

Inedible Mushrooms: A Good Source of Biologically Active Substances

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ABSTRACT: In the course of our investigation on biologically active substances from inedible mushrooms in Japan, Germany, and Vietnam, we studied the chemical constituents of 22 species belonging to five families: Scutigeraceae, Polyporaceae, Xylariaceae, Thelephoraceae, and Paxillaceae. Various types of chemical substances were purified and characterized based on the modern spectroscopic methods and also on chemical reactions. These metabolites have shown a broad activity in many biological systems, such as antimicrobial, nematocidal, inhibition of NO production, anti-human immunodeficiency virus, tumor necrosis factor- α , and antioxidant activities. These isolated metabolites did not only show interesting activities, but also are employed as chemical markers supported for chemosystematics of these families. This review paper deals with the chemical constituents of 22 species, their biological activities, and also a discussion on chemosystematics. © 2006 The Japan Chemical Journal Forum and Wiley Periodicals, Inc. Chem Rec 6: 79–99; 2006: Published online in Wiley InterScience (www.interscience.wiley.com) DOI 10.1002/tcr.20074

Key words: mushroom; Scutigeraceae; Polyporaceae; Xylariaceae; Thelephoraceae; Paxillaceae

Introduction

About 2900 fungal species have been recorded in Germany. But only about 350 of these species are known to be edible and good (about 50 species are allowed to be sold on markets according to German legislation), and about 120 species have been recorded to cause intoxications.¹ In addition, about 1500 species of mushrooms are now known in Japan, of which inedible, edible, and toxic mushrooms are 1200, 300, and 50 species, respectively.² Although there is no official detailed record on Vietnamese fungi, 2200 species were recently counted.³ So far, various types of secondary metabolites, which show characteristic pharmacological activities, have been isolated from mushrooms around the world.^{4a}

A lot of inedible mushrooms show bitter and pungent taste, and especially those belonging to Polyporaceae have been

used as medicinal drugs (anticancer, etc.) in China from ancient times.^{4b} The use of natural products isolated from mushrooms against infection and cancer diseases is one of the cornerstones of modern medicine.⁵ Examples of these mushrooms are *Ganoderma applanatum* and *Agaricus* spp., which have been claimed to have anticancer properties.⁶ Moreover, *Ganoderma lucidum* was reported to contain bitter-tasting triterpenes and many polysaccharides. The properties accorded to this mushroom included anti-hepatotoxic activity (*R,S*-

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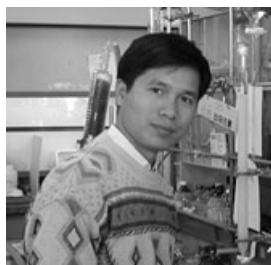
ganodermic acid), antitumor activity (polysaccharides and glucans), and cardiotoxic activity (alkaloids). Antimicrobial (activity) compounds were also reported from wood-rotting fungi such as *Fomes*, *Trametes*, and *Polyporus* spp.⁶ However, little attention has been paid to the chemical constituents of inedible mushrooms.

In the past recent years, we have investigated the chemical constituents of inedible mushrooms collected around the world. Some of these isolated metabolites showed valuable pharmacological activities, such as bitter drimane sesquiterpenoids, cryptoporin acids A—G from *Cryptoporus volvatus*,^{7–10} which showed the inhibitory activities of superoxide anion radical release,¹¹ colon and skin cancer development in mouse,^{12,13} and anti-human immunodeficiency virus (HIV)-1;¹⁴ 16 cytochalasins from *Daldinia eschscholzii*^{14–16} possessing

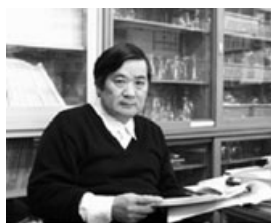
a strong cytotoxicity on the KB cell line,¹¹ apoptosis induction in HCT116 cells;¹⁷ daldinins A—C and daldinins A—C from *Daldinia childeae*,^{18,19} and entonaemins A—C from *Entonaema splendens*.²⁰ In continuation, we have collected a rather large amount of 22 species around the world, which allowed us to study their chemical constituents and biological activities. This review surveys all of these results, which were done by our group since 1998.

Biologically Active Compounds from *Albatrellus* sp. (Scutigeraceae)

Because grifolin (1) and neogrifolin (2) were isolated from the inedible mushroom *Albatrellus ovinus*,²¹ not only the chemical



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▶ Toshihiro Hashimoto was born in 1947 in Tokushima, Japan. He obtained a B.S. in 1971 and then Master of Pharmacy in 1973 at the Faculty of Pharmaceutical Sciences, Tokushima University. In 1982, he was awarded Ph.D. of Pharmacology at Kyoto University. He had been working at the Tokushima Bunri University (TBU) as Research Assistant from 1973 to 1988, where he researched on natural product chemistry. From 1983 to 1984, he moved to Oregon State University, USA and worked on cardiac glycoside as a postdoctoral. Then, he returned to TBU and has been working there since. He was appointed as lecturer in 1988, then Associate Professor in 1995. His major researches are phytochemistry and phytomedicine of inedible mushrooms and moss (liverwort), biotransformation of crude drugs by microorganisms, total synthesis of natural products, and medicinal chemistry of cardiac glycosides. He has published 150 international papers, 5 reviews, and 12 patents. ■

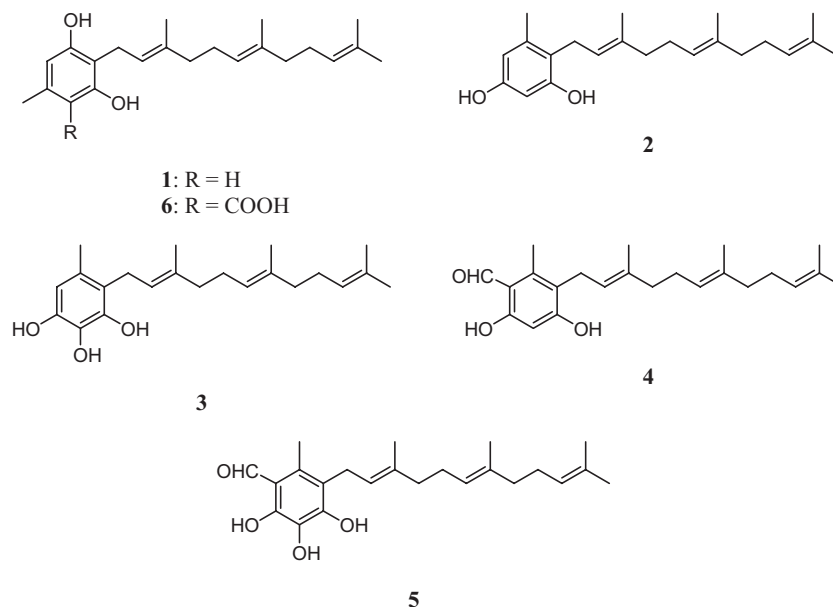


Fig. 1. Grifolin and neogrifolin derivatives from *Albatrellus* sp.

constitutions of other *Albatrellus* sp. but also their useful biological activities have been reported. Previous publications showed that grifolin derivatives possess a broad spectrum of biological activity, such as antimicrobial,^{22,23} plant growth inhibitory,²⁴ tyrosinase inhibitory,²⁵ anti-cholesteremic activity level in blood and liver,²⁶ promotion of melanin synthesis by B16 melanoma cells,²⁷ and activity on human and rat vanilloid receptor 1.²⁸ From the methanolic extract of the

Japanese fungus *A. ovinus*, 3-hydroxyneogrifolin (**3**), 1-formylneogrifolin (**4**), and 1-formyl-3-hydroxyneogrifolin (**5**) were purified (Fig. 1), of which **3** and **5** showed more potent antioxidative activity properties than either α -tocopherol or *tert*-butylhydroxyanisole (BHA).²⁹

Another Japanese *Albatrellus dispansus* was chemically studied, and grifolin (**1**), grifolic acid (**6**) as major components, grifolic acid methyl ester (**7**), and a new compound grifolinol



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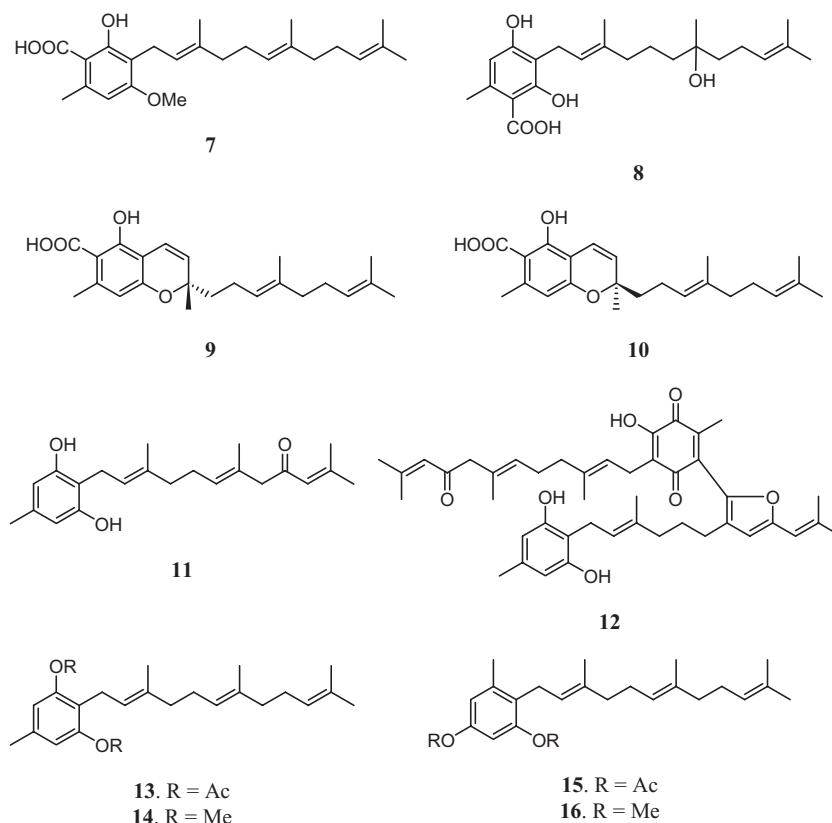


Fig. 2. Structures of grifolin derivatives (7–16).

(8) were isolated from its methanolic extract (Fig. 2). Their structures were established by a combination of two-dimensional (2-D) nuclear magnetic resonance (NMR), mass spectroscopy (MS), and chemical reactions.³⁰

In addition, (+) and (–)-daurichromenic acids (9, 10) were successfully prepared from grifolic acid (6), a major component from this fungus with only one step in the presence of dichlorodicyanobenzoquinone in benzene and stirred for 30 min at 90°C, in good yield (73%). Then, (+) and (–)-daurichromenic acids were separated by high-performance liquid chromatography (HPLC) equipped with chiral column. Previously, (+)-daurichromenic acid (9) was reported to show highly potent anti-HIV activity with a 50% effective concentration value of 0.00567 μg mL⁻¹.³¹ However, other compounds were devoid of anti-HIV activity (Table 1).

The suppression of the production of tumor necrosis factor (TNF)-α of grifolic acid derivatives was tested, and their results are shown in Table 2. Grifolin (1) strongly suppressed TNF-α production with 50% inhibitory concentration (IC₅₀) values of 3.98 μM, while the other compounds exhibited a weak and similar inhibitory activity. The finding suggested that the presence of the carboxylic group at C-1 in 6, 7 and 9, 10 decreased the inhibitory activity.

Table 1. Anti-human immunodeficiency virus effects of grifolic acid derivatives.

Samples	EC ₅₀ (μM)	CC ₅₀ (μM)
Grifolin (1)	>38	38
Grifolic acid (6)	>40	40
Grifolic acid methylester (7)	>53	53
(+)-Daurichromenic (9) ³¹	0.00567 μg mL ⁻¹ (0.15)	

EC₅₀ = 50% effective concentration; CC₅₀ = 50% cytotoxic concentration.

Table 2. Inhibitory activity of tumor necrosis factor-α of grifolic acid derivatives.

Samples	IC ₅₀ (μM)
Grifolin (1)	3.98
Grifolic acid (6)	20.3
Grifolic acid methylester (7)	28.0
(+)-Daurichromenic (9)	27.8
(–)-Daurichromenic (10)	28.4

IC₅₀ = 50% inhibitory concentration.

Table 3. Antimicrobial activity of grifolic acid derivatives [diameter of the zone of growth inhibition, bactericidal or fungicidal zone (in millimeters), including the diameter of disc, 6 mm].

Microorganism/Sample	1	6	7	9	10	Gentamicin	Nystatin
<i>Escherichia coli</i>	27	30	20	32	32	16	nt
<i>Klebsiella pneumoniae</i>	40	44	42	43	44	14	nt
<i>Pseudomonas aeruginosa</i>	20	27	16	24	22	16	nt
<i>Staphylococcus aureus</i>	19	27	17	24	25	15	nt
<i>Salmonella enteritidis</i>	18	28	26	26	38	18	nt
<i>Aspergillus niger</i>	18	29	23	28	29	nt	18
<i>Candida albicans</i>	20	32	26	30	28	nt	17

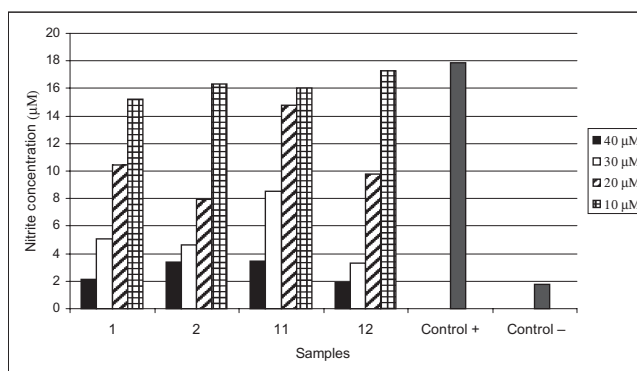
nt = not tested.

Previous studies repeatedly pointed out that grifolic acid derivatives possessed an interesting antimicrobial activity.^{22,23} However, only a few microorganisms have been tested. This time, grifolic acid derivatives at a dose of 50 µg per disk were tested against seven microorganisms, together with gentamicin (standard for bacteria) and nystatin (standard for fungi). Their antimicrobial activities are shown in Table 3. These samples have exceedingly good activity, in some cases three times stronger than that of standards. Especially, *Klebsiella pneumoniae*, an important medicinal pathogen, was the most susceptible to the samples.

Two new farnesyl phenols named grifolinones A and B (**11**, **12**), together with known grifolin (**1**) and neogrifolin (**2**), were isolated from the methanolic extract of the inedible fungus *Albatrellus caeruleoporus* (Fig. 2).³² Their structures are characterized by a combination of 2-D NMR, MS, infrared (IR), and ultraviolet (UV) spectra. This is the first report on the isolation of a dimeric grifolin, which is connected *via* carbon-carbon bond, and the second example of grifolin having the furane ring.²² Interestingly, the finding of this dimeric compound possessing a *para*-quinone supports the slightly purple color of the fruit bodies of this fungus.³³

Later, all four compounds (**1**, **2**, **11**, **12**) were tested for their inhibitory activity of NO production stimulated by lipopolysaccharide (LPS) in RAW 264.7 cells, a mouse macrophage cell line. They are strongly inhibiting the LPS-induced production of NO at 24 h with the IC₅₀ values of 23.4, 22.9, 29.0, and 23.3 µM, respectively. Especially, all four samples suppressed almost their NO production at the concentration of 40 µM, and were devoid of significant activity at 10 µM. Higher concentration (more than 50 µM) was also tested; however, cell viability was reduced due to their cytotoxicity (Fig. 3).

The distribution of grifolin (**1**), neogrifolin (**2**), and related compounds isolated from *Albatrellus* sp. around the world is listed in Table 4.²⁹ Accordingly, grifolin (**1**) and neogrifolin (**2**) were isolated from *Albatrellus confluens* and *A.*


Fig. 3. Inhibition of NO production of **1**, **2**, **11**, and **12**.

caeruleoporus as main products, while *A. dispansus* and *Albatrellus yasudai* mainly elaborated grifolic acid (**6**). Interestingly, only Japanese *A. ovinus* and *A. caeruleoporus* produced 1-formyl and 3-hydroxy as well as dimeric grifolin, respectively.

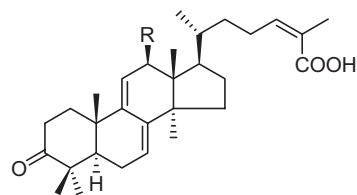
Triterpenoids from *Tyromyces fissilis* and *Fomitopsis spraguei* (Polyporaceae)

Tyromyces spp. belonging to the family Polyporaceae have been shown to be a good source of biologically active compounds. Previously, tyromycic acid (**17**), a new lanostane triterpenic acid, was isolated from *Tyromyces albidus*.³⁴ Later, tyromycin A, 1,16-bis-[4-methyl-2,5-dioxo-3-furyl]hexadecane was isolated from *Tyromyces lacteus* as an inhibitor of leucine and cysteine aminopeptidases.³⁵ 4-But-3-enoxymethyl benzoate was obtained by fermentation of a *Tyromyces* spp., and showed the inhibition of phospholipase A2.³⁶

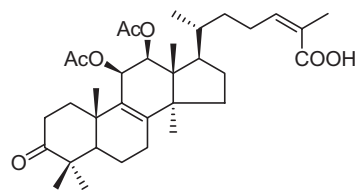
From a methanol-soluble extract of the fruit bodies of the Japanese inedible mushroom, *T. fissilis*, six new triterpenoids, tyromycic acids B—G (**18–23**), together with two known compounds, tyromycic acid (**17**) and trametenolic acid B (**24**), were isolated (Fig. 4). Tyromycic acids B—D and G possess a

Table 4. Distribution of grifolin derivatives from *Albatrellus* spp.

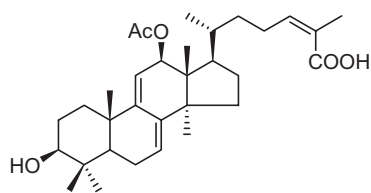
Species	1	2	3-5	6	11, 12	13-16
Japanese <i>Albatrellus</i> <i>ovinus</i>	+	++	++++			
European <i>A. ovinus</i>	++++	++++				++++
<i>Albatrellus confuens</i>	++++	++++				
<i>Albatrellus dispansus</i>	+++	+		++++		
<i>Albatrellus</i> <i>caeruleoporus</i>	++++	++++			++	
<i>Albatrellus yasudai</i>	+	+		++++		



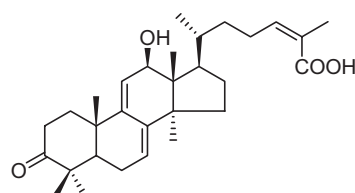
17. R = H
23. R = OAc



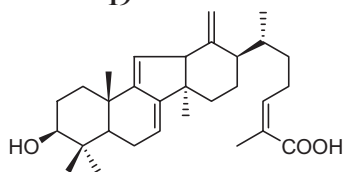
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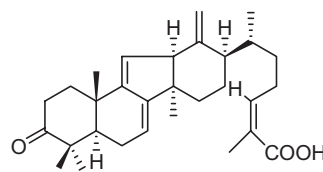
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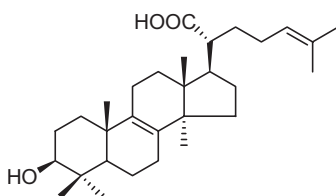
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Fig. 4. Triterpenoids from *Tyromyces fissilis* (17-24).

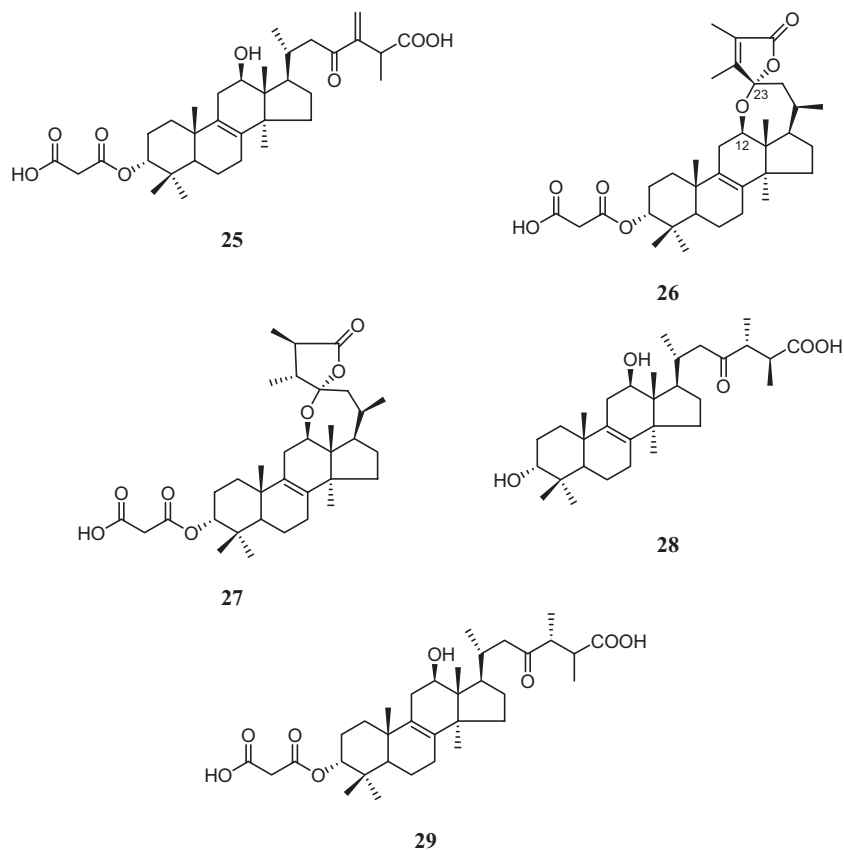


Fig. 5. Structures of lanostane triterpenoids from *Fomitopsis spraguei*.

lanostane skeleton, while tyromycic acids E, F (**21**, **22**) are based on a rare 14(13 → 12)*abeo*-lanostane skeleton. Their structures were determined by spectral data analysis and by single-crystal X-ray crystallography.^{37,38}

Fungal species of the Polyporaceae are known to produce 24-methyl-lanostanes with a carboxyl group at C-26, and some possess a carboxyacetoxyl group at C-3.^{39–43} Fomlactones A–C from *Fomes cajanderi* contain a 12,23 epoxy ring together with a 26,23-lactone.⁴⁴ So far, these compounds have been shown to possess interesting biological activities, such as anti-inflammatory,^{45,46} antimicrobial,^{47,48} anti-HIV,⁴⁹ and DNA polymerase and DNA topoisomerase inhibitory activity.⁵⁰

Investigation on the methanolic extract of the Japanese inedible mushroom *F. spraguei* (Polyporaceae) led to the isolation of five lanostane-type triterpenoids: three new compounds named fomitopsins A–C (**25–27**), and two known compounds, quercinic acid C (**28**) and 3 α -carboxyacetyl-12 β -hydroxyquercinic acid (**29**) (Fig. 5). Their structures were determined by 2-D NMR, MS, IR, UV spectra, and X-ray crystallographic analysis. An X-ray crystal structure analysis of quercinic acid C (**28**) established its stereochemistry as

3*R*,12*R*-dihydroxy-24*R*-methyl-23-oxo-25*S*-lanost-8-en-26-oic acid.⁵¹

Biologically Active Substances from Xylariaceous Fungi

The Xylariaceae is a large family, currently comprising more than 36 genera, of which two genera, *Hypoxylon* and *Daldinia*, are composed of about 150 and 20 species, respectively. They grow mostly on wood, seeds, and fruits. So far, at least one-third of these genera have been chemically investigated.⁵²

Biologically Active Compounds from *Hypoxylon* sp.

Hypoxylon is well known to contain many azaphilones, which are polyketides produced by ascomyceteous fungi.^{3,53} The first azaphilones, such as (+)-mitorubrinol (**30**) and mitorubrinic acid (**31**), were isolated from *Hypoxylon fragiforme*.⁵⁴ Recently, the stromata of young European *H. fragiforme* were reinvestigated, not only two azaphilones (**30**, **31**) but also three cytochalasins named fragiformins A and B (**32**, **33**) and

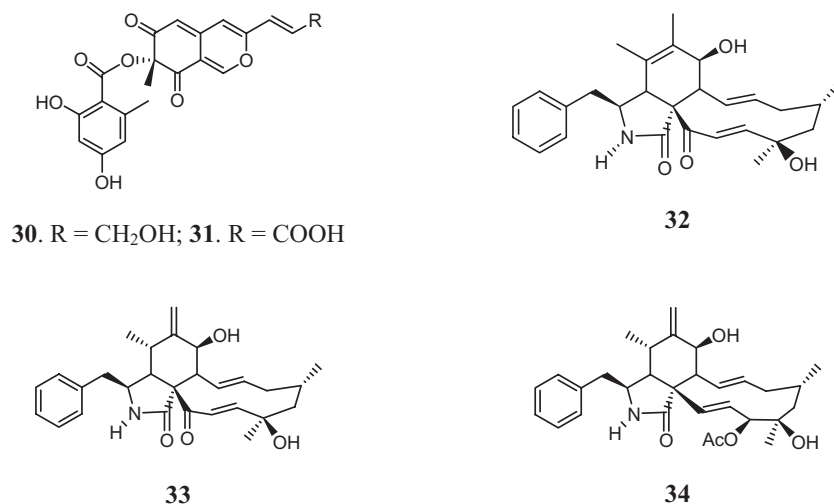


Fig. 6. Structures of azaphilones and cytochalasins isolated from *Hypoxylon fragiforme*.

cytochalasin H (**34**) were purified (Fig. 6).⁵⁵ These mitorubrin-type azaphilones can hardly be detected in mature stromata of this species and appear to be restricted to *H. fragiforme* and *Hypoxylon howeianum* while being absent in many related *Hypoxylon* spp.⁵⁵

Similar to mitorubrin derivatives are rubiginosins A and B (**35**, **36**) and entonaemin A (**37**), which are azaphilones with an orsellinic acid moiety attached to the bicyclic azaphilone backbone through an ester linkage. These metabolites along with rubiginosin C (**38**), rubiginosic acid (**39**), and daldinin C (**40**) were isolated from *Hypoxylon rubiginosum*.⁵⁶ The absolute configuration of compound **38** was established by circular dichroism (CD) spectroscopy, and the structure of **39** was confirmed by X-ray crystallographic analysis (Fig. 7).

Other group of azaphilones are daldinin C (**40**) and daldinins E, F (**41**, **42**) constituting *spiro*-tricyclic derivatives, which were purified and identified from *Hypoxylon fuscum*, together with a known compound, 4:5:4':5'-tetrahydroxy-1:1'-binaphthyl (BNT) (**43**).⁵⁷ The absolute configuration of daldinins E and F (**41**, **42**) at C-7 was determined to be *S* by comparing their CD spectral data with those of daldinin C (**40**). The antioxidant activity of these compounds was tested by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.⁵⁸ The antioxidative activity of BNT (**43**), which has never before been reported in the literature, was almost the same as that of ascorbic acid with the IC₅₀ values of 18.2 and 16.5 μM, respectively. In contrast, daldinins E and F, and daldinin C were devoid of significant activities with IC₅₀ values of 178.9, 212.3, and 412.0 μM, respectively.⁵⁷

One more group is cohaerins A and B (**44**, **45**), which are azaphilones with a fatty acid side chain linked with bicyclic azaphilone by an ester bond, isolated from *Hypoxylon cohaerens*.⁵⁹

The azaphilone compounds were also widespread in *Hypoxylon multiforme*; multiformins A—D (**46–49**) and BNT (**43**) were isolated from its methanolic extract.⁶⁰ These compounds, together with sassafrins A—D (**50–53**) from *Creosphaeria sassafras*, are azaphilones with a lactone ring in their molecule. The presence of a lactone ring in these azaphilones plays an important role in their pharmacological activity, which will be mentioned later in this paper. Furthermore, sassafrin D (**53**) possesses a new skeleton (Fig. 8).^{61a}

An HPLC profiling study comparing the extracts of *C. sassafras* with those of ca. 1000 specimens of Xylariaceae,^{59,61b,c,78} preferably of the genera *Hypoxylon* and *Daldinia*, was also carried out. According to the preliminary results, the sassafrins A—D (**50–53**) were not located in any of the other species of the family. On the other hand, various azaphilones and other metabolites which were identified from *Hypoxylon* and allied genera in the past years all proved absent in the extract of *C. sassafras*. These results support the taxonomic view of Ju et al.^{61d} that *Creosphaeria* is not a close ally of *Hypoxylon*. Furthermore, the results of the current paper provide another good example that chemotaxonomic data are well in agreement with recently established molecular phylogeny of this family, because they coincide with two recent independent polymerase chain reaction (PCR)-based studies on the ITS nrDNA gene^{61e} that also revealed the status of *Creosphaeria* as a rather isolated genus within the family.

Recently, two novel dimeric azaphilones, rutilins A and B (**54**, **55**), together with the known compounds, rubiginosins A and B (**35**, **36**) and entonaemin A (**37**), were obtained from *Hypoxylon rutilum*. Rutilins A and B are presumably synthesized via specific biogenic aldol condensations of mitorubrinol acetate moieties with rubiginosins A and B, respectively. The

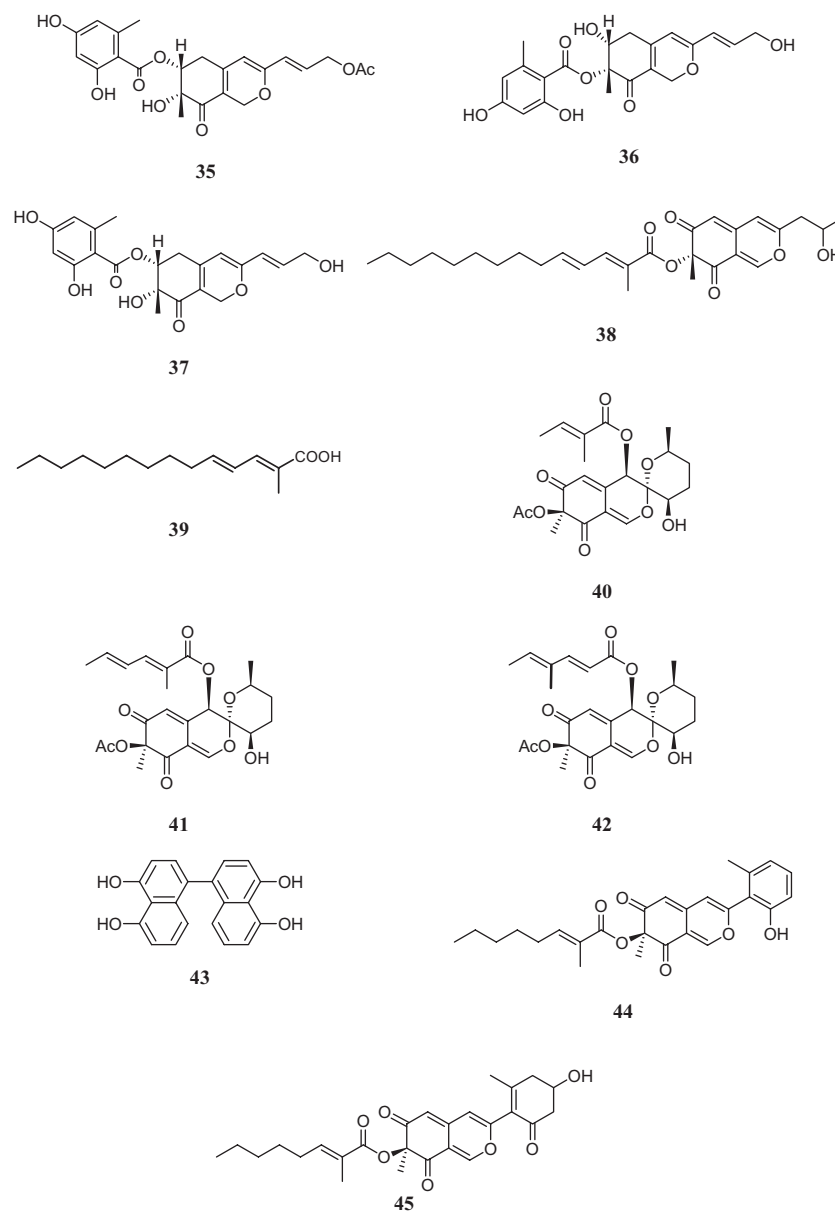


Fig. 7. Azaphilones from *Hypoxylon* sp.

resulting dimeric azaphilone scaffold of the rutilins is unprecedented in nature.⁶²

Finally, a very interesting result was an investigation of a large amount of female sex pheromones of the European spider *Linyphia triangularis* Clerck and related species,⁶³ 3*R*-hydroxybutyric acid (**56**), its dimer 3*R*-(3*R*-hydroxybutyryloxy)butyric acid (**57**) and trimer 3*R*-[3*R*-(3*R*-hydroxybutyryloxy)-butyryloxy]-butyric acid (**58**) from the EtOAc extract of the Japanese inedible mushroom *Hypoxylon truncatum*, which was later classified as *Hypoxylon annulatum*.⁵⁹ The absolute configurations of all asymmetric carbons of the spider sex pheromones were established to be *R* by chemical reactions and

gas chromatography (GC)–MS on a chiral column with authentic samples.⁶⁴ Further investigation of spider sex pheromones from 30 species of *Hypoxylon* and *Daldinia* was carried out; however, none of them contained these pheromones. More than 1 decade ago, truncatone (**59**) was also obtained from the Japanese *H. annulatum*.²⁰

Biologically Active Compounds from *Daldinia Concentrica*

Daldinia concentrica was first chemically studied by Allport and Bu'Lock in 1958 and reported (BNT) (**43**) and dihy-

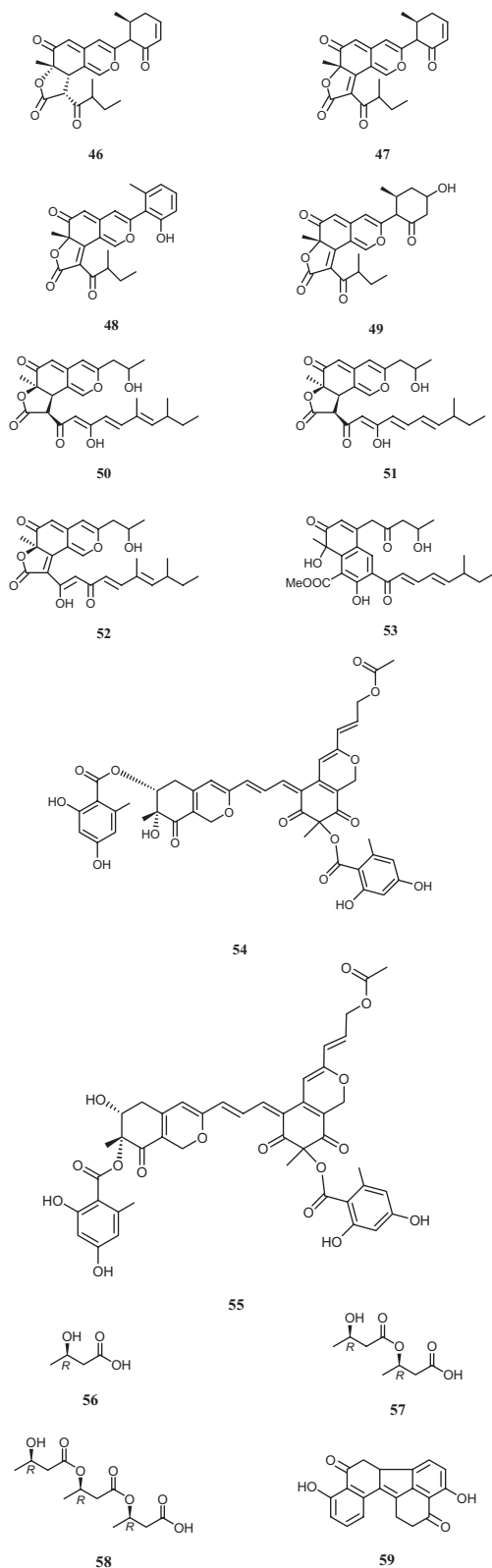


Fig. 8. Structures of azaphilones and spider sex pheromones.

droxyperylene quinone from the stromata of *D. concentrica*.⁶⁵ Recently, Qin and Liu reported that two new aromatic steroids, (17 β ,20*R*,22*E*,24*R*)-19-norergosta-1,3,5,7,9,14,22-heptaene and (17 β ,20*R*,22*E*,24*R*)-1-methyl-19-norergosta-1,3,5,7,9,14,22-heptaene,⁶⁶ and new homologous series of 3-alkyl-5-methoxy-2-methyl-1,4-benzoquinones were isolated from the fruit bodies of Chinese *D. concentrica*,⁶⁷ as well as a pair of novel hept-6-ene-2,4,5-triol stereoisomers from its culture broth.⁶⁸ One more Asian species was investigated, and a new compound, (3,4,5-trimethoxyphenyl)ethanol, was found together with a known compound, caruilignan C, from the fruit bodies of Korean *D. concentrica*. Both compounds exhibited neuroprotective effect against iron-induced neurodegeneration in a primary culture of mouse cortical neurons.⁶⁹

Meanwhile, the German *D. concentrica* elaborated not only BNT (43), but also daldinone A (60), daldinone B (61), daldiniapyrone (4-methoxy-5-carbomethoxy-6-pentyl-2*H*-pyran-2-one (62), daldinialanone (22*R*-hydroxylanosta-7,9(11),24-trien-3-one (63), 3,4,5-trihydroxy-1-tetralone (64), (+)-orthosporin (65), curuilignan D (66), (22*E*)-cholesta-4,6,8(14),22-tetraen-3-one (67), 3 β ,22-dihydroxylanosta-7,9(11),24-triene (68), concentricol (69),⁷⁰ concentricols B—D (70–72), and phenochalasin B (73). The absolute configurations of 60 and 63, 70–72 were determined by CD spectroscopy and the modified Mosher's method, respectively (Fig. 9).^{71,72}

Recently, we have obtained one species from Vietnam, which was *Xylaria intracolorata*, and two new compounds named coloratin A [3,5-dimethoxy-2-(6-oxo-5-pentyl-6*H*-pyran-3-carbonyl)-benzoic acid] (74) and coloratin B (2-carbomethoxyl-3,5-dimethoxybenzoic acid) (75) were isolated and identified (Fig. 10).⁷³

Antimicrobial Activity of Isolated Azaphilones from the Xylariaceae Family

Previous publications showed that azaphilones exhibited interesting biological activities, such as cholesteryl ester transfer protein,^{74a} monoamine oxidase inhibition,^{74b} endothelin receptor binding,^{74c} the inhibition of gp120–CD4 binding,^{74d} telomerase inhibition,^{74e} and the induction of Epstein–Barr virus.^{74f} In this review, we describe the antimicrobial, nematocidal, and inhibition of NO production by azaphilones, and also other isolated metabolites from xylariaceous fungi.

The *in vitro* antimicrobial activities of isolated azaphilones at a dose of 50 μ g per paper disc were tested against a panel of laboratory control strains belonging to the American Type Culture Collection, Manassas, VA, USA: *Staphylococcus aureus*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Escherichia coli* 95, and fungal organisms *Aspergillus niger* and *Candida albicans*. The disc diffusion method accord-

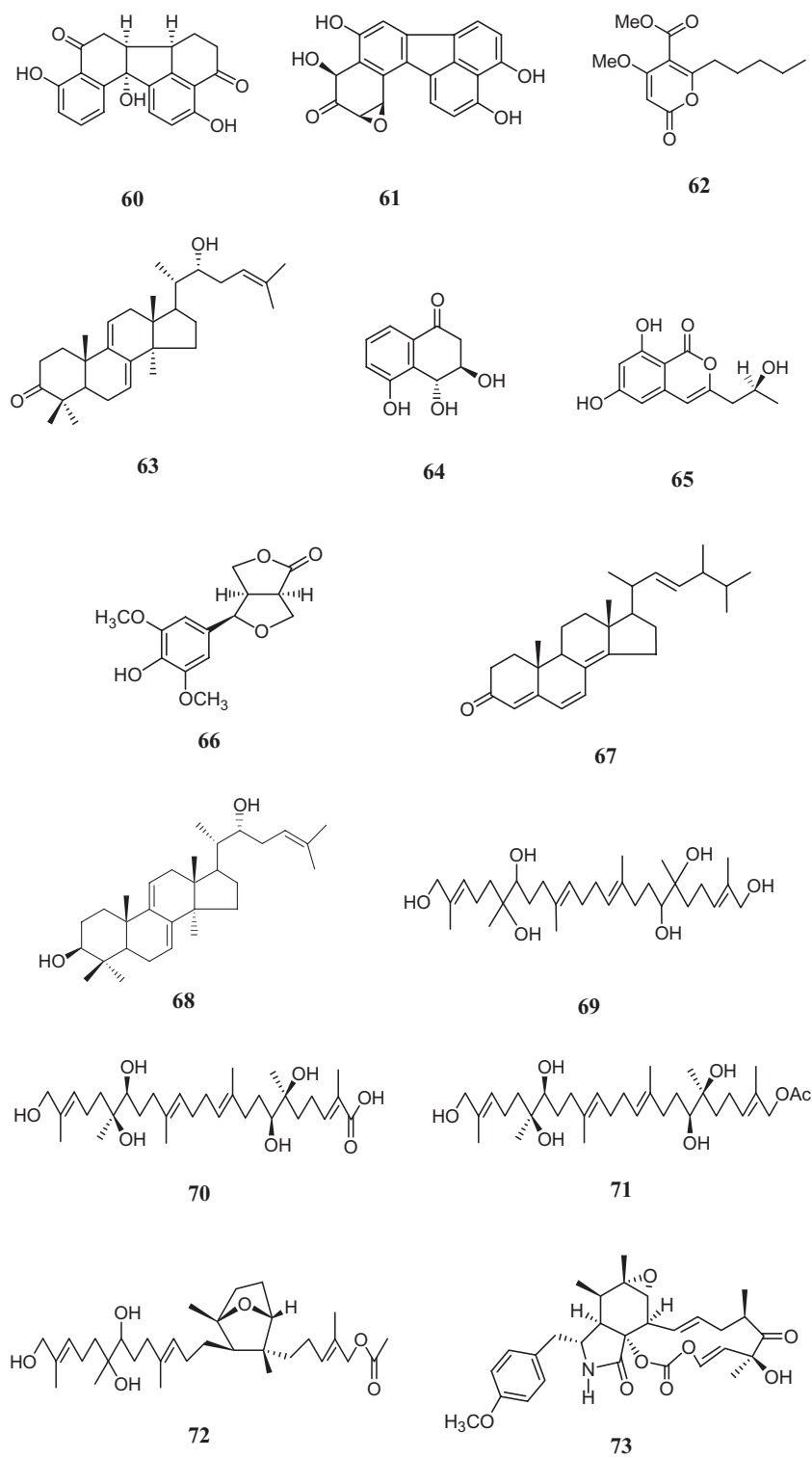
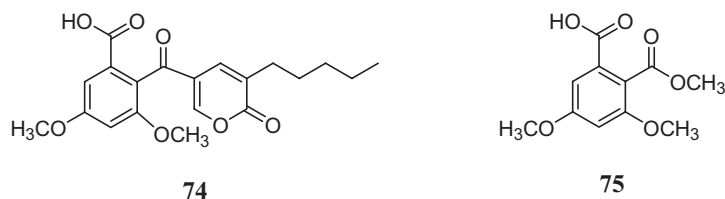


Fig. 9. Chemical constituents of *Daldinia concentrica*.

Fig. 10. Chemical constituents of *X. intracolorata*.**Table 5.** Antimicrobial activity of selected azaphilones [diameter of the zone of growth inhibition, bactericidal or fungicidal zone (in millimeters), including the diameter of disc, 12.7 mm].

Sample	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella enteritidis</i>	<i>Escherichia coli</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
Rubiginosin A (35)	13	7	9	8	13	15	15
Rubiginosin B (36)	17	16	15	15	19	16	16
Entonaemin A (37)	8	7	13	7	8	14	14
Rubiginosin C (38)	17	17	20	19	18	20	18
Daldinin C (40)	7	7	8	8	7	14	15
Daldinin E (41)	14	13	13	8	13	17	16
Daldinin F (42)	7	7	7	7	7	16	15
Multiformin A (46)	18	0 (+14)	18	0	0	0 (+19)	0 (+16)
Multiformin B (47)	20	19	20	19	19	18	19
Multiformin C (48)	18	18	18	16	18	17	17
Multiformin D (49)	0 (+15)*	0 (+17)	16	0 (+16)	0 (+15)	0	0 (+20)
Sassafrin A (50)	19	18	20	20	20	20	19
Sassafrin B (51)	18	19	20	21	14	20	19
Sassafrin C (52)	22	22	22	20	22	19	18
Sassafrin D (53)	17	19	17	17	19	18	17
Coloratin A (74)	15	16	22	16	16	15	17
A-1	23	22	23	21	20	—	—
A-2	—	—	—	—	—	19	16

*Values in parentheses represent diameters of bacteriostatic or fungistatic zones. Standards: A-1 = tetracycline; A-2 = nystatin; — = not tested.

ing to the National Committee for Clinical Laboratory Standards^{74g} was employed for the determination of antimicrobial activity of the compounds. Table 5 summarizes the antimicrobial properties of these azaphilones. Accordingly, moderate to strong activity was observed against all tested strains.

In a later study, a large number of representatives of *H. multifforme* and *H. cohaerens* had been analyzed by HPLC. It was found that the azaphilones appear to be present in particularly high concentrations in young, growing stromata, while they are not easily detected in fully mature and overmature specimens. Hence, the relatively high concentrations of these metabolites observed in the stromatal extracts (and their location in granules directly beneath the stromatal surface, see Fig. 11) suggest that these broad-spectrum antibiotics may act as a means of natural chemical defense against other microorganisms, or even other natural enemies such as insects and

nematodes that may attempt to feed on the growing stromata.^{59,75}

Nematicidal Activity of Isolated Metabolites from the Xylariaceae Family

The nematicidal activity of azaphilones, cytochalasins, and metabolites of other chemical types had been obtained in the past years during a chemotaxonomic survey of against *Caenorhabditis elegans* was evaluated and described in Table 6.⁵⁵ It clearly shows that all Xylariaceae metabolites tested exhibited much weaker bioactivities as compared to the standards. Several compounds were devoid of biological activities. Similar nematicidal activities were observed with phenochalasin B (73) and multiformin C (48). Daldinones A and B (60, 61) and

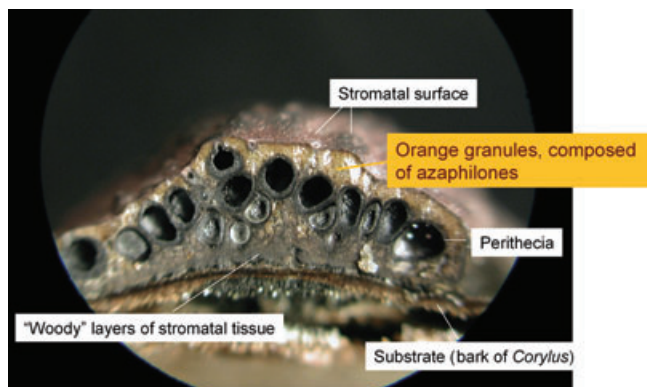


Fig. 11. Section through the stromata of *Hypoxylon fuscum*. Protection of the maturing stromata against enemies by deposit of large amounts of pigments (up to 5% of dry weight) in granula or waxy layers directly beneath the stromatal surface.

some further azaphilones from *C. sassafras* and *H. multifforme* also showed moderate effects.

Inhibition of NO Production in RAW 264.7 Cells by Azaphilones

We screened the inhibitory activity of 15 azaphilones, which were isolated from mushrooms belonging to the Xylariaceae family, and also elucidated the mechanism of inhibition of NO production stimulated by LPS in RAW 264.7 cells. The greatest inhibitors of NO production are rutilins A and B (**54**, **55**) consisting of the dimeric azaphilones with IC_{50} values of 1.76 and 1.80 μ M, respectively (Table 7).⁷⁶ The cytochalasins (**32**–**34**) exhibited rather high cytotoxic activity, and therefore, did not show any effect in this system. Their cytotoxicity was determined to be below the lowest test concentration employed (3 μ M). These results agree with those in several previous publications on cytochalasins in the literature, including that on the closely related cytochalasins and further congeners from *Daldinia*, where strong cytotoxicity had been reported.¹¹

Reverse transcription (RT)–PCR was used to examine how mRNA expression was affected by treatment with **35**. The results also showed a significant correlation with mRNA expression level as detected by electrophoresis on agarose gel (Fig. 12). Taking the results from RT–PCR and electrophoresis together, the inhibitory activity of the active azaphilone rubiginosin A on LPS-stimulated NO production in RAW cells is most likely due to a decrease in inducible NO synthase mRNA expression.⁷⁶

Chemosystematics of the Genus *Hypoxylon*

Based on isolated metabolites from *Hypoxylon* sp. as well as on other modern methods for classification, the genus *Hypoxylon*

Table 6. Nematicidal effects of xylariaceous metabolites.

Compound	LD ₉₀	LD ₅₀
Mitorubrinol (30)	>100	100
Mitorubrinic acid (31)	>100	>100
Fragiformin A (32)	>100	100
Fragiformin B (33)	>100	100
Cytochalasin H (34)	>100	100
Rubiginosin A (35)	50	25
Entonaemin A (37)	>100	100
Rubiginosin C (38)	50–100	25
Rubiginosic acid (39)	100	50
Daldinin C (40)	50	25
Daldinin E/F (41/42 ; 1:1 mixture)	100	50
Cohaerin A (44)	50	100
Cohaerin B (45)	50	100
Multiformin B (47)	50	10
Multiformin C (48)	25	10
Multiformin D (49)	>100	100
Sassafrin A (50)	100	50
Sassafrin B (51)	100	50
Sassafrin C (52)	50	25
Rutilin A (54)	>100	>100
Rutilin B (55)	>100	>100
Truncatone (59)	100	50
Daldinone A (60)	50	25
Daldinone B (61)	100	50
Daldiniapyrone (62)	100	10–25
Daldinialanone (63)	>100	>100
3,4,5-Trihydroxy-1-tetralone (64)	>100	>100
(+)-Orthosporin (65)	>100	>100
(22 <i>E</i>)-Cholesta-4,6,8(14), 22-tetraen-3-one (67)	100	100
Concentricol A (69)	>100	>100
Phenochalasin B (73)	25	10
Standard: Ivermectin	1–2.5	1

Note: The nematode suspension was diluted with phosphate-buffered saline (1000 nematodes/mL) and incubated with the test compounds. LD₅₀ and LD₉₀ were evaluated after 18 h, counting the percentages of motile and dead nematodes under a dissection microscope.

LD₉₀ = 90% lethal doses; LD₅₀ = 50% lethal doses.

is divided into two sections: section *Annulata* and section *Hypoxylon*. Within section *Hypoxylon*, the presence of four chemotypes now becomes evident (Fig. 13). *Hypoxylon macrocarpum* Pouz. contains BNT and macrocarpones belonging to chemotype 1.⁷⁸ While *H. fuscum* (chemotype 2) shows some affinities to *Daldinia childiae*, as revealed from the presence of daldinal A and daldinins (**40**–**42**) besides BNT (**43**). Species of the fourth chemotype, including, e.g., *H. fragiforme* (Pers.: Fr.) J. Kickx fil., lack BNT and contain mitorubrins instead. *H. rutilum* is probably placed in this group because it produced rubiginosins A and B, and entonaemin A (**35**–**37**).

Table 7. Inhibition of NO production by azaphilones.

Sample	IC ₅₀ (μM)
Mitorubrinol (30)	43.7
Mitorubrinic acid (31)	45.0
Rubiginosin A (35)	2.56
Rubiginosin B (36)	15.66
Entonaemin A (37)	14.24
Rubiginosin C (38)	39.16
Daldinin C (40)	55.66
Daldinin E (41)	43.68
Daldinin F (42)	30.62
Cohaerin A (44)	50.76
Cohaerin B (45)	54.55
Multiformin D (49)	13.81
Sassafrin A (50)	14.68
Sassafrin B (51)	15.66
Sassafrin C (52)	10.02
Rutilin A (54)	1.76
Rutilin B (55)	1.80
NG-methyl-L-arginine (standard)	88.4 ⁷⁷

IC₅₀ = 50% inhibitory concentration.

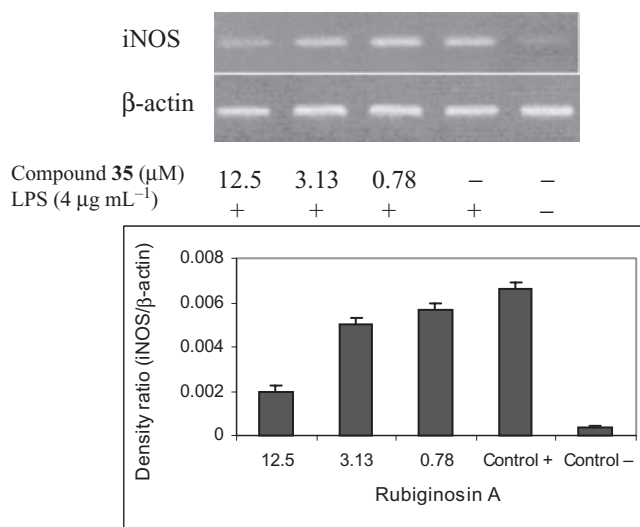


Fig. 12. Results of reverse transcriptase–polymerase chain reaction analysis of the inducible NO synthase (iNOS) mRNA level of **35**. LPS = lipopolysaccharide.

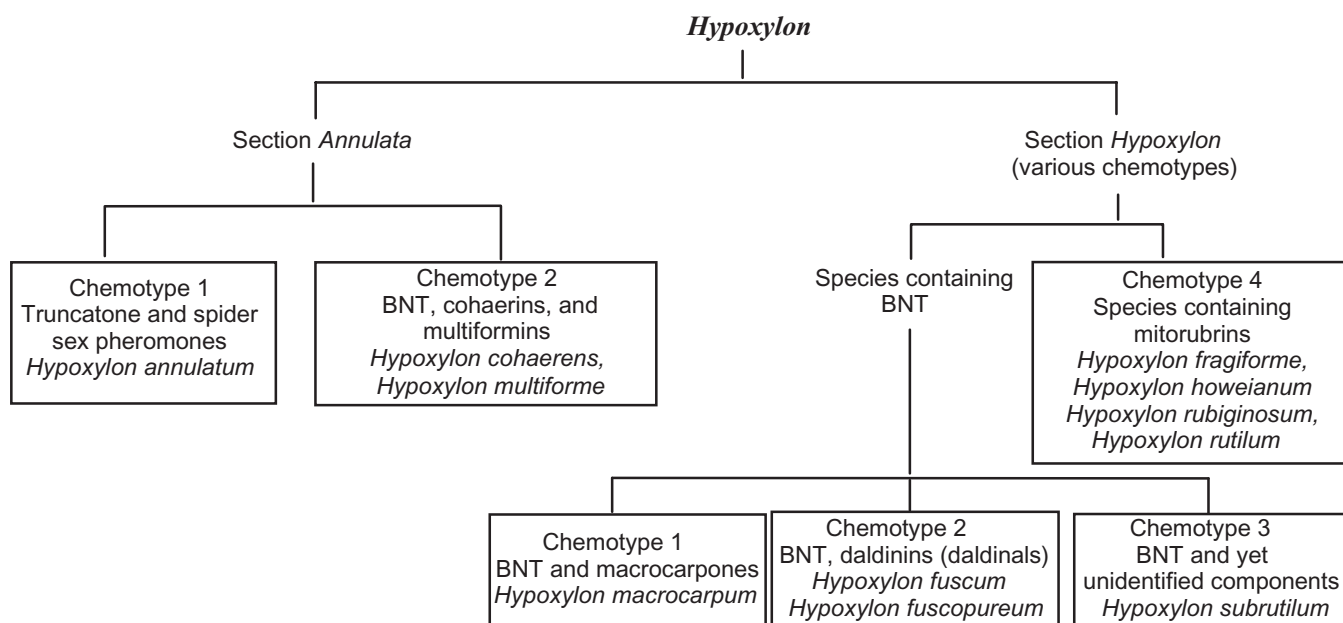
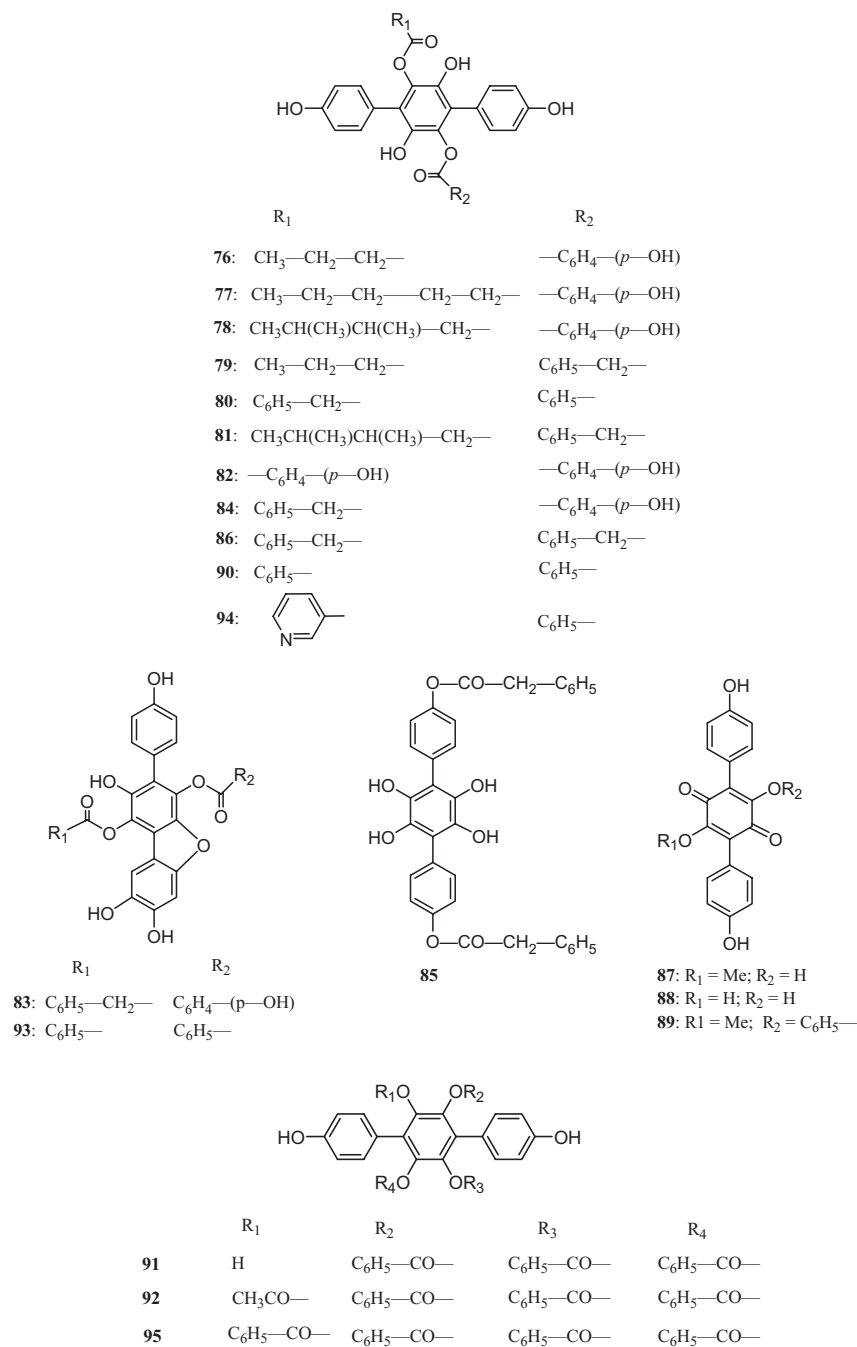


Fig. 13. Distribution of stromatal secondary metabolites in *Hypoxylon* sp. BNT = 4:5:4':5'-tetrahydroxy-1:1'-binaphthyl.

Species of section *Annulata* contain BNT (**43**) as a major component, in addition to yet unknown metabolites, and should be divided into two chemotypes (Fig. 13). *H. annulatum* is placed in chemotype 1 because it elaborated truncatone (**59**) and especially spider sex pheromones (**56–58**). Neither *Hypoxylon* nor *Daldinia* contains these pheromones, according

to the results of our investigation. Multiformins A–D (**46–49**) from *H. multiforme* also appear structurally related to the cohaerins A and B (**44, 45**), which we concurrently reported from *H. cohaerens*, a fungus that is known to be closely related to *H. multiforme*. Thus, they appear together in chemotype 2.


 Fig. 14. Structures of *p*-terphenyl compounds.

Antioxidative *p*-Terphenyls from Thelephoraceae and Paxillaceae Families

Mushrooms belonging to Thelephoraceae, which are widely distributed in East Asia, Australia, and America, are rich sources of biologically active compounds. *Thelephora auranti-*

otincta grows in symbiosis with pine trees. Previously, poly(phenylacetoxy)-substituted 1,1':4',1''-terphenyl derivatives named ganbajunins A—G and cycloleucomelone from *Thelephora ganbajunum*,^{79,80} and thelephorin A from *Thelephora vialis*⁸¹ were reported. Later, these *p*-terphenyl compounds exhibited a strong antioxidant activity.^{81–85}

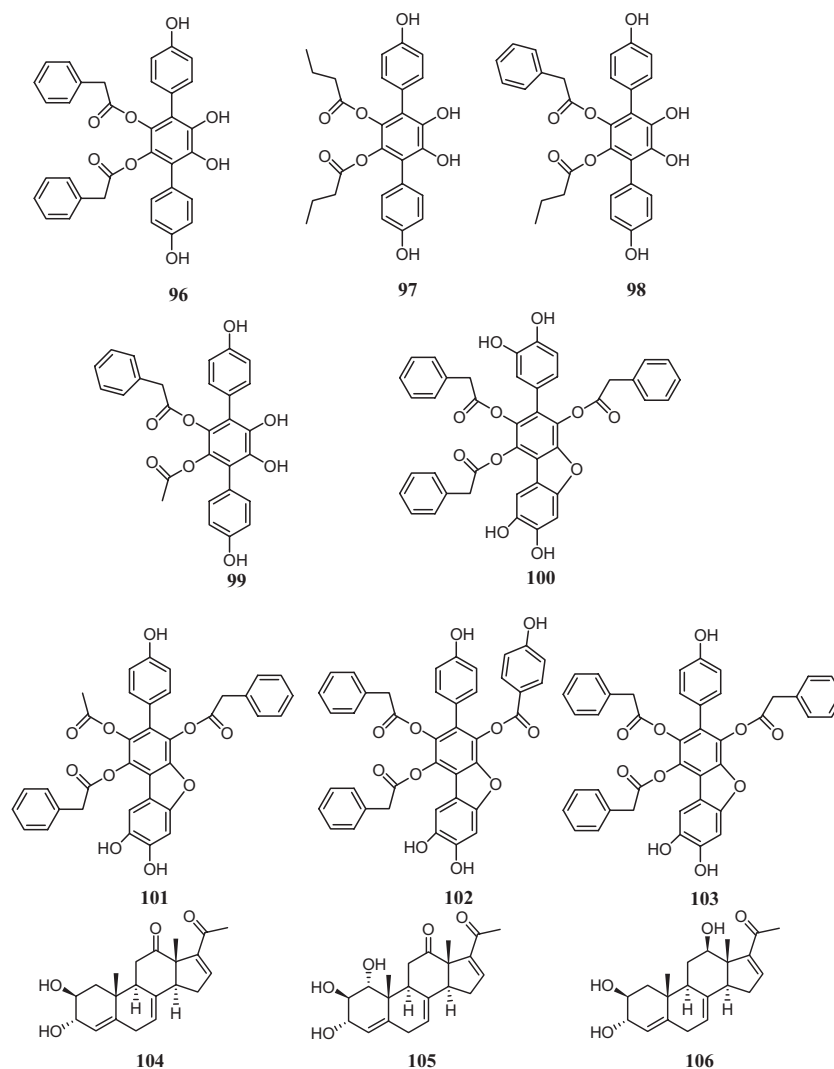


Fig. 15. Chemical constituents of Japanese *Thelephora terrestris*.

From Japanese *T. aurantiotincta*, thelephantins A—H (76–83), together with thelephorin A (84),⁸¹ ganbajunin C (85),⁷⁹ ganbajunin E (86), 2-*O*-methylatrometin (87),⁷⁹ atromentin (88), and *p*-hydroxybenzoic acid, were isolated and characterized from its methanolic extract (Fig. 14).^{84,85}

Similar to these compounds, thelephantins I—N (89–94),⁸⁶ together with dihydroaurantiacin dibenzoate (95),⁸⁷ were also isolated from Japanese *Hydnellum caeruleum*. Their structures were elucidated by spectroscopic methods and chemical reaction, which oxidized thelephorin A (84), a known compound, and thelephantin J (90) into their *para*-quinones and compared their UV and ¹³C NMR spectra.⁸⁶ All of the *p*-terphenyl compounds obtained from *T. aurantiotincta* and *H. caeruleum* possess two free hydroxyl groups at *para* position. In contrary, Japanese *Thelephora terrestris* elaborated

p-terphenyl derivatives with two substitution groups at *ortho* position to each other, terrestrins A—G (96–102), together with three known related compounds ganbajunin B (103),⁷⁹ thelephantin F (81), and thelephantin H (83).⁸⁸ Further fractionation of the methanolic extract of *T. terrestris* has led to the isolation and characterization of two unusual new pregnane-type steroids, 2 β ,3 α -dihydroxypregna-4,7,16-trien-12,20-dione (104) and 1 α ,2 β ,3 α -trihydroxypregna-4,7,16-trien-12,20-dione (105), which are named terresterones A and B (104, 105), as well as the previously known compound stizophyllin,⁹⁰ now assigned as 2 β ,3 α ,12 β -trihydroxypregna-4,7,16-trien-20-one (106) (Fig. 15).⁸⁹

Free radical-scavenging activities of isolated compounds from *T. aurantiotincta* were evaluated against the stable free radical DPPH.⁵⁸ Their antioxidant activities were defined as

the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% [IC₅₀ (μM)] as described in Table 8 and compared with those of known antioxidants, l-ascorbic acid, α-tocopherol, and BHA.

According to Table 8, *p*-terphenyl possessing *para*-dihydroxyl groups at the central aromatic ring showed stronger

antioxidative activities than those compounds with four substitution groups as **92** and **95**. Thus, the number of hydroxyl groups in these tested compounds is a very important factor to increase their activity.

Fungi of the genus *Paxillus* belonging to Paxillaceae grow widely in East Asia and North America on decayed pine trees.³³ Previously, leucometin-2, -4, -5, and -6 were reported from *Paxillus panuoides*,^{91,92} and the same authors later isolated curtisians A—D from *Paxillus curtisii*,⁹³ all of them were reported as new free radical scavengers.^{91–93} Meanwhile, Japanese *P. curtisii* produced *p*-terphenyl compounds, curtisians E—Q (**107–119**) with four substitution groups at the central aromatic ring (Fig. 16). Their structures were determined by a combination of 2-D NMR, especially rotating-frame Overhauser effect spectroscopy spectra and chemical reaction.

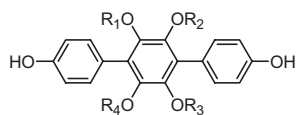
To determine the absolute configuration of curtisians E—H (**107–110**) and other curtisians, a mixture of curtisians E—H and also a crude methanol extract of *P. curtisii* were saponified with potassium hydroxide in methanol, followed by methylation and acetylation to afford 3-acetoxy-*n*-butyric acid methyl ester. This compound was analyzed by GC—MS on chiral column with authentic samples (each 3*R*- and 3*S*-acetoxy-*n*-butyric acid methyl ester) derived from 3*R*- and 3*S*-hydroxy-*n*-butyric acid. Consequently, the absolute configuration at C_{3a–3d} of the side chain of curtisians was established to be *S*.^{94–96}

Some of these curtisians (**111–119**) were later examined for their antioxidant activities by using the DPPH method, and the results are shown in Table 9. They showed moderate to strong activities as compared with the standards.

Table 8. Antioxidant activity of thelephantins and as compared with authentic samples.

Sample	IC ₅₀ (μM)
L-Ascorbic acid	16.5
<i>tert</i> -Butylhydroxyanisole	28.0
DL-α-Tocopherol	21.7
Thelephantin A (76)	12.1
Thelephantin B (77)	14.2
Thelephantin C (78)	7.6
Thelephantin D (79)	53.4
Thelephantin E (80)	20.0
Thelephantin F (81)	52.2
Thelephantin G (82)	14.5
Thelephantin H (83)	14.2
Thelephantin I (89)	319.6
Thelephantin J (90)	49.6
Thelephantin K (91)	124.7
Thelephantin L (92)	No effect
Thelephantin M (93)	46.9
Thelephantin N (94)	56.1
Dihydroaurantiacin dibenzoate (95)	No effect

IC₅₀ = 50% inhibitory concentration.



	R ₁	R ₂	R ₃	R ₄
107	C ₆ H ₅ CH ₂ CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—	CH ₃ CO—	CH ₃ CH(OH)CH ₂ CO—
108	CH ₃ CH(OAc)CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—	CH ₃ CH(OAc)CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—
109	C ₆ H ₅ CH ₂ CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—
110	C ₆ H ₅ CH ₂ CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—	CH ₃ CH(OH)CH ₂ CO—	CH ₃ CH(OAc)CH ₂ CO—
111	CH ₃ CH(OAc)CH ₂ CO—	H	CH ₃ CH(OH)CH ₂ CO—	H
112	CH ₃ CH(OAc)CH ₂ CO—	H	C ₆ H ₅ CH ₂ CH ₂ CO—	H
113	CH ₃ CH(OH)CH ₂ CO—	H	C ₆ H ₅ CH ₂ CH ₂ CO—	H
114	CH ₃ CH(OH)CH ₂ CO—	H	CH ₃ CO—	H
115	CH ₃ CH(OAc)CH ₂ CO—	H	CH ₃ CH(OAc)CH ₂ CO—	H
116	CH ₃ CH(OAc)CH ₂ CO—	H	CH ₃ CO—	H
117	CH ₃ CH ₂ CH ₂ CO—	H	CH ₃ CO—	H
118	C ₆ H ₅ CH ₂ CH ₂ CO—	H	CH ₃ CO—	H
119	C ₆ H ₅ CH ₂ CH ₂ CO—	H	C ₆ H ₅ CO—	H

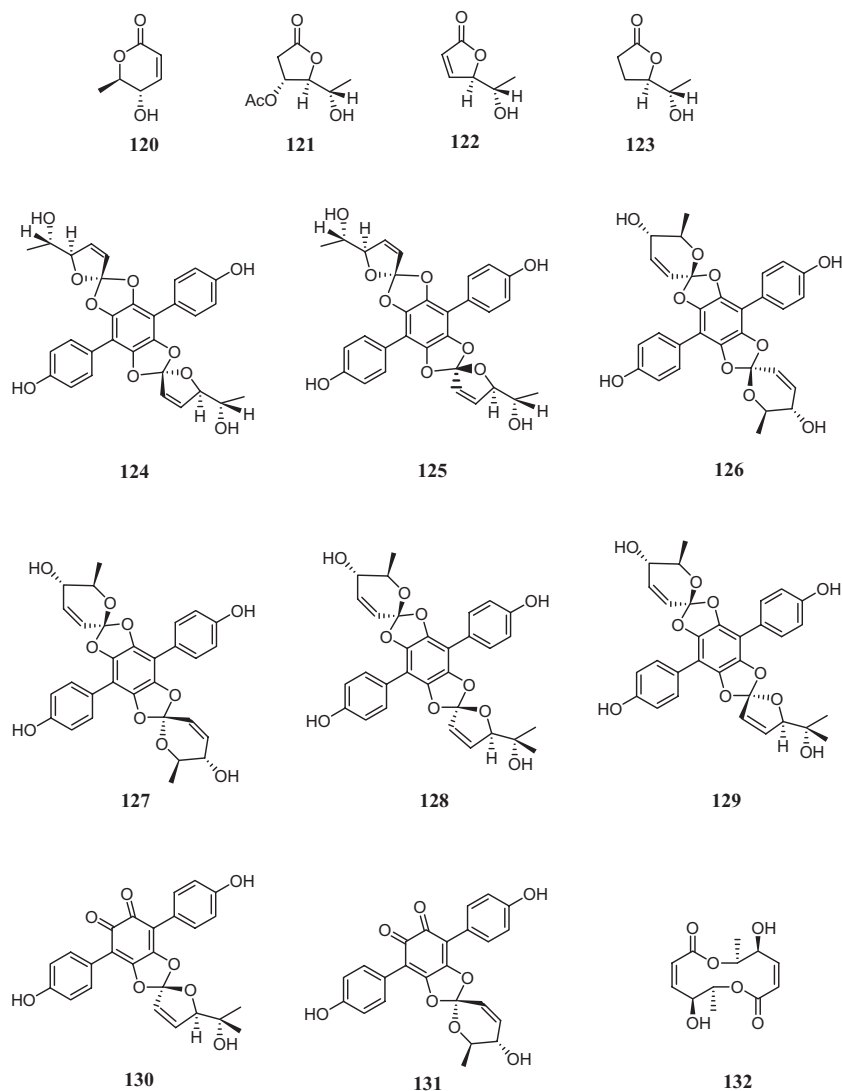
Fig. 16. *p*-Terphenyls from Japanese *Paxillus curtisii*.

Table 9. Free 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity of curtisians I—Q (111–119).

Sample curtisian	IC ₅₀ (μM)
I (111)	19.1
L (112)	24.0
K (113)	31.3
J (114)	117.8
M (115)	45.9
N (116)	48.8
O (117)	58.7
P (118)	44.0
Q (119)	43.4

IC₅₀ = 50% inhibitory concentration.

One more species belonging to this family is *Paxillus atrotomentosus*, an inedible mushroom with a large cap and which frequently appears on decayed pine trees. Previously, Holzapfel et al. and Besl et al. reported the isolation and structural characterization of leucomentin, flavomentin, and spiromentin derivatives from this fungus collected in Europe.^{3,97–98} Later, some antitumor components of the cultured mycelia of Korean *P. atrotomentosus*⁹⁹ and ergostane-type ecdysteroids from European *P. atrotomentosus* were purified.¹⁰⁰ Meanwhile, we investigated the chemical constituents of Japanese *P. atrotomentosus* and a new δ-lactone, osumundalactone (**120**), γ-lactone (**121**) along with two known γ-lactone (**122**, **123**), and six spiromentins E—J (**124–129**), together with spiromentins B and C (**130–131**)⁹⁷ and a novel dimeric lactone *bis*-osmundalactone (**132**) were isolated (Fig. 17).^{101,102} It is noteworthy that

**Fig. 17.** Isolated metabolites from Japanese *Paxillus atrotomentosus*.

neither leucomentin nor flavomentin, which were isolated from the European *P. atrotomentosus*, was detected in the Japanese species.

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