CHAPTER 2

LITERATURE REVIEW

2.1 Endophytic fungi

Endophytic fungi are fungi that form symptomless infections, for part of all their life cycle within tissue of healthy plants. The association of fungi with plant ranges from mutualistic symbiosis, or commensalism to borderline latent pathogen (Strobel and Long,1998). The major features of mutualistic symbiosis include the lack of destruction of most cells or tissues, nutrient or chemical cycling between the fungus and host, enhanced longevity and photosynthetic capacity of cells and tissues under the influence of Infection, enhanced survival of the fungus, and a tendency toward greater host specificity than seen in nectrophic infection (Lewis,1973).A comparison of the fitness of the host and fungus when living independently in contrast to their fitness when living in association is the major mean determining whether a specific symbiotic association is mutualistic or parasitic (Lewis,1974).The majority of the endophytic spicies which have been successfully identified are Ascomycetes and Deuteromycetes with a few Basidiomycetes and a very small number of Oomycetes (Issac,1992)

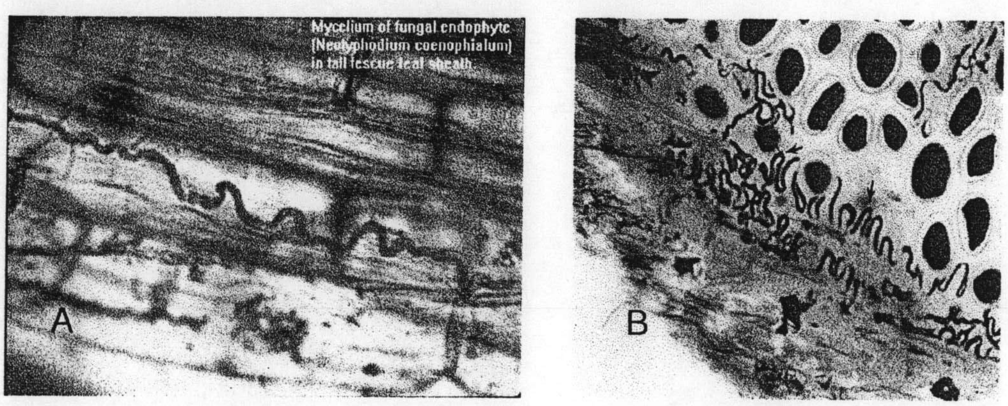


Figure 2.1 Vagetative growth in endophytic fungi of grasses. (A) Mycelium of fungal endophyte (*Neothyphodium coenophialum*) in tall festue leaf sheath. (B) Tangential section through seed of Festuca arundinaceae showing mycelium within seed (X800).

By definition, endophytic colonization or infection cannot be considered as causing disease, since a plant disease is an interaction between the host, parasite, vector and the environment over time, which results in the production of disease signs and/or symptoms. The distinction between and endophyte and a phathogen is not always clear. A mutation at a single genetic locus can change a phathogen to nonpathogenic endophytic organism with no effect on host specificity (Freeman and Rogriguez,1993). Many pathogens undergo an extensive phase of asymtopmatic growth corresponding to colonization and then latent infection before symptoms appear. Many pathogens of economically important crops many be endophytic or latent in weeds (Cerkauskas,1988; Cerkauskas et al.,1983;Harman et al.,1986; Hepperly et al.,1980; Kulik,1984; McLean and Roy,1988; Raid and Pennypacker,1987).Alternately, nonpathogenic endophytic organisms may play a role as biocontrol agents(Freeman and Rogriguez,1993). Both endophytic and latent infection fungi can infect plant tissues and become established after penetration. However,infection dose not imply the production of visible disease symptoms (Redin and Carris,1996).

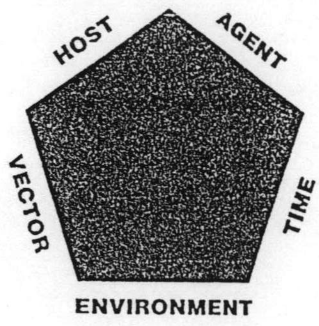


Figure 2.2 The five interactive components that can be involved in a symptomatic plant disease. (Redin and Carris,1996).

2.2 Grass endophytes

Many species of grasses (Gramineae) of the subfamily Pooideae (Festucoidea) are associated with intracellular fungi (White,1987; White and Cole,1985) These fungi grow endophytically within seeds,leaves, culms ,rhizomes,and meristems of grasses and never show external signs of infection or symptoms of disease.Clavicipitaceous endophytes have been known to exist in grasses since the discovery of an endophyte in seed of darnel (*Lolium temulentum* L.) by Vogl (1898 cited in wilson,1996).The oldest known specimens of damel with endophytic mycelium were seeds retrieved from a pharaohs tomb in an Egyptian pyramid dating back to 3400 B.C. . Most surveys for clavicipitaceous endophytes have concentrated on wild grasses,cultivated turfgrass and forage grass collections from Europe and North America.Clavicipitaceous endophyte of grasses can be divided into two natural groups: 1) the choke-including sexual forms or clavicipitaceous telemorphic endophytes,and 2) the muataulistic asexual forms or clavicipitaceous anamorphic endophytes (fig 2.3) which lack a sexual stage and are not considered to disease of host (Wilson,1996).

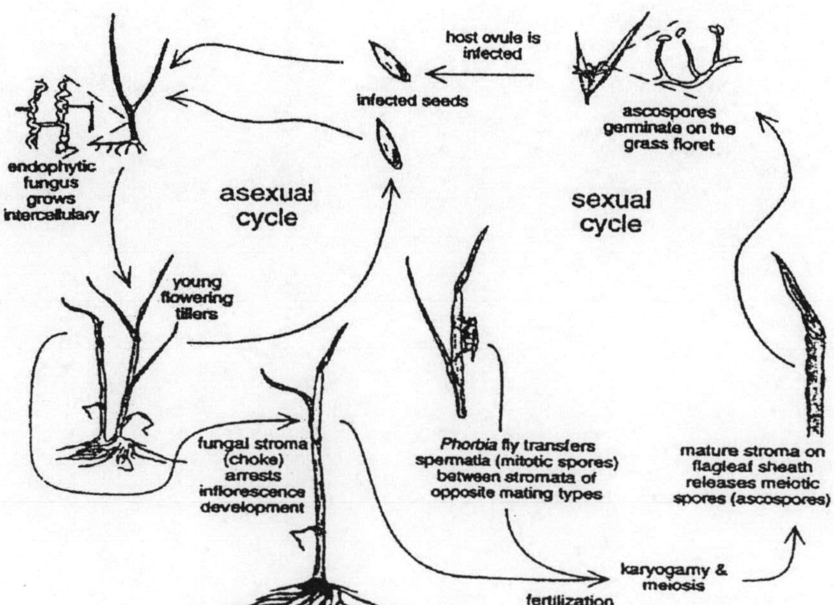


Fig 2.3 Alternative asexual and sexual life cycle of *Epichloe festacae* in symbiois with *Festuca* sp.(Bush et al.,1997)

Telemorphic endophytes are asomycetes (Clavicipitaceae: tribe Balansieae) that infect members of the grass family (Poaceae), sedge family (Cyperaceae) and rush family (Juncaceae) (Clay,1989). The Balensieae is one of three within the ascomycetes subfamily Clavicipitoideae(Clavicipitaceae). The other tribes are Clavicipiteae, containing *Claviceps* spp. and Ustilaginoideae,containing *Ustilaginoidea* spp. . Interest in the Balansieae is increasing due to a members of the group, particularly *Acermonium coenophialum*. These endophytes have been shown to give grasses increased insect resistance, drought to tolerance, and to cause toxic syndromes in cattle that consume infected grasses. (White and Morgan-Jones,1996).

Anamorphic endophyte are probably asexual derivatives of telemorphic endophytes, but they lack a sexual stage, rarely sporulate in their hosts, and form mutualistic associations with their hosts. They are imperfect fungi classified in the genus *Acremonium* sect.*Albo-lanosa*, appear to be closely related to the anamorph of *E.typhina*. They are restricted to cool-season grasses,often to genera that also are host for *E.typhina*. In culture,many produce conidia similar to those produce by *E.typhina*.(Clay,1989). Unlike the Balansiae, They do not sporurate or produce fruiting bodies on their host plants, but their hyphae occur intercellularly in leaf and stem tissue (Clay,1988). They are transimitted maternally by vegetative growth of hyphae into ovules and seeds. Because the strictly seed-borne endophytes do not produced symptoms on their hosts or suppress flowering and can be vertically transmitted from generation to generation,These anamorphic fungi with affinities to *E.typhina* offer the greatest potential for exploitation as biocontrol agents (Clay,1989).

2.3 Non-grasse endophytes

Fungal endophytes of grasses (Poaceae) and sedges (Cyperaceae and Juncaceae) are probably the most extensively studied group (Clay,1988). Report on the presence of endophytes in vascular plants, other than grasses, have focused mainly on ericaceous, dicotyledoneous plants and conifers.

Endophytes can be transmitted from one generation to next trougth the tissue of host seed or vegetative propagules. Except in the grasses, however, most endophytes appear to be transmitted horizontally, external to the host tissue, by spore; climate can greatly influence spore germination and resultant infection frequency of host plant tissue (Carroll,1988).

Rodrigues and Samuels (1990) isolated, for the first time, endophytic fungi from a tropical palm tree growing in the rainforest of Queenslnad, Australia. From this palm, which belong to the spicies *Licuala ramasayi*, eleven fungi were isolated. One of them was describe as a new spicies designated *Idriella licualae.*

In 1994, Rodrigues, recovering 57 spicies and six familial taxa from *Euterpe oleracea* an Amazon palm tree. Ascomycotina and Deuteromycotina were frequently isolated and *Xylaria cubensis* and *Letendraeopsis palmarum* were the most common endophytic spicies. The endophyte *Letendraeopsis palmarum* was described as a new genus and spicies (Rodrigues and Samuels, 1994).

Medeiros (1988) isolated endophytic and epiphytic fungi from leaves of cashew tree (*Anacardium occidentale*) growing in four Brazilian Northeastern states. Twenty-one spicies of endophytic fungi were reported, with some quantitative and qualilative differences found for different localities. *Collectrotrichum gloeosporioides*, *Pestalotia* sp., *Fusarium solani* and *Phomopsis* sp. were the predominant endophytes. In mango (*Mangifera indica*), several pathogenic fungi occur as endophytic, prior to inflorescence emergence *Dothiorella* spp. and *Phompsis mangifera* more frequently in tree not sprayed with copper. Endophytic colonization of inflorescence and pedicel tissue was considered to be primary route of infection for fruits that develop rot stem end during ripening (Johnson et al.,1992).

In 1992 Bill et al., isolated an endophytic *Phomopsis* sp. from woody host *Cavendishia pubescens*. The fungus produced paspalitrem A and C. Such compounds were only recorded at the time as being produced from selerotia of *Claviceps paspali*, causing neurological disorders in livestock.

Pereira et al., (1993) isolated endophytic fungi from young and old leaves of *Stylosanthes guianenisis*, a leguminous genus widely distributed in the tropical and subtropical regions of South America and used as forage plants. At least thirteen endophytic spicies were found. Most of them were rare isolated and *Glomerella cingulata*, *Phomopsis* sp. and *Xylaria* sp. were the most frequently found. The frequency of infection of leaves, as expected, increases with the increases of the plant age. The genus *Xylaria* is reported to be ferquent among endophytes from tropical hosts, as already mentioned.

2.4 Isolation technique of endophytic fungi

Probably no other step is as critical to obtaining good results as thorough but non-penetrating surface sterilization. The possibility that isolates have been initiated from propagules on the surface must be minimized. The choice of sterilization times,concentration, and volumes will be dictated by the thickness of sample, the relative permeability of its surface, and the texture of its surface. Selection of isolates that have emerged from the cut ends of surface sterilized grass leaves has been practiced to ensure that only internal fungi were selected. Such strict criteria usually have not been applied to isolations from plants.

Sterilization methods continue to vary widely, but the preferred method is three-step ethanol, sodium hypochlorite (NaOCl), ethanol treatment. The choice of sterilization times, concentrations, and volumes will be dictated by the thickness of sample, the relative permeability of its surface and the texture of its surface. Serial washing offers the advantage of eliminating the penetrating and killing effects of sterilizing chemicals Pre-washing with tap water can help reduce the time needed foe surface sterilization. This is especially important if vary tiny fragment of tissue are being used.(Bills,1996).

Surface-sterilized plant material may be examined with light microscope for the occurrence of internal hyphae. Fungal endophytes are stained with an aniline blue-lactic acid stain. Another stain used to detect endophytic fungi within plant tissue is rose bengal (Bacon and Hintonn,1997). Media used for culture of phytopatogens will be equally applicable to endophytic isolates. Generally prevalent among studies of endophytes are various formulations of malt ager. When handling many unknown species derived from isolation from vegetative growth a uesful approach is screen each isolate on several media simultaneously (e.g. cornmeal ager, oatmeal agar,V8 juice agar,etc.) in 60 mm plates. Antibacterial antibiotics should always be included in any primary isolation medium for fungi. Oxytetracycline, cholrotetracycline streptommycin sulfate and novobioicin have been used most frequently for endophyte isolation.

2.5 Botanical Aspects of *Croton sublyratus.*

Croton sublyratus (Fig 2.3) or Plau-noi (Thai name) is in the family of Euphorbiaceae(ภาควิชาเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย,2530; ลีนา ผู้พัฒนพงศ์ และ ธวัชชัย วงศ์ประเสริฐ,2530). This plant is a deciduous shrub or tree, 2-3.5 m high,shoots rusty scurfy. The leaves are simple, alternate,4-6 cm wide,10-15 cm long; chodate at the narrowed base, very shortly petioled obovate to almost lyrate oblong obtuse or acuminate repand-serrulate beneath glabrous or nerves and recenmes stellate-tomentose. Young leaves are dark brown and infloresscense. Petiole is stout, 6-12 mm long. The flowers are small, perfect and receme. Flowering is up the scar of the leaf with near shoot. Staminate flower has five lanceolate with acuminate sepal, five petal with stellate rim, long stellate base and stamens 15-20 glabrous. Pistillate flower is similar to staminate flower,no petal and ovary is densely stellate tomentose,brown-yellow with short styles. The fruits are capsules small 3 lobed crustaceuos sparsely pubescent and 3-5 mm long. The seed are 2-3 mm long, white-brown and smooth (ลีนา ผู้พัฒนพงศ์ ,2530; ลีนา ผู้พัฒนพงศ์ และ ธวัชชัย วงศ์ประเสริฐ ,2530;ลัดดาวัลย์ บุญรัตนกรกิจ,2535;Hooker,1973).

C. sublyratus grows extensively in tropical areas, especially those near by the Andaman sea such as Indonesia, Malaysia, Thailand, Burma and the south of China. Thai *C. sublyratus* or Plau-noi is found to be native to the Thai provinces of Prachin Buri, Prachuap Khiri Khun and the border near Burma of Kanchanaburi (ณรงค์ เพ็งปรีชา,2530; ลีนา ผู้พัฒนพงศ์ และธวัชชัย วงศ์ประเสริฐ,2530; ลัดดาวัลย์ บุญรัตนกรกิจ,2535; วีณา วิรัจฉริยากูล และ คณะ,2533).

2.5.1 The Uses of *C. sublyratus*.

C. sublyratus (Plau-noi) is a Thai folk medicine for anthelmintic and dermatologig agent for skin disease (ภาควิชาเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย,2530;ลัดดาวัลย์ บุญรัตนกรกิจ, 2535;). The plant parts of stem, bark, and leaf have been used as anthelmintic (คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล,1990). Firewood of Plau-noi is used for postpartum (เปรมจิตร นาคประสิทธิ์,บรรณธิการ,2528). In addition,it has been reported that Plau-noi and Plau-Yai (*C.oblongifolius* Roxb.) are used joinly in many Thai drug, such as stomachic, anthelmintic, emmenagogue, digestant, transquilizer, carminative, treatment of lymph, pruritic, leprocy, tumor and yews (ประเสริฐ พรหมมณี และคณะ,2531;นันทวัน บุญยะประภัศร,บรรณธิการ,2532).

2.5.2 Chemical Constituents of *C. sublyratus*.

Since 1987 , when Ogiso et al. Isolated and identified plaunotol as antipeptic ulcer substance from the stem of *C.sublyratus* (Ogiso et al.,1978),the reseach on isolation of the constituents from *C.sublyratus* has continued. The groups of compound found in this plant include diterpene lactone,furanoid diterpene,diterpene alcohols and esters of diterpene alcohol.

Figure 2.4 *Croton sublyratus* (A), Leaves of *Croton sublyratus* (B)

2.6 Study of secondary metabolite from the endophytic fungi

Natural products from endophytic microbes have been observed to inhibit or kill a wide variety of harmful disease-causing agents including, but not limited to, phytophytogen, as well as bacteria, fungi, viruses, and protozoa that affect humans and animals. Some example of bioactive products from endophytic fungi are Taxol an anticancer agents that are produced by endophytic fungus *Taxomyces andreanae* from Pacific yew *Taxus brevifolia*.

In 1996, Julie et al., found that a *Pestalotiosis microspora* an endophyte associated with the endangered tree *Torreya taifolia* (Florida torreya) produce Torreyanic acid, a selectively cytotoxic quinone dimer against human cancer cell lines.

Stierle et al. (1999) reported that sequoiatones A and B were isolated from the fungus *Aspergillus parasiticus*, an endophytic fungus of the coast redwood, *Sequoia sempervirens*. The compounds showed moderate and somewhat selective inhibition of human tumor cells, with greatest efficacy against breast cancer cell lines.

In 2000, Lu et al., found that a *Collectrotrichum* species, an endophyte from *Artemisia annua* can produce in vitro metabolites that were shown to be antimicrbial. These finding suggested the possibility that the endophytic *Collectotrichum* sp. in A. annua could protect the host by producing metabolites,which may be toxic or even lethal to phytopathogens.

Phomopsis longicolla, the endophytic fungus of the endangered mint *Dicerandra frutescens*, was found to produced dicerandrol A, B, C the xanthones with antimicrobial activities. (Wagenaar and Clardy,2001). Ambuic acid, a highly functionalized cyclohexanone produced by a number of isolate of Pestalotiopsis microspora found in rainforests around the world. This compound possesses antifungal activity (Li et al., 2001). The chemical compound, sources, biological activities of secondary metabolite of endophytic fungi were sumerized in Table 2.1 and Figure 2.5

Table 2.1 Source and biological activities of secondary metabolites of endophytic fungi

NO.	Compounds	Types	Endophytic fungi	Host plants	Activities	References
1	Peramine	Pyrrolopyrazine alkaloid	*Neotyphodium coenophialum* *N. lolli* *Epichloe festucae* *E. typhina*	Tall fescue, ryegrass	Insect Toxic	Schardl and Phillips,1997
2	Ergobalansine	Ergot alkaloid	*Neotyphodium* spp. *Clavicaeps purpurea*	*Festuca* spp.	Neurotoxic	Powell et al., 1992
3	Ergotamine					
4	Ergosine					
5	β-ergosine					
6	Ergovaline					
7	Ergostine					
8	Ergoptine					
9	β-ergoptine					
10	Ergonine					
11	Ergocristine					
12	α-ergocrptine					

Table 2.1 (continued)

NO.	Compounds	Types	Endophytic fungi	Host plants	Activities	References
13	β-ergocryptine	Ergot alkaloid	*Neotyphodium* spp. *Clavicaeps purpurea*	*Festuca* spp.	Neurotoxic	Powell et al., 1992
14	Ergocornine					
15	Ergonovine					
16	Lysergamind					
17	8-hydroxylsergamind					
18	Isolysergamide					
19	Phomopsichalasin	Cyclochalasan	*Phomasis* sp.	*Salix gracilostyla* var. *melanostachys*	Antibacterial and Antifungal	Horn et al., 1995
20	Cryptocin	Tetramic acid	*Cryptosporiopsis* cf. *quercina*	*Tripterygium wilfordii*	Antimycotic	Li et al., 2000
21	Lolitrem N	Indole diterpene alkaloid	*Neotyphodium lolli*	*Lolium perenne*	Neurotoxic	Munday-Finch et al.,1998
22	Lolitriol					
23	Lolicine A					
24	Lolicine B					

Table 2.1 (continued)

NO.	Compounds	Types	Endophytic fungi	Host plants	Activities	References
25	Loline	Saturated aminopyrrolizidine alkaloids	*Neotyphodium coenophialum* *N. unicinatum*	*Festuca arundinacea* *F. pratensis*	Insecticide	Schardl and Phillips,1997
26	Norloline					
27	*N*-methylloline					
28	*N*-formylnorloline					
29	*N*-acetylnorloline					
30	3β-hydroxyergosta-5-ene	Ergosterol	*Collectotrichum* sp.	*Artemisia annua*	Antifungal	Lu et al., 2000
31	3-oxoergosta-4,6,8(14),22-tetraene					
32	3β,5α-dihydroxy-6β-acetoxyergosta-7,22-diene					
33	3β,5α-dihydroxy-6β-phenylacetoxyergosta-7,22-diene					

Table 2.1 (continued)

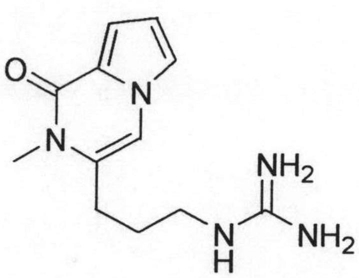
NO.	Compounds	Types	Endophytic fungi	Host plants	Activities	References
34 35	Heptelidic acid Hydroheptelidic acid	Sesquiterpenes	*Phyllostica* sp.	*Abies balsamea*	Toxic to spruce bud worm	Calhoun et al., 1992
36 37	Subglutinol A Subglutinol B	Diterpenes	*Fusarium subglutinans*	*Tripterygium wilfordii*	Immonosuppressive	Lee et al.,1995
38	Taxol	Diterpenes	*Taxomyces andrenea*	*Taxus brevifolia*	Anticancer	Strobel et al., 2003, Stierle and Strobel ,1995
			Stegolerium kukenani	*Stegolepis guianensis*	Anticancer	Strobel et al.,2001
			Aspergillus niger	*Taxus chinensis*		Wang et al., 2001
			Tubercularia sp.	*Taxus mairai*		Strobel et al., 2003 Wang et al., 2000
			Pestalotiopsis microspora	*Taxus wallachina* *Taxodium distichum*		Strobel et al., 2003, Li et al., 1998 Li et al., 1996
			Periconia sp.	*Torreya grandifolia*		Li et al., 1998
			Pestalotiopsis guepinii	*Wollemia nobilis*		Strobel et al., 1997

Table 2.1 (continued)

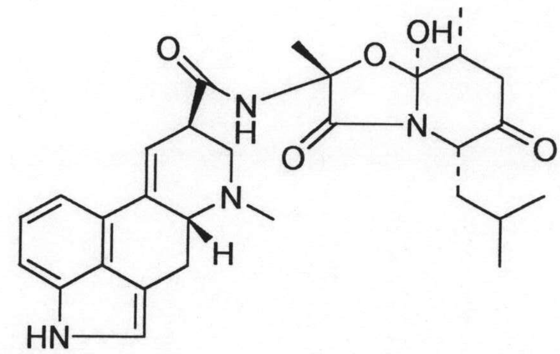
NO.	Compounds	Types	Endophytic fungi	Host plants	Activities	References
39	Leucinostatin A	Oligopeptide	*Acremonium* sp.	*Taxus baccata*	Phytotoxic, Anticancer, Antifungal	Strobel et al., 1997
40	Cryptocandin	Cyclopeptide	*Cryptosporiopsis* cf. *quercina*	Red wood	Antifungal	Strobel et al., 1999
41	Cytonic acids A	Tridepsides	*Cytonaema* sp.	*Quercus* sp.	Antiviral	Guo et al., 2000
42	Cytonic acid B					
43	Sequoiatone A	Ester	*Aspergillus parasiticus*	Red wood	Antitumor	Stierle et al., 1999
44	Sequoiatone B					
45	Dicerandrol A	2,2'-dimeric tetrahydroxanthones	*Phomopsis longicolla*	*Dicerandra frutescens*	Cytotoxic, Antibacterial	Wagenaar and Clardy, 2001
46	Dicerandrol B					
47	Dicerandrol C					

Table 2.1 (continued)

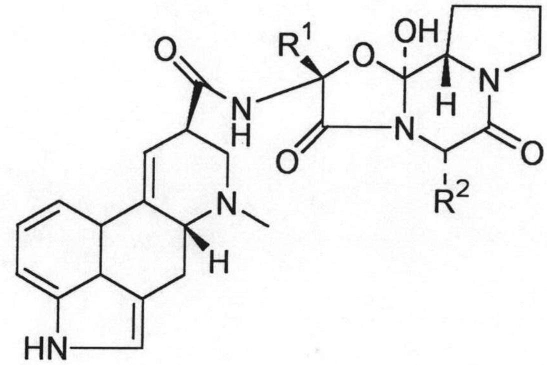
NO.	Compounds	Types	Endophytic fungi	Host plants	Activities	References
48	Ambuic acid	Cyclohexenone	*Pestalotiopsis microspora*	*Taxus spp.*	Antifungal	Li et al., 2001
49	CR377	Pentaketide	*Fusarium* sp.	*Selaginella pallescens*	Antifungal	Brandy and Clardy, 2000
50	Colletotric acid	Tridepside	*Collectrichum gloeosporiodes*	*Artemisia mongolica*	Antimicrobial	Zou et al., 2000
51	Pestacin	Isobenzofuran	*Pestalotiopsis microspora*	*Terminalia morobensis*	Antimicrobial, Antioxidant	Harper et al., 2003
52	Isopestacin	Isobenzofuranone	*Pestalotiosis microspora*	*Terminalia morobensis*	Antimicrobial, Antioxidant	Strobel et al., 2002
53	Naphthalene	Benzene	*Muscudor vitigenus*	*Paullina paullinioides*	Insect repellant	Daisy et al., 2002
54	Fusaricide	Pyridine alkaloid	*Fusarium* sp.	*Oxydendron arborcum*	Cytotoxic	Kimberly et al., 1996
55	Torreyanic acid	Quinone dimer	*Pestalotiopsis microspora*	*Torreya taxifolia*	Cytotoxic	Lee et al., 1996



1. Paramine



2. Ergobalansine



3. Ergotamine

4. Ergosine R^1= Me, R^2 = Bu^i

5. b-ergosine R^1= Me, R^2= *sec* Bu

6. Ergovoline R^1= Me, R^2= Pr^i

7. Ergostine R^1 = Et, R^2 = $PhCH_2$

8. Ergoptine R^1 =Et, R^2 = Bu^i

9. β-ergoptine R^1=Et, R^2 = *sec*-Bu

10. Ergonine R^1= Et, R^2 = Pr^i

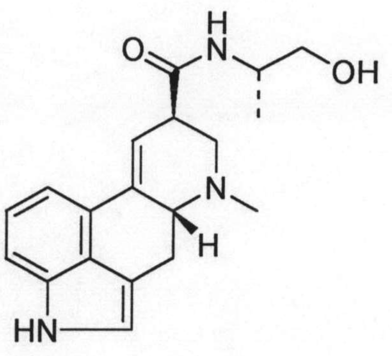
11. Ergocristine R^1=Pr^i, R^2 = $PhCH_2$

12. α-ergocryptine R^1= Pr^i, R^2= Bu^i

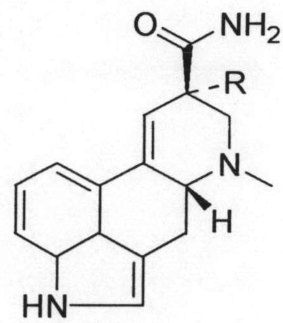
13. β-ergocryptine R^1= Pr^i, R^2= *sec* Bu

14. Ergocomine R^1=R^2=Pr^i

Figure 2.5 Sturcture of secondary metabolites of endophytic fungi

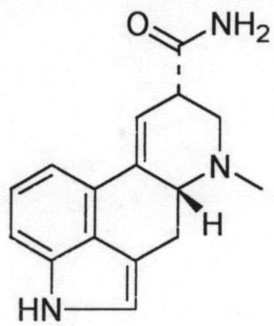


15. Ergonovine

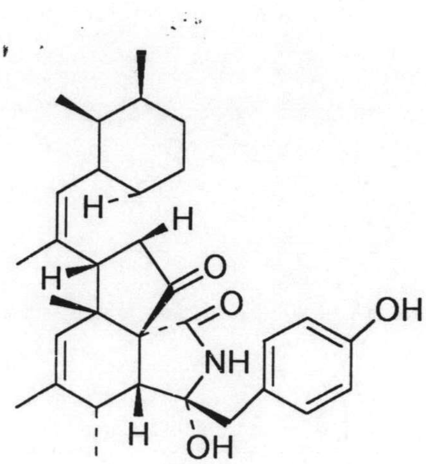


16. Lysergamind R=H

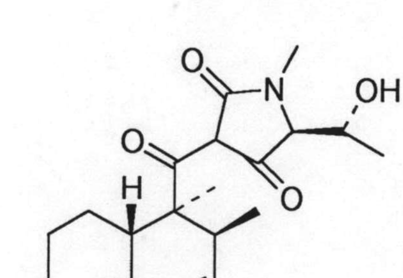
17. 8-hydroxylysergamind R=OH



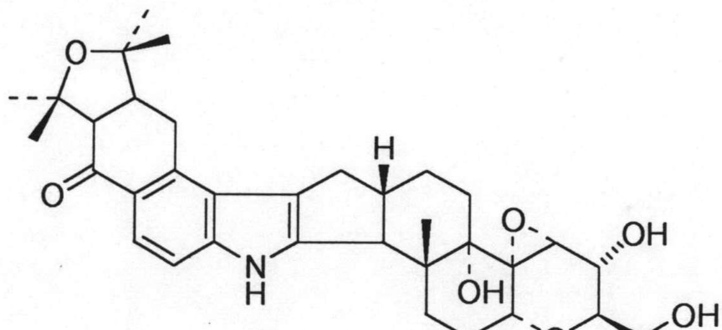
18.Isolysergamind



19. Phomopsichalasin



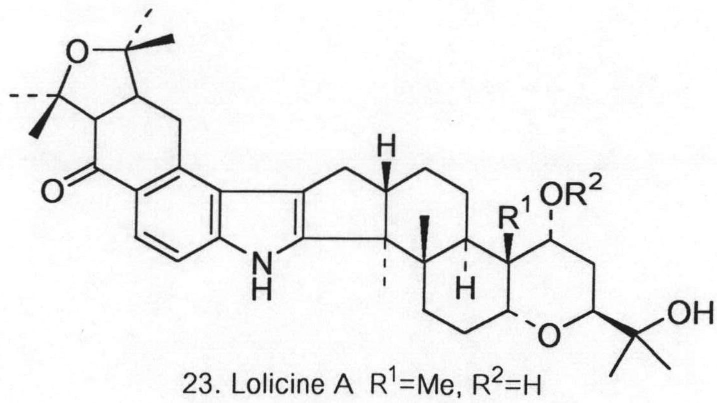
20. Cryptocin



21. Lolitrem N 31 beta,35 alpha

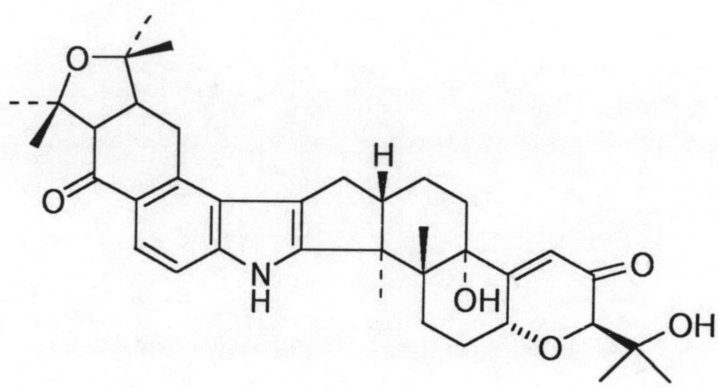
22. Lolitriol 31alpha,35 beta

Figure 2.5 (continued)

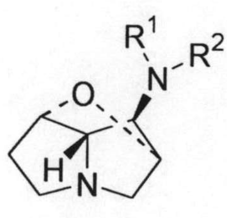


23. Lolicine A R^1=Me, R^2=H

24. Lolicine B R^1 = CHO, R^2 =H



25. Loliline

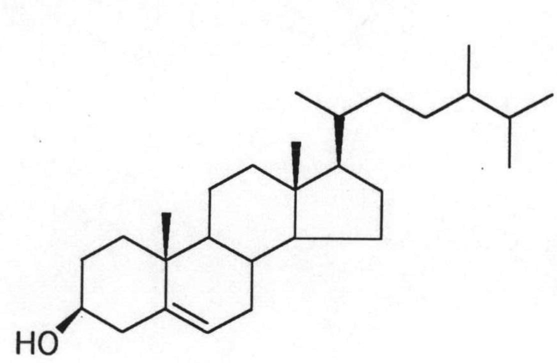


26. Norloline R^1=R^2=H

27. *N*-methylloline R^1=R^2=Me

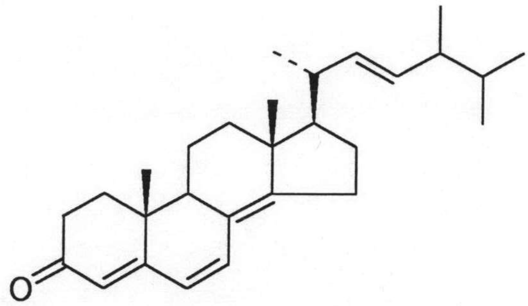
28. *N*-formylnorloline R^1=H, R^2 =CHO

29. *N*-acetylnorloline R^1=H, R^2=Ac

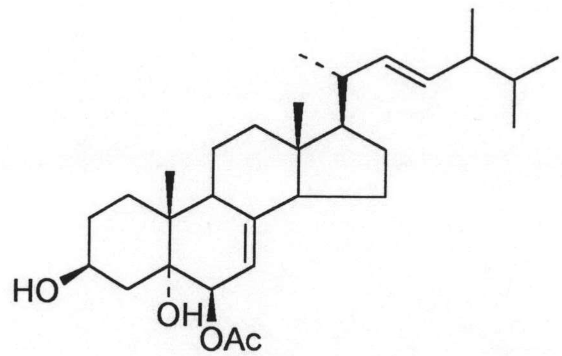


30. 3β-hydroxyergosta-5-ene

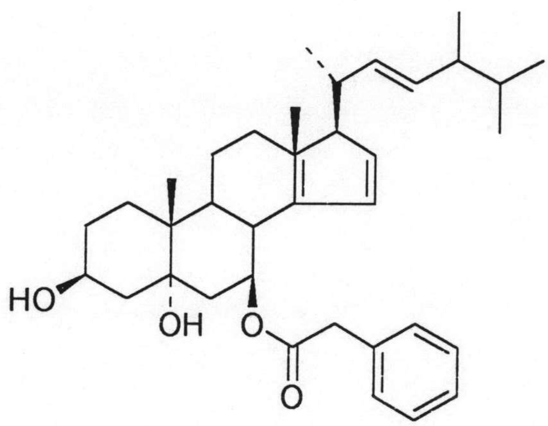
Figure 2.5 (continued)



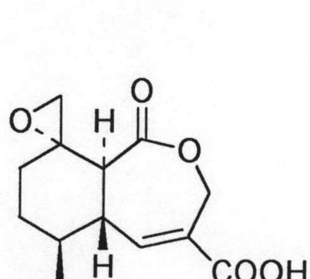
31. 3-oxoergosta-4,6,8(14),22-tetraene



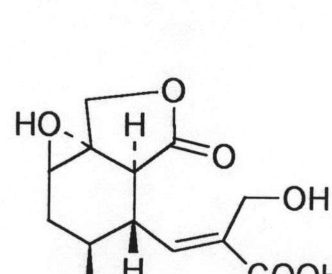
32. 3β,5α-dihydroxy-6β-acetoxyergosta-7,22-diene



33. 3β,5α-dihydroxy-6β-phynylacetoxyergosta-7,22-diene

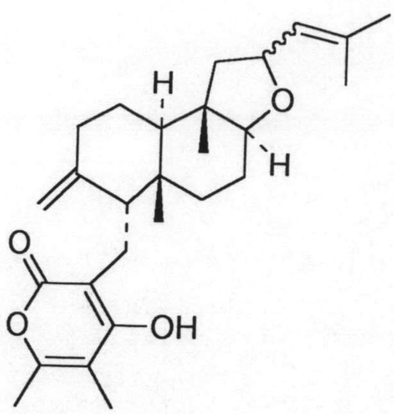


34. Heptelidic acid



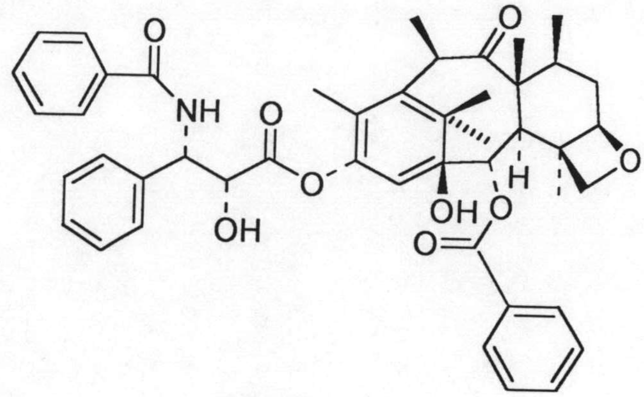
35. hydroheptelidic acid

Figure 2.5 (continued)

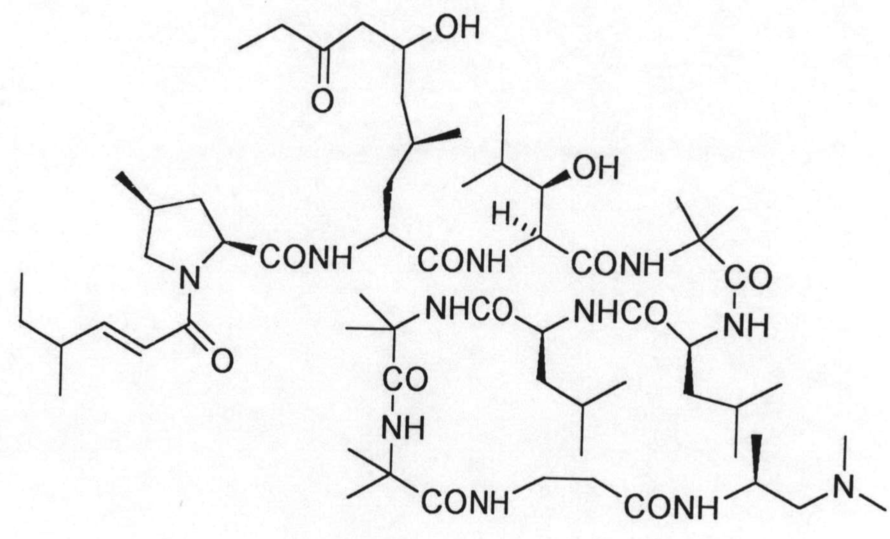


36. Sublutinol A 12 *S*

37. Sublutinol B 12 *R*

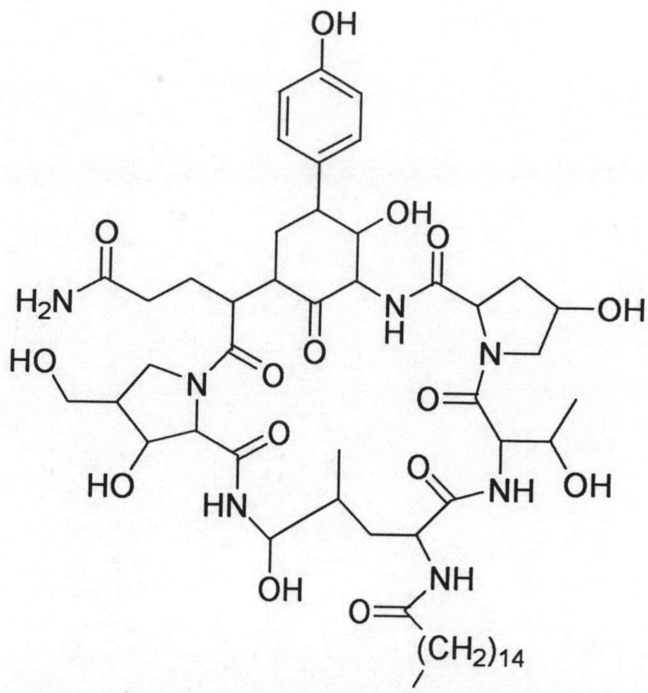


38. Taxol

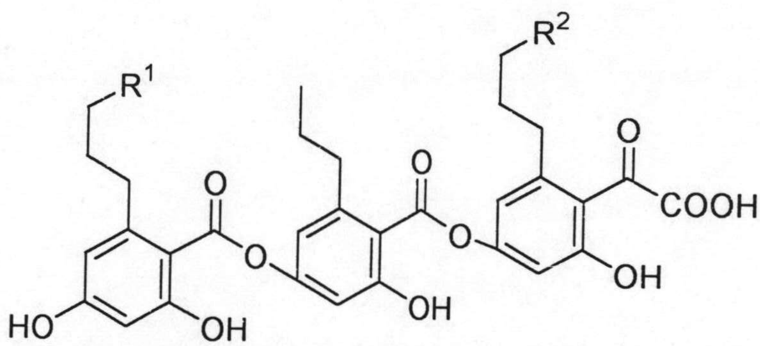


39. Leucinostatin

Figure 2.5 (continued)

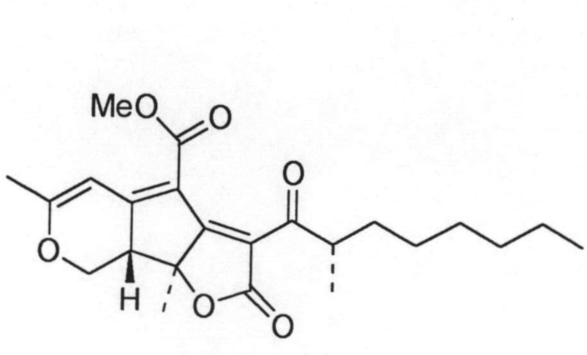


40. Cryptocandin

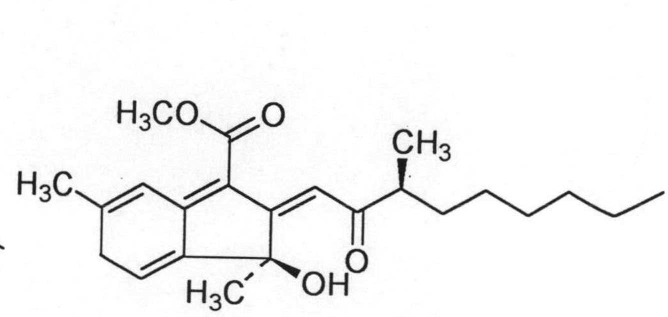


41. Cytonic acid A R^1= Et, R^2 = H

42. Cytonic acid B R^1= H, R^2 = Et

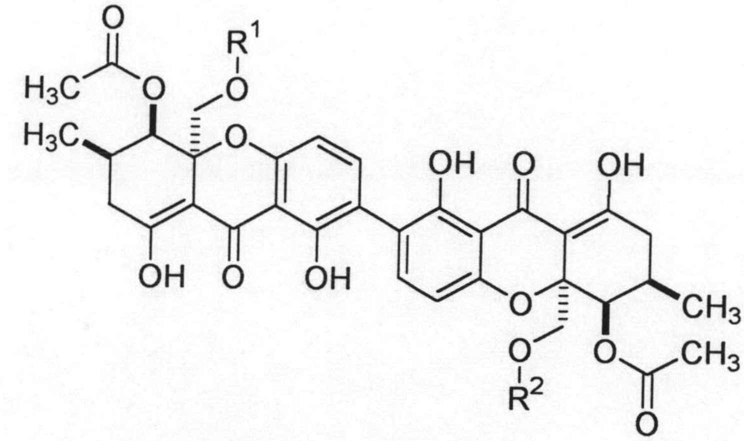


43. Sequoiatone A



44. Sequoiatone B

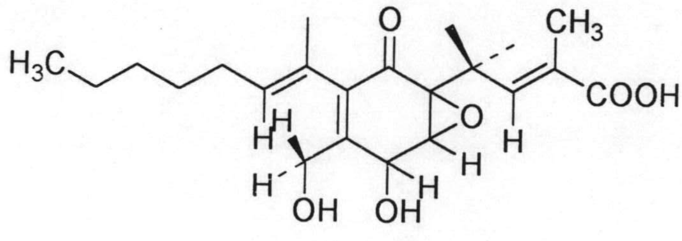
Figure 2.5 (continued)



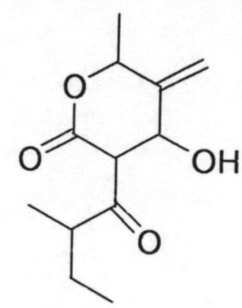
45. Dicerdrols A $R_1 = R_2 = H$

46. Dicerdrols B $R_1 = Ac$ $R_2 = H$

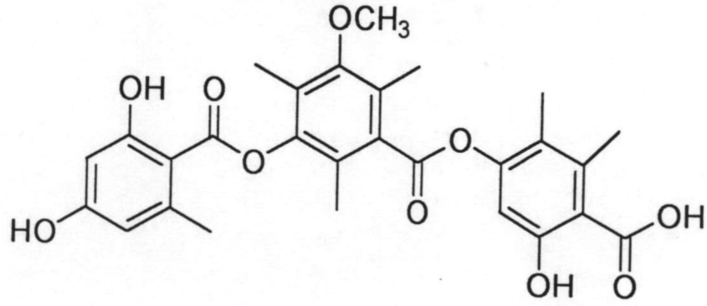
47. Dicerdrols C $R_1 = R_2 = Ac$



48. Ambuic acid

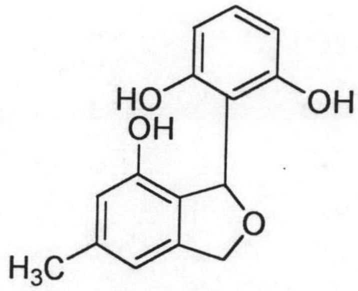


49. CR 377

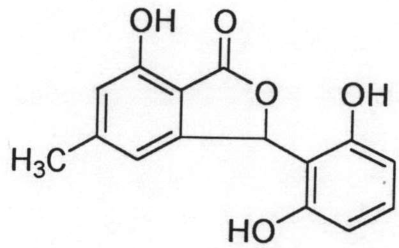


50. Colletoria acid

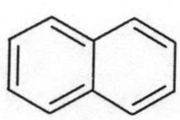
Figure 2.5 (continued)



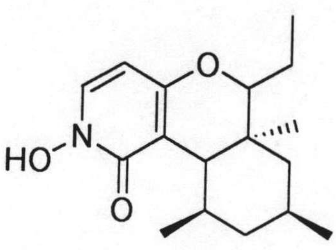
51. Pestacin



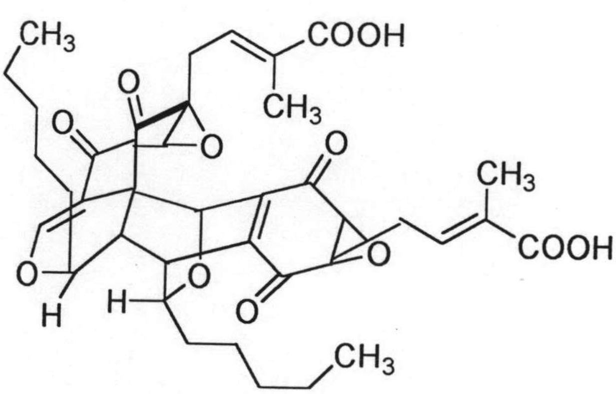
52. Isopestacin



53.Naphthalene



54. Fusaricide



55. Torreyanic acid

Figure 2.5 (continued)