Journal of Industrial Microbiology (1996) 17, 402–416

Pyrenomycetes and Loculoascomycetes as sources of secondary metabolites

LH Huang and T Kaneko

Department of Medicinal Chemistry and Natural Products Discovery, Central Research, Pfizer Inc, Eastern Point Road, Groton, CT 06340, USA

Over 400 secondary metabolites have been reported from members of the Pyrenomycetes and the Loculoascomycetes. Among these, members of the Hypocreaceae, the Clavicipitaceae, the Xylariaceae, the Melanosporaceae, and the Sordariaceae in the Pyrenomycetes, and those of the Pleosporaceae and the Sporormiaceae in the Loculoascomycetes have been explored frequently; and representative secondary metabolites produced by these fungi are illustrated. Many of them are reported to be phytotoxic and some of them have antibacterial or antifungal activities. Only recently were the compounds tested in screens targeted for specific enzyme inhibitors or receptor-agonists/antagonists. This group of fungi are attractive for screening for novel natural products because of the diversity of species and physiology.

Keywords: Ascomycetes; Pyrenomycetes; Loculoascomycetes; secondary metabolites; natural products

The pharmaceutical industry is increasing interest in screening fungi for secondary metabolites. Although interest is mounting, our knowledge of isolation methods, fungal physiology, and fungal diversity remains rather limited. The 69 000 fungal species currently recognized are estimated to be less than 5% of the total number (1.5×10^6) thought to exist [30]. Many groups of fungi, such as powdery mildews, some rusts and smuts, have eluded the efforts of fungal physiologists to cultivate them. Because of the time constraints imposed in the pharmaceutical industry, most investigators tend to concentrate on fast-growing fungi with similar physiological and biochemical requirements. In order to explore the potential of the less studied and more diverse groups of Ascomycetes to produce secondary metabolites, the Pyrenomycetes and Loculoascomycetes are reviewed in this article. The Ascomycetes consist mainly of the Plectomycetes, the Pyrenomycetes, the Loculoascomycetes, and the Discomycetes. The Plectomycetes which produce globose ascomata and the Discomycetes which produce cup-shaped ascomata are excluded from this review. Plectomycetes with anamorphic states *Aspergillus* and *Pencillium* have been reviewed extensively elsewhere. The grouping of genera into families is based on the schemes of Müller and von Arx and von Arx and Müller [9,46].

Excellent studies have been carried out on individual Pyrenomycetes and Loculomycetes. For example, Nair's group published a series of papers on metabolites of the Pyrenomycetes [48,49], while Turner's group worked on fungal metabolites including those of the Pyrenomycetes [2,24]. The Xylariaceae have been explored by Edwards and Whalley's groups with regard to secondary metabolite production [20,21,87]; Gloer and colleagues reported a series of novel compounds from coprophilous Ascomycetes including coniochaetones A and B from *Coniochaeta saccardoi* and terezines A–D from *Sporormiella teretispora* [78,80]. More recently, the squalene synthase inhibitors known as zaragozic acids (squalestatins) were found to be produced by members of the Pleosporaceae and their anamorphic states [10]. In contrast to the focus on fast growing organisms in many research programs, these researchers focused on certain types of slower-glowing, mid- to late-successional species.

Habitats

The two groups of fungi considered in this review live in various modes on many different substrates. Many of the Pyrenomycetes are saprophytes found in a variety of substrates such as soil, dung, wood, and decaying leaves or petioles. Some thrive in unusual environments, for example, on wood submerged in marine water. Many pyrenomycetes are parasitic to a wide range of organisms, including the marine red algae, lichens, other fungi, insects, and higher plants. Loculoascomycetes exist as superficial epiphytes, parasites, or hyperparasites of superficial fungi and insects, as internal parasites fruiting on green leaves and stems, lichens, mosses, as parasites fruiting on dead leaves, stems, or as saprophytes on dead leaves, herbaceous stems, wood, dung, and plant debris. In addition, they occur in the marine environments on such surfaces as submerged wood, sand, and algae.

Since stromata of the Xylariaceae are fairly large and are easily isolated, most investigators tend to work in this group of fungi in terms of secondary metabolites. Similarly, members of the Hypocreaceae and the Clavacipitaceae also produce more conspicuous stromata. Thus, the Xylariaceae produced the major compounds such as dihydroisocoumarins, punctaporonins, cytochalasins, butyrolactones, and succinic acid derivatives [7,87]. Marine environs are reservoirs for the fungi under review, and several novel compounds

Correspondence: LH Huang, Department of Medicinal Chemistry and Natural Products Discovery, Central Research, Pfizer Inc, Eastern Point Road, Groton, CT 06340, USA
Received 3 April 1996; accepted 28 August 1996

have been reported to be produced by the following marine fungi: auranticins A and B by *Preussia aurantiaca*; obionin A by *Leptosphaeria obiones*; leptosphaerin, leptosphaerodione, and leptosphaerolide by *Leptosphaeria oraemaris*; two lactides by *Hypoxylon oceanicum*, melinacidins III and IV and gancidin W by *Corollospora pulchella* and leptosins A–H by *Leptosphaeria* sp [43].

Members of the Pyrenomycetes and Loculoascomycetes can be obtained from culture collections such as American Type Culture Collection, Centraalbureau voor Schimmelcultures, International Mycological Institute, and Institute for Fermentation (Osaka). Taxonomists who are active in studying them in culture include Drs RT Hanlin, S Huhndorf, ES Luttrell, JD Rogers, AY Rossman, GJ Samuels, CH Shearer and EG Simmons.

Distribution of secondary metabolites in different taxa

Over 400 novel compounds have been discovered from fungi classified in the Pyrenomycetes and the Loculoascomycetes based on a survey of CA Selects: Novel Natural Products (published by the American Chemical Society) and the Dictionary of Natural Products (Chapman & Hall). The period covered in this review is from 1930 up to the present. The total number of the compounds produced would have been much larger if anamorphic states were taken into consideration. For example, members of the genera *Fusarium, Trichoderma* and *Gliocladium*, and the anamorphic states of the Hypocreales produce many toxic or other bioactive secondary metabolites.

Among the Pyrenomycetes, members of the Melanosporaceae, the Xylariaceae, the Hypocreaceae, and the Clavicipitaceae are the dominant producers of reported secondary metabolites, followed by members of the Sordariaceae and the Ophiostomataceae (Table 1). Among the Loculoascomycetes, members of the Pleosporaceae and the Sporormiaceae produce more secondary metabolites than those of the other families (Table 2). The genera whose members produce 17 or more secondary metabolites include *Gibberella, Claviceps, Chaetomium, Ceratocystis, Hypoxylon, Epichloe*, and *Neurospora*. Notable among them are *Gibberella fujikuroi*, which produces 65 gibberellins [26], and *Claviceps purpurea* and *C. paspali*, which elaborate 40 ergot alkaloids [71]. These have been reviewed in the references cited [26,71] and elsewhere and consequently are not included in this article.

The genera with more than two species that have been reported to produce secondary metabolites are listed in Table 3. All of the other genera not listed in Table 3 have either one or two species that are known to produce secondary metabolites. In terms of different types of secondary metabolites, the most productive genera in a decreasing order are *Chaetomium, Ceratocystis, Claviceps, Hypoxylon, Nectria*, and *Preussia*.

Table 1 Numbers of secondary metabolites produced by Pyrenomycetes

Family Genus	No. produced by genus	No. produced by family
Amphisphaeriaceae		2
Apiospora	2	
Clavicipitaceae		>83
Acrospermum	2	
Balansia	4	
Claviceps	>55	
Cordyceps	5	
Epichloe	17	
Diaporthaceae		8
Endothia	3	
Gnomonia	3	
Melanconis	2	
Diatrypaceae		2
Eutypa	2	
Halosphaeriaceae		3
Corollospora	3	
Hypocreaceae		>120
Calonectria	1	
Gibberella	>82	
Hypocrea	4	
Nectria	24	
Neocosmospora	9	
Hypomycetaceae		4
Hypomyces	4	
Melanosporaceae		60
Achaetomium	2	
Chaetomium	40	
Kernia	1	
Melanospora	1	
Microascus	1	
Petriella	2	
Thielavia	13	
Ophiostomataceae		20
Ceratocystis	20	
Polystigmataceae		5
Glomerella	5	
Sordariaceae		35
Coniochaeta	3	
Gelasinospora	4	
Neurospora	17	
Podospora	4	
Sordaria	7	
Xylariaceae		64
Biscogniauxia	2	
Bolinia	3	
Camarops	2	
Daldinia	6	
Engleromyces	1	
Hypoxylon	22	
Nummulariola	1	
Poronia	9	
Rosellinia	13	
Thamnomyces	2	
Xylaria	3	

Fermentation

Unlike bacteria, most fungi are acidiophilic and grow on solid substrates in their natural habitats. They are generally aerobic and mesophilic with regard to oxygen and temperature requirements. Fungal species and even strains of a single species vary considerably in their rate of growth. The variation of growth rate is especially evident in species of the Pyrenomycetes and the Loculoascomycetes. The periods of fermentation employed in producing the compounds reviewed here ranged from 2 days to 8 weeks. Most fermentations leading to all 400 compounds were done with stationary surface culture as the method of choice since this method provides better productions than either liquid or

Table 2 Numbers of secondary metabolites produced by Loculoascomycetes

Family Genus	No. produced by genus	No. produced by family
Botryosphaeriaceae		5
Botryosphaeria	1	
Guignardia	4	
Mycosphaerellaceae		3
Mycosphaerella	3	
Patellariaceae		4
Buellia	4	
Pleosporaceae		36
Cochliobolus	7	
Herpotrichia	1	
Leptosphaeria	18	
Ophiobolus	5	
Phaeosphaeria	3	
Pleospora	1	
Setosphaeria	1	
Pseudosphaeriaceae		8
Leptosphaerulina	1	
Pyrenophora	7	
Sporormiaceae		24
Preussia	11	
Sporormia	1	
Sporomiella	11	
Westerdykella	1	

Table 3 Number of species in a genus producing secondary metabolites and number of recognized species in a genus [29]

Genus	No. species which produce	Recognised species
Chaetomium	17	200
Ceratocystis	14	>80
Claviceps	8	35
Hypoxylon	8	120
Nectria	8	200
Preussia	6	10
Gibberella	5	10
Leptosphaeria	5	100
Cochliobolus	4	11
Cordyceps	4	100
Balansia	3	20
Endothia	3	10
Glomerella	3	4
Hypocrea	3	100
Hypomyces	3	30
Mycosphaerella	3	>500

solid fermentation. Many fast-growing fungi are fermented in liquid shaken culture while some slow-growing fungi are fermented on solid substrate. The amount of oxygen influences growth rate; when liquid culture was used, an rpm of shaker flasks below 300 was often employed. The pH of the fermentation media prior to sterilization ranged from 5.5 to 7.0. The temperature used to ferment cultures ranged from 24 to 30°C. In addition to aeration, pH and temperature, other factors affecting growth rate such as light, nutrient concentration, and age of the culture are also important, although these are seldom mentioned in the literature.

Secondary metabolites and biological activities

Representative secondary metabolites produced by these classes of organisms are listed in Table 4. The table is meant to be illustrative rather than comprehensive, and the compounds are listed in the alphabetical order of the producing organisms. The classification of secondary metabolites is based on their proven or probable biosynthetic origins. Some of them, for example entries 2, 8, and 19, are of mixed origin.

Polyketide-derived secondary metabolites share the biggest proportion among the compounds in the Table. They range from the relatively simple tetraketide (eg, isoepoxydon, entry 58) to undekaketide hypoxyxylerone (entry 39). Among the isoprene-derived compounds, the list includes the monoterpene isopulegol (entry 6) at one end and sesterterpene ophiobolin (entry 19) at the other. Although plants are better known sources of alkaloids, these two classes reviewed here produce unusually large number of alkaloids, including ergot alkaloids, cytochalasins, and epipolythiodioxopiperazines (eg, gliotoxin). There are relatively few examples of nucleosides and polypeptides reported to date. Bioactivity is described, when available, by using the term used in the original publication. For example, fungitoxic and antifungal activities might be considered the same, but both terms are retained according to the original papers. Most of the biological activities reported for these metabolites are either antifungal or antibacterial although some produce metabolites with antitumor, antiviral, or antiprotozoal activities. This may reflect the fact that these activities have been investigated most heavily since, besides the need for such agents, the assays were relatively straightforward and easy to carry out. There are also many compounds whose activity is described as phytotoxic. Here, again, phytotoxicity was often the only activity tested because the organism was initially isolated as a plant pathogen.

Other assays targeting specific enzymes or receptors are of relatively recent occurrence, and there may not be sufficient data accumulated to determine the numbers of metabolites with these activities. Some secondary metabolites bind to receptors (a tachykinin antagonist, cyclosporin C; and D_1-antagonist, obionin A) or inhibit enzymes (phospholipase A2 inhibitor, thielocin A1β; prostaglandin biosynthesis inhibitor, thielavin B; HIV protease inhibitor, cytochalasin analog L-696 474).

The antifungal activity of certain secondary metabolites is often cited as a possible ecological advantage for the producing organism over other fungi. However, the potency of the compound once purified does not appear to be high enough in many cases to result in a competitive edge. The actual concentration and synergy within the natural habitat needs to be examined to establish the ecological significance of such a compound [83].

Several hydroquinone and quinone derivatives have antibacterial activity (see entries 5, 34, 47, and 48). The compound in entry 47, for example, inhibited *Staphylococcus aureus* at 1 μg ml^{-1}. Although their biosynthetic origins

Table 4 Structures of the secondary metabolites produced by Pyrenomycetes and Loculoascomycetes

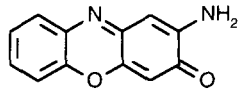
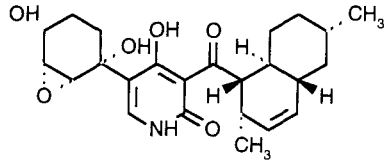
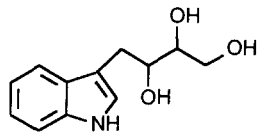
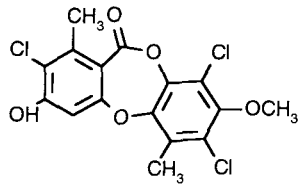
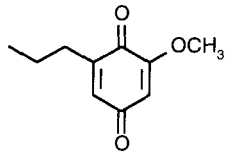
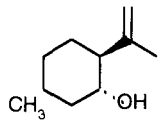
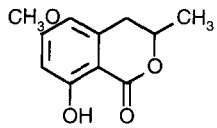
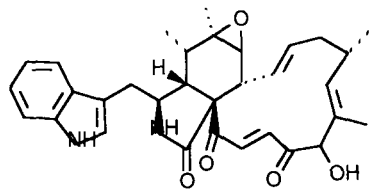
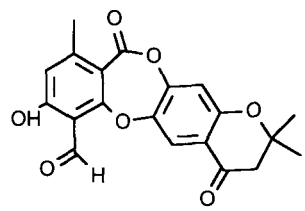
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
1. *Acrospermum viticola*	AV-toxin C		amino acid	phytotoxic	AV-toxins D and E also isolated	[37]
2. *Apiospora montagei*	Apiosporamide			antifungal		[4]
3. *Balansia epichloe*	A-1		amino acid	na	A-2 and B also isolated	[63]
4. *Buellia canescens*			polyketide	na		[68]
5. *Camarops microspora*				antibiotic		[77]
6. *Ceratocystis coerulescens*	isopulegol		monoterpine	na		[39]
7. *Ceratocystis fimbriata*			polyketide	na		[73]
8. *Chaetomium mollipilium*	chaetoglobosin A		cytochalasin	cytochalasin activity	Many species of this genus produce this compound	[75]
9. *Chaetomium mollicellum*	mollicellin		polyketide	antibacterial		[72]

Table 4 Continued

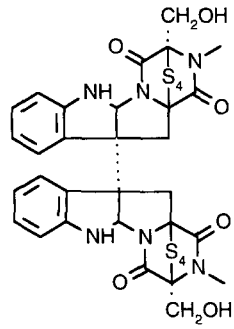
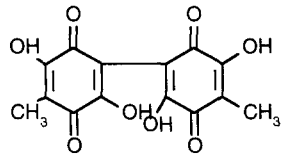
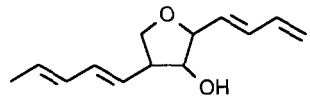
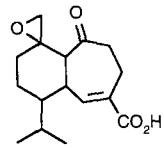
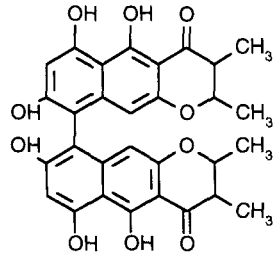
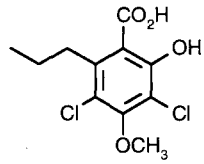
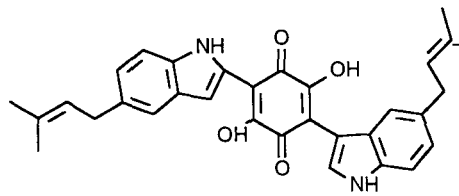
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
10. *Chaetomium nigricolor*	chetracin A		diketopiperazine	antibacterial and cytotoxic	B and C also isolated	[67]
11. *Chaetomium trilaterale*	oosporein		polyketide	phytotoxic		[18]
12. *Chaetomium coarctatum*				na		[14]
13. *Chaetomium globosum*	heptelidic acid		sesquiterpene	antibiotic and cytotoxic		[32]
14. *Chaetomium thielavioideum*	chaetochromin		polyketide			[69]
15. *Chaetomium* sp	differanisole		polyketide	cell differentiation		[57]
16. *Chaetomium elatum*	cochliodinol		shikimic acid pathway	na		[70]

Table 4 Continued

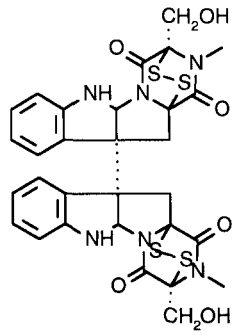
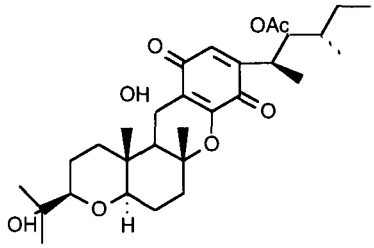
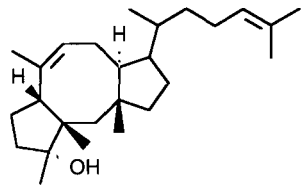
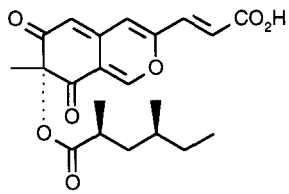
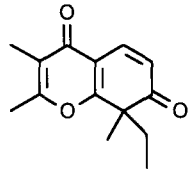
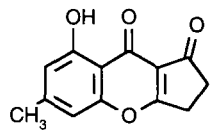
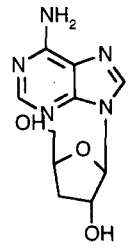
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
17. *Chaetomium minutum*	chaetocin		diketopiperazine	antibacterial and cytotoxic		[28]
18. *Cochliobolus miyabeanus*	cochlioquinone A		sesquiterpene mixed	na	B also isolated	[15]
19. *Cochliobolus heterostrophus*			sesterterpene	phytotoxic		[55]
20. *Cochliobolus lunata*	lunatoic acid A		polyketide	antifungal	Induces chlamydospore formation	[44,56]
21. *Cochliobolus spicifer*	spiciferone A			phytotoxic		[53]
22. *Coniochaeta saccardoi*	coniochaetone A			antifungal	Coniochaetone B also isolated	[78]
23. *Cordyceps militaris*	cordycepin		nucleoside	adenosine mimic		[42]

Table 4 Continued

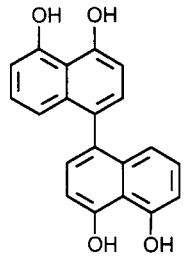
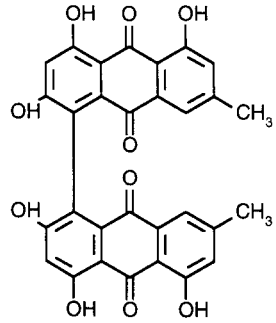
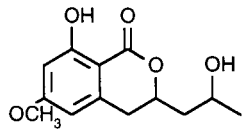
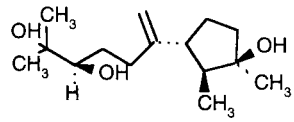
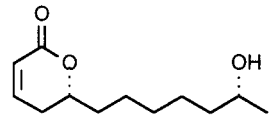
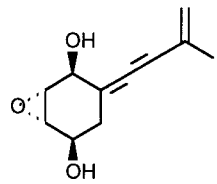
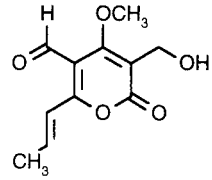
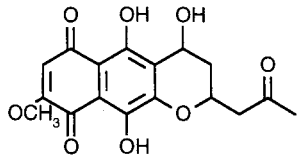
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
24. *Daldinia concentrica*			polyketide			[5]
25. *Endothia longirostris*	skyrin		polyketide		*E. gyrosa* and *E. fluens* also produce skyrin	[13]
26. *Endothia parasitica*	diaporthin		polyketide	na		[27]
27. *Epichloe typhina*	chokol E		sesquiterpene	fungitoxic	Chokols A–G also isolated	[40,91]
28. *Epichloe typhina*	gamahonolide A		fatty acid	antifungal	Gamahonolide B also isolated	[41]
29. *Eutypa lata*					Unusual allenic epoxide	[64,65]
30. *Gelasinospora multiforis*	multiforisin A			immuno-suppressive	Multiforisins B–E also isolated	[22]
31. *Gnomonia erythrostoma*	erythrostominoe		polyketide	na	Deoxyerythro-stominone and deoxyerythro-stominol also isolated	[19]

Table 4 Continued

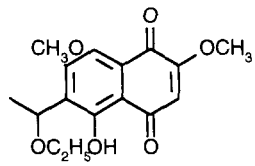
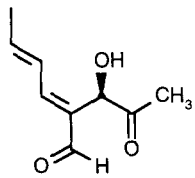
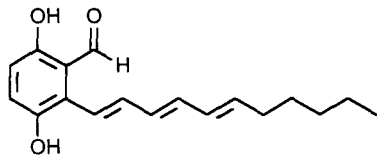
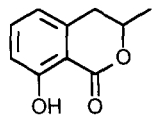
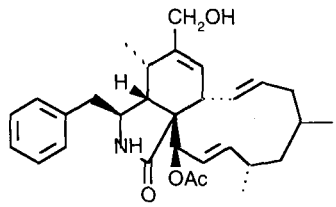
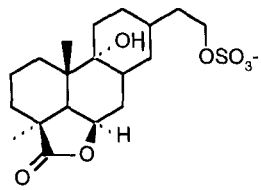
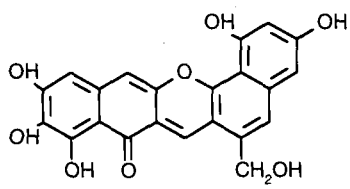
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
32. *Guignardia laricina*			polyketide	phytotoxic		[60]
33. *Hypocrea avellanea*	avellaneol		polyketide	antibiotic		[6,50]
34. *Hypocrea citrina*	auroctrin		polyketide	antibiotic		[48]
35. *Hypocrea peltata*	hypelcin A		peptide	antibacterial and antitumor		[23]
36. *Hypoxylon fragiforme*	mellein		polyketide	na		[7]
37. *Hypoxylon fragiforme*	L-69474		cytochalasin	HIV protease inhibitor		[58]
38. *Hypoxylon fragiforme*	mammatum		diterpine	na		[11]
39. *Hypoxylon fragiforme*	hypoxyxylerone		polyketide	na		[21]

Table 4 Continued

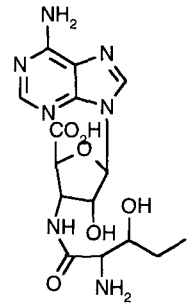
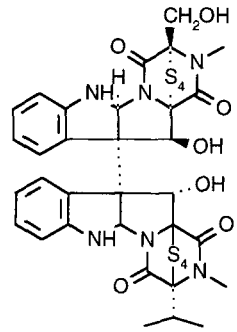
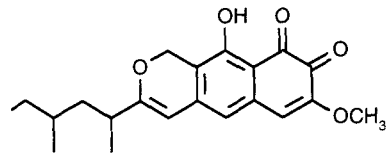
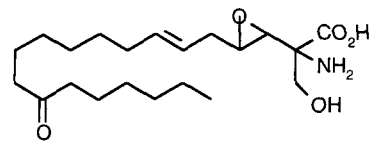
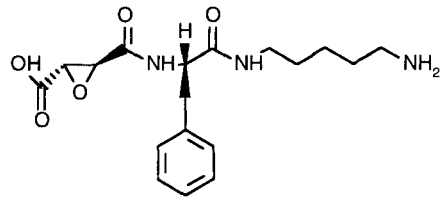
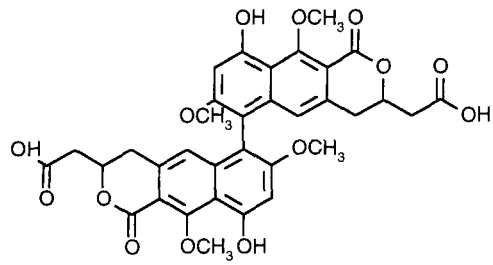
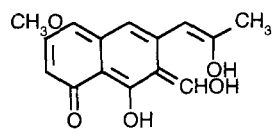
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
40. *Kernia* sp	FR90043		nucleoside	antifungal		[33]
41. *Leptosphaeria* sp	leptosin A		diketopiperazine	antitumor	B–J also isolated	[74]
42. *Leptosphaeria obiones*	obionin A		polyketide	D1 inhibitor		[62]
43. *Melanconis flavoviriens*	flavovirin		fatty acid	antifungal	Myriocin is also produced	[66]
44. *Microascus longirostris*	cathestatin A		amino acid	protease inhibitor	B and C also isolated	[92]
45. *Mycosphaerella astroma*	asteromine		polyketide	phytotoxic, antifungal, antibacterial		[8]
46. *Nectria haematococca*	nectriachrysone		polyketide	na		[61]

Table 4 Continued

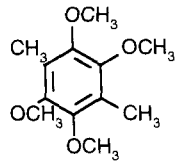
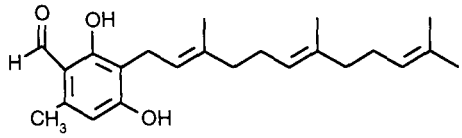
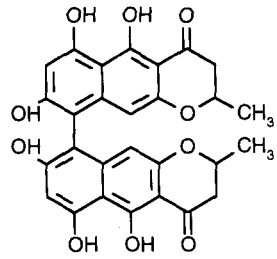
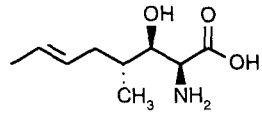
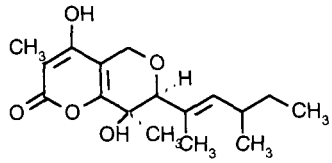
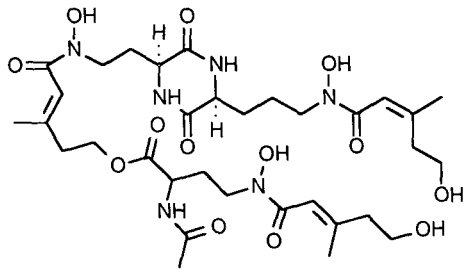
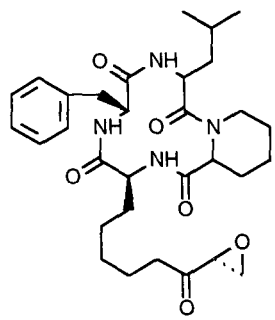
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
47. *Nectria coryli*			polyketide	antibacterial	Quinone and hydroquinone also isolated	[47]
48. *Nectria lucida*			terpene	antibacterial		[17]
49. *Nectria* sp	Cyclosporin C		peptide	tachykinin antagonist		[31]
50. *Nectria viridescens*	cephalochromin		polyketide	antibiotic		[16]
51. *Neocosmospora vasinfecta*	'C9 acid'		amino acid		Also produces cyclosporin A	[52]
52. *Neocosmospora vasinfecta*	neovasinin		polyketide	phytotoxic	Neovasinone also isolated	[51]
53. *Neurospora crassa*	deferri-coprogen		amino acid	siderophore	ferricrocin also isolated	[88]
54. *Petriella guttulata*	WF-3161		amino acid	antitumor		[76]

Table 4 Continued

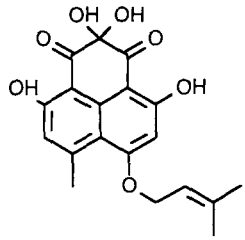
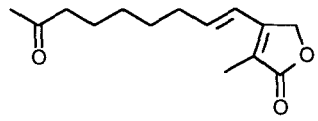
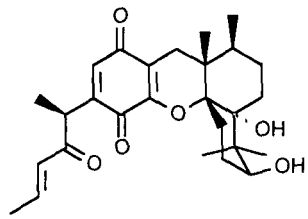
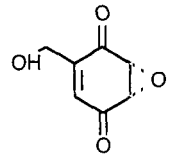
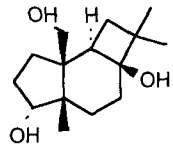
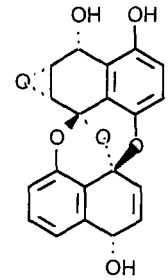
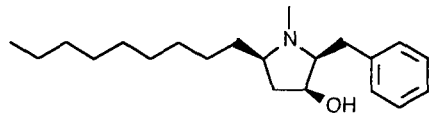
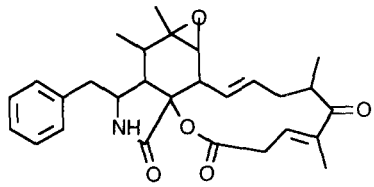
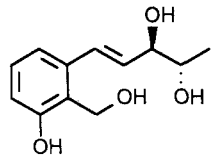
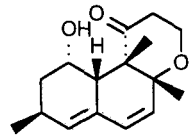
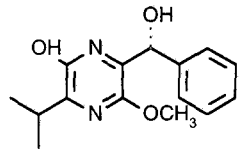
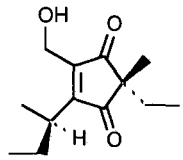
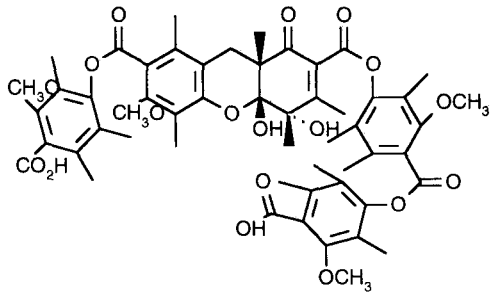
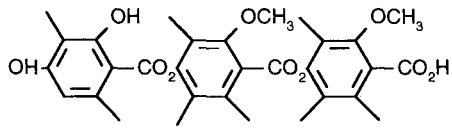
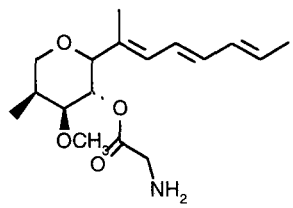
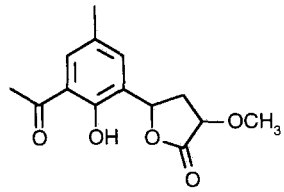
Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
55. *Phaeospharia rousseliana*	rousselianone A		polyketide	antifungal		[89]
56. *Podospora appendiculata*	appenolide A			antifungal	Appenolides B and C also isolated	[79]
57. *Podospora decipiens*	podosporin		Sesquiterpene mixed	antifungal and antibacterial		[82]
58. *Poronia punctata*	isoepoxydon		polyketide	'antifungal'		[25]
59. *Poronia punctata*	punctaporonin A		sesquiterpene	na	B–G also isolated	[20]
60. *Preussia isomera*	preussomerin A		polyketide	antifungal, antibacterial	B–F also isolated	[83,84]
61. *Preussia* sp	preussin			antifungal		[34]
62. *Rosellinia necatrix*	rosellichalasin		cytochalasin	phytotoxic	Cytochalasin E also produced	[3,35,36]

Table 4 Continued

Species	Name	Structure	Chemical class	Bioactivity	Notes	Ref
63. *Sordaria macrospora*	sordariol		polyketide	na		[12]
64. *Sporormia affinis*			polyketide	na		[45]
65. *Sporormiella tertispora*	terezine A		amino acid	weak antifungal, antibacterial	B–D also isolated	[80]
66. *Sporomiella similis*	similin A		polyketide	antifungal	B also isolated	[85]
67. *Thielavia terricola*	thielocin A1 beta		polyketide	Phospholipase A2 inhibitor		[90]
68. *Thielavia terricola*	thielavin B		polyketide	inhibitor of prostaglandins		[38]
69. *Westerdykella dispersa*	lanomycin		mixed	antifungal		[59]
70. *Xylaria globosa*	globoscin		polyketide			[1]

might be different, compounds in entries 34 and 48, share the same assortment of functional groups. An anisole derivative (entry 15) induces cell differentiation of mouse erythroleukemia cells to hemoglobin-producing erythrocyte-like cells at 5 $\mu g\ ml^{-1}$. The polyene aldehyde avellaneol (entry 33) is reported to have antibacterial and antileukemic activities. It is proposed to be derived from five acetate units. An α-pyrone multiforicin A (entry 30) can have an immunosuppressive effect since at 0.6 $\mu g\ ml^{-1}$ it suppresses the proliferation of mouse spleen lymphocytes stimulated by mitogens. Another small molecule, similin A (entry 66) is related to dehydropentenomycin and inhibits the growth of other coprophilous fungi.

While depsidones are primarily isolated from lichens as shown by entry 4, some were isolated from a fungus on food stuff, *Chaetomium mollicellum* (entry 9). A salicylic acid derivative thielavin B (entry 68) inhibits formation of prostaglandin E. It is also effective *in vivo* as an anti-inflammatory agent in the rat edema model. Thielocin A1β (entry 67) exhibits a potent inhibitory activity on rat phospholipase A_2-II with an IC_{50} of 50 nM. Against human PLA_2-II, the IC_{50} goes up to 12 μM.

There are several skyrin-type dimeric polyketides (entries 14, 25, 45, and 50). Skyrin was recently reported to be an antagonist of glucagon suggesting a potential application in the treatment of diabetes [86]. Cytochlasins [54] and epipolythiodioxopiperazines [81] are two groups whose numbers and the range of bioactivities are rapidly increasing. Here the challenge is how to find a compound with enough selectivity. This possibility is suggested by a cytochalasin analog L-696 474 [58]. It is reported to inhibit HIV protease with an IC_{50} of 3 μM. Although this value indicates that it is not particularly potent, the effect appears to be specific since the structurally related cytochlasin H does not have this inhibitory effect, and this compound is inactive against other proteases including stromelysin, papain, and human leucocyte elastase. This non-peptide natural product, therefore, can serve as a template to generate small-molecule HIV protease inhibitors with increased potency.

Future outlook

Members of the Sphaeriaceae, the Verrucariaceae, the Amphisphaeriaceae, the Diaporthaceae, and the Halosphaeriaceae in the Pyrenomycetes, and all of the families except the Pleosporaceae and the Sporormiaceae in the Loculoascomycetes are relatively unexplored with regard to production of secondary metabolites. Among them marine ascomycetes, lichenized ascomycetes, endophytic ascomycetes and fungi on twigs and stems and bark are particularly difficult to isolate because of their slow growth. Even among those genera that have been more frequently explored, there are still many additional species that remain to be investigated (Table 3). One of the problems why many fungi of the groups under review fail to be isolated seems to be the drying-up of samples collected which affects spore germination (GJ Samuels, personal communication). Thus, on-site isolation while collecting samples may increase the percentage of the fungi isolated. The challenges are to figure out how to access the diverse collection of such fungi and to have them grow well enough to express their metabolic potential and provide material for screening. An understanding of the physiology of these fungi including optimization of growth and fermentation conditions including temperature, aeration, pH, and nutrient concentration will be important in bringing out the potential. In addition, novel screens based on genomics and sample testing using automation should yield new applications of compounds derived from these microorganisms.

References

1 Adeboya M, RL Edwards, T Laessoe, DJ Maitland and ASJ Whalley. 1995. Metabolites of the higher fungi. Part 28. Globoscinic acid and globoscin, a labile acid-lactone system from *Xylaria globosa* and *Xylaria obovata*. J Chem Soc (Perkin I): 2067–2072.
2 Aldridge DC, A Borrow, RG Foster, MS Large, H Spencer and WB Turner. 1972. Metabolites of *Nectria coccinea*. J Chem Soc (Perkin Trans) 1: 2136–2141.
3 Aldridge DC, BF Burrows and WB Turner. 1972. The structures of the fungal metabolites cytochalasins E and F. J Chem Soc Chem Comm 3: 148–149.
4 Alfatafta AA, JB Gloer, JA Scott and D Malloch. 1994. Apiosporamide, a new antifungal agent from the coprophilous fungus *Apiospora montagnei*. J Nat Prod 57: 1696–1702.
5 Allport DC and JD Bu'Lock. 1960. Biosynthetic pathways in *Daldinia concentrica*. J Chem Soc Part I: 654–662.
6 Ananthasubramanian L, ST Carey and MSR Nair. 1978. Metabolites of Pyrenomycetes IX. Structure and absolute configuration of (+)-*R*-avellaneol, an antibiotic metabolite of *Hypocrea avellanea*. Tetrahedron Lett 38: 3527–3528.
7 Anderson JR, RL Edwards and AJS Whalley. 1983. Metabolites of the higher fungi. Part 21. 3-methyl-3,4-dihydroisocoumarins and related compounds from the ascomycete family Xylariaceae. J Chem Soc (Perkin Trans) 1: 2185–2192.
8 Arnone A, G Assante, M Montorsi and G Nasini. 1995. Asteromine, a bioactive secondary metabolite from a strain of *Mycosphaerella asteroma*. Phytochemistry 38: 595–597.
9 Arx JA von and E Müller.1975. A re-evaluation of the bitunicate ascomycetes with keys to families and genera. Studies Mycol 9: 1–159.
10 Bills GF, F Pelaez, JD Polishook, MT Diez-Matas, GH Harris, WH Clapp, C Dufresne, KM Byrne, M Nallin-Omstead, RG Jenkins, M Mojena, L Huang and JD Bergstrom. 1994. Distribution of zaragozic acids (squalestatins) among filamentous ascomycetes. Mycol Res 98: 733–739.
11 Borgschulte K, S Rebuffat, W Trowitzsch-Kienast, D Schomburg, J Pinon and B Bodo. 1991. Isolation and structure elucidation of hymatoxins B–E and other phytotoxins from *Hypoxylon mammatum* fungal pathogen of leuce poplars. Tetrahedron 47: 8351–8360.
12 Bouillant ML, J Bernillon, J Favre-Bonvin and N Salin. 1989. New hexaketides related to sordariol in *Sordaria macrospora*. Z Naturforsch 44c: 719–723.
13 Briggs LH and PW Le Quesne. 1965. Chemistry of Fungi. Part II. Constituents of three *Endothia* species. J Chem Soc Part II: 2290–2291.
14 Burrows BF. 1967. A new fungal tetrahydrofuran. Chem Commun 12: 597–598.
15 Canonica L, CG Casinovi, A Fiecchi, C Galeffi, GBM Bettolo, A Scala and AMV Torracca. 1976. The structure of cochlioquinones. Gazz Chim Ital 106: 147–166.
16 Carey ST and MSR Nair. 1975. Metabolites of Pyrenomycetes V. Identification of an antibiotic from two species of *Nectria*, as cephalochromin. J Nat Prod 38: 448—449.
17 Carey ST and MSR Nair. 1977. Metabolites from Pyrenomycetes VIII. Identification of three metabolites from *Nectria lucida* as antibiotic triprenyl phenols. J Nat Prod 40: 602–603.
18 Cole RJ, JW Kirksey, HG Cutler and EE Davis. 1974. Toxic effects of oosporein from *Chaetomium trilaterale*. J Agric Food Chem 22: 517–520.
19 Cross BE, MN Edinberry and WB Turner. 1972. Pigments of *Gnomonia erythrostoma*. Part I. The structures of erythrostominone, deox-

yerythrostominone, and deoxyerythrostominol. J Chem Soc (Perkin I): 380–390.
20 Edwards RL, DJ Maitland, JP Poyser and AJS Whalley. 1989. Metabolites of the higher fungi. Part 25. Punctaporonin G from the fungus *Poronia punctata* (Linnaeus: Fries) Fries. J Chem Soc (Perkin I): 1939–1941.
21 Edwards RL, V Fawcett, DJ Maitland, R Nettleton, L Shields and AJS Whalley. 1991. Hypoxyxylerone, a novel green pigment from the fungus *Hypoxylon fragiforme* (Pers: Fries) Kickx. J Chem Soc Chem Commun 15: 1009–1010.
22 Fujimoto H, Y Satoh, M Nakayama, T Takayama and M Yamazaki. 1995. Isolation of some immunosuppressive components from an ascomycete, *Gelasinospora multiforis*. Chem Pharm Bull 43: 547–552.
23 Fujita T, Y Takaishi, H Moritoki, T Ogawa and K Tokimoto. 1984. Fungal metabolites. I. Isolation and biological activities of hypelcins A and B (growth inhibitors against *Lentinus edodes*) from *Hypocrea peltata*. Chem Pharm Bull 32: 1822–1828.
24 Gardner D, AT Glen and WB Turner. 1972. Calonectrin and 15-deacetylcalonectrin, new trichothecanes from *Calonectria nivalis*. J Chem Soc (Perkin Trans) 1: 2576–2578.
25 Gloer JB and SM Truckenbrod. 1988. Interference competition among coprophilous fungi: production of (+)-isoepoxydon by *Poronia punctata*. Appl Environ Microbiol 54: 861–864.
26 Graebe JE. 1987. Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38: 419–465.
27 Hardegger E, W Rieder, A Walser and F Kugler. 1966. Konstitution des diaporthins und synthese der diaporthinsaure. Helv Chim Acta 49: 1283–1290.
28 Hauser D, HP Weber and HP Sigg. 1970. Isolierung und strukturaufklarung von chaetocin. Helv Chim Acta 53: 1061–1073.
29 Hawksworth DL, BC Sutton and GC Ainsworth. 1983. Ainsworth & Bisby's Dictionary of Fungi (7th edn). Commonwealth Mycological Institute, Kew, Surrey. 412 pp.
30 Hawksworth DL. 1991. Presidential address 1990. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol Res 95: 641–655.
31 Hayashi K, M Nishikawa, I Aramori, S Kiyoto and M Okuhara. 1996. Tachykinin antagonists screening from microbial origin. J Antibiot 49: 110–112.
32 Itoh Y, K Kodama, K Furuya, S Takahashi, T Haneishi, Y Takiguchi, and M Arai. 1980. A new sesquiterpene antibiotic, heptelidic acid producing organisms, fermentation, isolation and characterisation. J Antibiot 33: 468–473.
33 Iwamoto T, A Fujie, Y Tsurumi, K Nitta, S Hashimoto and M Okuhara. 1990. FR900403, a new antifungal antibiotic produced by a *Kernia* sp. J Antibiot 43: 1183–1185.
34 Johnson JH, DW Phillipson and AD Kahle. 1989. The relative and absolute stereochemistry of the antifungal agent preussin. J Antibiot 42: 1184–1185.
35 Kajimoto T, Y Imamura, M Yamashita, K Takahashi, M Shibata and T Nohara. 1989. Nuclear magnetic resonance studies of cytochalasin E and its decomposition product. Chem Pharm Bull 37: 2212–2213.
36 Kimura Y, H Nakajima and T Hamasaki. 1989. Structure of rosellichalasin, a new metabolite produced by *Rosellinia necatrix*. Agric Biol Chem 53: 1699–1701.
37 Kinjo J-E, K Yokomizo, Y Awata, M Shibata and T Nohara. 1987. Structures of phytotoxins, AV-toxins, C, D and E, produced by zonate leaf spot fungus of mulberry. Tetrahedron Lett 28: 3697–3698.
38 Kitahara N, A Endo, K Furuya and S Takahashi. 1981. Thielavin A and B, new inhibitors of prostaglandin biosynthesis produced by *Thielavia terricola*. J Antibiot 34: 1562–1568.
39 Koch W-G and V Sinnwell. 1987. Isopulegol from liquid cultures of the fungus *Ceratocystis coerulescens* (Ascomycotina). Z Naturforsch 42c: 159–161.
40 Koshino H, S Togiya, S-I Terada, T Yoshihara, S Sakamura, T Shimanuki, T Sato and A Tajimi. 1989. New fungitoxic sesquiterpenoids, chokols A–G, from stromata of *Epichloe typhina* and the absolute configuration of chokol E. Agric Biol Chem 53: 789–796.
41 Koshino H, T Yoshihara, M Okuno, S Sakamura, A Tajimi and T Shimanuki. 1992. Gamahonolides A, B, and Gamahorin, novel antifungal compounds from stromata of *Epichloe typhina* on *Phleum pratense*. Biosci Biotech Biochem 56: 1096–1099.
42 Kredich NM and AJ Guarino. 1960. An improved method of isolation and determination of cordycepin. Biochim Biophys Acta 41: 363–365.
43 Liberra K and U Lindequist. 1995. Marine fungi - a prolific resource of biologically active natural products? Pharmazie 50: 583—588.
44 Marumo S, M Nukina, S Kondo and K Tomiyama. 1982. Lunatoic acid A, a morphogenic substance inducing chlamydospore-like cells in some fungi. Agric Biol Chem 46: 2399–2401.
45 McGahren WJ, GA Ellestad, GO Morton and MP Kunstmann. 1976. Spectral and chemical characterisation of fungal metabolite LL-N313ζ. J Org Chem 41: 66–71.
46 Müller E and JA von Arx. 1973. Pyrenomycetes: Meliolales, Coronophorales, Sphaeriales. In: The Fungi, An Advanced Treatise, IV A (Ainsworth GC, FK Sparrow and AS Sussman, eds), pp 87–132, Academic Press, New York and London.
47 Nair MSR and M Anchel. 1972. An antibacterial quinone hydroquinone pair from the ascomycete, *Nectria coryli*. Tetrahedron Lett 9: 795–796.
48 Nair MSR, ST Carey. 1979. Metabolites of Pyrenomycetes XI. Structure of aurocitrin, a new antibacterial pigment from *Hypocrea citrina*. Tetrahdron Lett 35: 3233–3236.
49 Nair MSR, ST Carey and JC James. 1981. Metabolites of Pyrenomycetes XIV. Structure and partial stereochemistry of the antibiotic macrolides hypothemycin and dihydrohypothemycin. Tetrahedron 37: 2445–2449.
50 Nair MSR, ST Carey and L Ananthasubramanian. 1982. Metabolites or Pyrenomycetes. XV. Biogenesis of the antitumor antibiotic, (+) R-avellaneol. J Nat Prod 45: 644–645.
51 Nakajima H, K Nishimura, T Hamasaki, Y Kimura and S-I Udagawa. 1987. Structure of neovasinin, a new metabolite produced by the fungus, *Neocosmospora vasinfecta* EF Smith, and its biological activity to lettuce seedlings. Agric Biol Chem 51: 2831–2833.
52 Nakajima H, T Hamasaki, K Nishimura, T Kondo, Y Kimura, S-I Udagawa and S Sato. 1988. Isolation of 2-acetylamino-3-hydroxy-4-methyloct-6-enoic acid, a derivative of the 'C_9-amino acid' residue of cyclosporins, produced by the fungus *Neocosmospora vasinfecta* EF Smith. Agric Biol Chem 52: 1621–1623.
53 Nakajima H, T Hamasaki and Y Kimura. 1989. Structure of spiciferone A, a novel γ-pyrone plant growth inhibitor produced by the fungus *Cochliobolus spicifer* Nelson. Agric Biol Chem 53: 2297–2299.
54 Natori S and I Yahara. 1988. Cellular effects of cytochalasins. In: Microbial Toxins Foods Feeds: Cell Mol Modes Action (Pohland AE, VR Dowell Jr and JL Richard, eds), pp 395–402, Plenum, New York.
55 Nozoe S, M Morisaki, K Fukushima and S Okuda. 1968. The isolation of an acyclic C_{25}-isoprenoid alcohol, geranylnerolidol and a new ophiobolin. Tetrahedron Lett 42: 4457–4458.
56 Nukina M and S Marumo. 1977. Lunatoic acid A and B, Aversion factor and its related metabolite of *Cochliobolus lunata*. Tetrahedron Lett 30: 2603–2606.
57 Oka H, K Asahi, H Morishima, M Sanada, K Shiratori, Y Limura, T Sakurai, J Uzawa, S Iwadare and N Takahashi. 1985. Differanisole A, a new differentiation inducing substance. J Antibiot 38: 1100–1102.
58 Ondeyka J, OD Hensens, D Zink, R Ball, RB Lingham, G Bills, A Dombrowski and M Goetz. 1992. L-696,474, a novel cytochalasin as an inhibitor of HIV-1 protease. II. Isolation and structure. J Antibiot 45: 679–691.
59 O'Sullivan J, DW Phillipson, DR Kirsch, SM Fisher, MH Lai and WH Trejo. 1992. Lanomycin and glucolanomycin, antifungal agents produced by *Pycnidiophora dispersa* I. Discovery, isolation and biological activity. J Antibiot 45: 306–312.
60 Otomo N, H Sato and S Sakamura. 1983. Novel phytotoxins produced by the causal fungus of the shoot blight of larches. Agric Biol Chem 47: 1115–1119.
61 Parisot D, M Devys and M Barbier. 1991. Nectriachrysone, a new metabolite related to fusarubin produced by the fungus *Nectria haematococca*. J Chem Soc (Perkin I): 2280–2281.
62 Poch GK and JB Gloer. 1989. Obionin A: a new polyketide metabolite from the marine fungus *Leptosphaeria obiones*. Tetrahedron Lett 30: 3483–3486.
63 Porter JK, CW Bacon, JD Robbins, DS Himmelsbach and HC Higman. 1977. Indole alkaloids from *Balansia epichloe* (Weese). J Agric Food Chem 25: 88–93.
64 Renaud J-M, G Tsoupras and R Tabacchi. 1989. Biologically active natural acetylenic compounds from *Eutypa lata* (Pers: F) Tul Helv Chim Acta 72: 929–932.
65 Renaud J-M, G Tsoupras, H Stoeckii-Evans and R Tabacchi. 1989. A

novel allenic epoxycyclohexane and related compounds from *Eutypa lata* (Pers: F) Tul. Helv Chim Acta 72: 1262–1267.
66 Sailer M, V Sasek, J Sejbal, M Budesinky and V Musilek. 1989. Flavovirin — a new antifungal antibiotic produced by the pryenomycete *Melanoconis flavovirens*. J Basic Microbiol 29: 375–381.
67 Saito T, Y Suzuki, K Koyama, S Natori, Y Iitaka and T Kinoshita. 1988. Chetracin A and chaetocins B and C, three new epipolythiodioxo-piperazines from *Chaetomium* spp. Chem Pharm Bull 36: 1942–1956.
68 Sala T, MV Sargent and JA Elix. 1981. Depsidone synthesis. Part 15. New metabolites of the lichen *Buellia canescens* (Dicks) De Not: novel phthalide catabolites of depsidones. J Chem Soc (Perkin I): 849–854.
69 Sekita S, K Yoshihira and S Natori. 1980. Chaetochromin, a bis(naphthodihydropyran-4-one) mycotoxin from *Chaetomium thielavioideum*: application of ^{13}C-^{1}H long-range-coupling to the structure elucidation. Chem Pharm Bull 28: 2428–2435.
70 Sekita S. 1983. Isocochliodinol and neocochliodinol, bis(3-indolyl)-benzoquinones from *Chaetomium* spp. Chem Pharm Bull 31: 2998–3001.
71 Sicic H and V Gaberc-Porekar. 1992. Biosynthesis and physiology of ergot alkaloids. In: Handbook of Applied Mycology, Vol 4 Fungal Biotechnology (Arora DK, RP Elander and KG Mukerji, eds), pp 475–515, Marcel Dekker, New York.
72 Stark AA, B Kobbe, K Matsuo, G Buchi, GN Wogan and AL Demain. 1978. Mollicellins: mutagenic and antibacterial mycotoxins. Appl Environ Microbiol 36: 412–420.
73 Stoessl A. 1969. 8-Hydroxy-6-methoxy-3-methylisocoumarin and other metabolites of *Ceratocystis fimbriata*. Biochem Biophys Res Comm 35: 186–192.
74 Takahashi C, A Numata, Y Ito, E Matsumura, H Araki, H Iwaki and K Kushida. 1994. Leptosins, antitumor metabolites of a fungus isolated from a marine alga. J Chem Soc (Perkin I): 1859–1864.
75 Udagawa S, T Muroi, H Kurata, S Sekita, K Yoshihira, S Natori and M Umeda. 1979. The production of chaetoglobosins, sterigmatocystin, *O*-methylsterigmatocystin, and chaetocin by *Chaetomium* spp and related fungi. Can J Microbiol 25: 170–177.
76 Umehara K, K Nakahara, S Kiyoto, M Iwami, M Okamoto, H Tanaka, M Kohsaka, H Aoki and H Imanaka. 1983. Studies on WF-3161, a new antitumor antibiotic. J Antibiot 36: 478–483.
77 Volc J, P Sedmera, K Roy, V Sasek and J Vokoun. 1977. Two antibiotic benzoquinone-hydroquinone pairs from the pyrenomycete *Camarops microspora* (Karst) Shear. Coll Czechoslov Chem Commun 42: 2957–2961.
78 Wang H-J, JB Gloer, JA Scott and D Malloch. 1995. Coniochaetones A and B: new antifungal benzopyranones from the coprophilous fungus *Coniochaeta saccardoi*. Tetrahedron Lett 36: 5847–5850.
79 Wang Y, JB Gloer, JA Scott and D Malloch. 1993. Appenolides A–C: three new antifungal furanones from the coprophilous fungus *Podospora appendiculata*. J Nat Prod 56: 341–344.
80 Wang Y, JB Gloer, JA Scott and D Malloch.1995. Terezines A–D: new amino acid-derived bioactive metabolites from the coprophilous fungus *Sporormiella teretispora*. J Nat Prod 58: 93–99.
81 Waring P, RD Eichner A and Mullbacher. 1988. The molecular mechanism of toxicity of gliotoxin and related epipolythiodioxopiperazines. In: Microbial Toxins Foods Feeds: Cell Mol Modes Action (Pohland AE, VR Dowell Jr and JL Richard, eds), pp 505–512, Plenum, New York.
82 Weber HA, NC Baenziger and JB Gloer. 1988. Podosporin A: a novel antifungal metabolite from the coprophilous fungus *Podospora decipiens* (Wint) Niessl. J Org Chem 53: 4567–4569.
83 Weber HA and JB Gloer. 1988. Interference competition among natural fungal competitors: an antifungal metabolite from the coprophilous fungus *Preussia fleischhakii*. J Nat Prod 51: 879–883.
84 Weber HA and JB Gloer. 1991. The preussomerins: novel antifungal metabolites from the coprophilous fungus *Preussia isomera* Cain. J Org Chem 56: 4355–4360.
85 Weber HA, DC Swenson, JB Gloer and D Malloch. 1992. Similins A and B: new antifungal metabolites from the coprophilous fungus *Sporormiella similis*. Tetrahedron Lett 33: 1157–1160.
86 West RR, V Labroo, JR Piggott, RA Smith and PA McKernan. 1994. Use of skyrin and analogs for the treatment of diabetes mellitus and process for their preparation. 35 pp, PCT Int, Appl, WO 9414427 A2.
87 Whalley AJS and RL Edwards. 1995. Secondary metabolites and systematic arrangement within the Xylariaceae. Can J Bot S802–S810.
88 Wong GB, MJ Kappel, KN Raymond, B Matzanke and G Winkelmann. 1983. Co-ordination chemistry of microbial iron transport compounds. 24. Characterisation of coprogen and ferricrocin, two ferric hydroxamate siderophores. J Am Chem Soc 105: 810–815.
89 Xiao J-Z, S Kumazawa, H Tomita, N Yoshikawa, C Kimura and T Mikawa. 1993. Rousselianone A, novel antibiotic related to phenalenone produced by *Phaeosphaeria rousseliana*. J Antibiot 46: 1570–1574.
90 Yoshida T, S Nakamoto, R Sakazaki, K Matsumoto, Y Terui, T Sato, H Arita, S Matsutani, K Inoue and I Kudo. 1991. Thielocins A1α and A1β, novel phospholipase A2 inhibitors from ascomycetes. J Antibiot 44: 1467–1470.
91 Yoshihara T, S Togiya, H Koshino, S Sakamura, T Shimanuki, T Sato and A Tajimi. 1985. Three fungitoxic cyclopentanoid sesquiterpenes from stromata of *Epichloe typhina*. Tetrahedron Lett 26: 5551–5554.
92 Yu C-M, JM Curtis, JA Walter, JLC Wright, SW Ayer, J Kaleta, L Querengesser and ZR Fathi-Afshar. 1996. Potent inhibitors of cysteine proteases from the marine fungus *Microascus longirostris*. J Antibiot 49: 395–397.