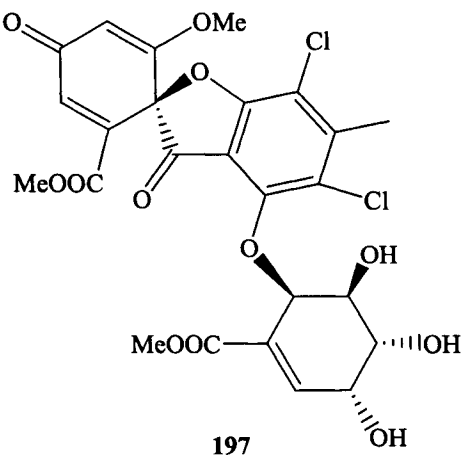
194 R = Cl



195 R = H

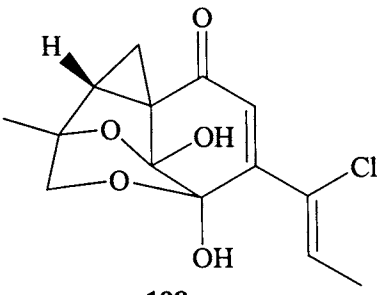
196 R = Cl

A novel fungal metabolite, Sch 202596 was discovered from the fermentation of a fungal culture *Aspergillus* sp. [220]. The fungus, *Aspergillus* sp. was isolated from the tailing piles of an abandoned uranium mine in Tuolemene County, California. Compound **197** was revealed to be a new spirocoumaranone by spectroscopy, related to the griseofulvin family of compounds. In the *in vitro* galanin receptor GALR1 assay compound (**197**) exhibited inhibitory activity with an IC_{50} value at 1.7 μM.



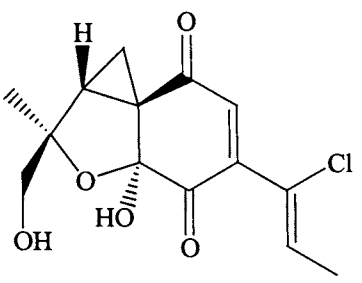
197

The structure and absolute configuration of microline, a new metabolite isolated from culture filtrates of *Gilmaniella humicola*, has been shown to be (**198**) by spectral data, chemical transformations and X-ray analysis [221].

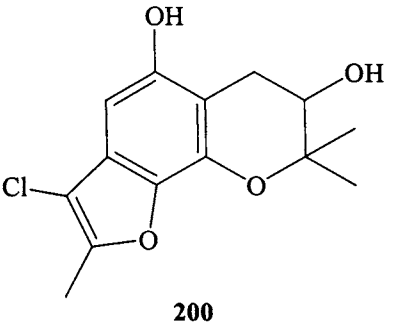


198

The structures of mikrolin and mycorrhizinol, two new metabolites isolated from culture filtrates of *Gilmaniella humicola,* have been shown to be (**199**) and (**200**), respectively, by spectral and chemical studies [222].

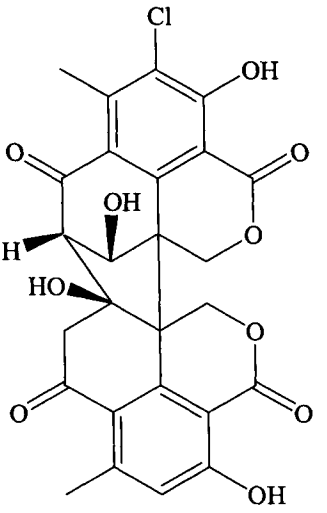


199



200

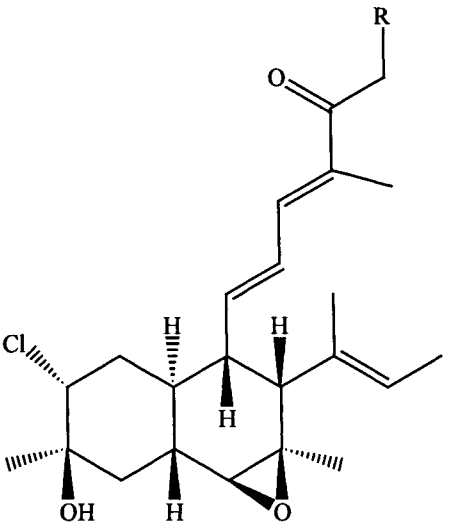
The structure and configuration of gilmaniellin, a metabolite of *Gilmaniella humicola*, has been shown to be (**201**) by X-ray analysis [223].



201

The novel substances designated as ICM0301 A-H were isolated from the culture broth of *Aspergillus* sp. F1491 [224, 225]. ICM0301s inhibited the growth of human umbilical vein endothelial cells induced by basic fibroblast growth factor with IC_{50} values of 2.2-9.3 μg/ml. ICM0301C (**202**) and ICM0301D (**203**) showed significant anti-angiogenic activity at a concentration lower than 101 μg/ml in the angiogenesis model using rat aorta cultured in fibrin gel. ICM0301s showed very low cytotoxicity against various tumor cells. The taxonomy of the producing organism, and the fermentation, isolation and biological activities of ICM0301s were described.

The structures were elucidated by spectroscopic analyses. ICM0301C and D have a substituted decalin skeleton containing one chlorine atom.



202 R = Me
203 R = H

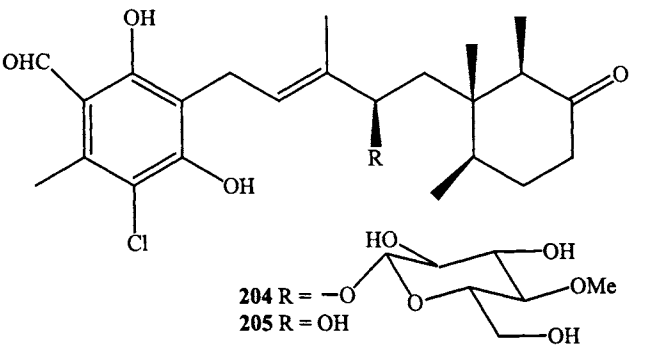
Vertihemipterin A, the ascochlorin glycoside, and its aglycone, 4′,5′-dihydro-4′-hydroxyascochlorin, and a new analog, 8′-hydroxyascochlorin, were isolated from the fermentation broth of the pathogenic fungus *Verticillium hemipterigenum* [226]. Structures of these compounds were elucidated by spectroscopic methods. Cytotoxic activities of these ascochlorin analogs were evaluated. All compounds were tested for their cytotoxic activities against three cancer cell-lines, KB, BC-1 and NCI-H187, as well as Vero cells. The compounds exhibited significant cytotoxicities against all cell lines, *e.g.* for compounds **204-206**, see Table 6.

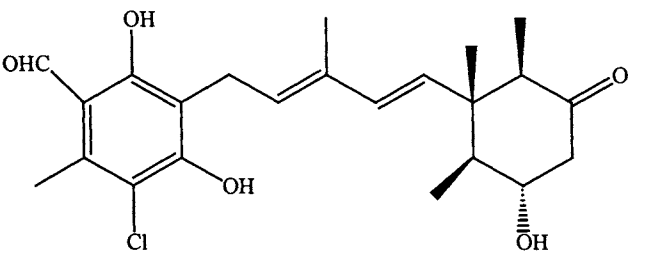
Table 6. Cytotoxic activities of compounds 204-206.

	Cytotoxicity (IC_{50}, μg/ml)			
Compound	KB[a]	BC-1[b]	NCI-H178[c]	Vero[d]
204	19	8.4	7.9	6.9
205	>20	>20	>20	38
206	2.7	1.4	2.2	3.4

[a] oral human epidermoid carcinoma; [b] human breast cancer;

[c] human small cell lung cancer; [d] African green monkey kidney fibroblast

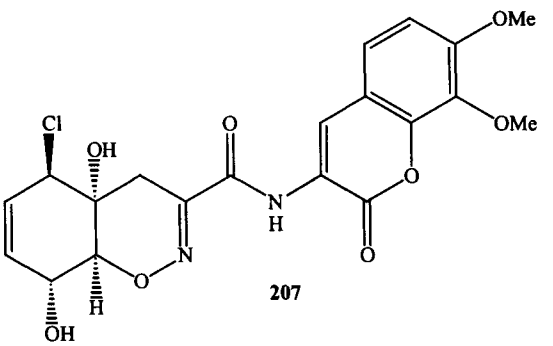




206

Trichodermamides A and B (**207**), two modified dipeptides, have been isolated from cultures of the marine-derived fungus *Trichoderma virens* [226]. Trichodermamide B displayed significant *in vitro* cytotoxicity against HCT-116 human colon carcinoma with an IC_{50} of 0.32 μg/ml. This metabolite also exhibited moderate antimicrobial activities against amphoterocin-resistant *C. albicans*, methacillin-resistant *S. aureus*, and vancomycin-resistant *E. faecium* with MIC values of ca. 15 μg/ml against

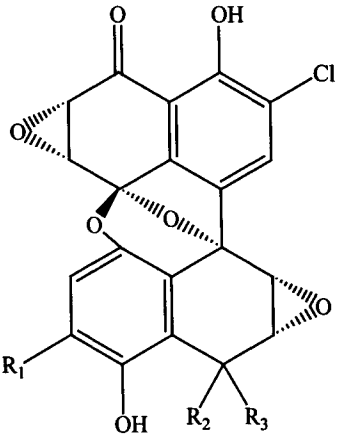
all three strains. Trichodermamide A was completely inactive in all of these bioassays, suggesting that the chlorine atom is an essential part of the pharmacophore. Chlorination is known to play an essential role in the activity of numerous, structurally diverse natural products including the antibiotics vancomycin and chloramphenicol and the antitumor compounds cryptophycin and rebeccamycin [227]. In the case of trichodermamide B (**207**), the chlorohydrin moiety at C4 and C5 might be a precursor of an epoxide, which could be the biologically active molecular form of this molecule.



207

The spiroxins **208-210** were purified from the culture extract of a marine-derived fungus, isolated from a soft orange coral collected from the waters near Vancouver Island, Canada [228]. Their unique *bis*-naphthospiroketal structures were established by spectroscopic methods. In addition to cytotoxicity, these compounds showed antibiotic activity and were active in a mouse xenograft model against human ovarian carcinoma. The mechanism of action of these compounds was shown to be due, in part, to their effect on DNA. Spiroxin A (**208**) showed some activity against Gram-positive bacteria but only marginal activity against Gram-negative bacteria. Compound **208** showed antitumor activity in nude mice against ovarian carcinoma (59 % inhibition after 21 days) at 1 mg/kg/dose given IP on day 1, 5 and 9 post staging. In a cytotoxicity assay, **208** exhibited a mean LC_{50} value of 0.09 μg/ml against a panel of 25 diverse cell lines. In evaluating its probable mechanism of action, it was observed that in the presence of *e.g.* 2-mercaptoethanol, **208** caused a concentration-dependent nicking of pBR322 DNA, suggesting that the compound partly exerts its cytotoxicity effect through a single-stranded DNA cleavage. Cytotoxicity of quinones has been attributed to DNA

modification, alkylation of essential protein thiol groups, oxidation of essential protein thiol groups by superoxide radicals or a combination of these mechanisms. The oxidation state of the spiroketal carbon, a masked ketone, could allow the spiroxins to behave chemically as quinone epoxides, possibly causing DNA cleavage under reducing conditions *via* an oxidative stress mechanism involving the formation of thiol conjugates. Thus, a variety of mechanisms may play a role in spiroxin-mediated cytotoxicity.

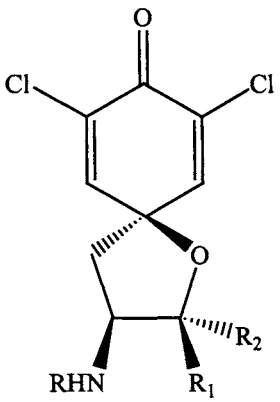


208 $R_1 = H, R_2 + R_3 = O$
209 $R_1 = Cl, R_2 + R_3 = O$
210 $R_1 = Cl, R_2 = OH, R_3 = H$

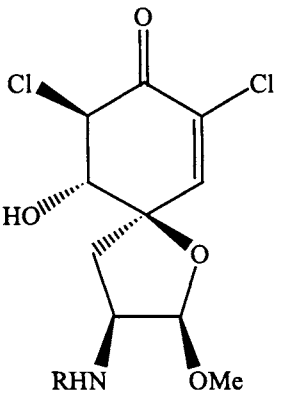
Gymnastatins A-E have been isolated from a strain of *Gymnascella dankaliensis* originally separated from the sponge *Halichondria japonica* [229]. Cytotoxic activities of compounds **211-215** were examined in the P388 lymphocytic leukemia test system in a cell culture [230]. The results showed that three of the compounds (**211-213**) exhibited potent cytotoxic activity and two (**214** and **215**) exhibited weak cytotoxic activity (ED_{50} 0.018, 0.108, 0.106, 10.5 and 10.8 μg/ml, respectively). Gymnastatin A of these compounds showed strongest cytotoxicity. This evidence suggested that conjugated ketones were important for the enhancement of cytotoxicity in gymnastatin analogs, and hence the cytotoxic activity of compound **213** resulted from a conjugated ketone, which might be derived from compound **213** in the test system.

Pericosines A (**216**) and B have been isolated from a strain of *Periconia byssoides,* originally separated from the sea hare *Aplysia*

kurodai, and their structures have been established based on spectral analyses. Pericosine A exhibited significant cytotoxicity (ED_{50} 0.12 μg/ml) in the P388 lymphocytic leukemia test system in cell culture.



211a R_1 = OH, R_2 = H
211b R_1 = H, R_2 = OH



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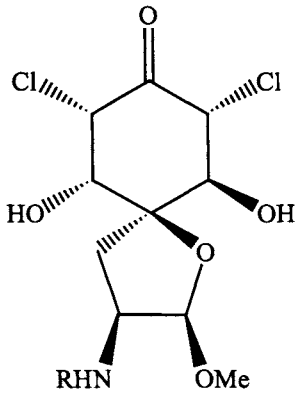
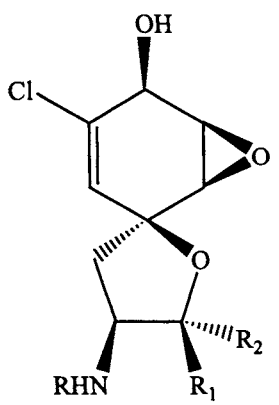
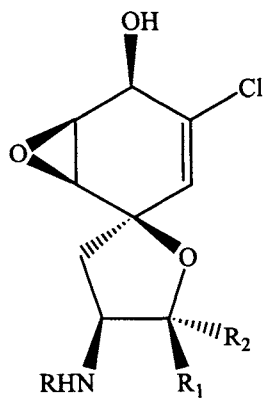


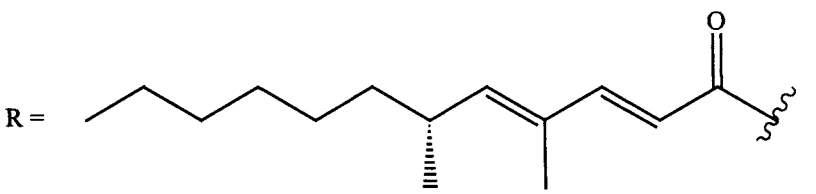
213



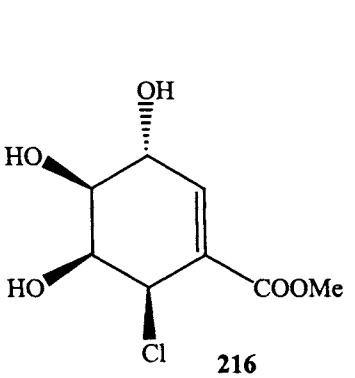
214a R_1 = OH, R_2 = H
214b R_1 = H, R_2 = OH



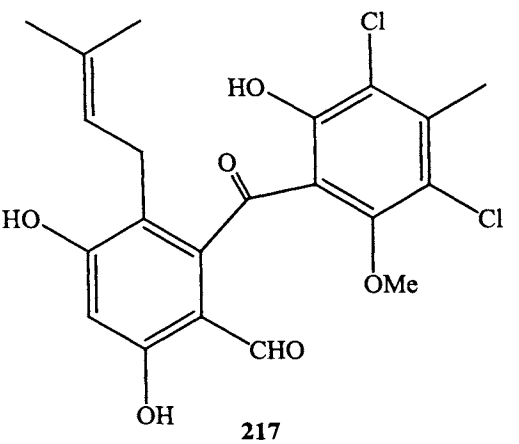
215a R_1 = OH, R_2 = H
215b R_1 = H, R_2 = OH



The isolation and structure determination of a new chlorinated benzophenone antibiotic, pestalone (**217**), is described [232]. The new compound was produced by a cultured marine fungus only when a unicellular marine bacterium strain, CNJ-328, was cocultured in the fungal fermentation. The fungus, isolated from the surface of the brown alga, *Rosenvingea* sp., collected in the Bahamas Islands, was identified as an undescribed member of the genus *Pestalotia*. Pestalone (**217**) exhibits moderate *in vitro* cytotoxicity in the National Cancer Institute's 60 human tumor cell line screen (mean GI_{50} = 6.0 μM). More importantly, pestalone showed potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MIC 37 ng/ml) and vancomycin-resistant *Enterococcus faecium* (MIC 78 ng/ml). The potency of this agent toward drug-resistant pathogens suggests that pestalone should be evaluated in more advanced, whole animal models of infectious disease.

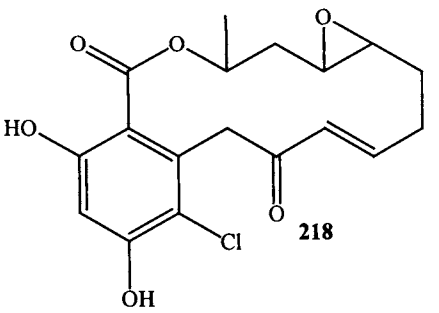


216

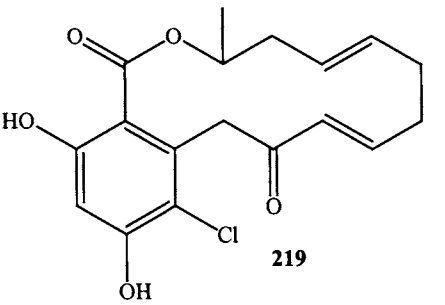


217

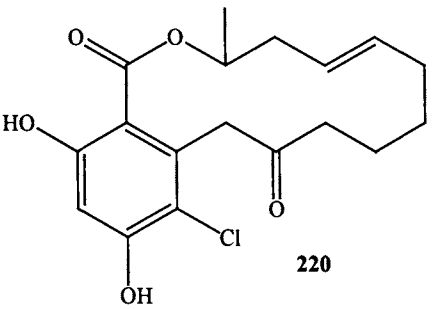
Structures of three novel compounds designated monordens C to E (**218-220**), isolated from the fermentation broth of amidepsine-producing fungus *Humicola* sp. FO-2942, were elucidated by spectroscopic evidence [233].



218

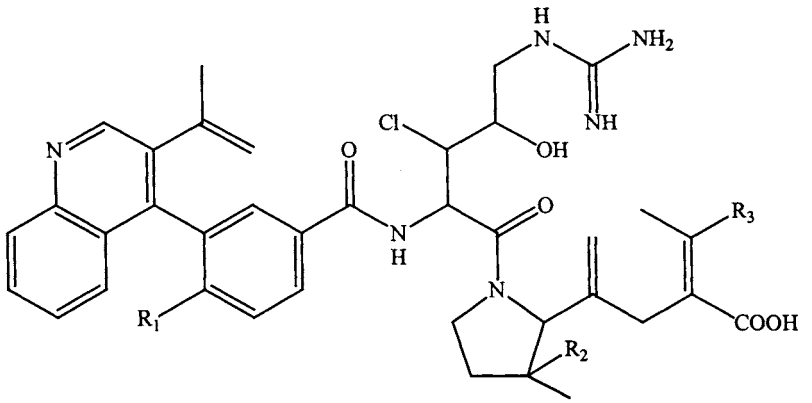


219



220

FR225659 and four related (**221-225**) compounds are novel gluconeogenesis inhibitors that consist of a novel acyl-group and three abnormal amino acids [234]. Spectroscopic analysis concluded that FR225659 is an N-acyl tripeptide consisting of a novel acyl, a 3-chloro-4-hydroxyarginine, a 3-hydroxy-3-methylproline, and a dehydrovaline. They were isolated from the culture broth of *Helicomyces* sp. No. 19353 and were purified by chromatography. These compounds inhibited the glucagon-stimulated gluconeogenesis of rat primary hepatocytes and had hypoglycemic effects in two different *in vivo* models [235].

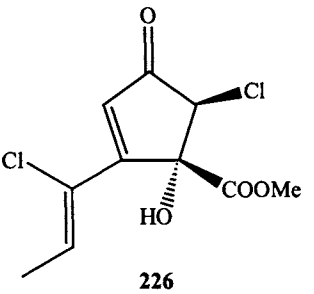


	R_1	R_2	R_3
221	OH	OH	Me
222	OH	OH	Et
223	OH	H	Me
224	OMe	OH	Et
225	OMe	OH	Me

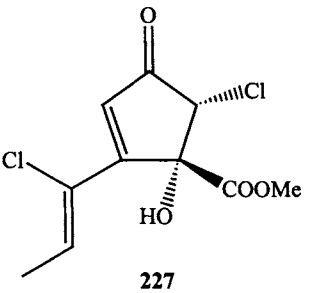
Two new biologically active cyclopentenones, VM 4798-la (**226**) and VM 4798-lb (**227**) were obtained [236] as a 3:1 inseparable mixture from fermentations of *Dasyscyphus* sp. A47-98. The mixture of the two isomers showed cytotoxic and weak antibacterial and antifungal properties (*e.g. Micrococcus luteus, Mycobacterium phlei, Candida parapsilosis, Rhodotorula glutinis, Aspergillus ochraceus* and *Zygorhynchus moelleri*).

In the serial dilution assay, **226** and **227** inhibited the growth of fungi and bacteria at 10-100 μg/disk. **226** and **227** caused a 50 % lysis of HeLa S3-, HL 60- and L1210-cells at a concentration of 10 μg/ml. The cytotoxic activity on Jurkat cells is quite significant with a 50 % lysis at 1.4 μM.

The 3:1 mixture of **226** and **227** completely inhibited the incorporation of the appropriate radioactive precursors into DNA, RNA and proteins in Jurkat cells at a concentration of 1.9 μM. This is suggested to be a consequence of the breakdown of the mitochondrias membrane potential.



226



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CONCLUSION

Fermentation of produced strains, purification, isolation and biosynthesis of griseofulvin were discussed in this review. In addition, compounds similar to griseofulvin were described. Griseofulvin is among the oldest antibiotics and a few that have been successfully used in the treatment of fungal diseases of the skin, nails, and hair. It inhibits *e.g. Trichophyton rubrum, T. mentagrophytes, T. tonsurans, Microsporum audouini, M. canis, M. gypseum,* and *Epidemophyton floccosum* and decreases growth of *Aspergillus* spp. and *Phialophora* spp. Usually, topical application is not sufficient and has to be accompanied by peroral application. In addition to its therapeutic uses, griseofulvin and its derivatives are interesting with respect to their biosynthesis, which has some specific features including introduction of the halogen atom in a reaction catalyzed probably by a halogen peroxidase, similarly to other chlorine containing antibiotics such as chlorotetracycline and chloramphenicol. It would be worth investigating, whether targeted genetic modification of production strains would lead to new derivatives of griseofulvin and related compounds and, possibly also to hybrid antibiotics with better biological activity or physico-chemical properties. In addition to antimicrobial activity, many compounds referred here exhibit other interesting biological activities, such as nematocidal, cytotoxic, antitumor and antiangiotensic *etc.* that might be used in the therapeutic practice.

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