CHAPTER II

LITERATURE REVIEW

The usual definition of natural products in the widest sense emphasizes that they are organic compounds isolated from diverse living things. These compounds may be derived by primary or rather secondary metabolism of living organisms. An important part of natural products, which are also classified as the group of small molecular secondary metabolites, usually exhibits some kinds of biological activities. The secondary metabolites isolated from microbes and exhibiting either antimicrobial (antibacterial, antifungal, antiprotozoal, and/or antiviral) activities were called as antibiotics (Bérdy, 2005).

One of the most prominent groups of microorganisms yet to be examined as a potential source of bioactive compounds are the actinomycetes of the genus *Micromonospora* (Sugawara *et al*, 1997).

1. Characteristics of *Micromonospora*

The genus *Micromonospora* (Ørskov 1923) belongs to the family Micromonosporaceae (Stackebrandt *et al*., 1997) and currently encompasses 30 validly described species (the type strain NTCT 4582^T of *Micromonospora gallica* is not available any longer, Kawamoto, 1989). This genus is well established according to its morphological and chemotaxonomic properties (Lechevalier and Lechevalier, 1970; Lechevalier *et al.*, 1977; Kroppenstedt *et al*., 1985) as well as through 16S rDNA-based phylogenetic analysis (Stackebrandt *et al*., 1997).

Micromonospora forms well-developed, branched, septate substrate mycelium and single conidia. This microorganism, normally lacking aerial mycelium, forming light yellow-orange to orange-red colonies (occasionally brown, maroon or blue-green) is composed of tightly woven, fine hyphae (0.2-0.6 μm in diameter). The dark brown to black spores are formed within and at the surface of the colonies which darken as a result of sporulation and usually turn black and may become viscid or mucoid (Sykes and Skinner, 1973). Most of *Micromonospora* strains are sensitive to pH below 6.0.

Growth occurs normally between 20 °C and 40 °C but not above 50 °C (Holt, 1989). This organism could grow in 1.5-5% NaCl concentration, normally in 3% NaCl. The temperature range for growth is 15-45 °C, and the optimal temperature is 25-30 °C.

The habitat of *Micromonospora* is generally in soil and aquatic system such as lake mud and river sediments. They were also isolated from marine environments, such as beach sand and deep marine sediment (Kawamoto, 1989).

2. Bioactive secondary metabolites from *Micromonospora*

Micromonospora is a genus of rare actinomycetes that can produce a large number of antibiotics. Approximately seven percent of actinomycetes antibiotics came from *Micromonospora* (Bérdy, 2005).

In case of the genus *Micromonospora*, there are many strains that could produce interesting bioactive compounds. The successes in discovery of secondary metabolites from such environments are shown as follows.

First of all is the famous Gentamicin **[1]**, an aminoglycoside antibiotic which can treat many types of bacterial infections, particularly gram-negative infection (Weinstein *et al.*, 1963). On that period, they just knew that gentamicin was produced by *Micromonospora*, but did not identify the species yet. After that, Berdy *et al.* (1977) gave us more information revealing that gentamicin was produced by *Micromonospora purpurea.*

Sisomicin **[2]** was the principal antibiotic produced in the fermentation of *M. inyoensis*. Sisomicin is an unsaturated aminocyclitol antibiotic having broad spectrum antibacterial activity, which was isolated from the fermentation broth by ion-exchange chromatography (Reimann *et al.*, 1973).

The fermentation broth of *M. peucetica* sp. has given an anthracycline complex whose glycosidic constituents represent a novel structural class within the family of doxorubicin **[3]** and daunorubicin **[4]** related anthracyclines, 11-deoxydaunorubicin **[5]**, 11-deoxydoxorubicin **[6]**, 11-deoxy-13-dihydrodaunorubicin **[7]**, and 11-deoxy-13-deoxodaunorubicin **[8]**. The four compounds were tested on an *in vitro* HeLa cell culture and showed IC_{50}'s ranging from 0.05 μg/ml for 11-deoxydaunorubicin **[5]** to

0.44 μg/ml for 11-deoxy-13-deoxodaunorubicin **[8]**. When tested *in vivo* on P388 leukemia, 11-deoxydaunorubicin **[5]** showed a T/C of 181 (100 mg/kg) and 11-deoxydoxorubicin **[6]** a T/C of 245 (66 mg/kg). For comparison, doxorubicin has a T/C of 213 (6.6 mg/kg) in the same test (Arcamone *et al.*, 1980).

Citreamicins α, β, γ, ζ, and η **[9-13]** (Carter *et al.*, 1989), the polycyclic xanthone structure type, were the antibacterial antibiotics isolated from *M. citrea*. As antimicrobial agents, these compounds were effective *in vitro* against a spectrum of gram-positive aerobic and anaerobic bacteria. Citreamicin η was the most potent congener with an *in vitro* MIC value <0.015μg/ml against several gram-negative strains. The compounds were structurally related to a small family of antibiotics including cervinomycin, siamomicin, lysolipin, and actinoplanone. The citreamicins were the first example of this class to be reported from *Micromonospora*, and were only the second example of the structural type to have a quinone ring fused to the pyrone.

A novel series of microbial metabolites were discovered in fermentation broths of two soil isolated. One was isolated from a soil sample collected on a construction site at Macquarie University in Sydney, Australia. The second producer was from soil collected in Henrico County, Virginia, U.S.A. Both cultures were identified as strains of *M. chalcea*. Production of the metabolites named macquarimicins A, B and C **[14-16]**. With MICs of 50 to 100 μg/ml, macquarimicin A has only very low activity against strain of *Bacteroides* and other anaerobes. Macquarimicins B and C were found to inhibit the leukemia cell line P-388 with macquarimicin B more potent than C (Jackson *et al.*, 1995; Hochlowski *et al.*, 1995).

Five novel ascomycin-like compounds, antascomicins A, B, C, D and E **[17-21]** were isolated from a strain of the genus *Micromonospora* n. sp. A92-306401, which was isolated from a soil sample collected in China. The antascomicins bind strongly to the FK506-binding protein FKBP12 and antagonize the immunosuppressive activity of FK506 and rapamycin (Fehr *et al.*, 1995).

Pyrrolosporin A **[22]** was a macrolide antitumor antibiotic processing an unusual spiro-α-acyltetronic acid moiety (Schroeder *et al.*, 1996). The antibiotic was isolated from the fermentation broth of *Micromonospora* sp. C39217-R109-7 (ATCC 53791) which was an actinomycete strain isolated from a soil sample collected at Puerto Viejo,

Peru. Pyrrolosporin A showed antimicrobial activity against gram-positive bacteria and it was weakly active against gram-negative bacteria. Pyrrolosporin A prolonged the life span of mice inoculated with P388 leukemia cells (Lam *et al.*, 1996).

A novel bioactive cyclic thiodepsipeptide, thiocoraline **[23]** (Baz, Cañedo and Puentes, 1997), was isolated from the mycelial cake of a marine actinomycetes strain L-13-ACM2-092. Based on morphological, cultural, physiological, and chemical characteristics, strain L-13-ACM2-092 was ascribed to the genus *Micromonospora.* This strain was isolated from a marine soft coral collected at the Indian Ocean near the coast of Mozambique. Thiocoraline showed a potent cytotoxic activity against P-388, A-549 and MEL-28 cell lines, and also strong antimicrobial activity against gram-positive microorganisms. This compound binds to supercoiled DNA and inhibits RNA synthesis (Romero *et al.*, 1997).

M. echinospora subsp. *echinospora* Y-03559J was found to produce a novel isonitrile compound, YM-47515 **[24]**. This strain was isolated from a soil sample collected in Chichibu-shi, Saitama, Japan. Compound YM-47515 showed promising antimicrobial activity against gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) (Sugawara *et al.*, 1997).

The antibiotic Ao58A **[25]**, which showed strong antifungal activity against some plant pathogenic fungi, was purified from the culture broth and mycelial mats of *M. coerulea* strain Ao58 using various chromatographic procedures. This antinomycete strain Ao58 was isolated from the sea-mud soil at Young-Jong Island in Korea. The antibiotic Ao58A was the glutarimide antibiotic streptimidone, 4-(2-hydroxy-5,7-dimethyl-4-oxo-6,8-nonadienyl)-2,6-piperidineione. This antibiotic Ao58A was very effective in inhibiting growth of *Phytophthora capsoci*, *Didymella bryoniae*, *Magnaporthe grisea*, and *Botrytis cinerea* in the range ~3-10 μg/ml of MICs. The antibiotic Ao58A was equally as effective as metalaxyl, vinclozolin, and tricyclazone in the control of these plant diseases. However, it did not show any phytotoxicity on the plants even when treated with 500 μg/ml (Kim, Moon, and Hwang, 1999).

Arisostatins A and B **[26-27]** have been found from a culture broth of *Micromonospora* sp. TP-A0316 isolated from the seawater sample collected in Toyama

Bay, Japan. They showed potent *in vitro* activity against gram-positive bacteria and some solid tumor call lines (Igarashi *et al.*, 2000).

A novel bioactive macrolide, IB-96212 **[28]** has been isolated from the fermentation broth of a marine actinomycete, L-25-ES25-008 that was isolated from a sponge collected at the Indian Ocean near the coast of Mozambique. The strain belongs to genus *Micromonospora.* The macrolide showed a very strong cytotoxic activity against P-388, and lower but significant activity against A-549, HT-29, and MEL-28 cell lines (Fernandez-Chimeno *et al.*, 2000).

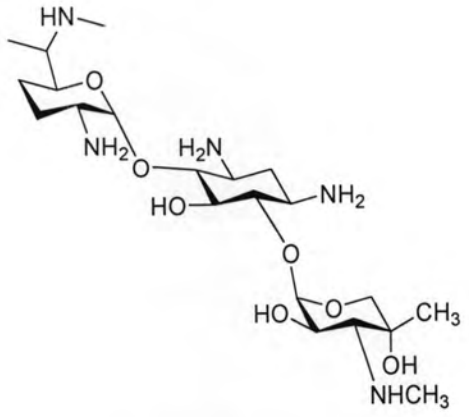
Two potent antitumor antibiotics, lomaiviticins A and B **[29-30]** with dimeric diazobenzofluorene glycoside structures, were isolated from the culture broth of *M. lomaivitiensis.* Lomaiviticins A and B were demonstrated to be DNA-damaging agents by BIA, both with a minimum induction concentration $\leq$ 0.1 ng/spot. The more abundant lomaiviticin A also showed cytotoxicity with IC_{50} values ranging from 0.01 to 98 ng/ml. Lomaiviticins A and B were also potent antibiotics against gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecium* (MIC's, 6-25 ng/spot) in a plate assay (He *et al.*, 2001).

Micromonospolides A-C **[31-33]** were isolated from a new species of the genus *Micromonospora* and their structures were elucidated to be bafilomycin-type macrolides which had a 16-membered lactone ring. Micromonospolides A-C inhibited gastrulation of starfish embryos at minimum inhibitory concentrations of 0.010, 0.011 and 1.6 μg/ml, respectively (Ohta *et al.*, 2001).

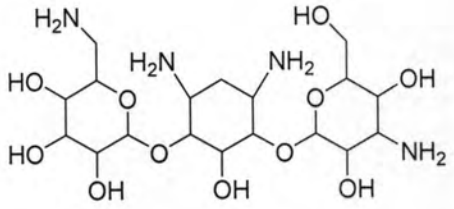
Mycinamicin II **[34]**, a 16-membered macrolide antibiotic, was produced by *M. griseorubida*, and has a strong antibacterial activity against gram-positive bacteria (Anzai *et al.*, 2003).

Diazepinomicin **[35]**, a new dibenzodiazepine alkaloid, was isolated from the culture of a marine actinomycete of the genus *Micromonospora* strain DPJ12. *Micromonospora* strain DPJ12 isolated from *Didemnum proliferum* Kott. collected by scuba at Shishijima Island, Japan. Diazepinomicin showed modest antimicrobial activity against selected gram-positive bacteria with MICs of about 32 μg/ml (Charan *et al.*, 2004).

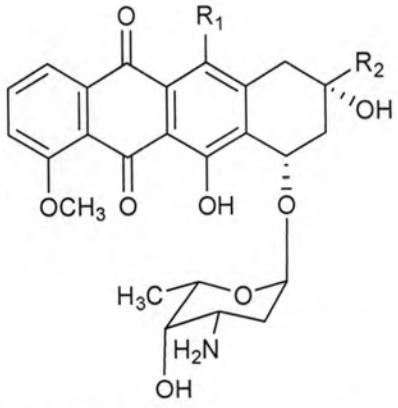
The structures of those bioactive metabolites from *Micromonospora* strains are shown below in Figure 2.1.



Gentamicin [1]



Sisomicin [2]



Doxorubicin [3]: R_1 = OH, R_2 = $COCH_2OH$

Daunorubicin [4]: R_1 = OH, R_2 = $COCH_3$

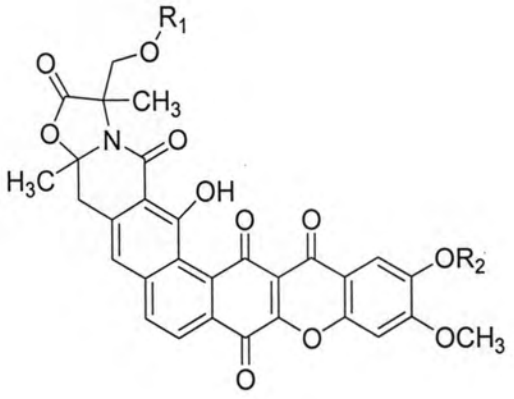
11-deoxydaunorubicin [5]: R_1 = H, R_2 = $COCH_3$

11-deoxydoxorubicin [6]: R_1 = H, R_2 = $COCH_2OH$

11-deoxy-13-dihydrodaunorubicin [7]: R_1 = H, R_2 = $CHOHCH_3$

11-deoxy-13-deoxodaunorubicin [8]: R_1 = H, R_2 = CH_2CH_3

Figure 2.1 Secondary metabolites from *Micromonospora* strains



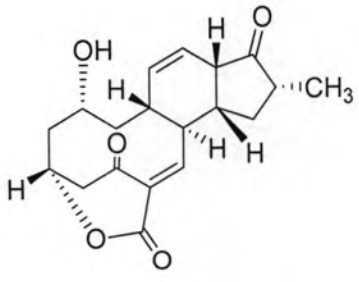
Citreamicin α [9]: $R_1 = COCH_2CH(CH_3)_2$, $R_2 = CH_3$

Citreamicin β [10]: $R_1 = COCH(CH_3)_2$, $R_2 = CH_3$

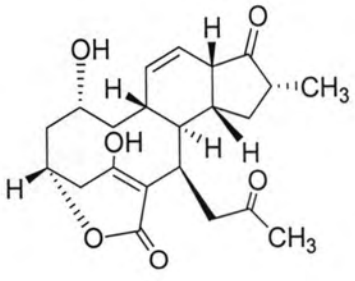
Citreamicin γ [11]: $R_1 = COCH_3$, $R_2 = CH_3$

Citreamicin ζ [12]: $R_1 = COCH_2CH(CH_3)_2$, $R_2 = H$

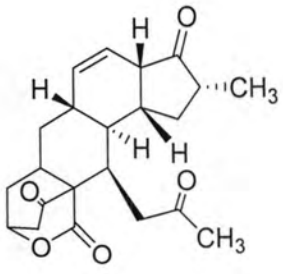
Citreamicin η [13]: $R_1 = H$, $R_2 = CH_3$



Macquarimicin A[14]

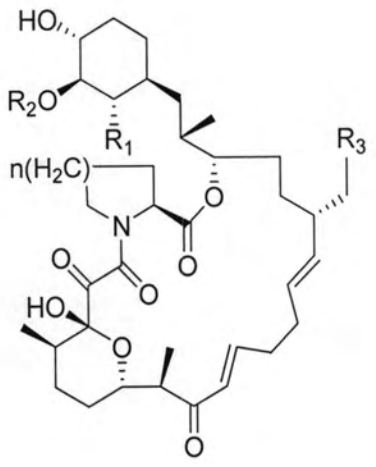


Macquarimicin B[15]



Macquarimicin C[16]

Figure 2.1 (continued)



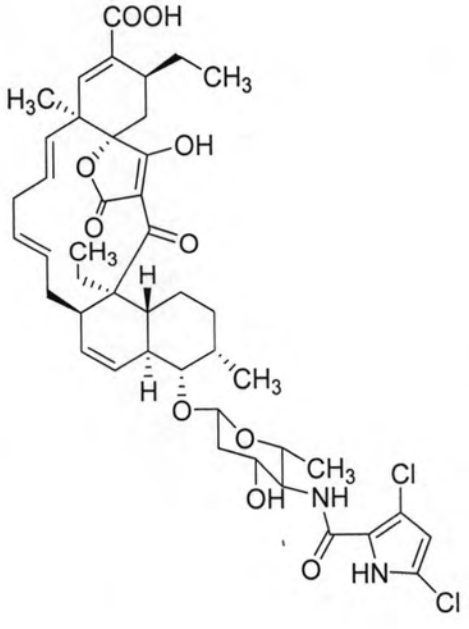
Antascomicin A [17]: R_1 = H, R_2 = H, R_3 = H, n = 2

Antascomicin B [18]: R_1 = OH, R_2 = H, R_3 = H, n = 2

Antascomicin C [19]: R_1 = OH, R_2 = CH_3, R_3 = H, n = 2

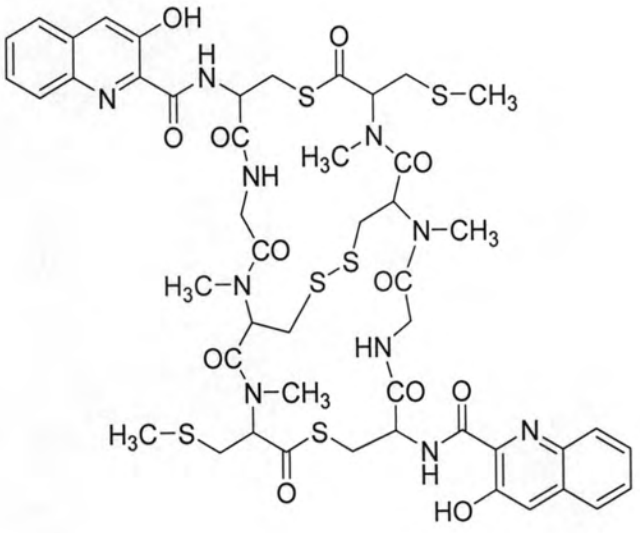
Antascomicin D [20]: R_1 = H, R_2 = H, R_3 = H, n = 1

Antascomicin E [21]: R_1 = H, R_2 = H, R_3 = OH, n = 2

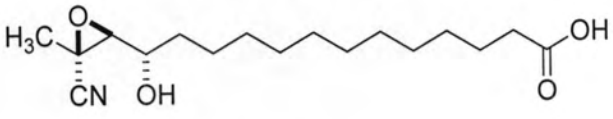


Pyrrolosporin A [22]

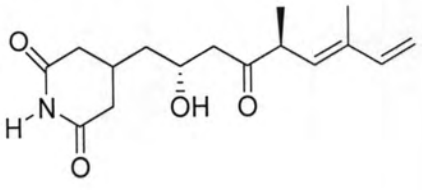
Figure 2.1 (continued)



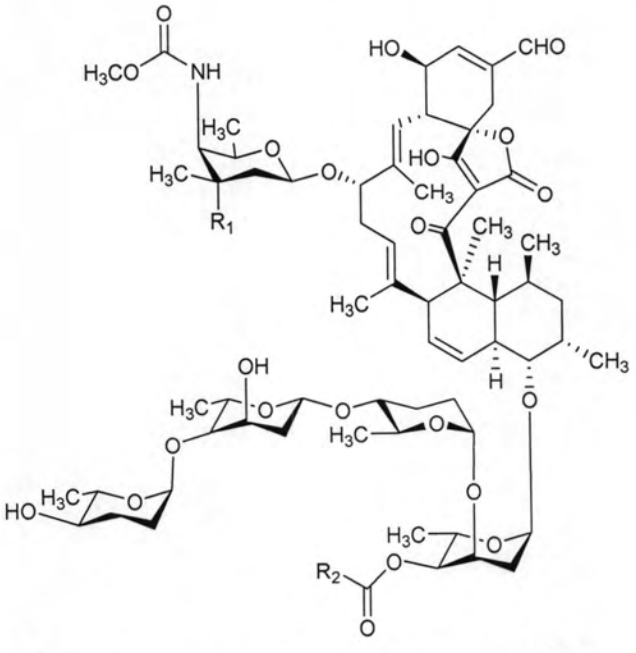
Thiocoraline [23]



YM-47515 [24]



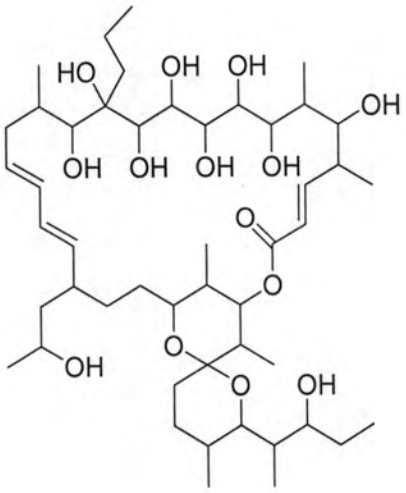
Ao58A [25]



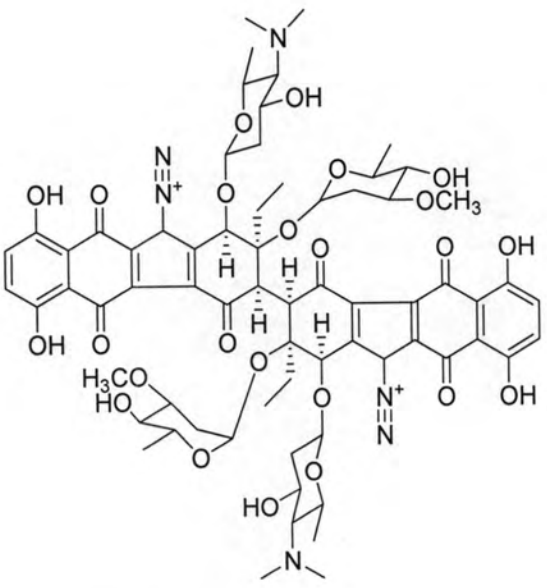
Arisostatin A [26]: $R_1 = NO_2$, $R_2 = CH(CH_3)_2$

Arisostatin B [27]: $R_1 = NH_2$, $R_2 = CH(CH_3)_2$

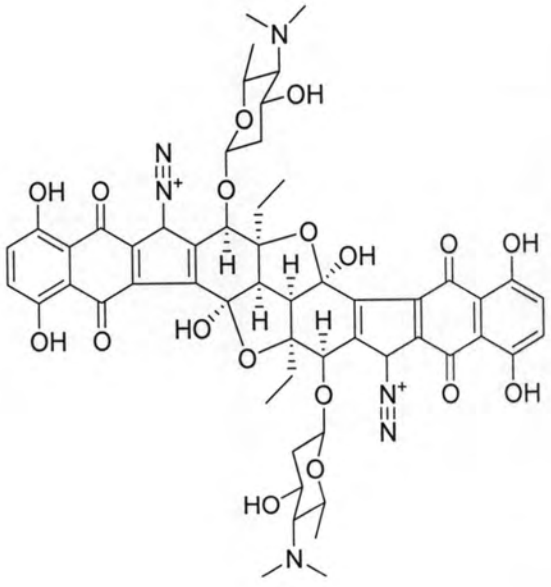
Figure 2.1 (continued)



IB-96212 [28]

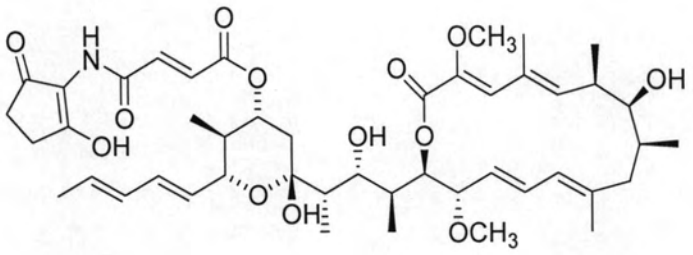


Lomaiviticins A [29]

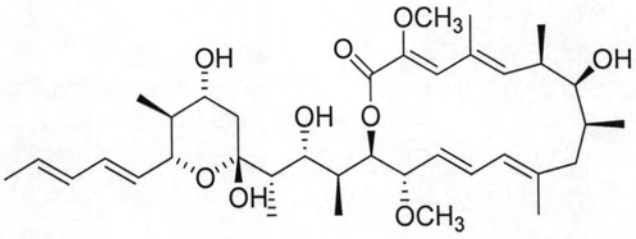


Lomaiviticins B [30]

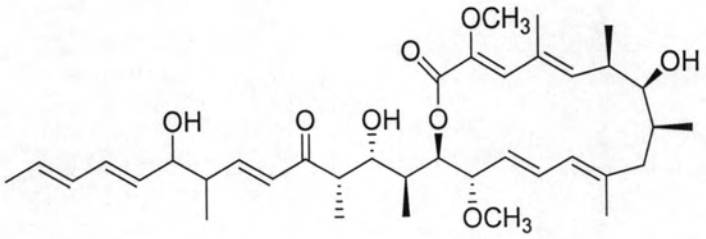
Figure 2.1 (continued)



Micromonospolide A [31]

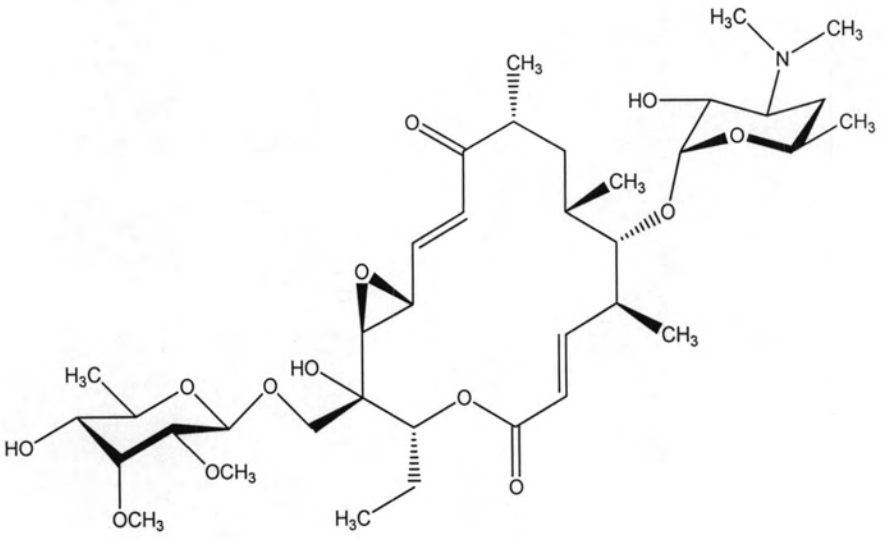


Micromonospolide B [32]

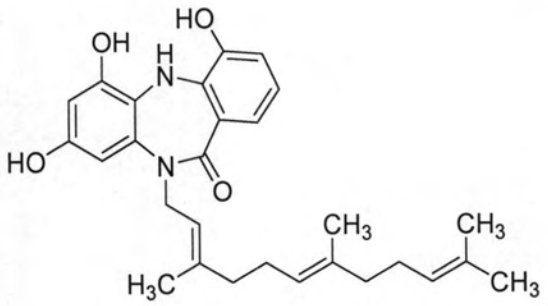


Micromonospolide C [33]

Figure 2.1 (continued)



Micinamicin II [33]



Diazepinomicin [34]

Figure 2.1 (continued)

3. Endophytic fungi

The term endophyte refers to a bacterial or fungal microorganism that colonizes interior organs of plants, but does not have pathogenic effects on its host(s). In their symbiotic association, the host plant (macrophyte) protects and feeds the endophyte, which "in return" produces bioactive metabolites to enhance the growth and competitiveness of the host and to protect it from herbivores and plant pathogens. Although the first discovery of an endophyte was made as far back as 1904, this group of microorganisms did not receive much attention until the recent realization of their ecological relevance and the potential of yielding metabolites with diverse structures and biological functions. Thus, during the past two decades over 100 endophytic microorganisms have been cultured and subjected to detailed investigations leading to the chemical characterization and biological evaluation of a large number of natural products, many of which have been shown to have novel structures and interesting biological activities.

Among endophytic microorganisms, endophytic fungi represent one of the largest conservatively and relatively untapped resources of biologically active small molecule natural products (Gunatilaka, 2006).

4. Bioactive secondary metabolites from endophytic fungi

Taxus wallachiana, Nepalese yew, has many associated endophytic fungi. One of these is a *Phoma* sp. that lives primarily in the intercellular spaces of tissues in the phloem/cambial region of the tree limb producing two antibiotic substances: altersolanol A **[36]** and 2-hydroxy-6-methylbenzoic acid **[37]**. Limb samples of *Taxus wallachiana* were gathered at a site near Singhe-To, Nepal, about 40 miles northwest of Kathmandu. Both altersolanol A and 2-hydroxy-6-methylbenzoic acid possessed antibiotic activity, having MIC values of 20 and 100 μg/ml, respectively, against *Bacillus subtilis*. The spectrum of the antibacterial activity of altersolanol A is quite broad and includes both gram-negative and gram-positive organisms such as *Pseudomonas aeruginosa* and *Bacillus* sp. (Young *et al*, 1994).

In 1971, a novel compound isolated from the bark of the northwest Pacific yew tree, *Taxus brevifolia* Nutt., was described. This compound, named taxol **[38]** (also known in the literature as paclitaxel) demonstrated moderate *in vivo* activity against the

P-388, P-1534, and L-1210 murine leukemia, the Walker 256 carcinosarcoma, sarcoma 180, and Lewis lung tumor test systems. Endophytic microbes associated with the Pacific yew tree were examined as potential sources of the anticancer drug taxol, a secondary metabolite of the host organism. The first promising organism found was the novel fungus, *Taxomyces andreanae*, which was isolated from the inner bark of a yew tree growing in the northwestern Montana (Stierle and Strobel, 1995).

Inhibitors of farnesyl-protein transferase (FPTase) had the potential of being anticancer agents for tumors in which *ras* was found mutated and contributed to cell transformation. The extracts of an endophytic fungus isolated from living leaves of *Berberis oregana* produced the tricarboxylated alkylsulfated, oreganic acid **[39]**, as a potent (IC_{50} = 14 nM) and specific inhibitor of FPTase. Its desulfated analog **[40]** was less active (IC_{50} = 3.3 nM). The trimethylester **[41]** and its desulfated analog **[42]** were inactive (Jayasuriya *et al.*, 1996).

Acremonium sp. occurs as an endophytic fungus in European yew (*Taxus baccata*). It produced a series of peptide antifungal-anticancer agents known as the leucinostatins. Leucinostatin A **[43]** was especially active against the oomycetous-plant pathogenic fungus-*Pythium ultimum* with an effective 1 day 50% inhibitory concentration of < 1μmol. Leucinostatin A also possessed activity against certain human cancer cell lines, for instance, its IC_{50} value was 2.3 nM for the breast cancer cell line BT-20, contrasted with 640 nM for a normal mammary cell line (Strobel, Torczynski, and Bollon, 1997).

Fusarium moniliforme, a fungus that commonly produced a symptomless endophytic association with corn (*Zea mays* L.) cultivars and inbred lines, was shown to catabolize 6-methoxy-benzoxazolinone (MBOA) **[44]** and 2-benzoxazolinone (BOA) **[45]**, biologically active compounds known to be fungistatic (Yue, Bacon, and Richardson, 1998).

Two novel human cytomegalovirus (hCMV) protease inhibitors, cytonic acids A **[46]** and B **[47]**, were isolated from the solid-state fermentation of endophytic fungi *Cytonaema* sp. which had been isolated from *Quercus* sp. in the U.K. (Guo *et al.*, 2000).

CR377 **[48]**, a new pentaketide antifungal agent, was isolated from the culture broth of a fungus, CR377 (*Fusarium* sp.), that showed potent activity against *Candida*

albicans in agar diffusion assays. Cultures of endophytic fungi collected in the Guanacaste Conservation Area of Costa Rica (Brady and Clardy, 2000).

Three new antimicrobial metabolites were characterized from the culture of *Colletotrichum* sp., an endophytic fungus isolated from inside the stem of *Artemisia annua*. These compounds were 6-isoprenylindole-3-carboxylic acid **[49]**, 3β,5α-dihydroxy-6β-acetoxy-ergosta-7,22-diene **[50]** and 3β,5α-dihydroxy-6β-phenylacetyloxy-7,22-diene **[51]**. The compounds **[49-51]** inhibited the growth of all the test bacteria (*Bacillus subtilis*, *Staphylococcus aureus, Sarcina lutea* and *Pseudomonas* sp.) with minimal inhibitory concentrations (MICs) ranging from 25-75 μg/ml. At 200 μg/ml, compounds **[49-51]** were shown to be fungistatic to the crop pathogenic fungi *Guenmannomyces graminis* var. *tritici*, *Rhizoctonia cerealis*, *Helminthosporium sativum*, and *Phytophthora capisici* (Lu *et al.*, 2000).

Ambuic acid **[52]**, a highly functionalized cyclohexenone with antifungal activity, was isolated endophytic fungi associated with many tropical plant species. This compound was found in representative isolates of these fungal species obtained from rainforest plants located on several continents (Li *et al.*, 2001).

Jesterone **[53]** and hydroxyl-jesterone **[54]** are novel highly functionalized cyclohexenone epoxides isolated from an endophytic fungus, *Pestalotiopsis jesteri*. The fungus was isolated from the inner bark of small limbs of a *Fragraca bodenii* (family Gentianaceae). This particular plant species was common to the forests of the tropical New Guinea Highland. Jesterone, in particular, displayed selective antimycotic activity against the oomycetous fungi which are some of the most plant pathogenic of all disease causing fungi (Li and Strobel, 2001).

Preussomerins G-L **[55-60]** were isolated from an endophytic fungus, *Mycelia sterile*, from the root of *Atropa belladonna*. These six compounds showed the antibacterial activities and the preussomerins G-I also inhibited farnesyl-protein transferase (FPTase) (Krohn *et al.*, 2001).

Isopestacin **[61]** is an isobenzofuranone obtained from the endophytic fungus *Pestalotioosis microspora* that was isolated from a combretaceaous plant, *Terminalia morobensis* growing in the Sepik river drainage of Papua New Guinea. While a few other isobenzofuranones are known from natural sources, isopestacin is the only one

having a substituted benzene ring attached. This compound possessed antifungal activity and it also behaved as an antioxidant scavenging both superoxide and hydroxyl free radicals (Strobel *et al.*, 2002).

Additional analysis of the culture fluid of *Pestalotiopsis microspora*, an endophytic fungus native to the rainforest of Papua New Guinea, provided a second compounds, pestacin **[62]**. This product was 1,5,7-trisubstituted and exhibited moderate antifungal properties and antioxidant activity 11 times greater than the vitamin E derivative trolox (Harper *et al.*, 2003).

The marine endophytic fungus No. 1893 was isolated from the dropper of *Kandilia candel* from an estuarine mangrove on the South China Sea coast. The ethyl acetate extract of the fermentation broth of this fungus strain exhibited cytotoxicity toward NCI4460 and Bel7402, and high activity against *Heliothis armigera* (Hühner) and *Sinergasilus* spp. Two lactones, 1893A **[63]** and B **[64]**, were isolated from the extract (Chen *et al.*, 2003).

Three novel dihydroisocoumarin derivatives with antimalarial, antituberculous, and antifungal activities were isolated from an endophytic fungus, *Geotrichum* sp., collected from *Crassocephalum crepidioides* S. Moore (Compositae). Structures were established as 7-butyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one **[65]**, 7-but-15-enyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one **[66]**, and 7-butyl-6,8-dihydroxy-3(*R*)-pentylisochroman-1-one **[67]** (Kongsaeree *et al.*, 2003).

An endophytic fungus isolate of *Gliocladium* sp. was obtained from the Patagonian Eucryphiacean tree, *Eucryphia cordifolia*, known locally as "ulmo". This fungus produced a mixture of volatile organic compounds (VOC's) lethal to such plant pathogenic fungi as *Pythium ultimum* and *Vertricillim dahliae*, while other pathogens were only inhibited by its volatiles. The primary volatile compound produced by *Gliocladium* sp. was 1,3,5,7-cyclooctatetraene or annulene **[68]**, which by itself, was an effective inhibitor of fungal growth (Stinson *et al.*, 2003).

The benzoquilone derivatives, rhizoctonic acid **[69]** and monomethylsulochrin **[70]**, were isolated from the culture of *Rhizotocnia* sp. (Cy064), an endophytic fungus in the leaf of *Cynodon dactylon* (Graminae) located in Jiangsu Province, China. The *in vitro* growth inhibition assay against *Helicobacter pylori* by agar dilution method

indicated that the minimum inhibitory concentrations (MICs) of compounds **69** and **70** against all of the five clinical and a reference (ATCC 43504) strains were 25.0 and 10.0 μg/ml, respectively. The MIC of ampicillin used as the positive control against these strain was 2.0 μg/ml (Ma *et al.*, 2004).

Aspergillus fumigatus CY018 was recognized as an endophytic fungus for the first time in the leaf of *Cynodon dactylon*. The ethyl acetate extract of a solid-matrix steady culture of this fungus afforded to two new metabolites, named asperfumoid **[71]** and asperfumin **[72]**. Asperfumoid was shown to inhibit *Candida albicans* with MIC of 75.0 μg/ml (Liu *et al.*, 2004).

An isolate of *Curvularia* sp. was obtained from the leaves of *Ocotea corymbosa* (Lauraceae), a native plant of the Brazilian Cerrado. The ethyl acetate extract from culture of this fungus afforded the benzopyran derivatives: 2-methyl-5-methoxy-benzopyran-4-one **[73]**, (2'*S*)-2-(propan-2'-ol)-5-hydroxy-benzopyran-4-one **[74]**, (2*R*)-2,3-dihydro-2-ethyl-5-methoxy-benzopyran-4-one **[75]**, 2,3-dihydro-2-methyl-benzopyran-4,5-diol **[76]**. The benzopyrans **73** and **74** showed weak *in vitro* antifungal activity against *Cladosporium sphaerospermum* and *C. cladosporioides*. Compound **74** was able to induce cell proliferation: 70% on human cervix tumor (Hela cells) and 25% on Chinese hamster ovary (CHO cells) (Teles *et al.*, 2005).

Phomoxins B and C **[77-78]**, as well as the previously reported fungal metabolite eupenoxide **[79]**, were isolated from an endophytic fungus *Eupenicillium* sp. obtained from a local rainforest tree in Australia, *Glochidion ferdinandi* (family Euphorbiaceae). The compounds **77-79** were all tested in a number of antimicrobial assays. No microbial growth inhibition was observed (Davis *et al.*, 2005).

Two new metabolites named 6-oxo-de-*O*-methyllasioplodin **[80]** and (*E*)-9-etheno-lasiodiplodin **[81]**, with three known compounds lasiodiplodin **[82]**, de-*O*-methyllasioplidin **[83]**, and 5-hydroxy-de-*O*-methyllasioplodin **[84]**, were isolated from the mycelium extracts of a brown alga (*Sagassum* sp.) endophytic fungus (No. ZZF36) obtained from Zhanjiang sea area, China. Lasiodioplodin and its relatives were known to be very efficient inhibitors of prostaglandin biosynthesis, and exhibited significant anti-leukemic and potato micro-tuber inducing activities. Compound **83** exhibited the inhibitory activities against the microorganisms, especially to *Staphylococcus aureus*

with MIC = 6.25 μg/ml. Compound **82** inhibited the *in vitro* growth of *S. aureus*, *Bacillus subtilis*, and *Fusarium oxysporum* with MICs = 25, 50, and 100 μg/ml, respectively. But compound **84** showed effect against *S. aureus* at 100 μg/ml only and compound **80** exhibited no activity (Yang *et al.*, 2006).

Five cadinane sesquiterpenoid derivatives were isolated from *Phomopis cassiae*, an endophytic fungus isolated from *Cassia spectabilis*. They are two diastereoisomeric 3,9,12-trihydroxycadalene **[85-86]**, 3,12-dihydroxycalamenene **[87]**, 3,12-dihydroxycadalene **[88]**, and 3,11,12-trihydroxycadalene **[89]**. Antifungal activity of the isolated compound was evaluated against *Cladosporium sphaerospermum* and *C. cladosporioide*, revealing **89** as the most active compound (Silva *et al.*, 2006).

Colletotrichum gloeosporiodes, an endophytic fungus in *Cryptocarya mandioccana* Nees (Lauraceae), produced two antifungal metabolites, (-)*cis*-4-hydroxy-6-deoxyscytalone **[90]** and (4*R*)-4,8-dihydroxy-α-tetralone **[91]**. The antifungal activity of **90** and **91** was evaluated by means of direct bioautography on TLC plate. The detection limit of these compounds required to inhibit growth of the phytopathogenic fungi *C. sphaerospermum* and *C. cladosporioide* was 5.0 μg, comparable with the positive control nystatin (Inácio *et al.*, 2006).

6,8-Dimethoxy-3-(2'-oxo-propyl)-coumarin **[92]** and 2,4-dihydroxy-6-[(1'*E*,3'*E*)-penta-1',3'-dienyl]-benzaldehyde **[93]**, in addition to known compound periconicin B **[94]**, were isolated from the ethyl acetate extract of *Periconia atropurpurea*, and endophytic fungus obtained from the leaves of *Xylopia aromatica*, a native plant of the Brazilian Cerrado. Biological analyses were performed using two mammalian cell lines, human cervix carcinoma (HeLa) and Chinese hamster ovary (CHO). The results showed that compound **93** was able to induce a slight increase in cell proliferation of Hela (37% of increase) and CHO (38% of increase) cell lines. Analysis of compound **93** showed that it has potent cytotoxic activity against both cell lines, with IC_{50} of 8.0 μM. Biological analyses using phytopathogenic fungi *C. sphaerospermum* and *C. cladosporioide* revealed that also **93** showed potent antifungal activity as compared with nystatin (Teles *et al.*, 2006).

Two new natural products, 2-hydroxymethyl-3-methylcyclopent-2-enone **[95]**, and *cis*-2-hydroxymethyl-3-methylcyclopentanone **[96]**, and known compound, asterric

acid **[97]**, were isolated from the endophytic fungus mitosporic *Dothideomycin*sp. LRUB20, which was isolated from stems of the Thai medicinal plant *Leea rubra*. Compound **96** was separated and identified in the form of its 2,4-dinitrophenylhydrazone derivative **[98-99]**. Compounds **95**, **97** and hydrazone **99** exhibited mild antimicrobial activity, both with MIC values of 200 μg/ml. Compounds **95**, **97**, and **98** were inactive (at 50 μg/ml) toward Vero, KB, NCI-H187, and BC cell lines. Hydrazone **99** showed only mild cytotoxicity against the Vero cell line with an IC_{50} value of 21.7 μg/ml (Chomcheon *et al.*, 2006).

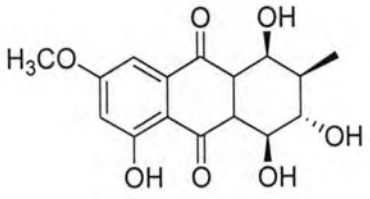
Cultivation of the endophytic fungus *Chaetomium globosum*, which was isolated from the inner tissue of the marine red alga *Polysiphonia urceolata*, resulted in the isolation of chaetopyranin **[100]**, a new benzaldehyde secondary metabolite. Compound **100** represents the first example of a 2*H*-benzopyran derivative of marine algal-derived fungi as well as of genus *Chaetomium*. Compound **100** was found to have moderate activity for its DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging property and also showed moderate to weak cytotoxic activity toward several cell lines (Wang *et al.*, 2006).

The endophytic fungus *Penicillium* sp. isolated from the mangrove plant *Aegiceras corniculatum* produced eight new indole triterpenes named shearinines D-K **[101-108]**. Shearinines D **[101]**, E **[102]**, and (with reduced potency) G **[104]** exhibited significant *in vitro* blocking activity on large-conductance calcium-activated potassium channels (Xu *et al.*, 2007).

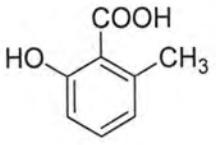
Recently, there was a study to find the anti-*Candida* metabolites from endophytic fungi, particularly from Ascomycota and Basidiomycota isolated from their fruit-bodies or as soil-borne, coprophilous or endophytic fungi. They were screened for activity against *Candida albicans* and a range of other pathogenic and saprotropic fungi. Considerably more Ascomycota (11-16%) than Basidiomycota (3.5%) produced metabolites with activity against *C. albicans*. From five species of endophytes, six bioactive compounds were isolated and identified, *viz.* cerrulin **[109]**, arundifungin **[110]**, shaereoosidin A **[111]**, 5-(1,3-butadiene-1-yl)-3-(propene-1-yl)-2-(5H)-furanone **[112]**, ascostroside A **[113]**, and a derivative of **113**, ascosteroside B [114]. There were the antifungal activities of **110** and **112-114** in agar diffusion test, comparable with those of amphotericin B. Compound **114** exhibited a similar antifungal activity as **113**

but cytotoxicity towards Hep G2 cells was considerable lower. This study points to endophytic fungi related to hemibiotrophic or latent plant pathogens as an important source of bio- and chemodiversity (Weber *et al.*, 2007).

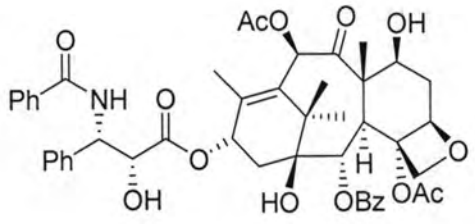
The structures of these bioactive metabolites from endophytic fungi are shown below in Figure 2.2.



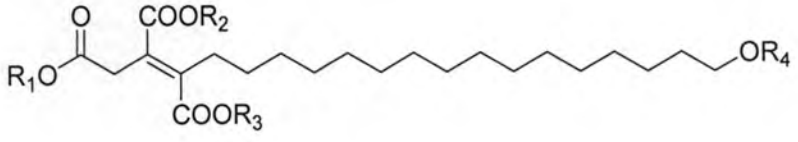
Altersolanol A [36]



2-hydroxy-6-methylbenzoic acid [37]

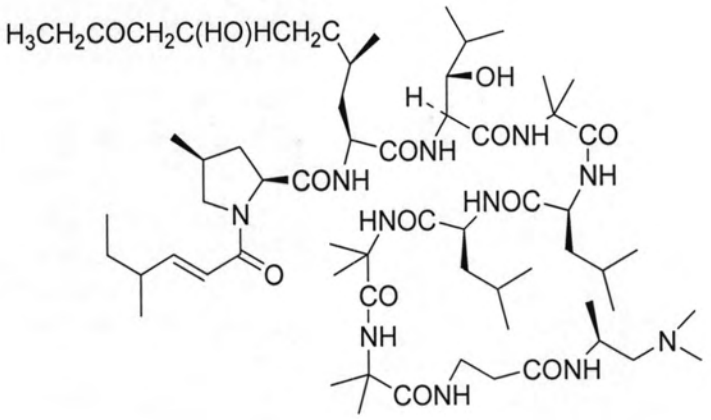


Taxol [38]

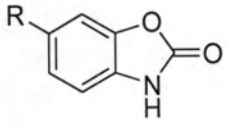


Oreganic acid [39]	**: $R_1 = R_2 = R_3 = H$, $R_4 = SO_3H$**
Oreganic acid desulfated [40]	**: $R_1 = R_2 = R_3 = R_4 = H$**
Trimethylester [41]	**: $R_1 = R_2 = R_3 = CH_3$, $R_4 = SO_3H$**
Trimethylester desulfated [42]	**: $R_1 = R_2 = R_3 = CH_3$, $R_4 = H$**

Figure 2.2 Structures of bioactive secondary metabolites from endophytic fungi.

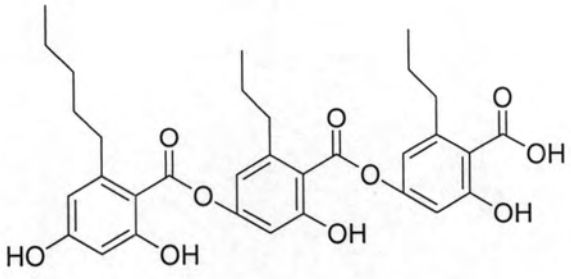


Leucinostatin A [43]

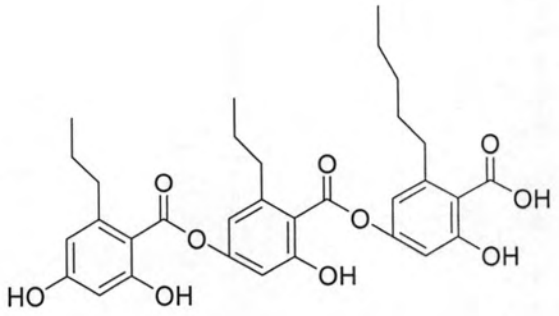


6-methoxy-benzolinone (MBOA) [44] : **R = OCH_3**

2-benzoxazolinone (BOA) [45] : **R = H**

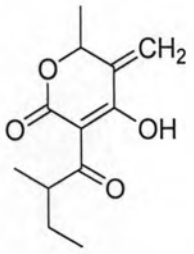


Cytonic acid A [46]

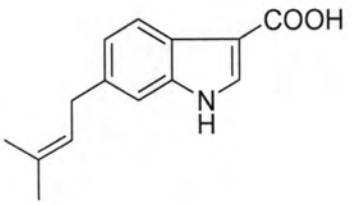


Cytonic acid B [47]

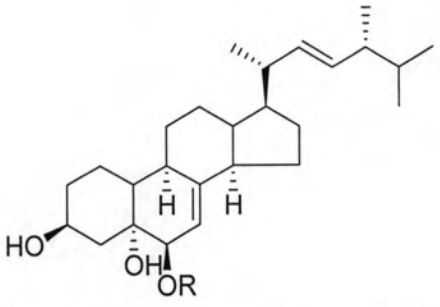
Figure 2.2 (continued)



CR377 [48]

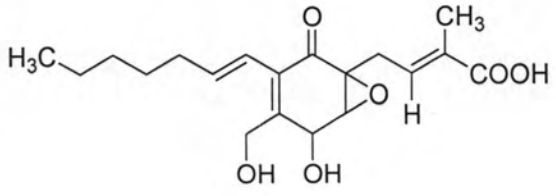


6-isoprenylindole-3-carboxylic acid [49]

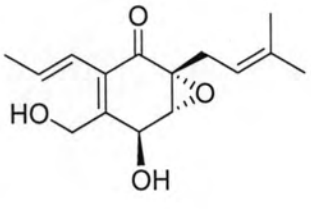


3β,5α-dihydroxy-6β-acetoxy-ergosta-7,22-diene [50] : **R = $COCH_3$**

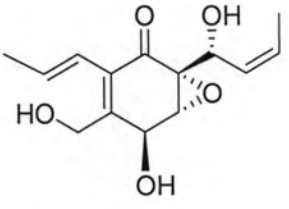
3β,5α-dihydroxy-6β-phenylacetyloxy-7,22-diene [51] : **R = $COCH_2C_6H_5$**



Ambuic acid [52]

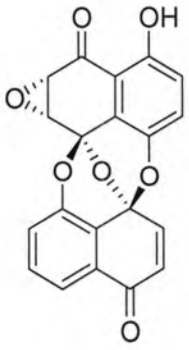


Jesterone [53]

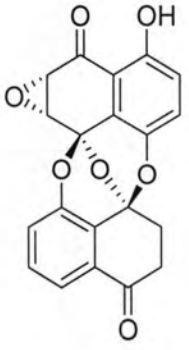


Hydroxyl-jesterone [54]

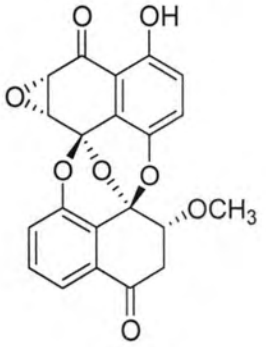
Figure 2.2 (continued)



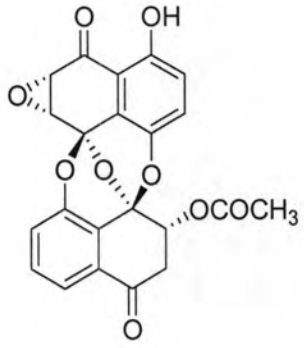
Preussomerins G [55]



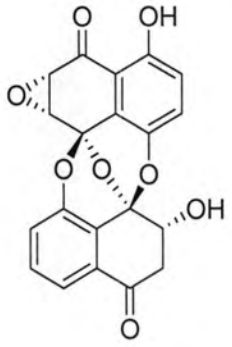
Preussomerins H [56]



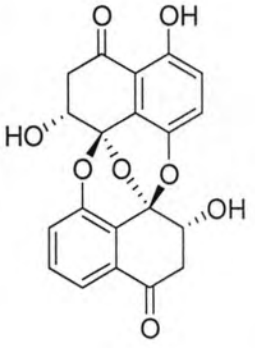
Preussomerins I [57]



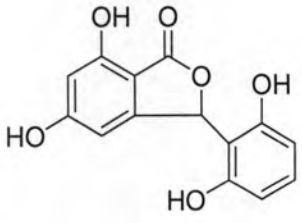
Preussomerins J [58]



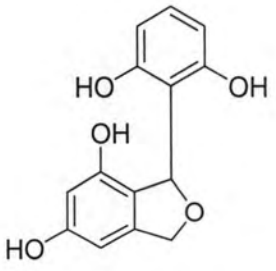
Preussomerins K [59]



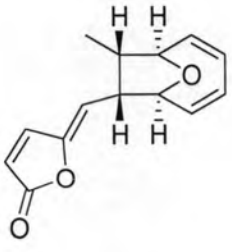
Preussomerins L [60]



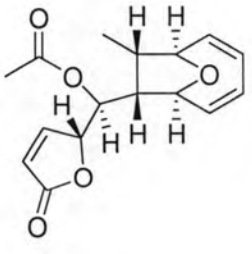
Isopestacin [61]



Pestacin [62]

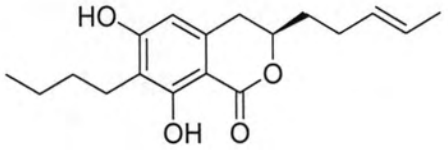


1893 A [63]

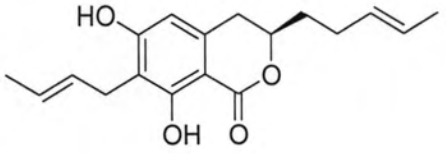


1893 B [64]

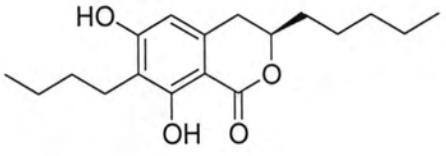
Figure 2.2 (continued)



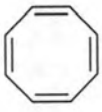
7-butyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one [65]



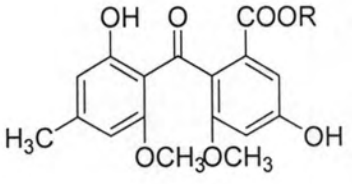
7-but-15-enyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one [66]



7-butyl-6,8-dihydroxy-3(*R*)-pentylisochroman-1-one [67]

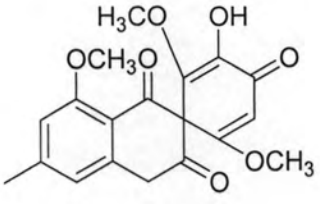


1,3,5,7-cyclooctatetraene or annulene [68]

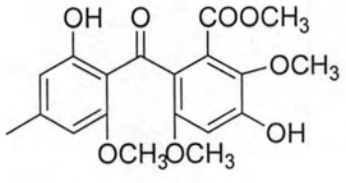


Rhizoctonic acid [69]

Monomethylsulochrin [70]

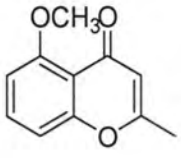


Asperfumoid [71]

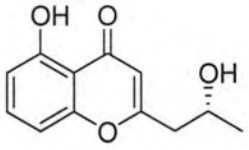


Asperfumin [72]

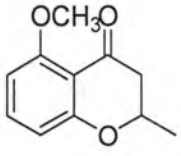
Figure 2.2 (continued)



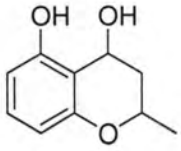
2-methyl-5-methoxy-benzopyran-4-one [73]



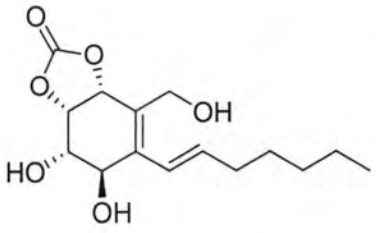
(2'*S*)-2-(propan-2'-ol)-5-hydroxy-benzopyran-4-one [74]



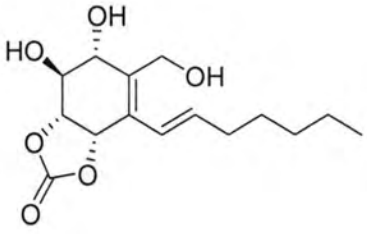
(2*R*)-2,3-dihydro-2-ethyl-5-methoxy-benzopyran-4-one [75]



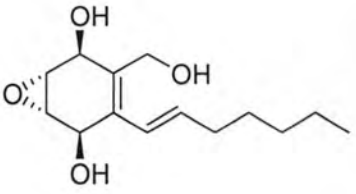
2,3-dihydro-2-methyl-benzopyran-4,5-diol [76]



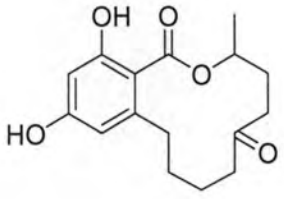
Phomoxins B [77]



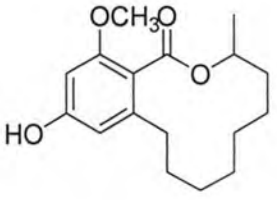
Phomoxins C [78]



Eupenoxide [79]

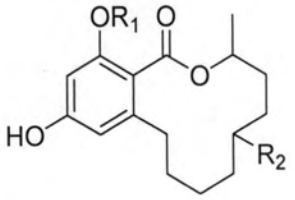


6-oxo-de-*O*-methyllasioplodin [80]



(*E*)-9-etheno-lasiodiplodin [81]

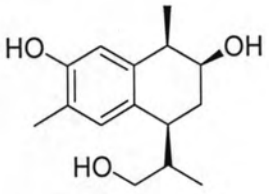
Figure 2.2 (continued)

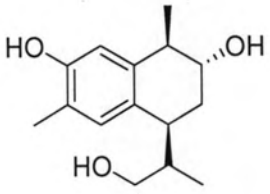


Lasiodiplodin [82] : $R_1 = CH_3$, $R_2 = H$

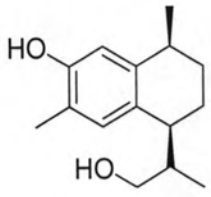
de-*O*-methyllasioplidin [83] : $R_1 = H$, $R_2 = H$

5-hydroxy-de-*O*-methyllasioplodin [84] : $R_1 = H$, $R_2 = OH$

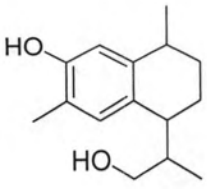




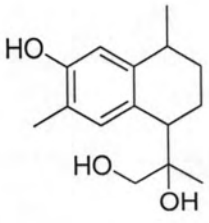
3,9,12-trihydroxycadalene [85-86]



3,12-dihydroxycalamenene [87]

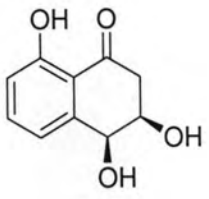


3,12-dihydroxycadalene [88]

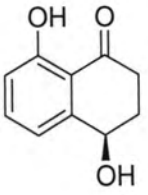


3,11,12-trihydroxycadalene [89]

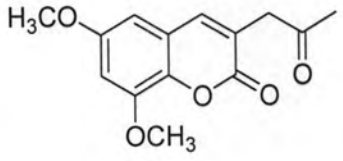
Figure 2.2 (continued)



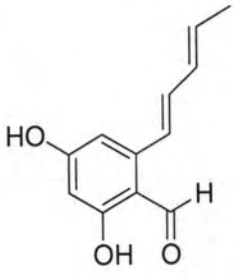
(-)*cis*-4-hydroxy-6-deoxyscytalone [90]



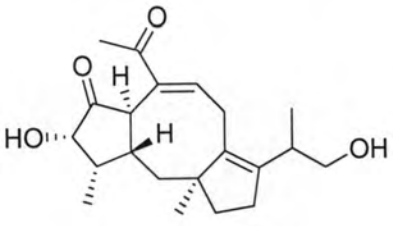
(4*R*)-4,8-dihydroxy-α-tetralone [91]



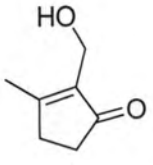
6,8-Dimethoxy-3-(2'-oxo-propyl)-coumarin [92]



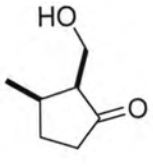
2,4-dihydroxy-6-[(1'*E*,3'*E*)-penta-1',3'-dienyl]-benzaldehyde [93]



Periconicin B [94]

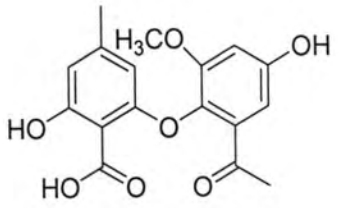


2-hydroxymethyl-3-methylcyclopent-2-enone [95]

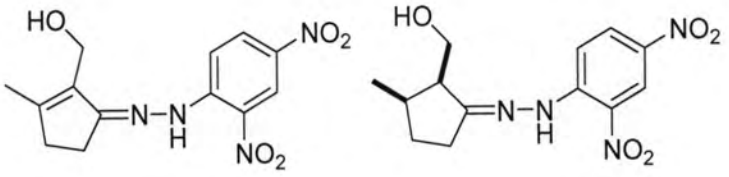


cis-2-hydroxymethyl-3-methylcyclopentanone [96]

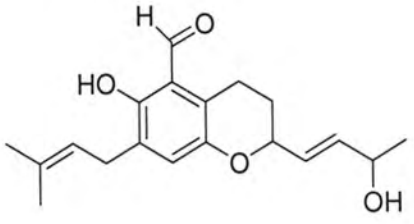
Figure 2.2 (continued)



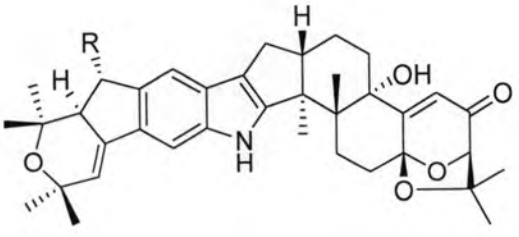
Asterric acid [97]



2,4-dinitrophenylhydrazone derivative [98-99]

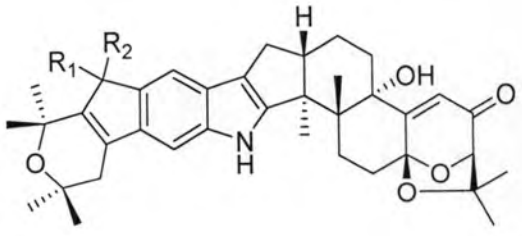


Chaetopyranin [100]



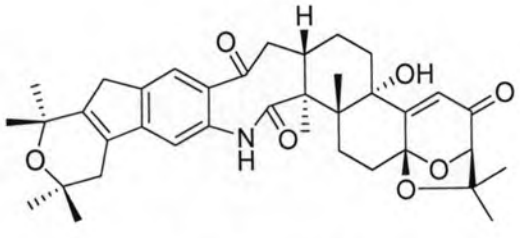
Shearinine D [101] : R = OH

Shearinine E [102] : R = OCH_3

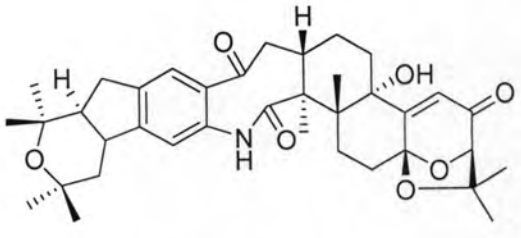


Shearinine F [103] : $R_1 = R_2 = H$

Shearinine G [104] : $R_1 + R_2 = O$

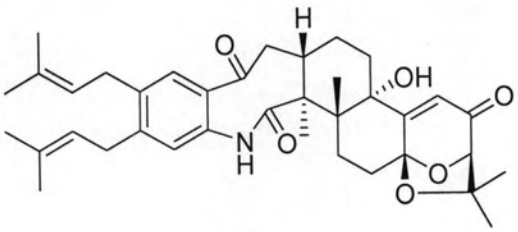


Shearinine H [105]

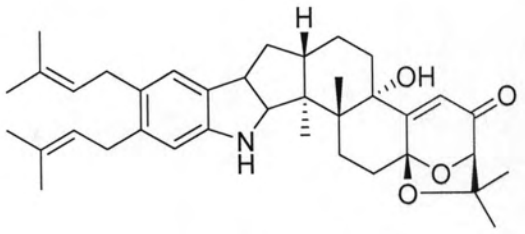


Shearinine I [106]

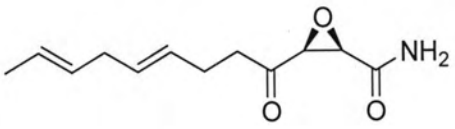
Figure 2.2 (continued)



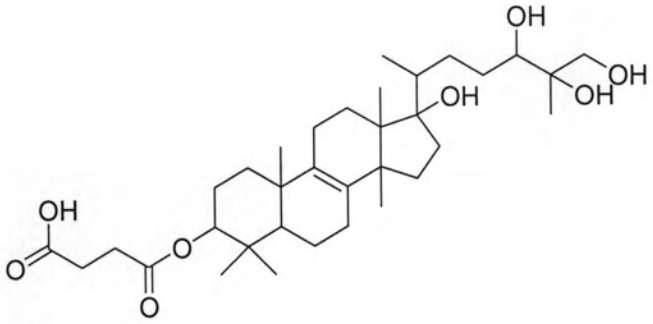
Shearinine J [107]



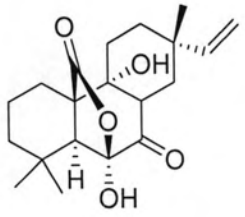
Shearinine K [108]



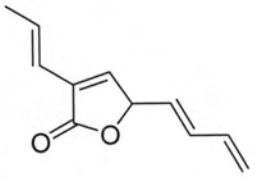
Cerrulin [109]



Arundifungin [110]

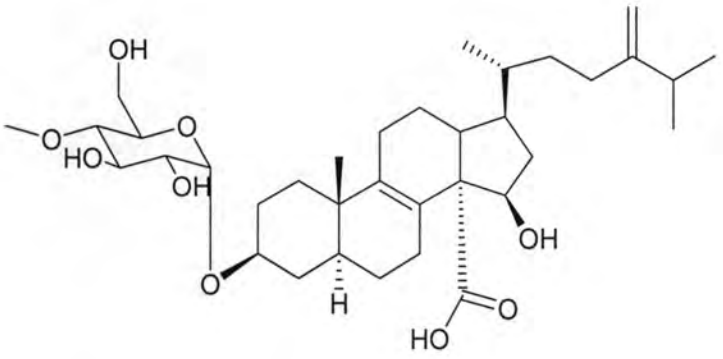


Sheareoosidin A [111]

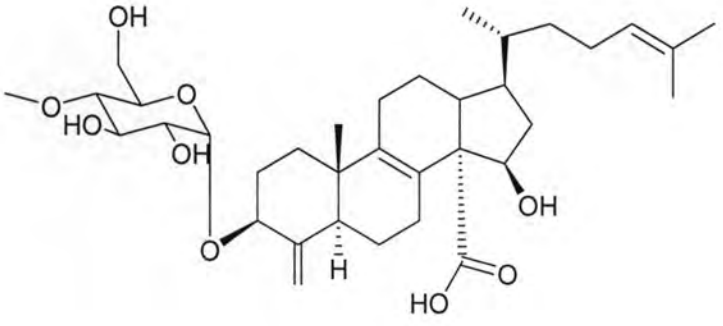


5-(1,3-butadiene-1-yl)-3-(propene-1-yl)-2-(5H)-furanone [112]

Figure 2.2 (continued)



Ascostroside A [113]



Ascostroside B [114]

Figure 2.2 (continued)

5. Characteristics of the endophytic fungus *Exserohilum* species

The genus *Exserohilum* belongs to Family Pleosporaceae, Order Pleosporales, Class Euascomycetes, Phylum Ascomycota, Kingdom Fungi. *Exserohilum* is a phaeoid or dematiaceous filamentous fungus. It is cosmopolitan in nature inhabiting plant material, particularly grasses, and soil. Colonies are gray to blackish-brown, suede-like to floccose in texture, and have an olivaceous black reverse. Conidia are straight, curved or slightly bent, ellipsoidal to fusiform and formed apically through a pore (poroconidia) on a sympodially elongating geniculate conidiophore. Conodia have a strongly protruding, truncate hilum and the septum above the hilum is usually thickened and dark. The end cells are often paler than the other cells and the walls are often finely roughened. Conidial germination is bipolar (Domsch, Gams, and Anderson, 1980). The hilum is defined as "a scar on a conidium at the point of attachment to the conidiophore" (McGinnis, Rinaldi, and Winn, 1986).

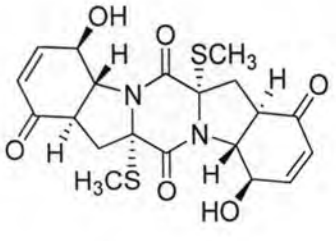
6. Bioactive secondary metabolites from *Exserohilum* species

The novel phytotoxins, exserohilone **[115]** and its dihydro derivative, 9,10-dihydroexserohilone **[116]**, were isolated from the culture broth of *Exserohilum holmii*, a pathogenic fungus of the weedy plant *Dactyloctenium aegyptium* (crowfoot grass) which was a serious grasseous weed in all major tropical and semitropical agricultural areas of the world. Compounds **115** and **116** may possess antimicrobial or antiviral activity since structurally related compounds such as aranotine, gliotoxin, sporidesmin, chaetocin, chetonin, and epicorazins had comparable properties (Sugawara, Sugawara, and Strobel, 1985).

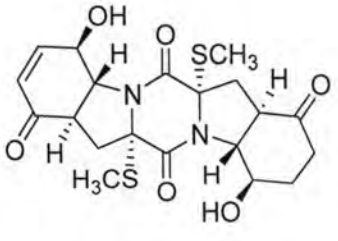
Three *Exserohilum turcicum* strains from France, isolated from maize, produced a lipophilic phytotoxin that was structurally characterized as monocerin **[117]**. This molecule caused brown necrotic lesions on pregerminated seeds: the median dose (ID_{50}) being around 10^{-3} M. It decreased the viability of maize root cap cells and mesophyll protoplast suspensions: the ID_{50} was 2.5-5 x 10^{-4} M for cells and around 8 x 10^{-5} M for protoplast (Cuq *et al.*, 1993).

Recently, four new cytotoxic disulfides, rostratins A-D **[118-121]**, were isolated from the whole broth of the marine derived fungus *Exserohilum rostratum*, a fungus strain found associated with a marine cyanobacterial mat collected off the island of Lanai, Hawaii. Rostratins A, B, C, and D showed *in vitro* cytotoxicity against human colon carcinoma (HCT-116) with IC_{50} values of 8.5, 1.9, 0.76, and 16.5 μg/ml, respectively (Tan *et al.*, 2004).

The structures of bioactive secondary metabolites are shown in Figure 2.3.

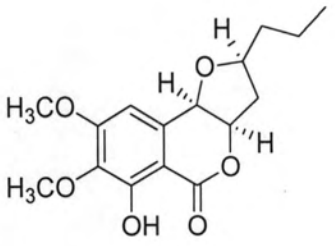


Exserohilone [115]

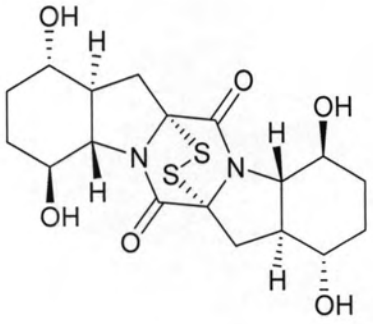


9,10-dihydroexserohilone [116]

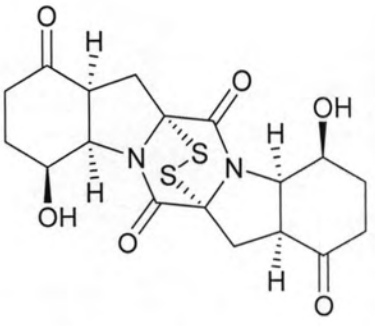
Figure 2.3 Structures of bioactive secondary metabolites from the fungi *Exserohilum*.



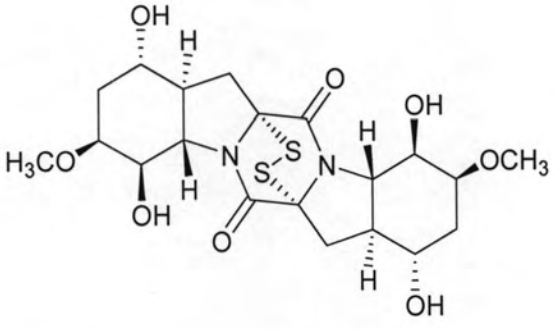
Monocerin [117]



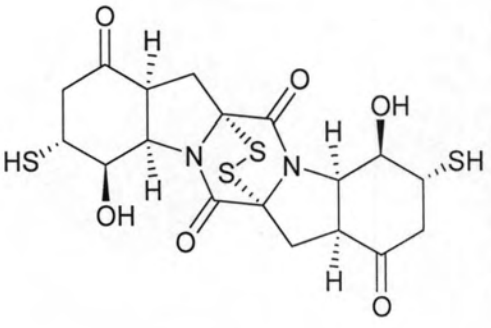
Rostratin A [118]



Rostratin B [119]



Rostratin C [120]



Rostratin D [121]

Figure 2.3 (continued)