Endophytes: a rich source of functional metabolites

R. X. Tan* and W. X. Zou

Institute of Functional Biomolecule, School of Life Sciences, Nanjing University, Nanjing 210093, P. R. China. E-mail: rxtan@netra.nju.edu.cn

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1   Introduction

An endophyte is a bacterial (including actinomycete) or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant (Fig. 1), typically causing no apparent symptoms of disease.[1,2] The endophytic population of a given species varies from several to a few hundreds of bacterial and fungal strains. Endophytes can be isolated from mildly surface-sterilized plant tissues and cultivated on nutrient agar (Fig. 2). The relationship between the endophyte and its host plant may range from latent phytopathogenesis to mutualistic symbiosis.[3] Presumably owing to their specialised niches, no substantial body of work has accumulated since the first discovery of endophytic fungus in darnel in 1904.[4] However, much renewed
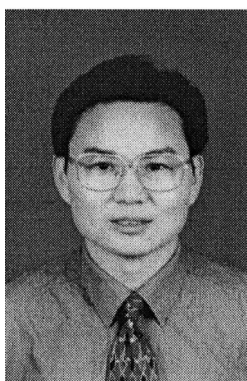
Ren Xiang Tan, born in Jiangsu (1960), received his BS in Pharmacy in 1983 and his MS in Medicinal Chemistry in 1986 from the China Pharmaceutical University, and his PhD in Organic Chemistry in 1990 from Lanzhou University where he spent his two-year postdoctoral research period. After his joining SLSNU (School of Life Sciences, Nanjing University) as associate professor in 1992, he was promoted as Professor of Botany (1994), Chair Professor (1999), department head (1996) and vice dean (1999). He has been successively visiting scholars to the Institute of Organic Chemistry, Technical University of Berlin, Berlin, Germany (Professor F. Bohlmann), Institute of Pharmacognosy and Phytochemistry, University of Lausanne, Lausanne, Switzerland (Professor K. Hostettmann), and the Institution of Oceanography, University of California, San Diego, USA (Professor W. Fenical). His work interest has focused on the structure determination and functions of biomolecules originating from medicinal plants and specially inhabiting microorganisms. He has authored some 100 scientific publications and three monographs.
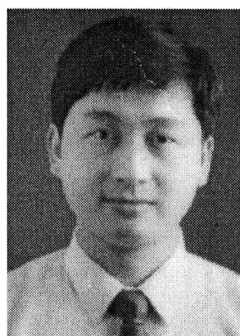
Wen Xin Zou, born in Jiangsu (1967), received his BS in Microbiology in 1988 from Jiangxi University and his MS in Botany in 1991 from Nanjing University. After joining SLSNU, he was promoted to lecturer (1993) and associate professor (2001). Since 1997, he has been working in Professor Tan's group on the biology and chemistry of endophytic fungi originating from Artemisia annua and A. monogolica, two traditional Chinese medicinal plants.
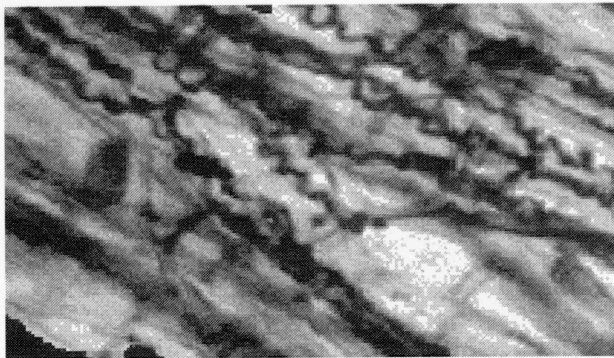
Fig. 1 Endophytic fungal hyphae between the cells of tall fescue stems (reproduced from http://pss.ag.utk.edu/fescue with permission from Professor H. A. Fribourg, University of Tennessee).

Fig. 2 Endophytic mycelia growing out from cut areas of surface-sterilized plant segments on antibiotics-supplemented WA plate. Upper: endophyte on *Artemisia annua* L. stems; lower: endophyte on spruce wood (reproduced from http://www.uni-tuebingen.de/uni/bbm/Forsch/OiEndoE.html (no longer available) with permission from Professor F. Oberwinkler, University Tübingen).

attention is now being paid to the chemistry and bioactivity of endophyte metabolites, and to endophytic biodiversity and related ecological functions. This review, beginning with a brief survey of the biological aspects of endophytes, describes endophyte metabolites characterized before 2000.

2 Biological survey of endophytes

2.1 Distribution and biodiversity

Almost all vascular plant species examined to date were found to harbor endophytic bacteria and/or fungi.[1,5] Moreover, the colonization of endophytes in marine algae,[6,7] mosses and ferns[8,9] has also been recorded. As a matter of fact, endophytes are important components of microbial biodiversity.[10] Commonly, several to hundreds of endophyte species can be isolated from a single plant, among them, at least one species showing host specificity. The environmental conditions under which the host is growing also affect the endophyte population,[11] and the endophyte profile may be more diversified in tropical areas. Arnold *et al.*[5] isolated 418 endophyte morphospecies (estimated 347 genetically distinct taxa) from 83 healthy leaves of *Heisteria concinna* and *Ouratea lucens* in a lowland tropical forest of central Panama, and proposed that tropical endophytes themselves could be hyperdiverse with host preference and spatial heterogeneity. Moreover, genotypic diversity has been observed in single endophyte species originating from conifers,[12,13] birch[14] and grasses.[15] Accordingly, endophytes are presumably ubiquitous in the plant kingdom with the population being dependent on host species and location.

2.2 Origin and evolution

Some phytopathogens in the environment are of endophyte origins.[16] Many innocuous fungal endophytes are quiescent phytopathogens which may cause infectious symptoms when the host plant is aged and/or stressed. On the other hand, during the long co-evolution of the phytopathogen and its host plant, an endophytic mutant may result from balanced antagonism and/or gene mutation. Dual cultures of the host calli and endophytes demonstrated that both the endophytes and the host calli excrete metabolites toxic to each other.[17,18] Further investigation led to the development of a hypothesis that the endophyte–host interaction could be a balanced pathogen–host antagonism.[19] Freeman and Rodriguez[20] found that a naturally occurring nonpathogenic endophytic mutant developed from the mutation of a single locus in the genome of the wild-type *Colletotrichum magna*, a pathogen causing anthracnose in cucurbit plants. This mutant is able to grow systemically inside the host plant without pathogenic symptoms, but retaining wild-type levels of *in vitro* sporulation, spore adhesion, appressoria formation, infection and host specificity.

The *Acremonium* (asexual fungi now reclassified in the genus *Neotyphodium* Glenn, Bacon and Hanlin[21]) endophytes, which usually inhabit tall fescue, perennial ryegrass (*Lolium perenne* L.), and many cool-season grasses, are considered mutualistic symbionts of the host grasses. The grass and the endophytic fungus are so intimately associated that they act 'as a whole', much like 'a single organism'. And, indeed, some of these endophytic *Neotyphodium* species can only spread by infecting seeds from the mother plants (Fig. 3).[22]

2.3 Physiological and ecological roles

Endophytes colonizing inside plant tissues usually get nutrition and protection from the host plant. In return, they confer profoundly enhanced fitness to the host plants by producing certain functional metabolites.

2.3.1 Growth promotion of the host plant

Endophyte-infected plants often grow faster than non-infected ones.[23] This effect is at least in part due to the endophytes' production of phytohormones such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances,[24] and/or partly owing to the fact that endophytes could have enhanced the hosts' uptake of nutritional elements such as nitrogen[25] and phosphorus.[26,27] A culture broth of *Colletotrichum gloeosporioides*, an endophyte fungus of *Artemisia annua* L., has also been found in our laboratory to be able to promote the growth of the host callus (Fig. 4, unpublished work).

2.3.2 Improvement of the hosts' ecological adaptability

Certain endophytes improve the ecological adaptability of hosts by enhancing their tolerance to environmental stresses
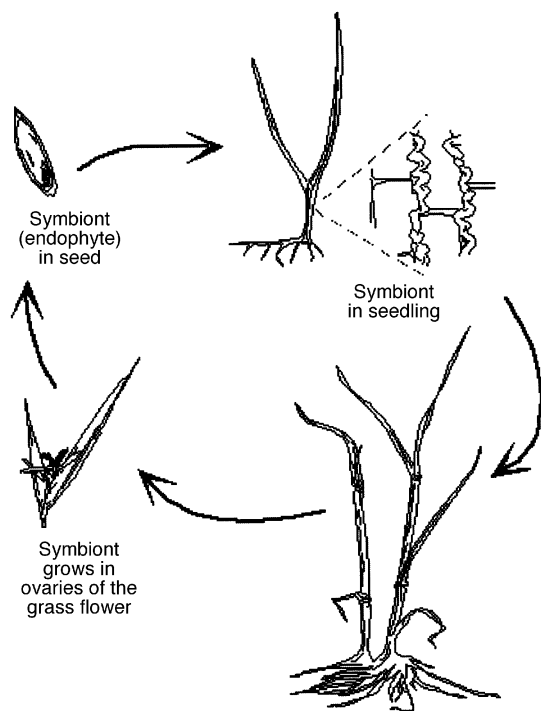
Fig. 3 Schematic illustration of endophyte–grass symbiont (reproduced from http://www.ca.uky.edu/agcollege/plantpathology/schardl/schardl.htm with permission from Professor C. L. Schardl, University of Kentucky).
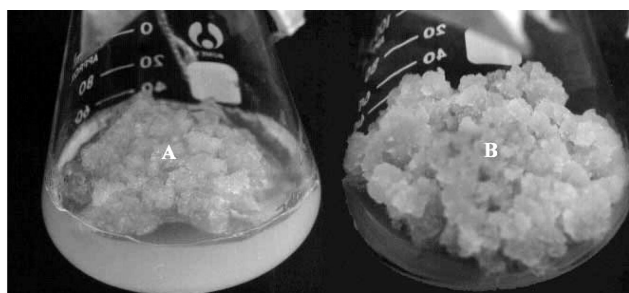

Fig. 4 An *Artemisia annua* endophyte culture liquid can significantly promote the growth of host callus incubated on MS medium without (A) and with (B) 10% endophyte culture liquid.

and resistance to phytopathogens and/or herbivores including some insects feeding on the host plant. Endophyte-infected grasses usually possess an increased tolerance to drought[28,29] and aluminium toxicity.[30] Furthermore, some endophytes are able to provide the host plant with protection against some nematodes,[31,32] mammal[33] and insect herbivores[34,35] as well as bacterial and fungal pathogens.[36,37] Some endophytes are capable of enhancing the hosts' allelopathic effects on other species co-growing nearby, usually being competitor(s) for the nutrition and the space.[38-40] This could be the reason why some plants with special endophytes are usually competitive enough to become dominant species in successional fields.[41]

3 Alkaloids

3.1 Amines and amides

Amines and amides are quite common secondary metabolites of *Acremonium* endophytes, the anamorphic *Epichloë* species reclassified as genus *Neotyphodium* Glenn, Bacon and Hanlin.[21] These endophytic fungi inhabit tall fescue, perennial ryegrass and many cool-season grasses.[22] Peramine 1, a pyrrolopyrazine alkaloid, was characterized both in culture and *in planta* from *Neotyphodium coenophialum*, *N. lolli*, *Epichloë festucae* and *E. typhina* present in the stem and leaf of tall fescue, ryegrass and other grasses.[22] This secondary metabolite was shown to be
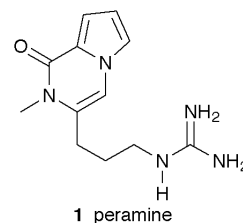
1 peramine

toxic to insects (extremely to Argentine stem weevil) without any harmful impact on mammals.[42,43] Biosynthetically proline, arginine and a methyl donor have been proposed to be involved in the biosynthesis of the compound.[44] Peramine is currently accepted as a unique chemical marker for the fungi in the *Neotyphodium/Epichloë* complex.[45]

The ergot alkaloids 2–18 were the second group of amine and amide alkaloids discovered in cultures of *Neotyphodium* endophytes, all being also characterized previously from ergot sclerotia.[46] In addition, these metabolites were demonstrated later to be neurotoxic to insects and mammal herbivores.[22] Ergovaline 6 and other structurally related ergopeptines have been assumed to be most likely responsible for the toxicosis of livestock after consuming endophyte-infected tall fescue.[44] Biosynthesis of ergot alkaloids such as ergovaline is better understood with the ergot fungus *Claviceps purpurea* (Fig. 5).[45] Tryptophan and a mevalonic acid derivative dimethylallyl diphosphate were demonstrated to be precursors of these metabolites.[47] The novel amide 19 was characterized as a ras-farnesyltransferase inhibitor from the culture broth of an endophytic *Phoma* sp.[48] Furthermore, three new cytotoxic cytochalasans 20–22, together with the known metabolite cytochalasin E 23, were purified from the culture broth of *Rhinocladiella* sp., an endophyte present in the perennial twining vine of *Tripterygium wilfordii*.[49]

2 ergobalansine
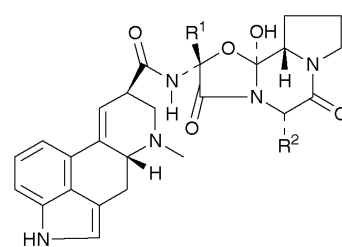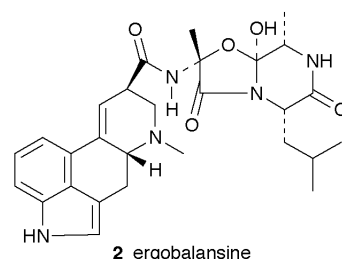
3 ergotamine R¹ = Me, R² = PhCH₂
4 ergosine R¹ = Me, R² = Buⁱ
5 β-ergosine R¹ = Me, R² = *sec*-Bu
6 ergovaline R¹ = Me, R² = Prⁱ
7 ergostine R¹ = Et, R² = PhCH₂
8 ergoptine R¹ = Et, R² = Buⁱ
9 β-ergoptine R¹ = Et, R² = *sec*-Bu
10 ergonine R¹ = Et, R² = Prⁱ
11 ergocristine R¹ = Prⁱ, R² = PhCH₂
12 α-ergocryptine R¹ = Prⁱ, R² = Buⁱ
13 β-ergocryptine R¹ = Prⁱ, R² = *sec*-Bu
14 ergocornine R¹ = R² = Prⁱ

Phomopsichalasin 24, a novel cytochalasan with an isoindolone moiety fused to a 13-membered tricyclic system, was characterized from the culture of an endophytic *Phomopsis*
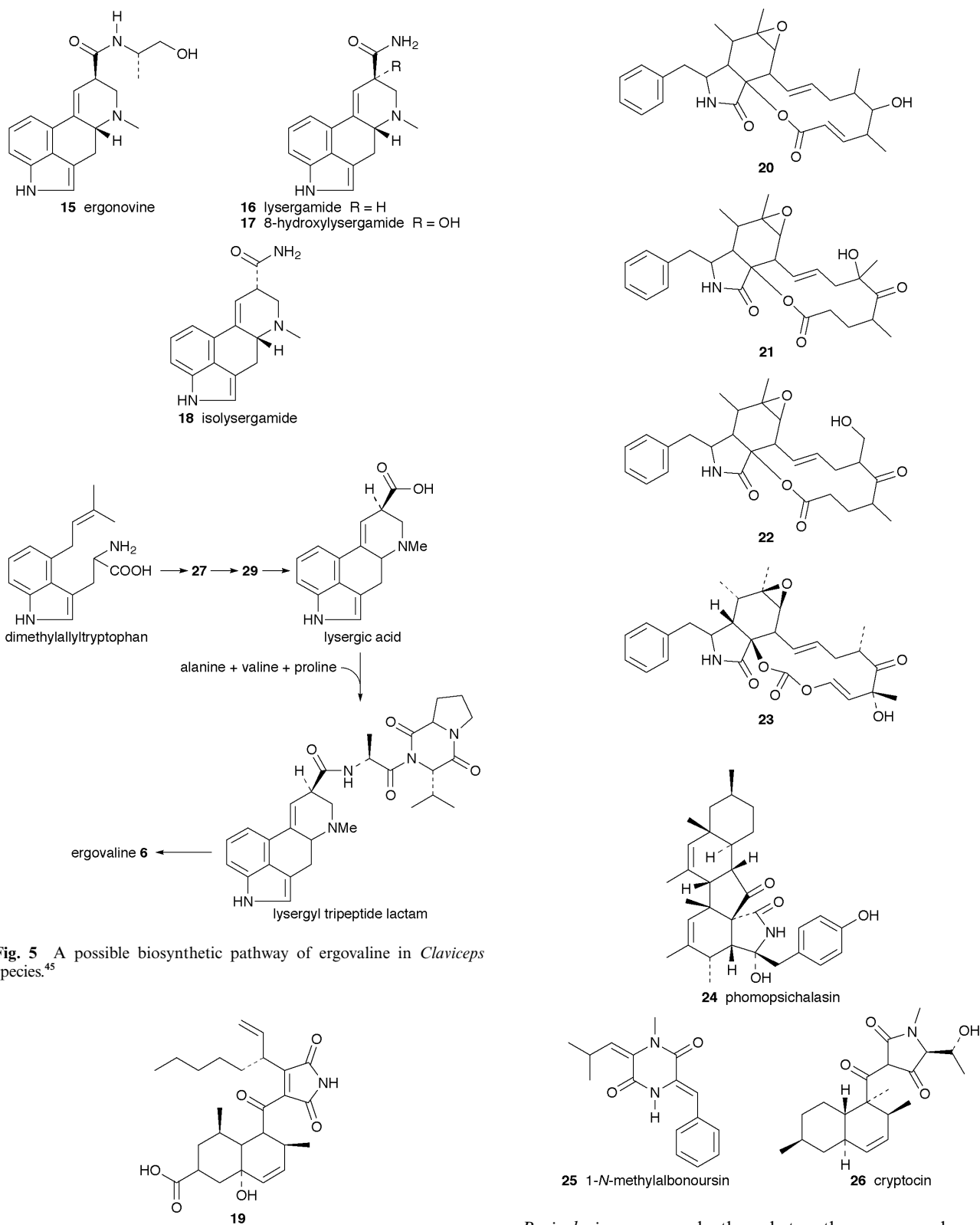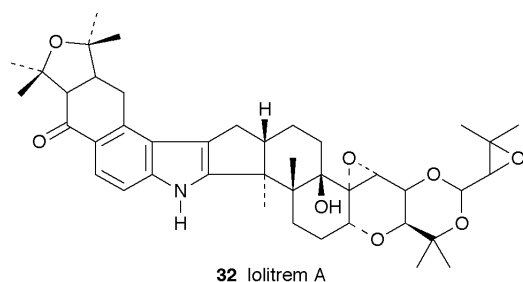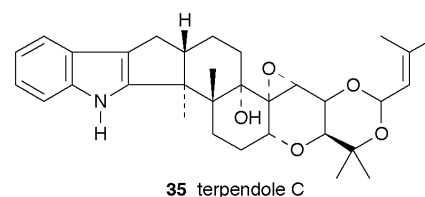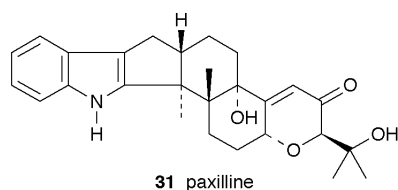
15 ergonovine

16 lysergamide R = H
17 8-hydroxylysergamide R = OH

18 isolysergamide

dimethylallyltryptophan → **27** → **29** → lysergic acid

alanine + valine + proline

ergovaline **6** ←
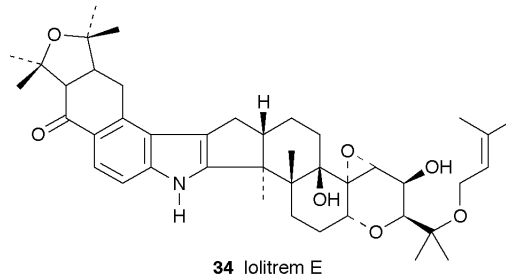
lysergyl tripeptide lactam

Fig. 5 A possible biosynthetic pathway of ergovaline in *Claviceps* species.[45]

19

20

21

22

23

24 phomopsichalasin

25 1-*N*-methylalbonoursin   **26** cryptocin

sp. originating from twigs of *Salix gracilostyla* var. *melanostachys*.[50] In disk diffusion assays, this metabolite was shown to be antibacterial against *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella gallinarum*, and antifungal against the human pathogenic yeast *Candida albicans*. 1-*N*-Methylalbonoursin **25**, an unusually fluorescent and weakly antibiotic alkaloid of prokaryote origin, was also isolated from the liquid culture of an *Acremonium*-like *Streptomyces* sp., an endophyte living in perennial ryegrass seedling tissues. Biosynthetically, the diketopiperazine skeleton of the compound was demonstrated to originate from leucine and phenylalanine.[51] Most recently, a tetramic acid analog cryptocin **26**, a potent antimycotic against

Pyricularia oryzae and other phytopathogens, was characterized from the culture of an endophytic fungus *Cryptosporiopsis* cf. *quercina*[52] present in the inner bark of the stems of *Tripterygium wilfordii*.[53]

3.2 Indole derivatives

Indole alkaloids such as chanoclavine **27**, agroclavine **28** and elymoclavine **29**, previously characterized from ergot sclerotia, were reisolated from a culture of *Neotyphodium* endophytes.[46] They are toxic to some insects and mammals.[22] A non-tremorgenic lolilline **30** and tremorgenic indolediterpenes paxilline **31**, lolitrem A **32**, B **33** and E **34**, and terpendole C **35** were detected not only in cultures of *N. lolli* and *E. festucae*, but

27 chanoclavine

28 agroclavine R = H
29 elymoclavine R = OH

30 lolilline

31 paxilline

32 lolitrem A

33 lolitrem B

34 lolitrem E

35 terpendole C

also in the endophyte-infected plants *Lolium perenne* and *Festuca* spp. These tremorgenic metabolites were ascertained as the causative agents of ryegrass staggers in livestock. Attention to the biosynthesis of the lolitrems and paxilline demonstrated that geranylgeranyl pyrophosphate and tryptophan were primary precursors[44] while alkaloids **30** and **35** were two intermediates.[54,55] Recently, four additional new neurotoxic metabolites of this type, lolitrem N **36**, lolitriol **37**, lolicine A **38** and B **39**, were isolated from *L. perenne* infected with *N. lolii* and the finding confirmed the proposal that lolitriol **37** is the biosynthetic precursor of lolitrem A, B and E (**32–34**).[56] A metabolic grid for the biosynthesis of lolitrems was proposed as in Fig. 6 by Munday-Finch.[45] Besides grass endophytes and *Aspergillus flavus*, tremorgenic paspalitrems A **40** and C **41** can also be produced by *Phomopsis* sp., an endophytic fungus isolated from the living bark of *Cavendishia pubescens*.[57]

Some endophytes can produce plant hormones with an indole framework. The growth-promoting phytohormone indole-3-acetic acid (IAA, **42**) was reisolated from cultures of a root-associated endophytic bacterium *Azospirillum brasilense* SP 7,[58] the tall fescue fungal endophyte *Acremonium coenophialum*,[59] *Aureobasidium pullulans* and *Epicoccum purpurascens*[60] and *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*.[61] Together with IAA and indole-3-acetonitrile **43**, cytokinins could also be produced by an endophytic strain of *Hypoxylon serpens* isolated from tobacco.[60] The alternative production of endophytes for phytohormones is assumed to be related to the plant growth-promoting effect allowed by the endophyte infection. On the other hand, a new indole derivative 6-isoprenylindole-3-carboxylic acid **44** was characterized

recently from the *A. annua* endophyte *Colletotrichum* sp. It shows moderate antibacterial activity against the Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea* and the Gram-negative bacterium *Pseudomonas* sp. Furthermore this new product is also inhibitory to the growth of some crop phytopathogenic fungi *Phytophthora capisici*, *Rhizoctonia cerealis* and *Gaeumannomyces graminis* var. *tritici*.[61]

3.3 Pyrrolizidines

Lolines **45–51**, saturated aminopyrrolizidine alkaloids, were exclusively found in endophyte-infected grasses such as *Festuca arundinacea* (infected with *N. coenophialum*) and *F. pratensis* (with *N. unicinatum*). Surprisingly, these alkaloids could be detected neither in endophyte cultures nor in non-infected grasses.[22] Lolines are potent broad-spectrum insecticides, acting both as metabolic toxins and feeding deterrents depending on the specific insect species. Unlike ergot and indole diterpene alkaloids, these loline derivatives are much less toxic to mammals although some biological activity in small mammals and mammalian tissues has been reported.[62,63] Ecologically, certain loline analogs have been demonstrated to contribute to the allelopathic properties of host grasses.[44] The biosynthetic pathway of loline alkaloids is not to date very clear. However, the pyrrolizidine ring system of lolines could originate from spermidine.[44]

In addition to loline **45**, *N*-methylloline **47**, *N*-formylloline **50** and *N*-acetylloline **51**, a new 5,6-dehydro-*N*-acetylloline **52** was isolated from endophyte-infected *Festuca argentina*. Injection (ip) of these compounds into mice allowed no obvious toxication confirming that they were independent of the toxicity of the endophyte-infected plant.[64]

4 Steroids

Along with ergosterol **53**, 3β,5α,6β-trihydroxyergosta-7,22-diene **54**, 3β-hydroxyergosta-5-ene **55**, 3-oxoergosta-4,6,8(14),22-tetraene **56**, 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene **57**, 3β-hydroxy-5α,8α-epidioxyergosta-6,9(11),22-triene **58** and 3-oxoergosta-4-ene **59**, two new steroids, 3β,5α-dihydroxy-6β-acetoxyergosta-7,22-diene **60** and 3β,5α-dihydroxy-6β-phenyl-

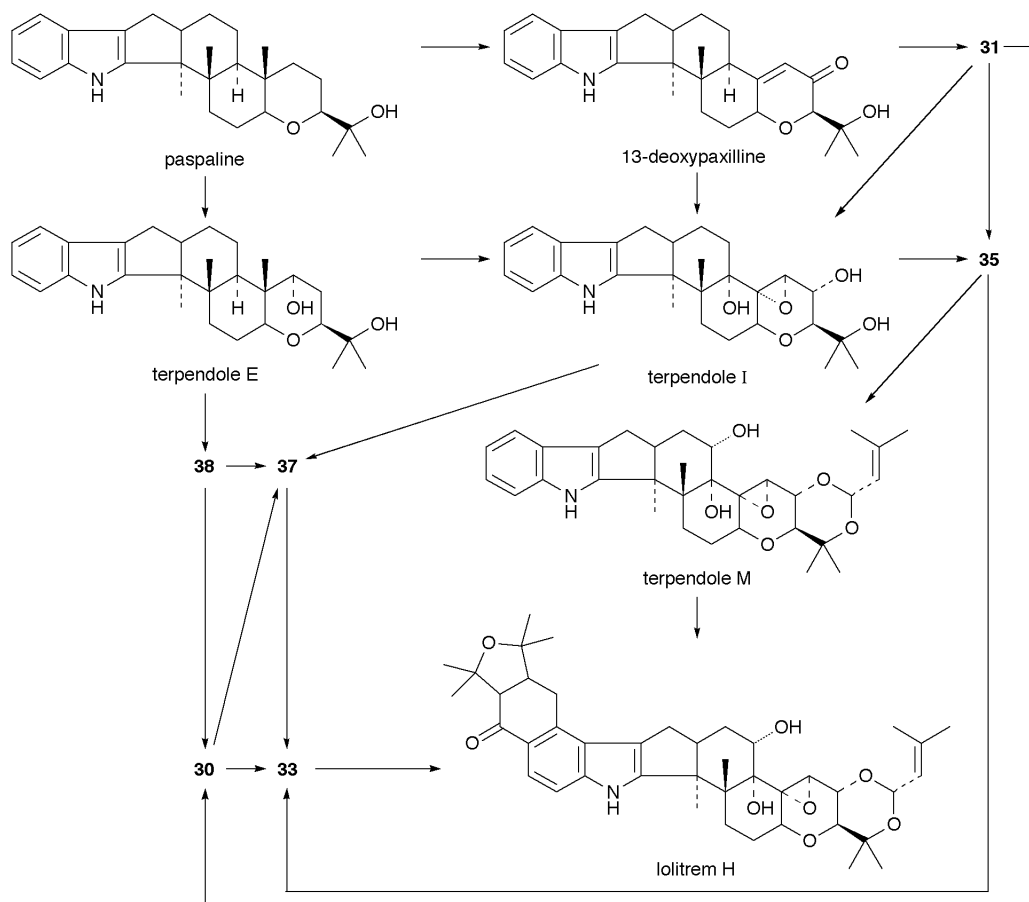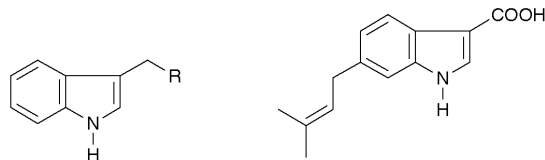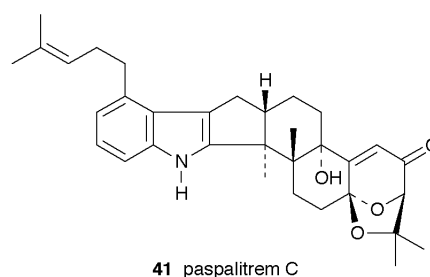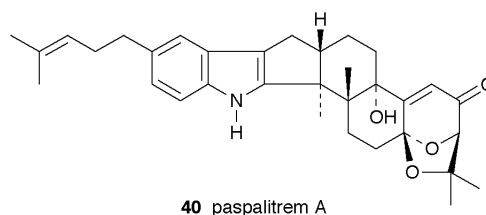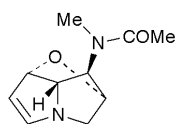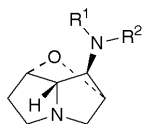**Fig. 6** A possible metabolic grid for biosynthesis of lolitrems.[45]

36 lolitrem N 31β,35α
37 lolitriol 31α,35β

38 lolicine A R¹ = Me, R² = H
39 lolicine B R¹ = CHO, R² = H

40 paspalitrem A

41 paspalitrem C

42 indole-3-acetic acid R = COOH
43 indole-3-acetonitrile R = CN

44 6-isoprenylindole-3-carboxylic acid

acetoxyergosta-7,22-diene **61** were characterized from the liquid culture of an fungal endophyte *Colletotrichum* sp. of *A. annua*. Metabolites **55**, **56**, **60** and **61** were shown to be anti-fungal against some crop pathogens *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis*, *Helminthosporium sativum* and *Phytophthora capisici*.[61]
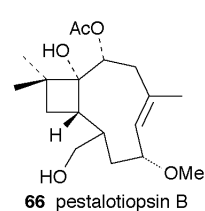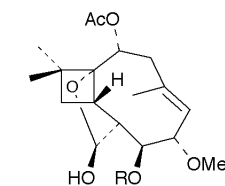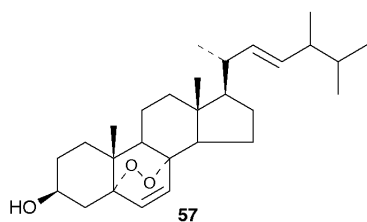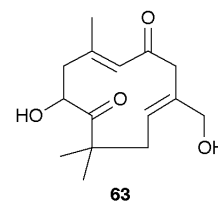
5 Terpenoids

Terpenoids have often been isolated from some endophyte cultures originating from a variety of host plants. Those identified so far are mainly sesqui- and diterpenes, some of which are partly degraded.

5.1 Sesquiterpenes

2α-Hydroxydimeninol **62**, **63** and pestalotiopsins A–C **64–66** are sesquiterpenes characterized from endophytic *Pestalotiopsis* spp. associated with *T. brevifolia*.[65–68] In particular, the new sesquiterpene **63** is a highly functionalized humulane derivative, the first of fungal origin. Heptelidic acid **67** and hydroheptelidic acid **68** isolated from *Phyllosticta* sp., an endophytic fungus of

45 loline R¹ = H, R² = Me
46 norloline R¹ = R² = H
47 *N*-methylloline R¹ = R² = Me
48 *N*-formylnorloline R¹ = H, R² = CHO
49 *N*-acetylnorloline R¹ = H, R² = Ac
50 *N*-formylloline R¹ = Me, R² = CHO
51 *N*-acetylloline R¹ = Me, R² = Ac

52 5,6-dehydro-*N*-acetylloline

53

54

55

56

57

58

59

60

61

62 2α-hydroxydimeninol

63
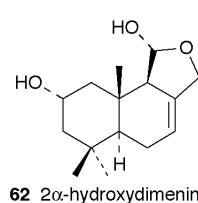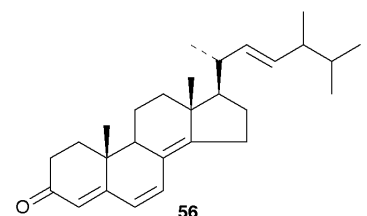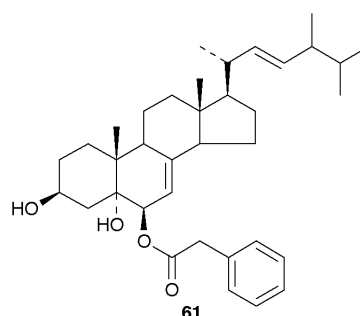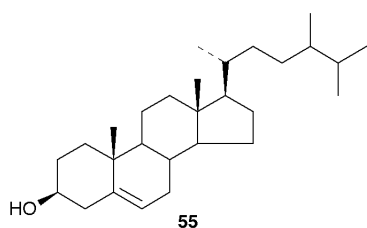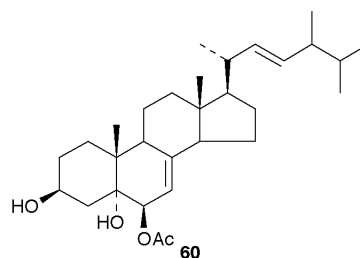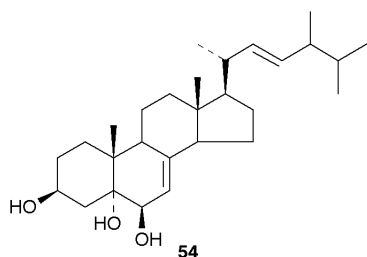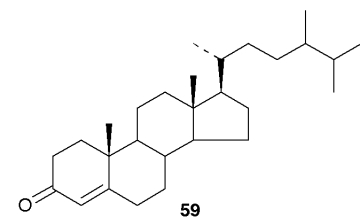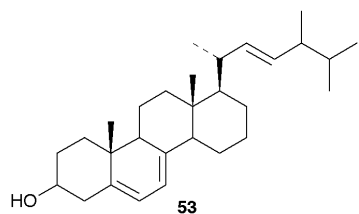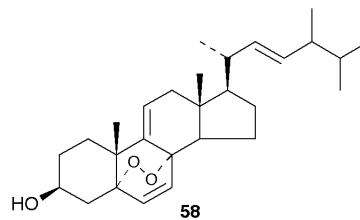
64 pestalotiopsin A R = H
65 pestalotiopsin C R = Me

66 pestalotiopsin B

67 heptelidic acid

68 hydroheptelidic acid

69 X = O
70 X = OH, H

Abies balsamea, have been shown to be toxic to spruce bud worm (*Choristoneura fumiferana*) larvae.[69] Two new benzo-furan-carrying normonoterpene derivatives **69** and **70**, toxic to spruce bud worm larvae and/or cells, have been characterized from a culture of an unidentified endophytic fungus obtained from wintergreen *Gaultheria procumbens*.[70] Some of the first reported sesquiterpenes produced by fungal endophytes were chokols A–G **71**–**77**, which were isolated from *Epichloë typhina* on *Phleum pratense* and were found to be fungitoxic to the leaf spot disease pathogen *Cladosporium phlei*.[71] Obviously, chokols A, F and G were partly degraded.
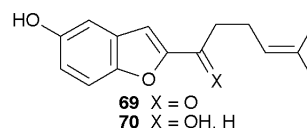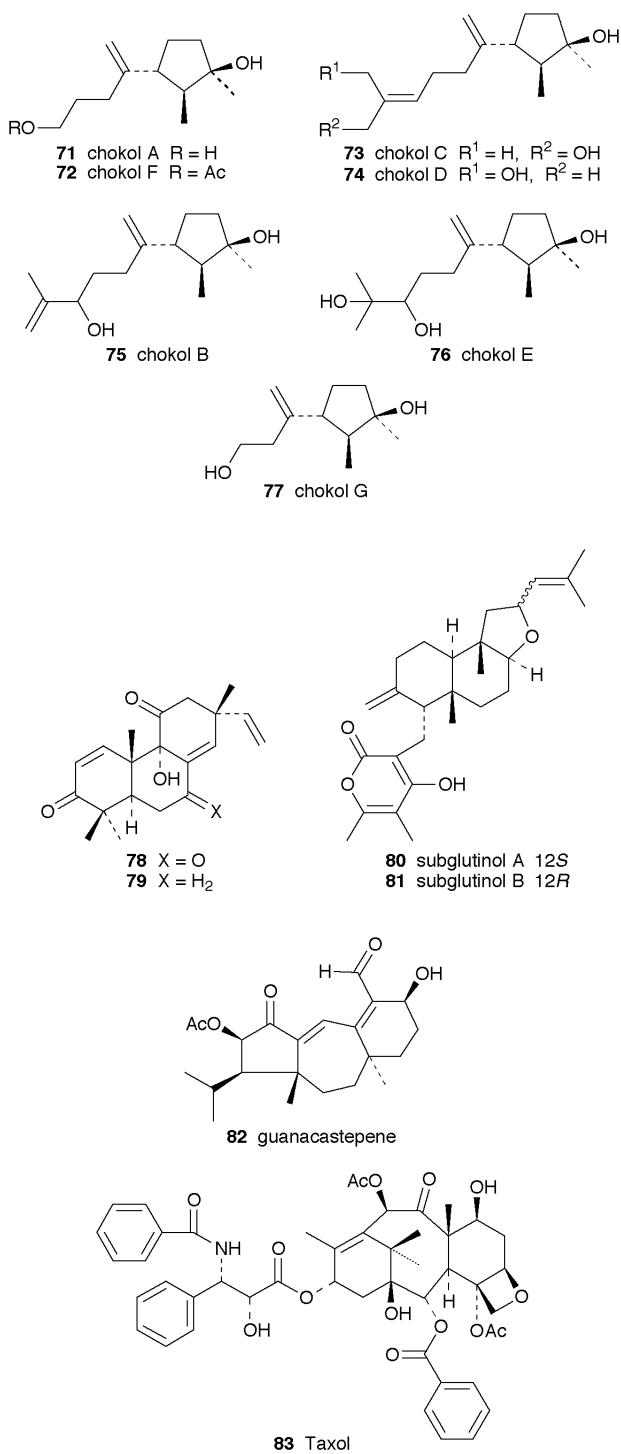
5.2 Diterpenes

Two new insect toxins **78** and **79** of a pimarane diterpene framework were isolated from the broth of an unidentified

71 chokol A R = H
72 chokol F R = Ac

73 chokol C R¹ = H, R² = OH
74 chokol D R¹ = OH, R² = H

75 chokol B

76 chokol E

77 chokol G

78 X = O
79 X = H₂

80 subglutinol A 12S
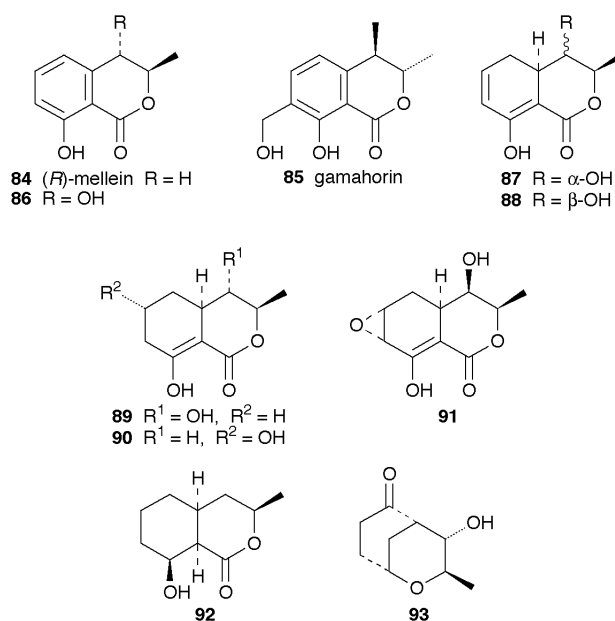81 subglutinol B 12R

82 guanacastepene

83 Taxol

endophyte from a needle of the balsam fir *Abies balsamea*.[72] Subglutinol A **80** and B **81**, immunosuppressive but noncyto-toxic, were produced by *Fusarium subglutinans*, an endophytic fungus from the perennial twining vine *Tripterygium wilfordii*.[73] Guanacastepene **82**, a novel diterpenoid produced by an unidentified fungus from the branch of *Daphnopsis americana* growing in Guanacaste, Costa Rica, was shown to be anti-bacterial against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*.[74] Taxol **83** originally characterized from the inner bark of the Pacific yew, *Taxus brevifolia*, is an efficacious anticancer diterpene found in extremely small quantities in slowly growing *Taxus* species.[75,76] Its unique mode of action, of preventing the depolymerization of tubulin during the processes of cell division, made it a huge success in both clinic and market. However, the source of Taxol is a frustrating problem all over the world owing to the difficulty and unacceptably low yield in its total synthesis.[77] In 1993,

Taxol was reported to be produced in *in vitro* culture by a new endophytic fungus, *Taxomyces andreanae*, which was isolated from a Pacific yew *T. brevifolia* in Montana, USA.[78] Since then, a variety of endophytic fungi belonging to different categories isolated from *T. brevifolia*,[79] *T. wallachiana*,[80] *T. yunnanensis*,[81] *T. baccata*,[82] *T. mairei*,[83] *Taxodium distichum*,[84] *Torreya grandifolia*,[85] and *Wollemia nobilis*[86] have been reported to be capable of producing Taxol and/or taxane derivatives in some endo-phyte cultures. However, the biosynthetic route to Taxol and other taxanes in endophytic fungi is still not very clear. It would be fascinating if Taxol could be supplied through a scaled-up fermentation of such an endophyte.

6 Isocoumarin derivatives

(*R*)-Mellein **84**, an isocoumarin isolated from *Pezicula* spp.,[87] is strongly fungicidal, herbicidal and algicidal. Gamahorin **85** is a new isocoumarin from stromata of *E. typhina* on *P. pratense*.[88] New isocoumarin-related metabolites **86–93** were isolated from the conifer endophyte cultures.[89] Among the products **87–89** and **91–93** described for the first time, compounds **87** and **92** were toxic to spruce budworm larvae with the former also toxic to spruce budworm cells.

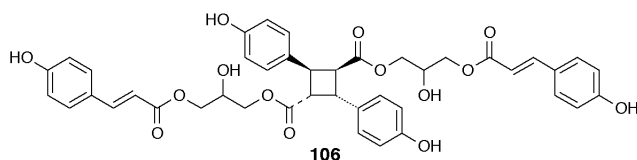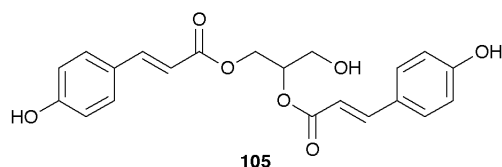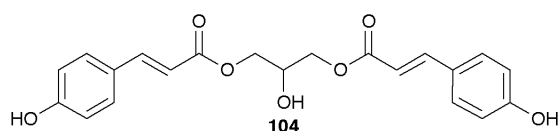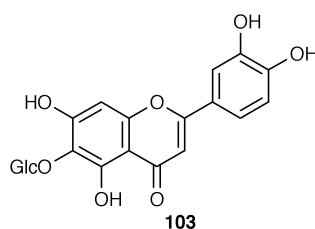84 (*R*)-mellein R = H
86 R = OH

85 gamahorin

87 R = α-OH
88 R = β-OH

89 R¹ = OH, R² = H
90 R¹ = H, R² = OH

91

92

93

7 Quinones

Insecticidal rugulosin **94** was characterized from *Hormonema dematioides*, an endophytic fungus of balsam fir.[69] From cultures of an unidentified endophyte obtained from an eastern larch (*Larix laricina*) needle, 8,1′,5′-trihydroxy-3′,4′dihydro-1′*H*-[2,4′]binaphthalenyl-1,4,2′-trione **95** was characterized as a toxin to spruce budworm larvae.[90] A highly hydroxylated quinone altersolanol A **96**, characterized from phytopathogenic *Alternaria* spp., was reisolated from an endophytic *Phoma* sp. with its antibacterial activity disclosed.[91] Preussomerin N₁ **97**, palmarumycin CP₄ₐ **98**, and palmarumycin CP₅ **99** were new ras farnesyl-protein transferase inhibitors produced by an endophytic *Coniothyrium* sp.[92]

8 Flavonoids

Tricin **100** and related flavone glycosides **101–103**, toxic to mosquito larvae, have been isolated from endophyte-infected blue grass (*Poa ampla*).[93]

9 Phenylpropanoids and lignans

Three new phenylpropanoids **104–105** and lignan **106** were characterized from stromata of *Epichloë typhina* on *Phleum*

94 rugulosin

95

96 altersolanol A

97 preussomerin N_1 **98** palmarumycin CP_{4a} **99** palmarumycin CP_5

100 tricin R = H
101 R = Glc
102 R = Rha-Glc

103

104

105

106

107 coniferin R = H
108 syringin R = OMe

109 leucinostatin A

110 echinocandin A $R^1 = R^2 = R^3 = OH$
111 echinocandin B $R^1 = H$, $R^2 = R^3 = OH$
112 echinocandin D $R^1 = R^2 = R^3 = H$
113 echinocandin H $R^1 = R^2 = OH$, $R^3 = OMe$
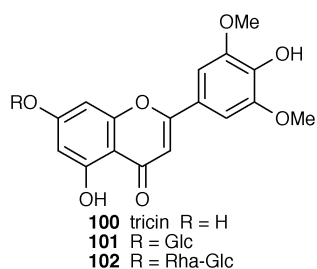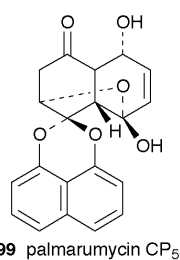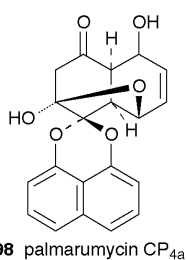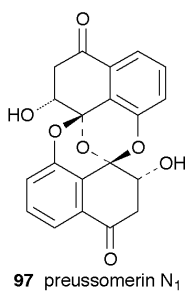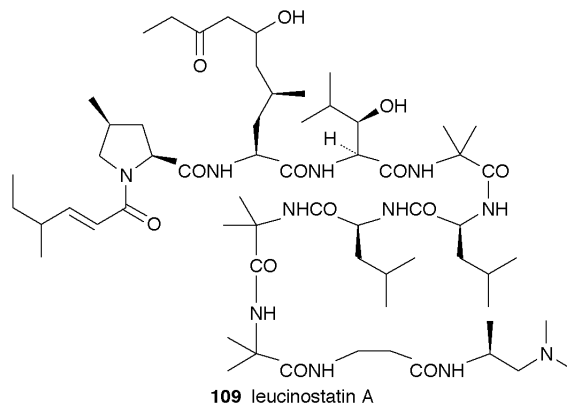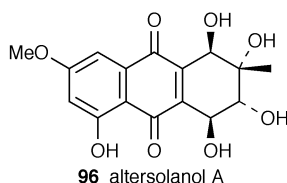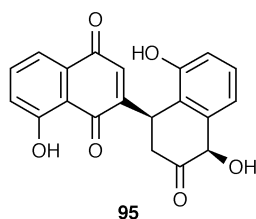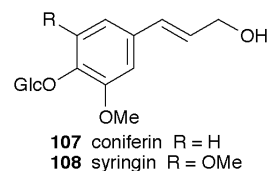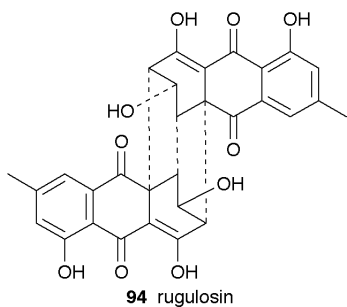
pratense.[94] Interestingly, coniferin **107** and syringin **108**, two monolignol glucosides produced by the host plant, were ascertained to be specifically recognized by the endophytic *Xylariaceae* species as chemical signals during the establishment of fungus–plant interactions.[95]

10 Peptides

Leucinostatin A **109**, an oligopeptide with phytotoxic, anticancer and antifungal properties characterized originally from *Penicillium lilacinum*,[96] was reisolated from culture of *Acremonium* sp., an endophytic fungus from *Taxus baccata*.[97] This mycotoxin causes necrotic symptoms in many non-host plants presumably because they cannot transform it into the much less toxic leucinostatin A-β-di-*O*-glucoside as can the host plant *T. baccata*.[98] The cyclopeptides echinocandins A **110**, B **111**, D **112** and H **113**, produced by *Aspergillus rugulosus* and *A. nidulans* var. *echinulatus*,[99,100] were reisolated from endophytic *Cryptosporiopsis* sp. and *Pezicula* sp. in *Pinus sylvestris* and *Fagus sylvatica* and shown to be antimicrobial.[101] Cryptocandin **114**, a cyclopeptide with potent antifungal activities, is a metabolite of endophytic *Cryptosporiopsis* cf. *quercina* of redwood.[53]
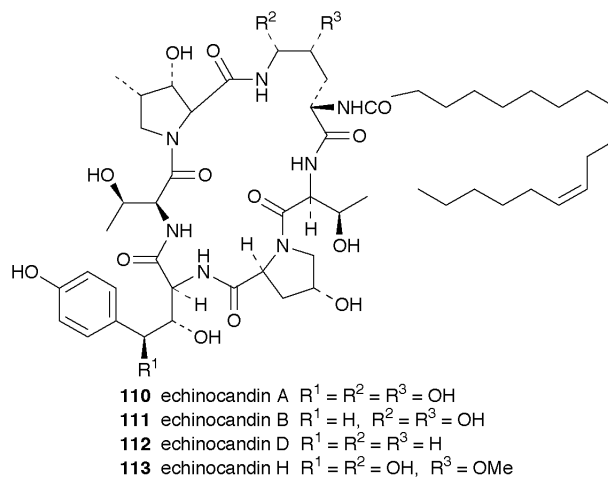
11 Phenol and phenolic acids

Phenol and phenolic acids, frequently detected in cultures of endophytes, often have pronounced biological activities. 2-Hydroxy-6-methylbenzoic acid was isolated from endophytic *Phoma* sp. and shown to be antibacterial.[91] 2-Methoxy-4-hydroxy-6-methoxymethylbenzaldehyde, produced by a tree endophyte *Pezicula* sp. strain 553, was shown to be antifungal by the bioautography assay against phytopathogen *Cladosporium cucumerinum*.[87] Five known antifungal phenolic acids (*p*-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, tyrosol, *cis*- and *trans*-*p*-coumaric acids) were isolated from stromata of *E. typhina* on *P. pratense* together with compounds **104–106**.[94] From *Colletotrichum gloeosporioides*, an endophytic fungus of

114 cryptocandin
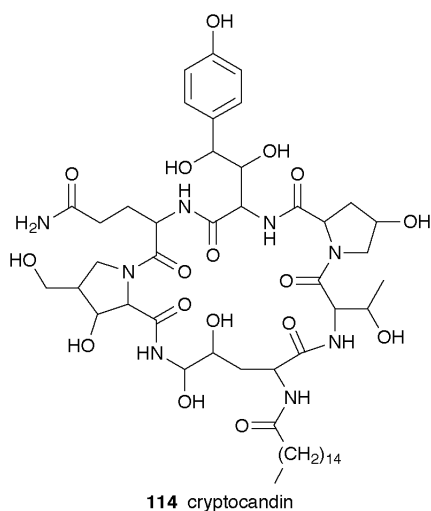
Artemisia mongolica, a new antimicrobial tridepside colletotric acid **115** was characterized in our laboratory.[102] Furthermore, two isomeric novel tridepsides cytonic acids A **116** and B **117** were reported as human cytomegalovirus (an ubiquitous opportunistic pathogen) protease inhibitors from the culture of the endophytic fungus *Cytonaema* sp. isolated from *Quercus* sp.[103]

115 colletotric acid

116 cytonic acid A R¹ = Et, R² = H
117 cytonic acid B R¹ = H, R² = Et
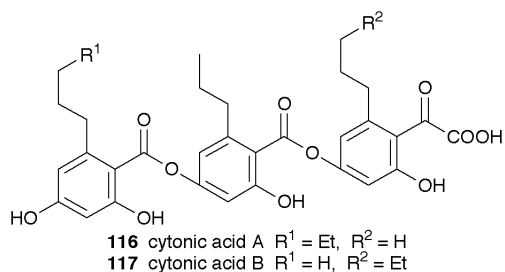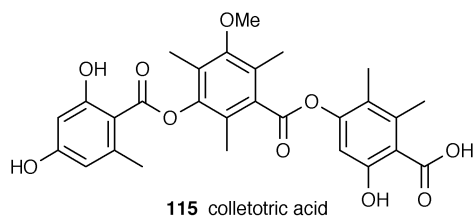
12   Aliphatic compounds

Four antifungal aliphatic compounds **118–121** were characterized from stromata of *E. typhina* on *P. pratense*.[104] From an endophyte of the eastern larch, a novel ester metabolite **122** was isolated as antibacterial agent against *Vibrio salmonicida*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[90] Phomodiol **123** and phomopsolide B **124**, metabolites of endophytic *Phomopsis* spp. present in the genus *Salix* and non-willow plants, may serve as potential markers for taxonomy of these fungi.[105]

13   Chlorinated metabolites
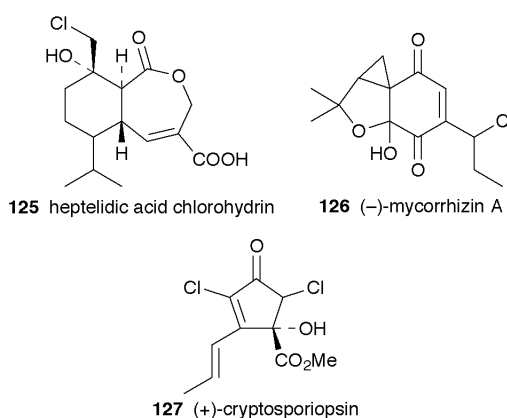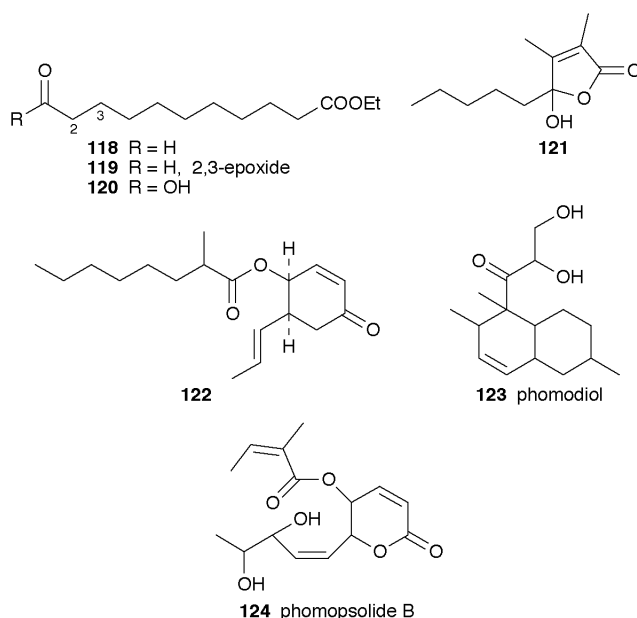
Three chlorinated metabolites from endophytic fungi were reported including an insect-toxic heptelidic acid chlorohydrin **125**, and the two antimicrobial and algicidal compounds (−)-mycorrhizin A **126** and (+)-cryptosporiopsin **127**. They were isolated from cultures of balsam fir needle endophyte *Phyllosticta* sp. strain 76,[69] tree endophytes *Pezicula* sp. and *P. livida* strain 1156,[87] respectively. Compounds **125** and **126** possess sesquiterpene skeletons, the latter being partly degraded.

118 R = H
119 R = H, 2,3-epoxide
120 R = OH

121

122

123 phomodiol

124 phomopsolide B

125 heptelidic acid chlorohydrin

126 (−)-mycorrhizin A

127 (+)-cryptosporiopsin

14   Others

A new antifungal pentaketide **128** was recently characterized from a *Fusarium* sp., an endophytic fungus living in the interior part of *Selaginella pallescens* stem.[106] Other macromolecules such as sulfated oligosaccharides were reported to be capable of mediating the interaction between a marine red alga and its endophyte.[107] Two novel antitumor metabolites sequoiatones A **129** and B **130** were isolated from an endophytic fungus *Aspergillus parasiticus* of redwood.[108] Three new monoterpenes, C-methylated acetogenins **131–133**, were produced by *Pestalotiopsis* spp., endophytic fungi of *Taxus brevfolia*, which were shown to be of chemotaxonomic significance.[68] Two possible pathways related to the biosynthesis of these metabolites from certain polyketide precursors were also put forward. In addition to gamahorin **85**, three new antifungal metabolites gamahonolide A **134** and B **135** and 5-hydroxy-4-phenylfuran-2(5*H*)-one **136** were characterized from stromata of *E. typhina* on *P. pratense*.[88] Chaetomellic acids A **137** and B **138** originally isolated from the culture of an endophytic *Chaetomella acutisea* (MF5686) were found to be specific inhibitors of farnesyl-protein transferase (FPTase).[109]

128

129 sequoiatone A

130 sequoiatone B

131

132 R = CH₂OH
133 R = CHO
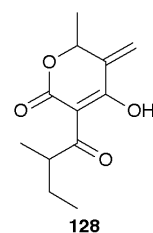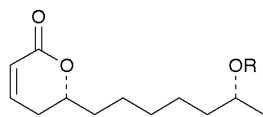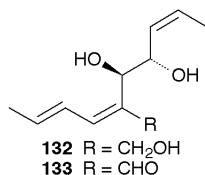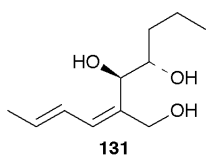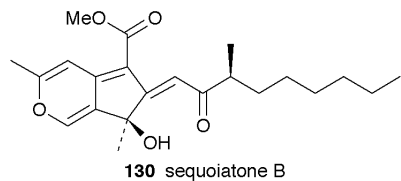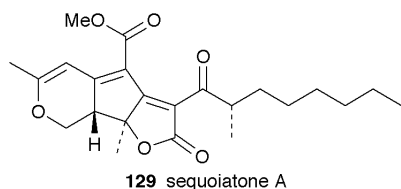
134 gamahonolide A R = H
135 gamahonolide B R = COCH₂CH₂COOCH₂CH

136

137

138
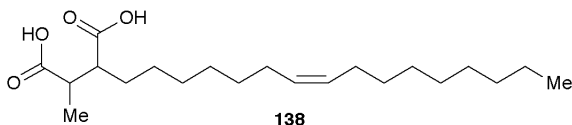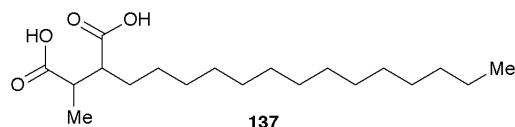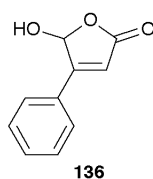
15   Comment

Like other microorganisms invading plant tissues, endophytes produce extracellular hydrolyases as a resistance mechanism to overcome attack by the host against pathogenic invasion and/or to get nutrition from the host. Such enzymes including pectinases, esterases, cellulases and lipases,[60] proteinase,[110] α-1,4-glucan lyase[111] and phosphatases[112] have been documented with different endophytes. Enzymatic activities closely related to the host-specificity of the endophytes were demonstrated.[60] The action of these enzymes gives rise to the possibility that the 'genetic recombination' of the endophyte with the host may occur in evolutionary time. This could be the reason why some endophytes can produce some phytochemicals originally characteristic of the host. The extended significance of the productivity of endophytes for some important phytochemicals such as Taxol lies in that it provides an alternative strategy for easing the impact of the growing population on plants which are needed as well for the preservation of biodiversity and the ecosystem.

As a poorly investigated store of microorganisms 'hidden' within the host plants, endophytes are obviously a rich and reliable source of bioactive and chemically novel compounds with huge medicinal and agricultural potential. Further research at the molecular level in this field is clearly required for a better understanding of the host–endophyte interaction which may lead to a quicker recognition of genetically particular and/or host gene carrying endophytes.

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