BIOLOGICALLY ACTIVE SUBSTANCES OF JAPANESE INEDIBLE MUSHROOMS[§]

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Abstract - As a part of our systematic investigation of biologically active substances of inedible mushrooms, we studied the chemical constituents of three Basidiomycetes fungi (*Cryptoporus volvatus*, *Lentinellus ursinus* and P*axillus atrotomentosus*), five inedible Ascomycetes fungi (*Daldinia concentrica*, *Daldinia vernicosa*, *Entonaema splendens*, *Hypoxylon truncatum* and *Ascoclavulina sakaii*), and isolated a number of novel terpenoids, phenolics and nitrogen-containing compounds. The isolation, structural determination and biological activity of the new compounds will be discussed.

1. Introduction

About 1500 species of mushrooms are now known in Japan. Edible and inedible mushrooms are about 300 and 1200 species, including 50 toxic species.[1] A lot of inedible mushrooms show bitter and pungent tastes and specially those belonging to Polyporaceae have been used as medicinal drugs (anti-cancer *etc.*) in China from ancient times.[2] The use of natural products isolated from mushrooms against infection and cancer deseases is one of the cornerstones of modern medicine.[3] Little attention has been paid to the chemical constituents of inedible mushrooms in Japan, except for the poisonous species. We have been interested in the biologically active substances present in inedible mushroom without toxicity and have studied the chemistry and pharmacology as well

[§]Dedicated to the memory of Professor Koji Nakanishi

as their application as a sourse of medicinal or agricultural drugs.

Recently, we found that an inedible mushroom, *Cryptoporus volvatus* (Polyporaceae) contained a large amount of novel bitter drimane sesquiterpenoids, cryptoporic acids (CPAs) A～G (**1**～**7**) which showed strong inhibition of superoxide anion radical release.[4-7] The isolation, structural characterization, and biological activity of several unique terpenoids, aromatic compounds, and nitrogen-containing compounds from three inedible Basidiomycetes fungi (*Cryptoporus volvatus*, *Lentinellus ursinus* and *Paxillus atrotomentosus*), five inedible Ascomycetes fungi (*Daldinia concentrica, Daldinia vernicosa*, *Entonaema splendens*, *Hypoxylon truncatum* and *Ascoclavulina sakaii*) are discussed in this review.

2. Biologically Active Cryptoporic Acids from a Fungus *Cryptoporus volvatus*

The fungus *Cryptoporus volvatus* grows on decayed pine trees, and specially after a forest fire. Its fruit body emits a resinous odor which attracts adults insect such as *Parabolitophagus felix* and *Ischnodactylus loripes*. In China, this fruit body (Chinese name; 隐孔菌) has been used as the ablactation (weaning) for infants because of powerfully bitter taste, and as bronchodilating drug. Previouly, ergosterol[8] and an atitumor-active protein-polysaccharide,[9] and volatile components including 1-(*E*, *Z*)-3, 5- and 1-(*E*, *E*)-3, 5-undecatrienes and a few mono- and sesquiterpenoids[10] have been found in the fruit body of *C. volvatus*. We were also aware that *C. volvatus* contained a large amount of bitter sesquiterpenoids, and subsequently isolated and identified cryptoporic acids (CPAs) A～G (**1**～**7**).

2-1. Isolation of Cryptoporic Acids A～G

Fresh *C. volvatus* (1.16 Kg), collected in Tokushima was macerated and extracted with ethyl acetate (3 L) overnight. The ethyl acetate extract (75.6 g) was subjeted repeatedly to column chromatography of Sephadex LH-20 ($CHCl_3$-MeOH=1:1) and of silica gel ($CHCl_3$-MeOH gradient) to afford ergosterol (2.351 g), cryptoporic acid A (CPA-A, **1**, 3.291 g), CPA-B (**2**, 1.772 g), CPA-C (**3**, 5.063 g), CPA-D (**4**, 5.692 g), CPA-E (**5**, 5.950 g), CPA-F (**6**, 0.875 g), and CPA-G (**7**, 2.018 g).

2-2. Structue Elucidation of Cryptoporic Acids A ~ G

The spectral data of cryptoporic acid A (CPA-A, **1**), $C_{23}H_{36}O_7$ indicated the presence of carboxyl (3450-2500 and 1710 cm^{-1}; δ_c 177.6) and two methoxycarbonyl (1740 and 1730 cm^{-1}; δ_H 3.68 and 3.75) groups. ^{1}H-^{1}H COSY spectrum of **1** suggested the presence of the partial structures A and B as shown in Figure 1. The ^{1}H and ^{13}C NMR spectra of the sesquiterpene part of **1** are very similar to those of a known drimane sesquiterpene, albicanol (**8**) isolated from the liverworts *Diplophyllum albicans*[11] and *Bazzania* species[12] as shown in Figure 2. This indicated that **1** might be composed of albicanol linked through the primary alcohol to the dimethyl ester of isocitric acid (partial structure B).

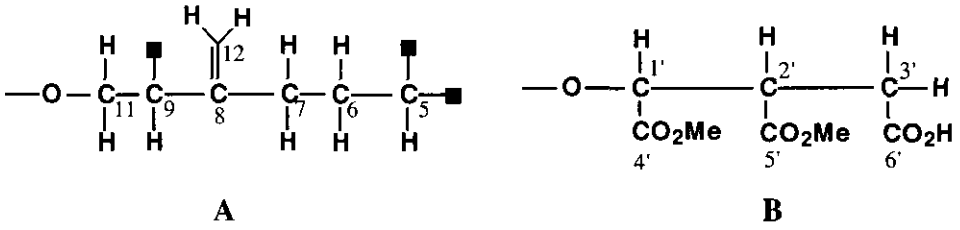
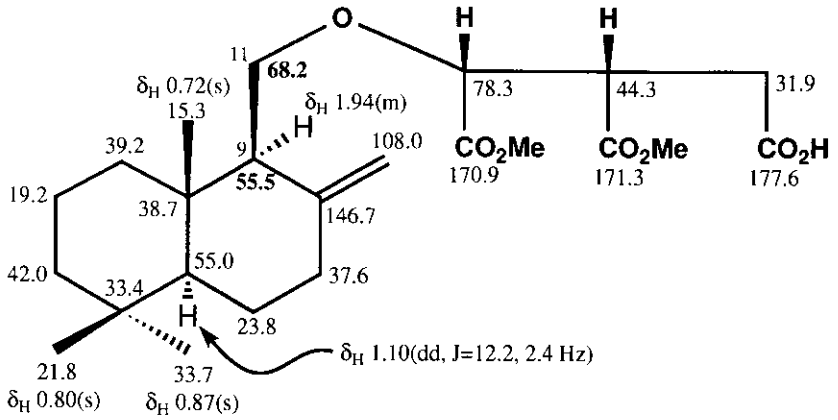


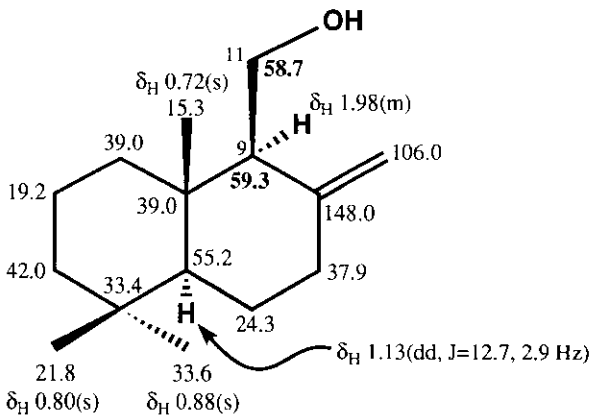
Figure 1. Partial structures (A and B) of cryptoporic acid A (**1**)

The positions of ether linkage between C-11 and C-1' and the carboxylic acid group at C-3' of isocitric acid dimethyl ester and the stereostructure of CPA-A (**1**) were confirmed by the analysis of the long-range ^{13}C-^{1}H 2D-COSY NMR and NOE difference spectra of CPA-A (**1**) as shown in Figure 3.

The absolute configuration of the sesquiterpene part was determined as the same as that of albicanol, because the ketone (**10**) obtained by ozonolysis of trimethyl ester (CPA-A Me_3, **9**) of **1** showed the the negative Cotton effect at 274 nm ([θ] -2933) as that of ketone (**11**)[12] derived from ozonolysis of **8** as shown in Scheme 1. The absolute configuration of isocitric acid dimethyl ester part was solved as follows. Kaneko *et al.*[13] had previouly reported that the (+)-tetraacetate (**13'**) {$[\alpha]_D$+13.6°(acetone)} was obtained from the (-)-isocitric acid (**14**) *via* reduction of (+)-isocitric acid lactone (**15**) by $LiAlH_4$ and subsequent acetylation as shown in scheme 2. The triacetate (**12**) obtained from **9** by lithium aluminium hydride ($LiAlH_4$) reduction and acetylation



CPA-A (1)



albicanol (8)

Figure 2. ^{1}H and ^{13}C NMR spectra of CPA-A (**1**) and albicanol (**8**)

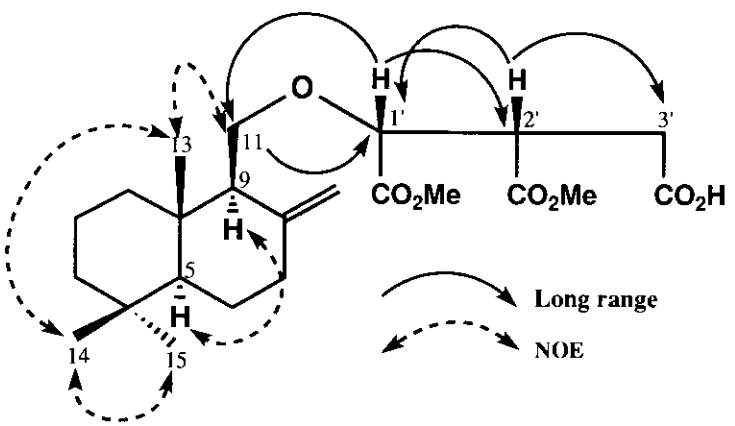
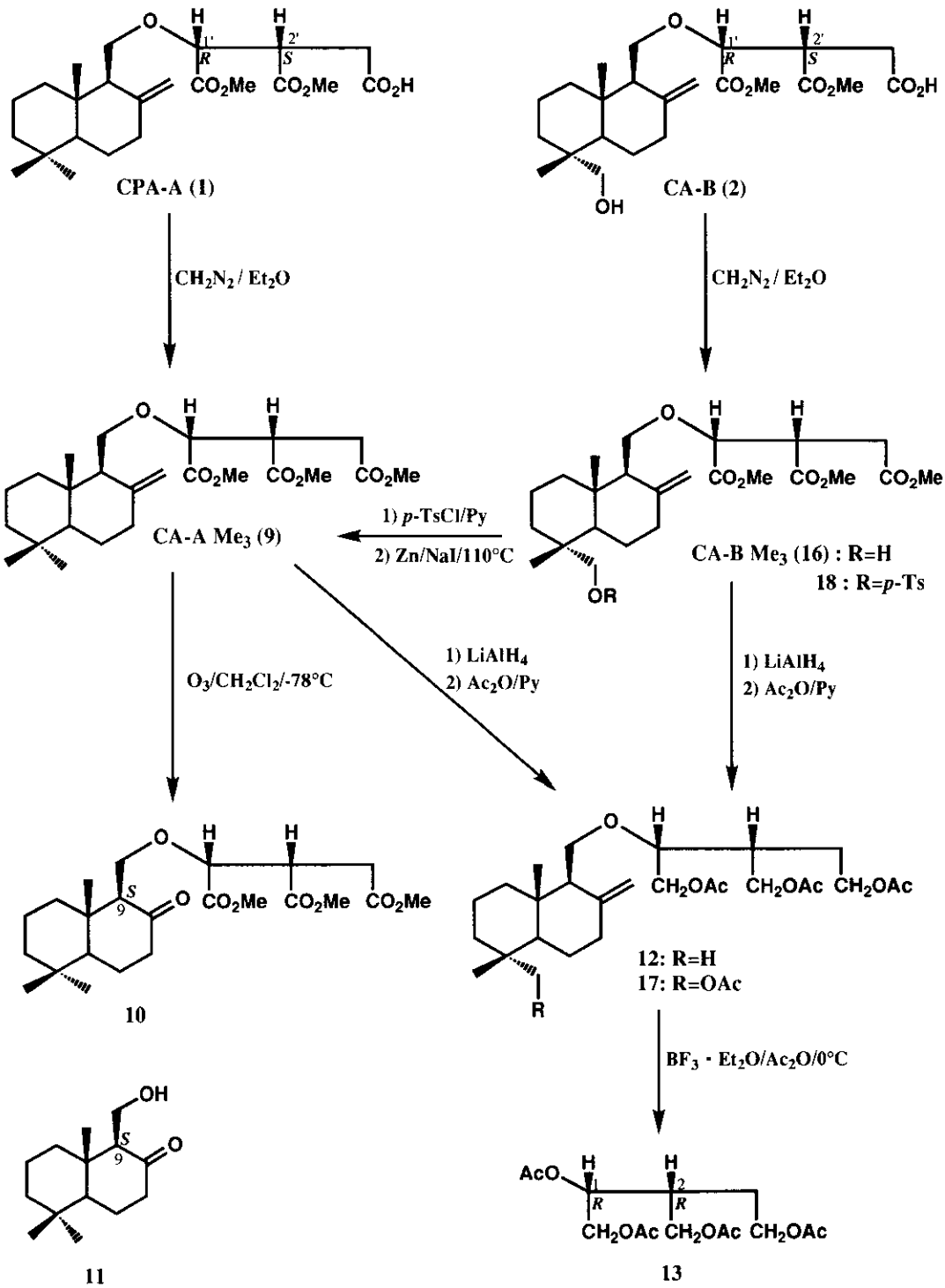
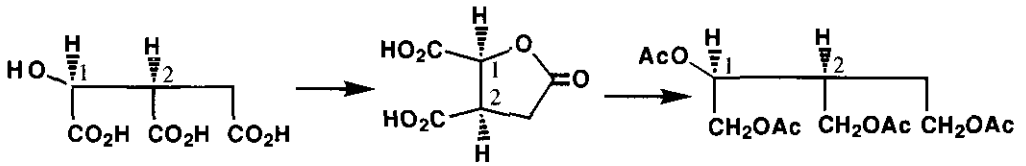


Figure 3. Long range ^{13}C-^{1}H correlation and NOE difference spectra of CPA-A (**1**)



Scheme 1. Chemical transformation of cryptoporic acids A (1) and B (2)

(Ac_2O, Py) was treated with $BF_3 \cdot Et_2O$ and then with Ac_2O to give (-)-tetraacetate (**13**) {$[\alpha]_D$ -12.9° (acetone)}. The spectral data (IR, ^1H and ^{13}C NMR) of compound (**13**) were identical with those of the (±)-tetraacetate (**13''**) prepared from the commercially available (±)-isocitric acid lactone (**15'**). Thus, the absolute configuration at C-1' and C-2' of CPA-A (**1**) were determined as *R* and *S*, respectively.



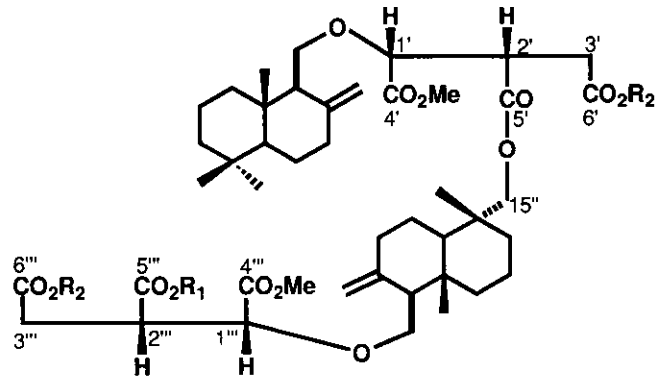
14 (1*S*, 2*R*) **15** (1*S*, 2*R*) **13'** (1*S*, 2*R*)

15' (1*S*, 2*R*+1*R*, 2*S*) **13''** (1*S*, 2*R*+1*R*, 2*S*)

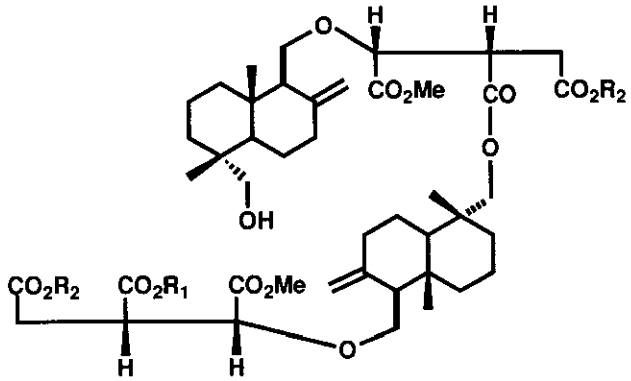
Scheme 2. Chemical transformation of (-)-isocitric acid (**14**)

The ^1H and ^{13}C NMR spectra of cryptoporic acid B (CPA-B) (**2**), $C_{23}H_{36}O_8$ were quite similar to those of CPA-A (**1**) except for the NMR signals of the 15-positions [**2**: δ_H 3.04 and 3.39 (2H, d, J=11 Hz), δ_C 71.3 (t); **1**: δ_H 0.87 (3H, s), δ_C 33.7 (q)], suggesting that **2** was 15-hydroxylated compound of **1**. Reduction ($LiAlH_4$) of the methyl ester (CPA-B Me_3, **16**) followed by acetylation (Ac_2O, Py) gave the tetraacetate (**17**). The absolute stereochemistry of **2** was the same as that of **1**, because the tosylate (**18**) prepared from CPA-B Me_3 (**16**) with *p*-tosyl chloride was converted to CPA-A Me_3 (**9**) by reduction with NaI-Zn as shown in Scheme 1.[14]

The spectral data of cryptoporic acid C (CPA-C, **3**), $C_{45}H_{68}O_{14}$ indicated the presence of carboxyl (3450-2400 and 1715 cm^{-1}; δ_c 177.6) and three methoxycarbonyl (1740 and 1730 cm^{-1}; δ_H 3.69, 3.76 and 3.77) groups. Reduction ($LiAlH_4$) of pentamethyl ester (CA-C Me_5; **19**) followed by acetylation afforded triacetate (**12**) and tetraacetate (**17**) which were identical with the compounds derived from CPA-A (**1**) and CPA-B (**2**). Thus the gross structure of **3** was deduced as depicted. The positions of the two carboxyl groups and the ester linkage between the two sesquiterpene units of CPA-C (**3**) were determined to be 3' and 3''', and 5', respectively by the long-range ^{13}C-^1H correlation spectrum.



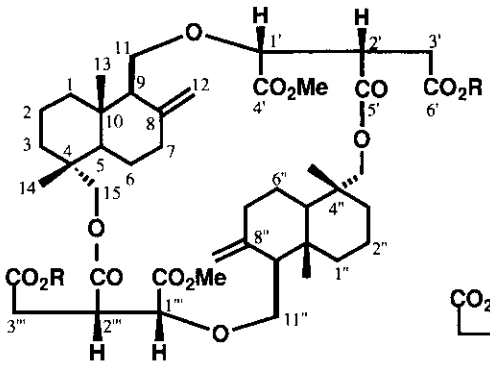
CPA-C (3) : R_1=Me; R_2=H
CPA-F (6) : R_1=R_2=H
CPA-C Me_5 (19) : R_1=R_2=Me



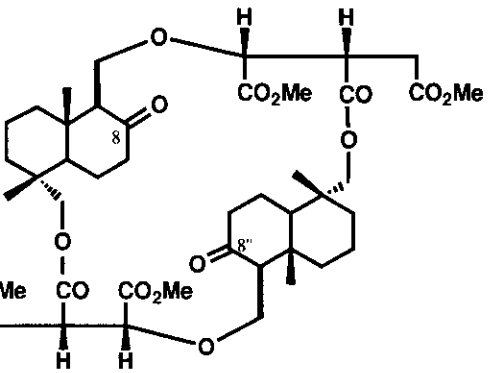
CPA-E (5) : R_1=Me; R_2=H
CPA-G (7) : R_1=R_2=H
CPA-E Me_5 (22) : R_1=R_2=Me

As the ^1H and ^{13}C NMR spectra of cryptoporic acid D (CPA-D) (**4**), $C_{44}H_{64}O_{14}$ was simple and only half of the carbon signals appeared, **4** was deduced to be a symmetric dimer. The tetramethyl ester (CPA-D Me_4, **20**) was converted into the only tetraacetate (**17**) by reduction with $LiAlH_4$, followed by acetylation with Ac_2O and Py. X-Ray crystallographic analysis (Figure 4) of diketone (**21**) [ozonolysis of **20** followed by Zn/AcOH] was carried out to establish the stereochemistry (C_2 Synmetry). Recently, X-Ray crystallographic analysis of CPA-D (**4**) was done as shown in Figure 5. The absolute configuration of CPA-D (**4**) was determined with the CD spectrum of **21**, which showed a negative Cotton effect at 276 nm ([θ] -1876).

The ^1H and ^{13}C NMR spectra of cryptoporic acid E (CPA-E) (**5**), $C_{45}H_{68}O_{15}$ suggested that this



CPA-D (4) : R=H
CPA-D Me_4 (20) : R=Me



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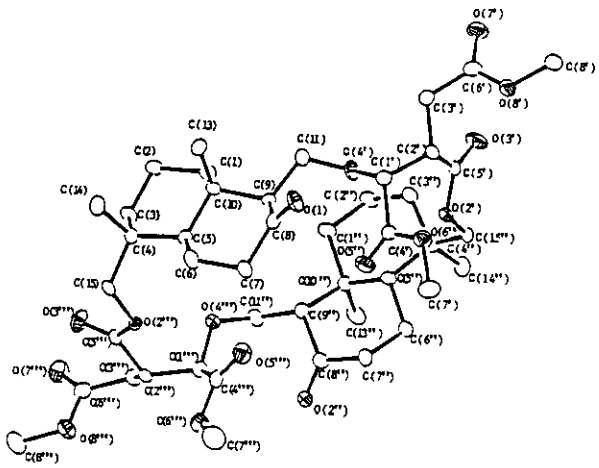


Figure 4. ORTEP drawing of molecular structure of compound (**21**)

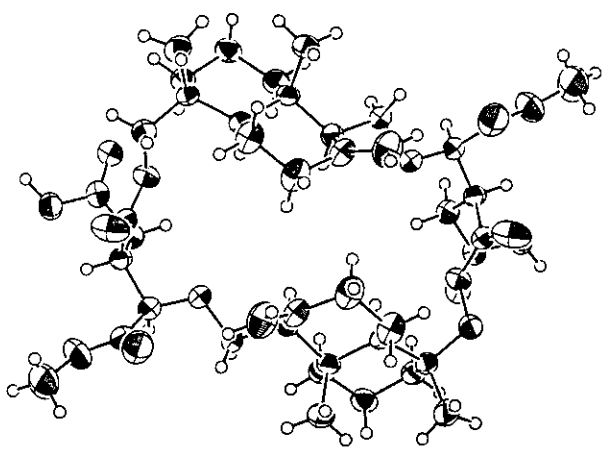


Figure 5. ORTEP drawing of molecular structure of CPA-D (**4**)

compound was ansymmetrical dimer of CPA-B (**2**). Pentamethyl ester (CPA-E Me_5, **22**) was treated in a similar manner as described above to give a tetraacetate (**17**). The long-range ^{13}C-^{1}H correlation spectrum of **5** revealed the position of the carboxyl and the ester groups as depicted.

Cryptoporic acid F (CPA-F) (**6**), $C_{44}H_{66}O_{14}$ was converted to CPA-C Me_5 (**19**) by methylation with diazomethane, suggesting that CPA-F might be a mono-demethyl derivative of CPA-C (**3**). The positions of three carboxylic acid groups were further established to be at C-3', C-2''' and C-3''', by analyses of the long range ^{13}C-^{1}H 2D spectrum of the tri-*p*-bromophenacyl ester (**23**) obtained by reaction with *p*-bromophenacyl bromide.

Cryptoporic acid G (CPA-G) (**7**), $C_{44}H_{66}O_{15}$ was converted to CPA-E Me_5 (**22**) by methylation with diazomethane, suggesting that CPA-G might be a monomethyl derivative of CPA-E. The position of three carboxylic acid groups were further established to be at C-3', C-2''' and C-3''', like CPA-F by analyses of the long range ^{13}C-^{1}H 2D spectrum of the tri-*p*-bromophenacyl ester (**24**).

2-3. Biological Activity of Cryptoporic Acids A-E[7]

Cryptoporic acids (CPAs) showed intensely bitter taste and completely inhibited elongation of the second coleoptile and germination of rice in husk at a concentration of 200 ppm.

Superoxide anion radical (O_2^{-} •), which is produced by inflammation and injury due to radiation, etc., is now recognized to play an important role in hepatic, cardiac, renal, and pancreatic insufficiency and injury to other organs.[15] Inhibitors of superoxide anion radical release and radical scavengers are necessary to prevent human diseases caused by ischemia and inflammation.[16] CPAs strongly inhinbited the release of superoxide anion radical from guinea pig peritoneal macrophages, induced by O_2^{-} • stimulant FMLP (formyl methionyl leucyl phenylalanine: 10^{-7} M), at concentrations from 0.05 to 25 μg/mL (IC_{50}), as shown in Table 1. The inhibitory activity of CPAs-C~G with dimeric structures appeared to be about 100 to 500 times as potent as CPAs-A and B with monomeric structures. CPA-C also inhibited the release of O_2^{-} • from rabbit polymorphonuclear leukocytes, induced by O_2^{-} • stimulant FMLP (10^{-7} M), at a concentration of 2 μg/mL (IC_{50}), as shown in Table 2.

Table 1. Inhibitory Effects of CPAs A~G on Superoxide Anion Radical Release from Gunia-Pig Peritoneal Macrophages Induced by $O_2^{-}\cdot$ Stimulant FMLP[a]

Experimental Samples	IC_{50} (μg / mL)
CPA-A	13.0
CPA-B	25.0
CPA-C	0.07
CPA-D	0.10
CPA-E	0.05
CPA-F	0.30
CPA-D	0.15

a. FMLP=FormylMethionyl Leucyl Phenylalanine concentration= 10^{-7} M

Table 2. Inhibitory Effect of Cryptoporic Acid C on Superoxide Anion Radical Release from Rabbit Polymorphonuclear Leukocytes Induced by $O_2^{-}\cdot$ Stimulant FMLP

Experimental Samples	$O_2^{-}\cdot$ release inhibition (%)
Cryptoporic acid C	6 μg / mL 88
	3 μg / mL 70
	1.5 μg / mL 20
	IC_{50} : 2.0 μg / mL

CPA-E inhibited the tumor-promotion activity of okadaic acid, in two stage carcinogenesis experiments[17] on mouse skin at a concentration of 1.2 to 5.9 μM, while CPA-D slightly enhanced tumor as shown in Figure 6. CPA-D activated protein kinase C and other protein kinases at concentrations of up to 100 μM.[18]

The antitumorigenic effect of CPA-E on colon carcinogenesis was investigated as shown in Table 3. The incidence and the number of tumors per animal were reduced in CPA-E-fed animals compared to the controls: 31% vs 75% in female rats, and 31% vs 63 % in female mice, respectivelly. Intrarectal deoxycholic acid-induced colonic mucosal ornithine decarboxylase activity was significantly lowered in CPA-E-fed animals compared to controls. This shows an antipromoting

activity of CPA-E against colon carcinogenesis. Thus, it was concluded that CPA-E inhibits colon cancer development in both rats and mice treated with 2 different colon carcinogens.[19]

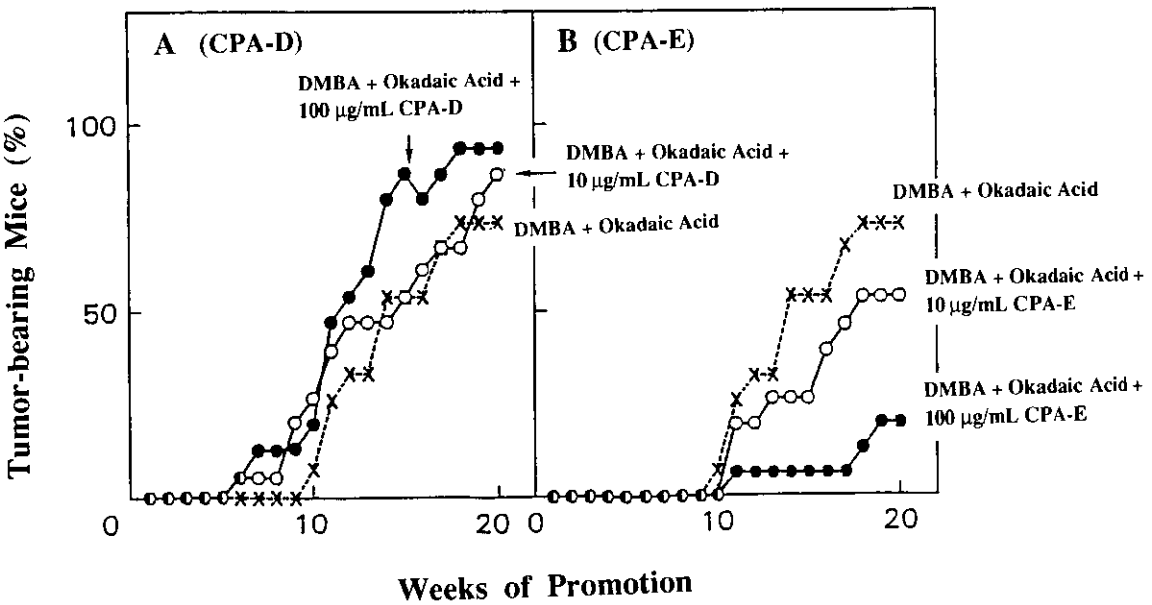


Figure 6. Anti-tumor promotion activities of cryptoporic acid D (CPA-D)(**4**)(A) and E (CPA-E)(**5**)(B) on the percentage of tumor-bearing mice of the group treated with 7,12-dimethylbenz[*a*]anthracene (DMBA; cancer initiator) plus okadaic acid (cancer promoter).

Table 3. Effect of Cryptoporic Acid E (CPA-E) on Colon Tumor

Experimental Groups	Female F344 rats[a]	Colon tumor Incidences
Control	CE-2	12/16 (75%)
Cryptoporic acid E (CPA-E)	CE-2 + 0.2% CPA-E	5/16 (31%)

Experimental Groups	female ICR mice[b]	Colon tumor Incidences
Control	CE-2	10/16 (63%)
CPA-E	CE-2 + 0.06% CPA-E	5/16 (31%)

a. All rats received an intrarectal dose of 2 mg of methylnitrosourea 3 times weekly for week1-2. Rats in Group CA-E started to have CA-E diet at week 3, and experiment was terminated at week 35.

b. All mice received a subcutaneous injection of 20 mg of dimethylhydrazine · 2HCl/Kg body weight once weekly 1-15. Mice in group CA-E started to have CA-E diet at week 1, and experiment was terminated at week 25.

The cytotoxicity of CPAs-A~G and their methyl esters was investigated as shown in Table 4. The methyl ester (CPA-A Me_3) inhibited KB, KB-V in the presence of vinblastine (VLB), KB-V in the absence of VLB and ZR-75-1 cells at a concentration of ED_{50} 2.3, 2.6, 3.4 and 12.2 μg/mL, respectively. The methyl ester (CPA-B Me_3) inhibited KB-V cell in the presence of VLB at a concentration of ED_{50} 3.8 μg/mL . CPAs-C and -D inhibited ZR-75-1 cell at a concentration of ED_{50} 12.9 and 14.5 μg/mL, respectively. The methyl ester (CPA-C Me_5) inhibited KB, LNCap and ZR-75-1 cells at a concentration of ED_{50} 14.2, 15.7 and 13.0 μg/mL, respectively. The other CPAs and methyl esters were inactive on the cytotoxicity testing.

Table 4. Cytotoxicity Testing (ED_{50}: μg/mL) of Cryptoporic Acids A~G and Their Derivatives on KB, KB-V, Lu1, LNCaP and ZR-75-1 Cell Lines

Samples	KB	KB-V(+VLB)[a]	KB-V(-VLB)[b]	Lu1	LNCap	ZR-75-1
CPA-A	>20	>20	>20	>20	>20	>20
CPA-A Me_3	2.3	2.6	3.4	>20	>20	12.2
CPA-B	>20	>20	>20	>20	>20	>20
CPA-B Me_3	>20	3.8	>20	>20	>20	>20
CPA-C	>20	>20	>20	>20	>20	12.9
CPA-C Me_5	14.2	3.8	>20	>20	15.7	13.0
CPA-D	>20	>20	>20	>20	>20	14.5
CPA-D Me_4	>20	>20	>20	>20	>20	>20
CPA-E	>20	>20	>20	>20	>20	>20
CPA-E Me_5	>20	>20	>20	>20	>20	>20
CPA-F	>20	>20	>20	>20	>20	>20
CPA-G	>20	>20	>20	>20	>20	>20

a. +VLB= The presence of vinblastine
b. -VLB= The absence of vinblastine

The anti-HIV-I (Human Immunodeficiency Virus-I) assay of CPA-A~G and their methyl esters was investigated as shown in Table 5. CPA-C and CPA-F showed 99.6% (IC_{50} 61.0 μg/mL) and 99.7 % (IC_{50} 42.2 μg/mL) inhibition of HIV-1 at a concentration of 200 μg/mL. The other CPAs and methyl esters were inactive on the anti-HIV-1 assay.

Table 5. Inhibitory Effects of Cryptoporic Acids A~G and Their Derivatives on HIV-1 RT (p66)

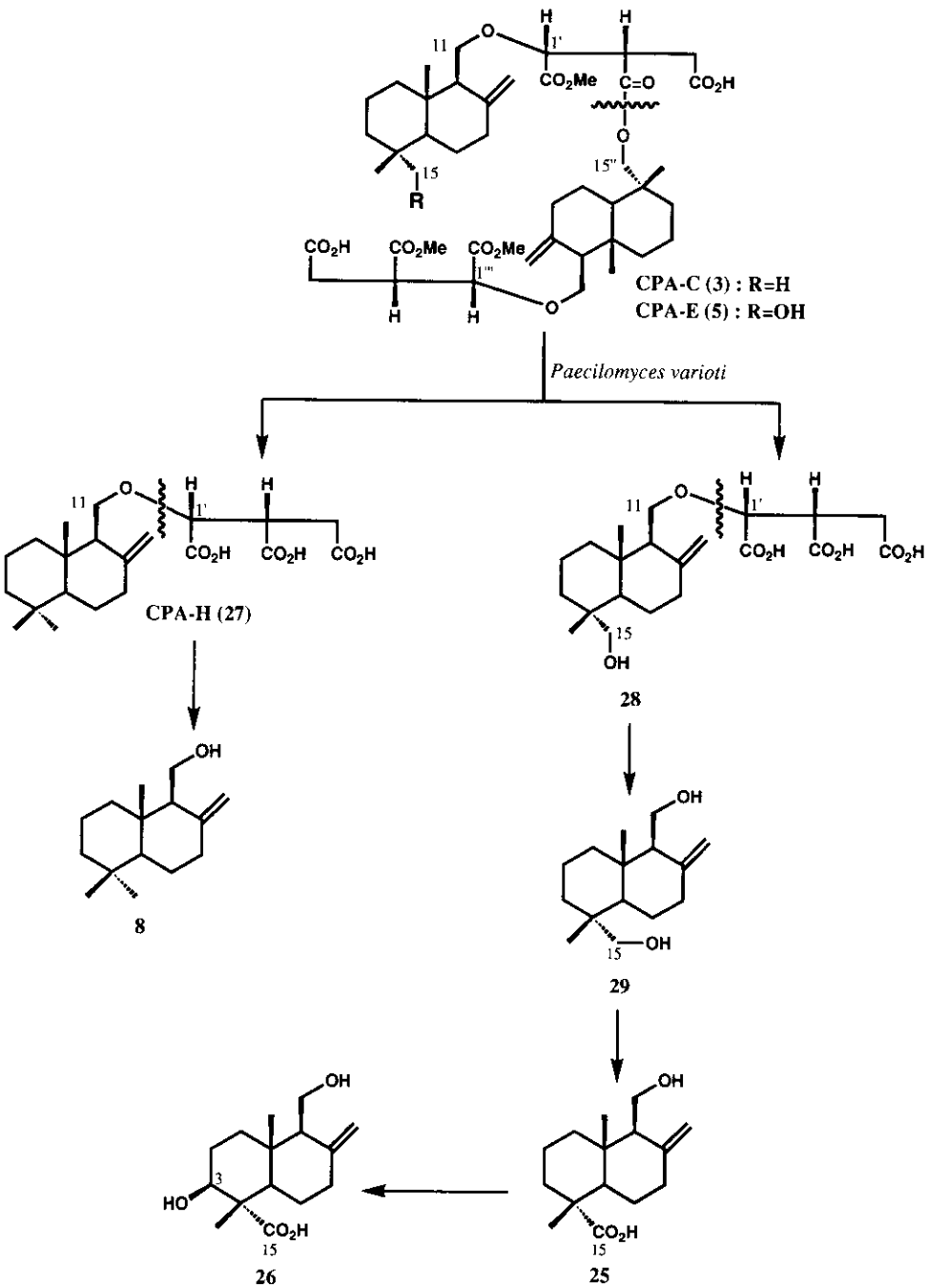
Experimental Samples	% Inhibition at 200 μg/mL	IC50 (μg/mL), r^2
CPA-A	16.1	Inactive
CPA-A Me_3	2.23	Inactive
CPA-B	5.77	Inactive
CPA-B Me_3	2.23	Inactive
CPA-C	**99.6**	61.0 μg/mL, r^2=0.992
CPA-C Me_5	0.00	Inactive
CPA-D	25.4	Inactive
CPA-D Me_4	0.00	Inactive
CPA-E	19.0	Inactive
CPA-E Me_5	3.73	Inactive
CPA-E	19.0	Inactive
CPA-F	**99.7**	42.2 μg/mL, r^2=0.889
CPA G	37.3	Inactive

2-4. Biotrasformation of *C. volvatus* by the Fungus *Paecilomyces varioti*[20]

Fresh *C. volvatus* was infected by the fungus *Paecilomyces varioti* maintaining humidity and room temperature for one month. The methanolic extract of the mushroom was partitioned between n-hexane, chloroform, *n*-butanol and water. The *n*-hexane and chloroform extracts were purified by column chromatography on silica gel to give albicanol (**8**) and **25** as a major compound. The *n*-butanol and water extracts were further chromatographed on reverse phase silica gel to give **26** from the former, and **27** and **28** from the latter, respectively. Fresh *C. volvatus* which had been autoclaved and then infected with *P. varioti* gave **25**, **26** and **29**. However, neither the degraded product nor demethyl cryptoporic acid was obtained when cryptoporic acids D (**4**) and E (**5**) were incubated with *P. varioti*.

On the basis of the above results, the possible biotransformation pathways of cryptoporic acids contained in fresh *C. volvatus* by the fungus *P. varioti* are proposed as shown in Scheme 3. Hydrolysis of the ester groups of cryptoporic acids occurs firstly to give demethyl compounds (**27**) and (**28**), then the ether bond at C-1' is cleaved to afford sesquiterpenoids (**8**) and (**29**). Compound (**29**) is further oxidized to furnish **25** and **26**. The yield of the degradation product (**8**)

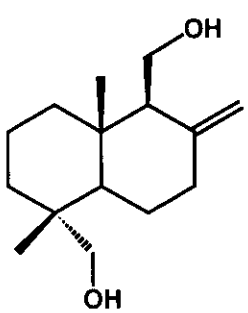
of the triacetate (**12**) of CPA-A (**1**) with boron trifluoride-ether at C-1' of isocitric acid part was very low, but the fungus *P. varioti* readily converted cryptoporic acids into the compounds (**8**) and (**25**) in good yields.



Scheme 3. Methabolic pathways of cryptoporic acids by the fungus *Paecilomyces varioti*

2-5. Biotrasformention of *C. volvatus* by the beetle *Tibolium castaneum*

Dry powders of *C. volvatus* was ingested and biotransformed by the beetle *Tibolium castaneum* at room temperature for one month to afford five novel metabolites, sesqui- (**29**, **30**), nor-sesqui- (**31, 32**) and bis-norsesquiterpenoids (**33**).[21] Their stereostructures were established by a combination of the extensive 2D NMR, X-Ray crystallographic analysis (Figure 6) and chemical degradation. The absolute structures of compounds (**29**) and (**30**) were determined by the same method as earier mentioned in albicanol (**8**); the ketones (**34**) and (**35**) obtained by acetylation and the subsequent ozonolysis of **29** and **30** showed the negative Cotton effects at 290 nm as that of ketone (**11**) derived from ozonolysis of **8**. The absolute structures of compounds **32** and **33** were determined by the Kusumi'method[22] for determining the absolute configuration of carboxylic acids with new chiral anisotropic reagent, phenylglycine methyl ester (PGME). (*R*)- and (*S*)-PGME were condensed (PyBOP, BOHT and *N*-methylmorphorine) with (*S*)-2-methylbutanoic acid and (*S*)-2-methylpentanoic acid to afford **36** and **37**, respectively. The $\Delta\delta$ (δ_S-δ_R) values having opposite signs are arranged on the right and left-hand sides of the PGME plane of **36** and **37** as shown in Figure 7. (*R*)- and (*S*)-PGME were condensed with compounds (**32**) and (**33**) to afford **38** and **39**, respectively. From the $\Delta\delta$ (δ_S-δ_R) values of **38** and **39** as shown in Figure 7, the absolute structure of C-8 of **32** and **33** were determined as *R*, respectively. Cryptoporic acids (CPA-E *etc.*) could be metabolized by cleavage of the ether bond at C-1' to give compound (**29**), and subsequently by oxidation to compounds (**30**) and (**31**), and converted into compound (**33**) *via* Baeyer-Villiger reaction as shown in Scheme 4.



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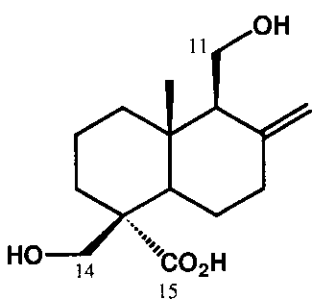

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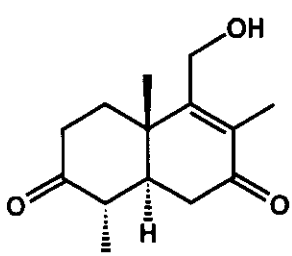

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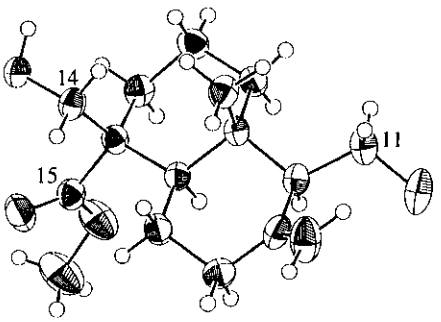
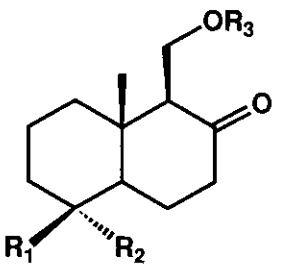
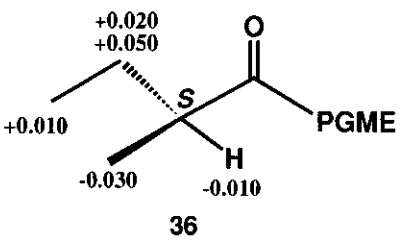


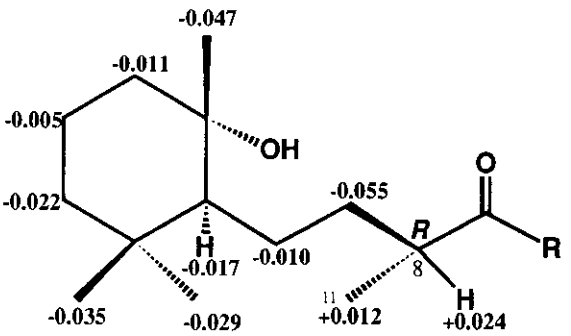
Figure 6. ORTEP drawing of **30**



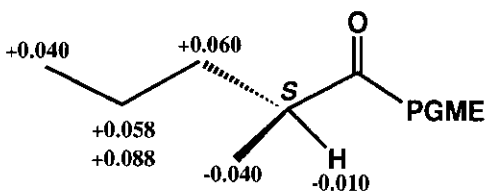
11 : R_1=R_2=Me, R_3=Ac
34 : R_1=Me, R_2=CH_2OAc, R_3=Ac
35 : R_1=CH_2OAc, R_2=CO_2H, R_3=Ac



36



32 : R=OH
38 : R=PGME



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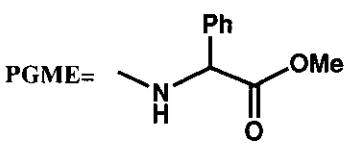
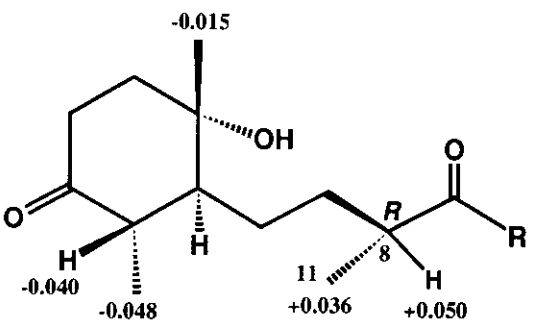
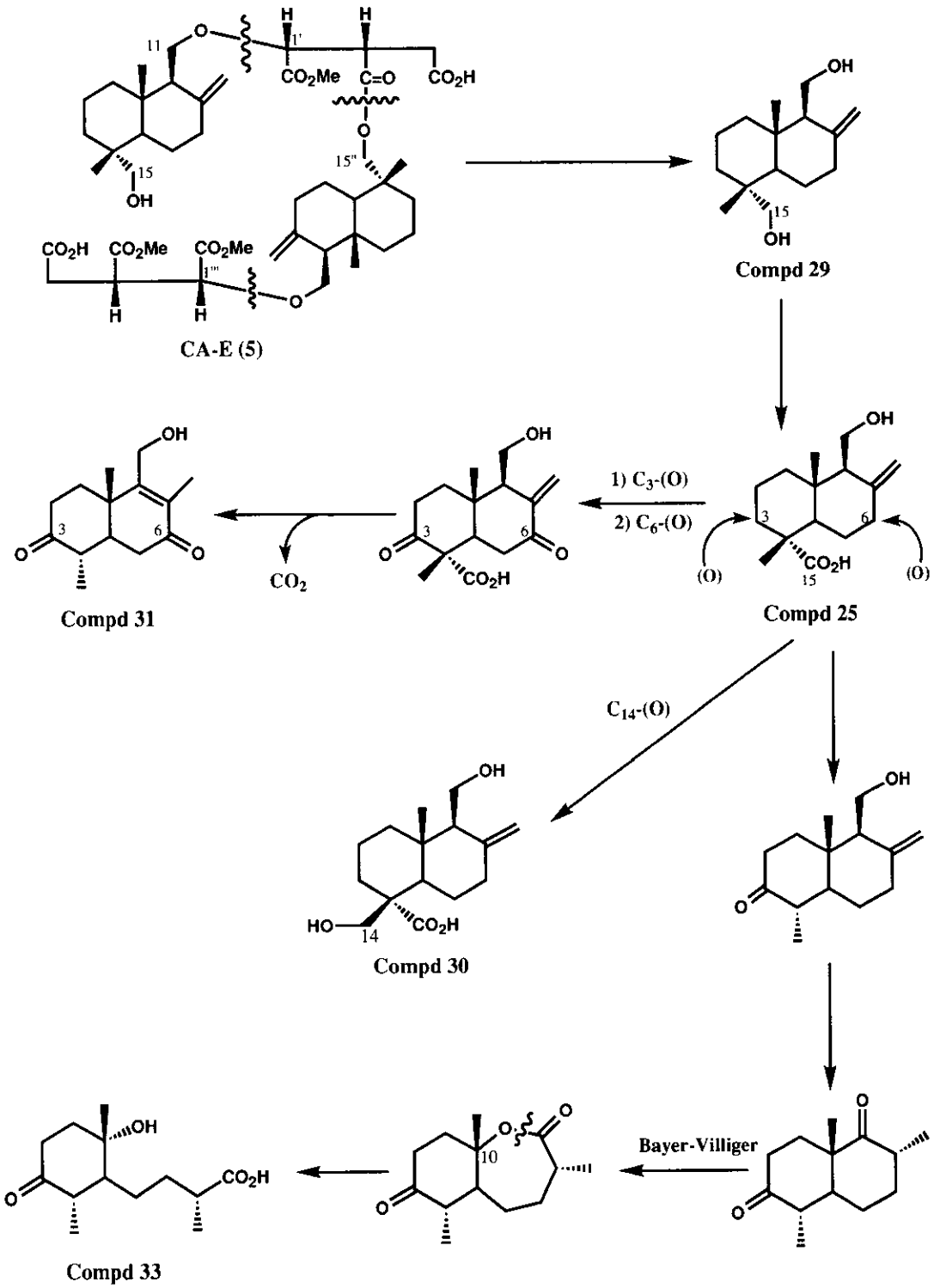




33 : R=OH
39 : R=PGME

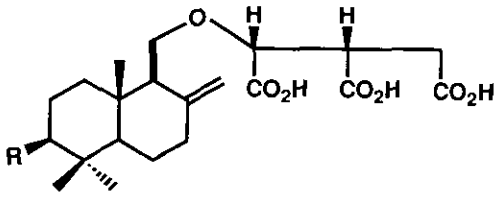
Figure 7. $\Delta\delta$ values ($\delta_{S\text{-PGME}} - \delta_{R\text{-PGME}}$) of compounds (**36**~**39**) in 600 MHz ^{1}H NMR



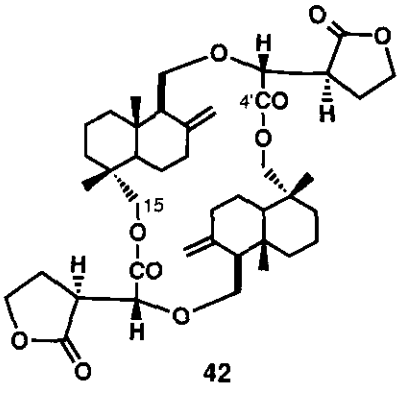
Scheme 4. Methabolic pathways of cryptoporic acids by the beetle *Tribolum castaneum*

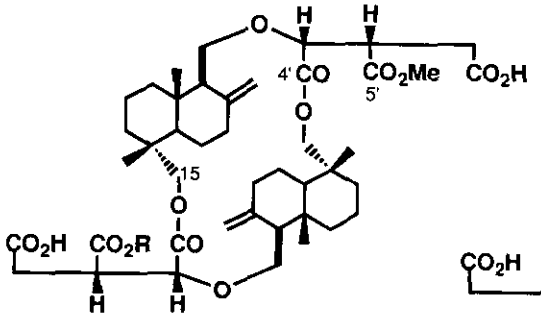
2-3. Cryptoporic Acid Analogues from the Basidomycetes Fungi

After we reported the absolute structures of cryptoporic acids (CPAs) A~G from *C. volvatus*, three research groups have reported the isolation and structural elucidation of cryptoporic acid analogues from the Basidomycetes fungi. Hirotani *et al.*[23] reported the structural characterization of cryptoporic acids H (CPA-H) (**40**) from the cultured broth of *C. volvatus* and CPA-I (**41**) in conjunction with CPA-H from *Ganoderma neo-japonicum* culture. None of the CPAs isolated from the fruit body of *C. volvatus* have been detected in the cultured broth of both fungi. Nozoe *et al.*[24] reported the structure elucidation of roseolide A (**42**) from the fungus *Roseoformes subflexibilis*. The position of dimerization in **42** was placed between C-15 and C-4', instead of C-15 and C-5' in CPA-D. Morita *et al.*[25-26] reported the structure determination of haploporic acids (HAs) A~C (**43~45**) isolated from the fungus *Haploporus odorus* whose structures were similar to those of CPAs and **42**.

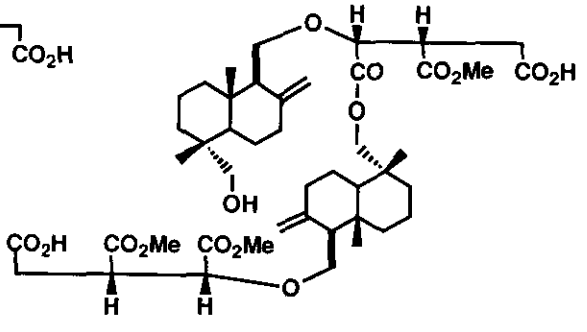


CPA-H (40) : R=H
CPA-I (41) : R=OH





HA-A (43) : R=Me
HA-C (45) : R=H

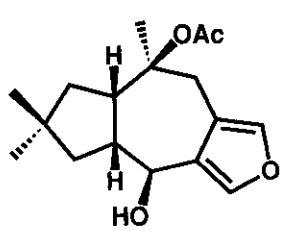


HA-B (44)

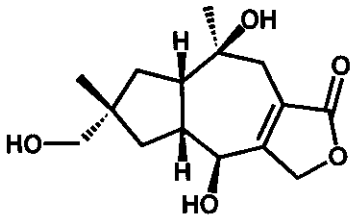
3. Novel Sesquiterpenoids from Basidiomycetes Fungus *Lentinellus ursinus* [27]

The inedible mushroom *Lentinellus ursinus* belonging to the Aphyllophorales-Lentinellaceae is known to grow mainly on dead oak trees and shows an intense pungent taste. We investigated the chemical constituents of the ethyl acetate extracts of *L. ursinus* and isolated three novel lactarane-type [3-acetyl-8-hydroxylactariusfuran (**46**), isolactarorufin B (**47**) and lentinellusfuran (**48**)] and one novel marasmane-type [lentinellol (**49**)] sesquiterpenoids along with five known lactarane-type [3, 8-dihydroxylactariusfuran (**50**),[28] 1, 8-dihydrolactariusfuran (**51**),[29] lactarorufin A (**52**),[30] furosardonin A (**53**)[31] and 5, 13-epoxy-2, 5, 7-lactaratrien-8-ol (**54**)[32]] and one known seco-lactarane [lactardial (**55**)[33]] as shown in Figure 8. This pungent taste is due to lactardial (**55**). The stereostructure of **46** was deduced from careful analysis of HMBC and NOSY spectra of 600 MHz NMR, and finally established by X-Ray crystallography as shown in Figure 9. The absolute structure of **46** was established by the modified Mosher's method[34] with 2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA) esters (**56**) of **46** , and the absolute configuration of the secondary hydroxyl group at C-8 was determined as *S*-configuration as shown in Figure 10. The stereostructures of isolactarorufin B (**47**), lentinellusfuran (**48**) and lentinellol (**49**) were established by a combination of the extensive 2D NMR (DQF-COSY, HMBC and NOESY) and chemical degradation.

Lactarane-, secolactarane- and marasmane-types sesquiterpenoids have been reported from a large number of both *Lactarius* and *Russula* species belonging to the Russulaceae, and all these sesquiterpenoids are believed to be biosynthesized from farnesyl pyrophosphate *via* humulene according to Scheme 6.[35] The lactaranes, secolactaranes and marasmanes constitute the majority of the Russulaceae sesquiterpenoids. The lactaranes from *Lentinellus ursinus* are the first example from mushroom of Lentinellaceae. *L. ursinus* chemically resembles *Lactarius* and *Russula* species, The pungent sesquiterpenoids such as lactardial (**55**), velleral (**57**),[36] piperdial (**58**)[33] and isovelleral (**59**)[37] have been identified in extract of the Russulaceae species (*Lactarius piperatus*, *L. vellerus etc.*), and showed antimicrobial, insect antifeedant and antitumor activities. Stearoylvelutinal (**60**) isolated from *L. vellerus* was treated with human saliva to give a pungent unsaturated dialdehyde, velleral (**57**) (32%) as shown in Scheme 5.[38]



46*



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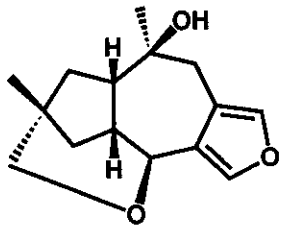

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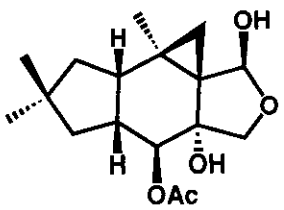

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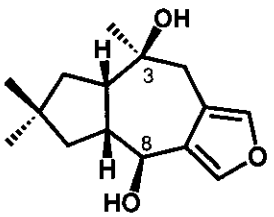

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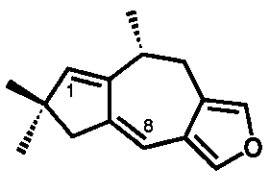

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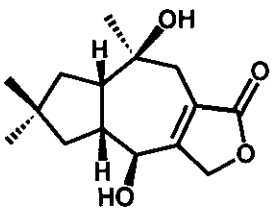

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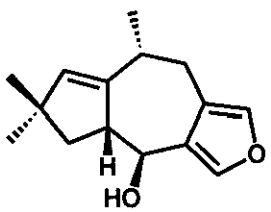

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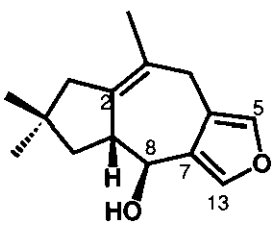

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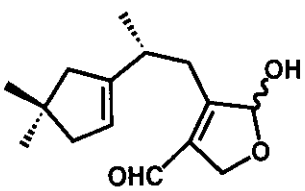


55

pungent taste

*New compounds

Figure 8. Sesquiterpenoids from Basidiomycetes fungus *Lentinellus ursinus*

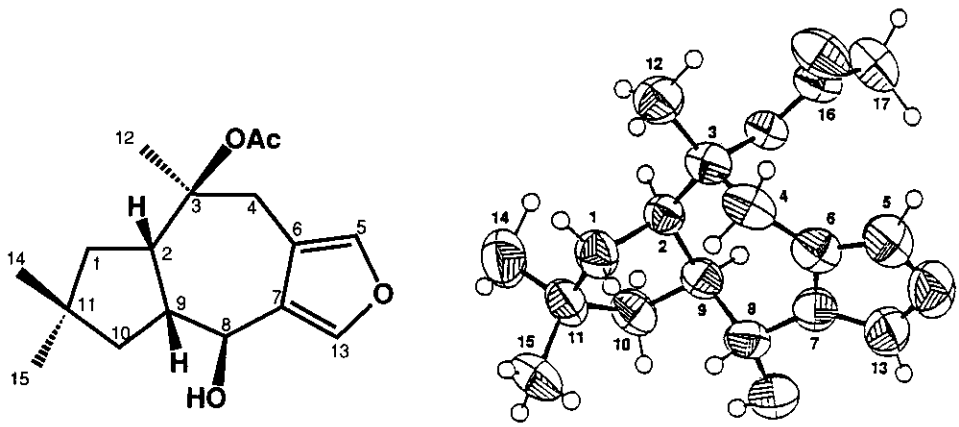
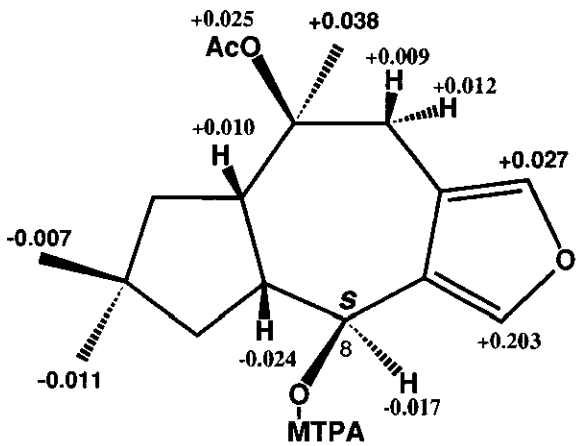
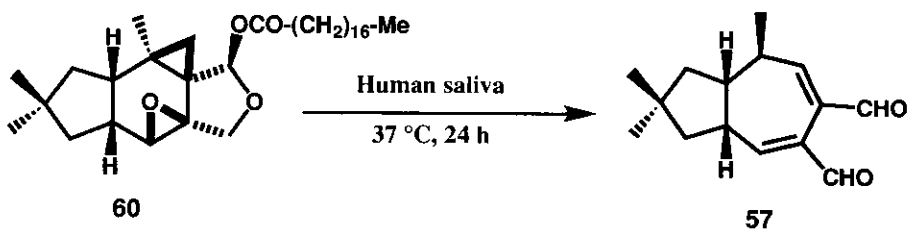


Figure 9. X-Ray crystallographic analysis of **46** (ORTEP drawing)

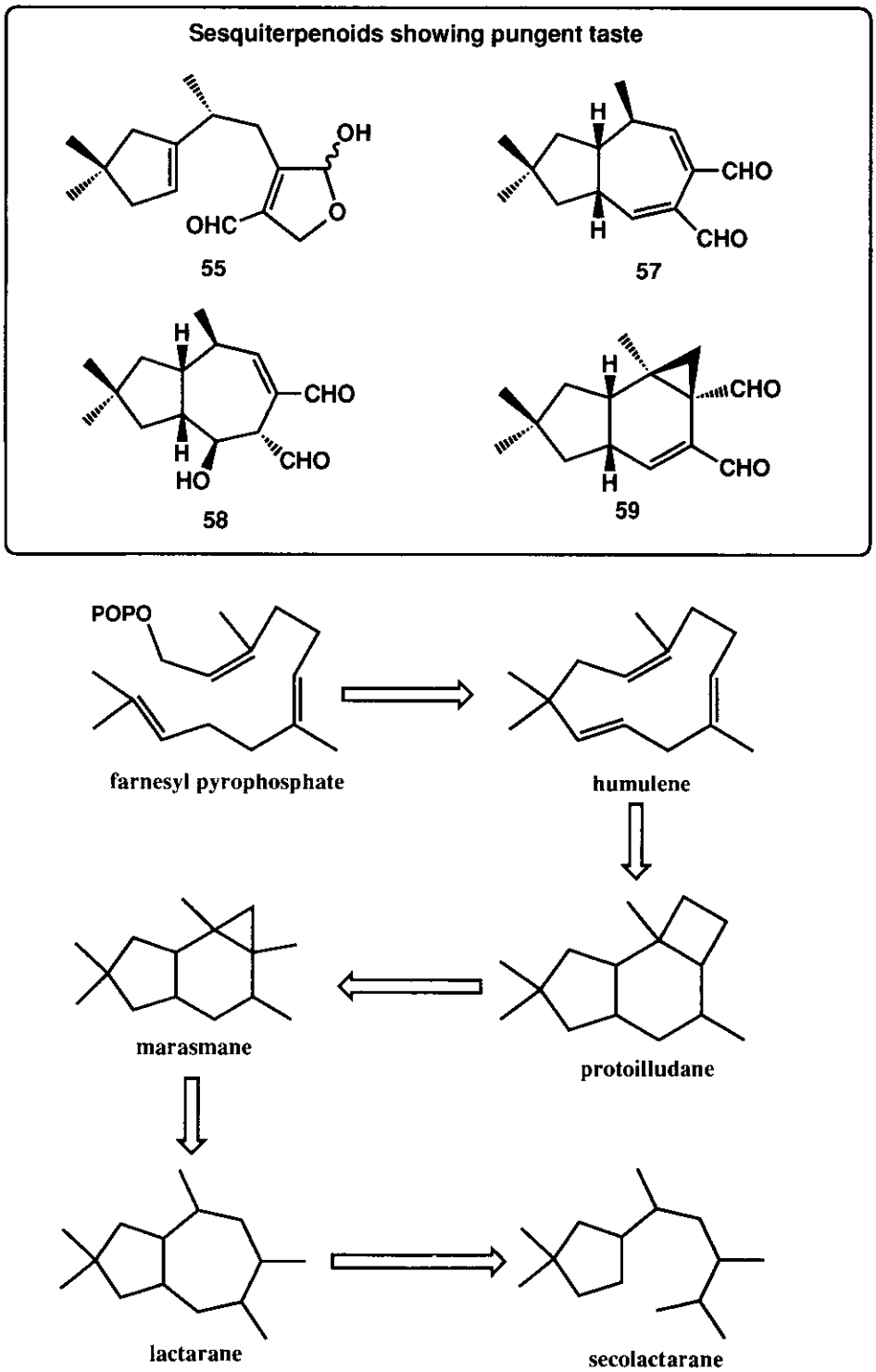


Δδ [(-)-MTPA - (+)-MTPA]

Figure 10. Modified Mosher's method of MTPA ester (**56**)



Scheme 5.



Scheme 6. The biogenesis of lactarane-, secolactarane-, and marasmane-type sesquiterpenoids

4. Novel γ- and δ-Lactones and Spiromentins from Basidiomycetes Fungus *Paxillus atrotomentosus*[39]

Paxillus atromentosus belonging to the Paxillaceae family is a lignicolous inedible mushroom with a large cap and frequently appears on decayed pine trees. Previously, Steglich *et al.*[40-42] reported the isolation and structural characterization of novel leucomentin, flavomentin and spiromentin derivatives from a European species of *P. atrotomentosus*. Further fractionation of the ethyl acetate extracts of the Japanese *P. atrotomentosus* resulted in the isolation of (+)-osumundalactone (**61**) as a new δ-lactone and a new γ-lactone (**62**), along with two known γ-lactone (**63** and **64**) (Figure 11) and six new spiromentins E-J (**65-70**) along with two known spiromentins B (**71**)[40] and C (**72**)[40]. (+)-(4*S*, 5*R*)-Osumundalactone ($[\alpha]_D$ +70.9°) has previously been obtained from both leucomentin 3[39] and flavomentin D[40] by chemical reaction. (-)-(4*R*, 5*S*)-Osumundalactone ($[\alpha]_D$ +70.9°) has been obtained as the hydrolysis product of osmundalin.[43] It is noteworthy that neither leucomentin nor flavomentin, which were isolated from the European *P. atrotomentosus* was detected in the Japanese species.

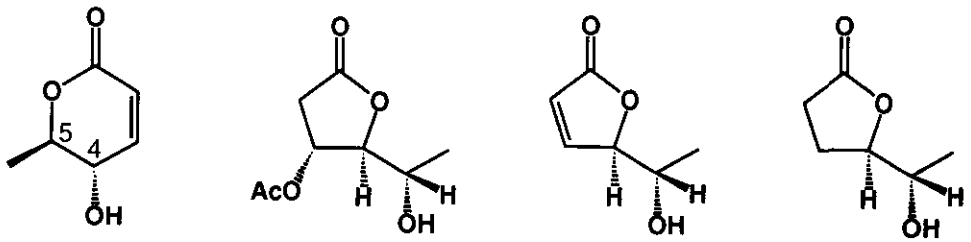


Figure 11. γ– and δ-Lactones from the fungus *Paxillus atrotomentosus*

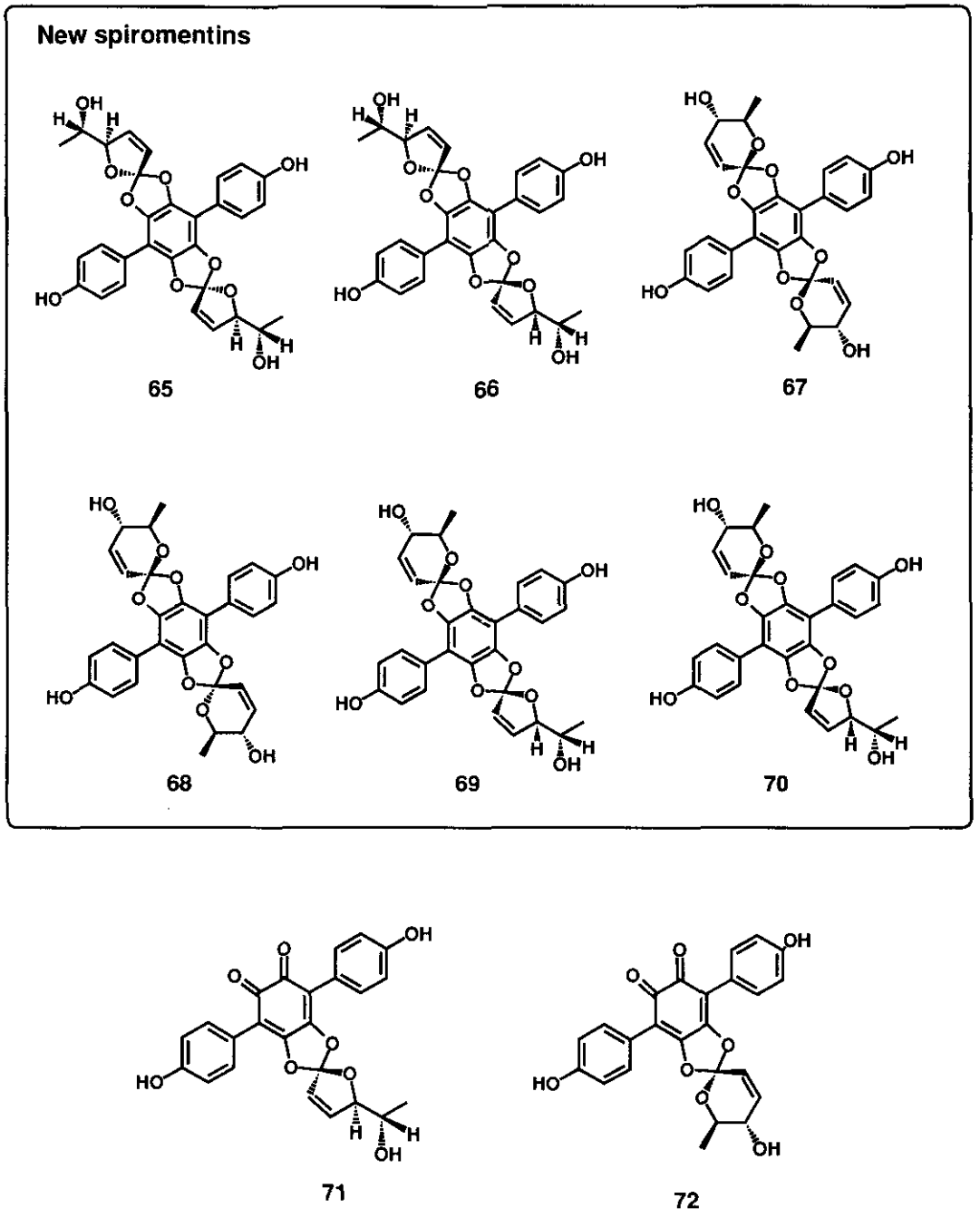


Figure 12. Spiromentins from the fungus *Paxillus atrotomentosus*

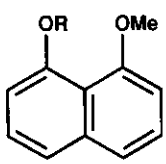
5. Chemical Constituents of the Ascomycetes Fungus *Daldinia concentrica*

The Xylariaceous fungi form one of the many families of the Ascomycetes, which are an assemblage of genera with obscurity, and almost inedible. The family consists of a central core of genera, namely *Daldinia*, *Hypoxylon*, *Entonaema*, *Xylaria*, *Rosellinia*, *Poronia*, *Podosodaria*, *Hycocopra*, *Nummularia*, *Kretzschmaria*, *Camillea*, and *Penzigia*.[46]

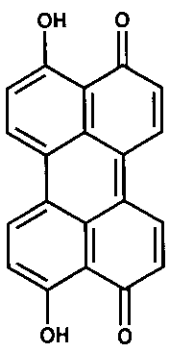
Chemically the Xylariaceous fungi have been little investigated. The fruit bodies of an European fungus *Daldinia concentrica* have been shown to contain the dihyroxyperylene quinone (**73**) which is derived from the dinaphthyl (**74**).[47-48] The same fungus in culture produces the naphthalene derivatives (**75**) and (**76**).[49]

In the course of the investigation of the biologically active substances from the Xylariaceous fungi, a novel binaphtyl (**77**), three novel benzophenone derivatives (**78-80**) three novel azaphilone derivatives named daldinins A-C (**81-83**) were isolated from the AcOEt extract of Japanese *Daldinia concentrica*, together with the known binaphthyl (**74**).

The EtOAc extract (26.7 g) of the dry material (424 g) of *D. concentrica* collected in Tokushima in 1993 was subjected repeatedly to column chromatography on Sephadex LH-20 ($CHCl_3$: MeOH = 1:1) and on silica gel ($CHCl_3$-AcOEt gradient) to afford **77** (1.67 g), **78** (2.58 g), **79** (1.67 g), **80** (0.13 g), **81** (1.366 g), **82** (37 mg), and **83** (1.662 g) as well as a known binaphthyl, chromagen (**74**) (67mg).



75 : R=H
76 : R=Me



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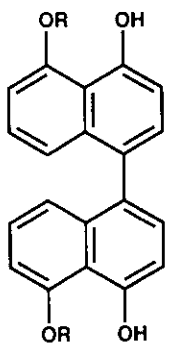
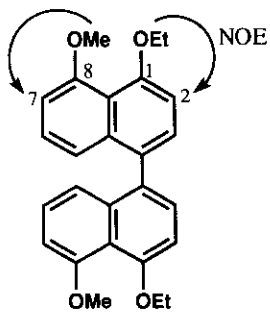


74 : R=H
77 : R=Me



84

The IR and ^1H NMR spectra of **77** ($C_{22}H_{18}O_4$) indicated the presence of a hydrogen-bonded

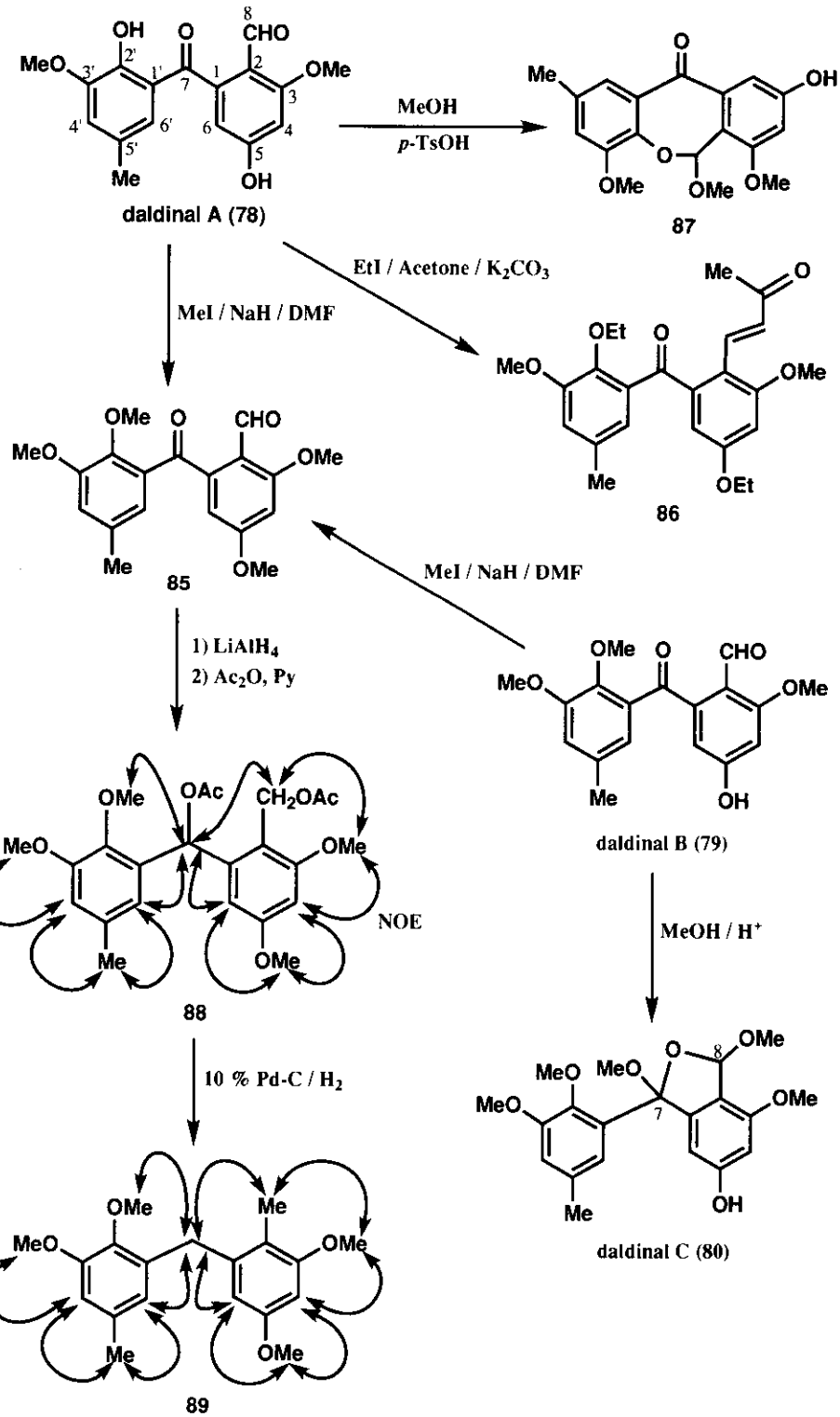
phenolic hydroxyl [3360 cm^{-1}; δ_H 9.53 (s)] and a methoxyl [δ_H 4.10 (s)] group. As **77** showed only half carbon signals of molecular formula in the ^{13}C NMR spectrum **77** might be a synmetrical dimer. Compound (**77**) was treated with BBr_3 in CH_2Cl_2 at -78°C to give **74**, which has identical data with those of the authentic **74.**[47] The structure of **77** was finally established as 1, 1'-dihyroxy-8, 8'-dimethoxybinaphthyl with NOE difference spectrum of the diethyl ether (**84**) of **77**.

The IR and ^1H NMR (acetone-d_6) spectra of daldinal A (**78**)($C_{17}H_{16}O_6$) indicated the presence of two phenolic hydroxyl [3350 cm^{-1} ; δ_H 10.06, 11.94 (each 1H, s)], two methoxyl [δ_H 3.85, 4.00 (each 3H, s)], and one hydrogen bonded conjugated formyl [1650 cm^{-1} ;δ_H 10.16 (1H, s)] groups. Methylation (MeI/NaH/DMF) gave the dimethyl ether (**85**) indicating the presence of two phenolic hydroxyl groups. Ethylation (EtI / K_2CO_3 / acetone, reflux) of **78** gave the diethyl ether (**86**) [$C_{24}H_{28}O_6$; EI-MS: m/z 412 (M^+); ^1H NMR ($CDCl_3$): δ 2.16 (s, -COMe)] as an aldol condensation product. Compound (**78**) was treated with *p*-TsOH/MeOH to afford an acetal derivative (**87**) [δ_H 6.51 (1H, s, H-8)]. Compound (**85**) was reduced with $LiAlH_4$, followed by acetylation to afford **88**. Hydrogenolysis (H_2 /20%Pd-C) of **88** afforded the diphenyl methane derivative (**89**). The structure of **78** was determined as 2-formyl-2', 5-dihydroxy-5'-methyl-3, 3'-dimethoxybenzophenone from the above results and NOE difference spectra of **78**, **86**, **88** and **89**, as shown in Scheme 7.

The ^1H and ^{13}C NMR spectra of daldinal B (**79**)($C_{18}H_{18}O_6$) were similar to those of **78**, except for the presence of three methoxyl groups [δ_H 3.40, 3.83 and 3.97 (each 3H, s)] in place of a hydrogen-bonded phenolic hydroxyl group [δ_H 11.94 (1H, s)]. Methylation of **79** gave a compound (**85**). The structure of **79** was finally established as C-2' methyl ether of **78** by X-Ray crystallographic analysis.

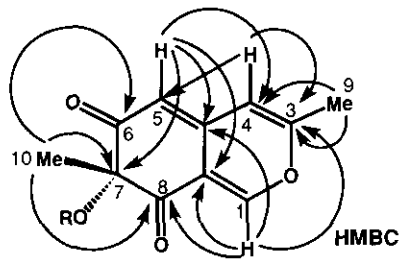
The ^1H and ^{13}C NMR spectra of daldinal C (**80**) ($C_{20}H_{24}O_7$) indicated the presence of five methoxyl [δ_H 3.22, 3.43, 3.60, 3.63, and 3.76 (each 3H, s)], diacetals [δ_H 6.11(IH, s, H-8); δ_c 99.6 (d, C-8) and 109.3 (s, C-7)] signals. Compound (**80**) may be racemate since the optical rotation is $[\alpha]_D \pm 0°(CHCl_3)$. Compound (**79**) was treated with *p*-TsOH/MeOH to afford **80** easily. As MeOH was used in the process of isolation, compound (**80**) might be an artifact arising from **79**.

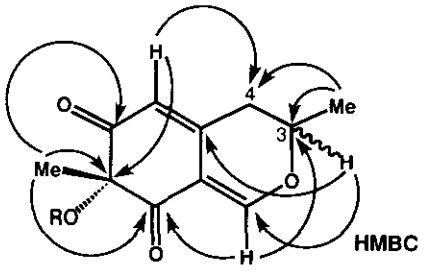
The IR and UV spectra of daldinin A (**81**) indicated the presence of an ester [1718 cm^{-1}] and an α, β, γ, δ-conjugated carbonyl group [1657 cm^{-1} ; λ_{max} 330 nm]. The ^1H and ^{13}C NMR spectra of **81** showed the presence of two tertiary methyl groups [δ_H 1.52(s, 7-Me), 2.17 (s, 3-Me)], three



Scheme 7. Chemical transformation of daldinals A-C (**78-80**)

olefinic protons [δ_H 5.50 (s, 5-H), 6.12 (s, 4-H), 7.86 (s, 1-H)], and two carbonyl carbons (δ_c 192.7, 193.3), which were similar to those of a known azaphilone (**90**)[50] [δ_H 1.49 (s, 7-Me), 2.17 (s, 3-Me), 6.17 (s, 4-H), 7.76 (s, 1-H)]. Compond (**81**) was hydrolyzed with lipase (porcine pancreas) at 37 °C, followed by methylation with CH_2N_2 to afford azaphilone (**91**) and methyl esters, the latter of which consisted of **92** (37%), **93** (8%), **94** (29%) and **95** (25%) judging from GC-MS analysis. The planar structure of **81** was derived from a careful analysis of the 2D NMR spectra including HMBC (Figure 13) of **81**. The absolute configuration at C-7 of **81** was confirmed as *S* by comparison of the CD spectrum of **81** with that of **90**[37]. The CD spectrum of **81** showed negative first (350 nm) and positive second Cotton effects (271 nm), while that of **90** showed positive first (357 nm) and negative second Cotton effects (270 nm), indicating that the absolute configuration at C-7 of **81** was represented as *R*.





81 : R= -CO(CH$_2$)$_n$-Me n=14, 16

-CO(CH$_2$)$_7$CH=CH-(CH$_2$)$_7$-Me (Z)

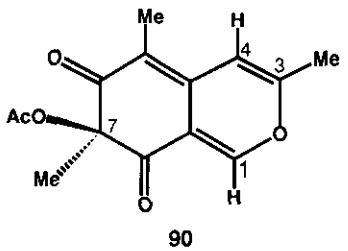
-CO(CH$_2$)$_7$CH=CH-CH$_2$-CH=CH-(CH$_2$)$_4$-Me (Z, Z)

82 : R= -CO(CH$_2$)$_n$-Me n=14, 16

-CO(CH$_2$)$_7$CH=CH-(CH$_2$)$_7$-Me (Z)

-CO(CH$_2$)$_7$CH=CH-CH$_2$-CH=CH-(CH$_2$)$_4$-Me (Z, Z)

91 : R=H



90

Me-(CH$_2$)$_n$-COOMe **92** : n=14 ; **93** : n=16

Me-(CH$_2$)$_7$-CH=CH-(CH$_2$)$_7$-COOMe (Z)

94

Me-(CH$_2$)$_4$-CH=CH-CH$_2$-CH=CH-(CH$_2$)$_7$-COOMe (Z, Z)

95

Figure 13. New azphilones, daldinins A (**81**) and B (**82**) isolated from the fungus *Daldinia concentrica*

The spectral data (^{1}H and ^{13}C NMR, and IR) of daldinin B (**82**) were similar to those of daldinin A (**81**), except for the presence of signals of one secondary methyl [δ 1.51(d, J=6.4 Hz)], one methylene [δ 2.75 (m)] and one methine [δ 4.38 (m)] bearing an oxygen function. Compond (**82**) was heated with DDQ in benzene to afford **81**. Thus, the structure of daldinin B (**82**) was determined as 3, 4-dihydro derivative of daldinin A (**81**) from the above results and careful analysis of the HMBC spectrum (Figure 13) except for the absolute configuration at C-3.

The IR and UV spectra of daldinin C (**83**, $C_{22}H_{26}O_9$) indicated the presence of a hydroxyl group (3522 cm^{-1}), an α, β-conjugated ester (1715 cm^{-1} ; λ_{max} 216 nm) and an α, β, γ, δ- conjugated ketone (1678 cm^{-1} ; λ_{max} 303 nm). The ^{1}H and ^{13}C NMR of **83** indicated two olefinic methyls [δ_H 1.53 (s), 1.78 (d, J=5.3 Hz)], an acetoxyl [δ_H 2.17 (s)], two methines [δ_H 3.31 (br s), 3.84 (m)] bearing oxygen functions and an acetal group (δ_c 102.5). Acetylation (Ac_2O, Py) of **83** afforded a monoacetate (**96**). Hydrolysis (KOH/MeOH, rt, 24 h) of **83** gave a diol (**97**) and tiglic acid. The structure of **83** was deduced from careful analysis of the 2D NMR spectra including HMBC (Figure 14) and NOESY, and finally established by X-Ray crystallography as shown in Figure 15. The absolute configuration of **83** was deduced by comparison of the CD spectrum of daldinin B (**82**) with that of the diol (**97**), and was further confirmed by the CD spectrum of dibenzoate (**98**) derived from **97** with *p*-Br-BzCl/Py/DMAP. The CD spectrum of **82** showed the positive first (351 nm) and the negative second Cotton effects (304 nm), while that of **97** showed the negative first (350 nm) and the positive second Cotton effects (302 nm). The CD spectrum of **98** exhibited the negative (254 nm) and the positive Cotton effects (237 nm). Thus, the whole structure of daldinin C including the absolute configuration was established as depicted in formula (**83**). Daldinin C (**83**) is the first example from nature of an azaphilone derivative with spiro-acetal structure between the B and C rings. It is very interesting that two enantiomers at C-7 (daldinins A, B: *R* configuration; daldinin C: *S* configuration) were isolated from the same fungus. Compounds (**78**) and (**79**) completely inhibited germination of the root of rice in husk at 5 ppm.

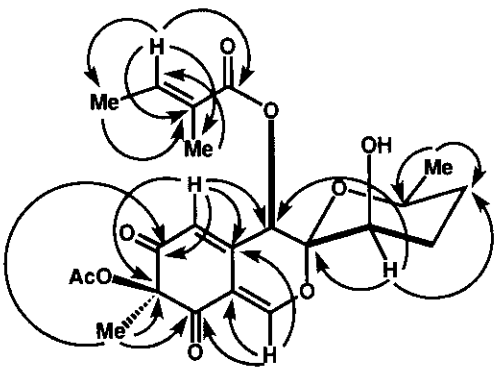


Figure 14. The HMBC correlation of daldinin C (**83**)

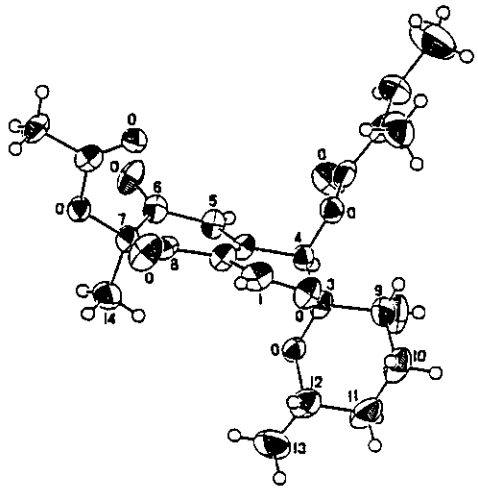
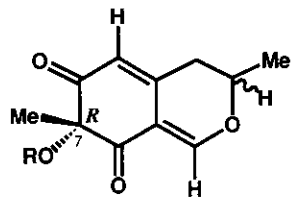


Figure 15. ORTEP drawing of **83**



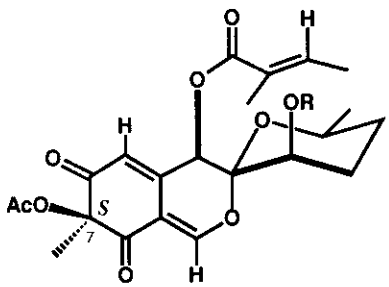
Daldinin B (82)

R= -CO(CH$_2$)$_n$-Me n=14, 16

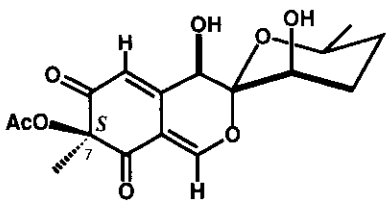
-CO(CH$_2$)$_7$CH=CH-(CH$_2$)$_7$-Me

-CO(CH$_2$)$_7$CH=CH-CH$_2$-CH=CH-(CH$_2$)$_4$-Me

CD (EtOH)
351 nm : Δε +2.29
304 nm : Δε -13.72

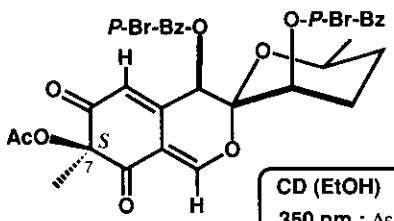


83 : R=H	96 : R=Ac
CD (EtOH)	CD (EtOH)
350 nm : Δε -1.55	353 nm : Δε -2.63
304 nm : Δε +13.92	301 nm : Δε +15.23
234 nm : Δε -12.37	240 nm : Δε -13.13



97

CD (EtOH)
350 nm : Δε -1.38
302 nm : Δε +13.44



98

CD (EtOH)
350 nm : Δε -1.26
303 nm : Δε +8.18
254 nm : Δε -33.34
237 nm : Δε +6.92

Figure 16. The CD spectra of daldinins B (**82**) , C (**83**) and **96**~**98**

6. Novel Cytochalasins of the Ascomycetes Fungus *Daldinia vernicosa*

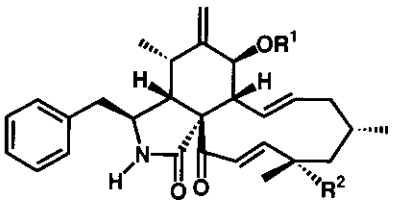
Chemical investigation of ethyl acetate extract of an *Daldinia vernicosa* belonging to the Xylariaceae has led to the isolation of sixteen novel 10-phenyl-[11]-cytochalasans (**99-114**).[51-53]

Fresh *C. volvatus* (774 g), collected in Tokushima was macerated and extracted with ethyl acetate for 1 week. The ethyl acetate extract (34.23 g) was subjeted repeatedly to column chromatography on silica gel (*n*-hexane-AcOEt and CH_2Cl_2-MeOH gradients), and each fraction was purified by HPLC (CH_2Cl_2-MeOH=98:2) to afford cytochalasins **99** (375 mg), **100** (37 mg), **101** (4 mg), **102** (487 mg), **103** (320 mg), **104** (390 mg), **105** (100 mg), **106** (4 mg), **107** (37 mg), **108** (7 mg), **109** (34 mg), **110** (2 mg), **111** (16 mg), **112** (5 mg), **113** (9 mg), and **114** (2 mg), respectively.

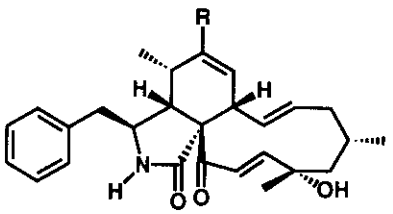
The IR and UV spectra of cytochalasin (**99**) ($C_{28}H_{35}O_4N$) indicated the presence of two hydroxyl (3488 and 3385 cm^{-1}), an amide (3208 and 1703 cm^{-1}) and an α, β-unsaturated ketone groups [1667 cm^{-1} ; λ_{max} 210 nm (log ε = 4.20)]. The ^1H and ^{13}C NMR of **99** contained the signals corresponding to two secondary [δ_H 0.94 (d, J=6.6 Hz), 1.05 (d, J=7.1 Hz)] and a tertiary methyl [δ_H 0.94 (s)], an exomethylene [δ_H 5.07, 5.26 (both br s)], a *trans*-enone [δ_H 6.59 (d, J=15.9 Hz), 7.08 (d, J=15.9 Hz)], an amide [δ_H 6.29 (NH, br s); δ_C 174.0 (s)], a tertiary hydroxyl [δ_C 72.8 (s)], and a secondary hydroxyl [δ_H 4.06 (br d, J=10.0 Hz)] groups. Acetylation of **99** afforded the mono-acetate (**115**) suggesting the presence of a secondary alcohol. The structure of **99** was deduced from careful analysis of ^1H-^1H COSY (Figure 17), HMBC (Figure 18) and NOESY (Figure 19) spectra.

Cytochalasin (**106**) was obtained as suitabale single crystals, and the X-Ray crystallographic analysis unequivocally confirmed the complete structure and relative stereostructure as shown in Figure 20. Structures of the other cytochalasins were determined by 600 MHz 2D NMR spectra and comparison of spectroscopic data with cytochalasins (**99** and **106**).

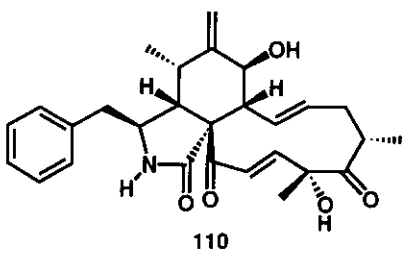
The cytochalasans are a group of secondary metabolites generated by cultures of a various molds found in cereal crops. The cytochalasans produce various important biological effects including inhibition of cell movement, inhibition of cytoplasmic cleavage, nuclear extrusion of cultured cell, and plant growth inhibitory activity. It is noteworthy that recently, new cytochalasin was identified as an HIV-I protease inhibitor.[54]



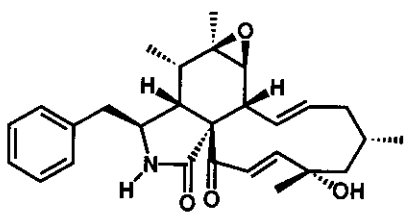
99 : R^1=H, R^2= OH
100 : R^1 = H, R^2=H
115 : R^1 = Ac, R^2=OH



107 : R = Me
108 : R = CH_2OH



110



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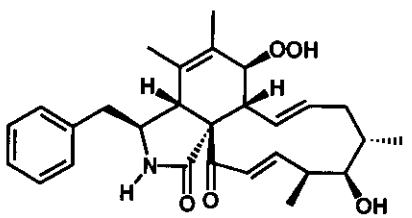

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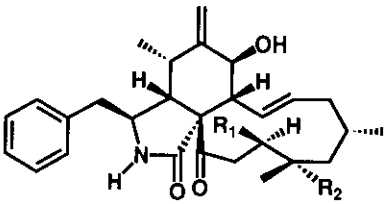
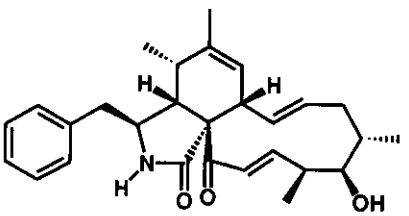


101 : R_1 = H, R_2 = OH
102 : R_1 =OH, R_2 = OH
103 : R_1 =OH, R_2 = OH
104 : R_1 = OAc, R_2 = OH
105 : R_1 = H, R_2 = H
106 : R_1 = OH, R_2 = H



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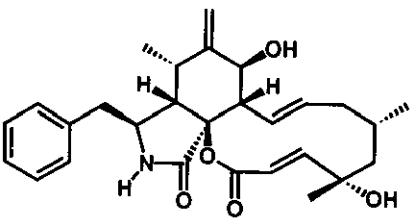

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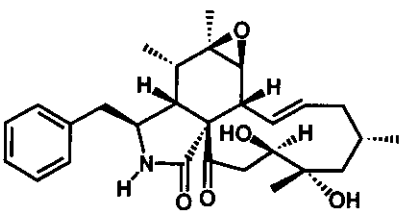
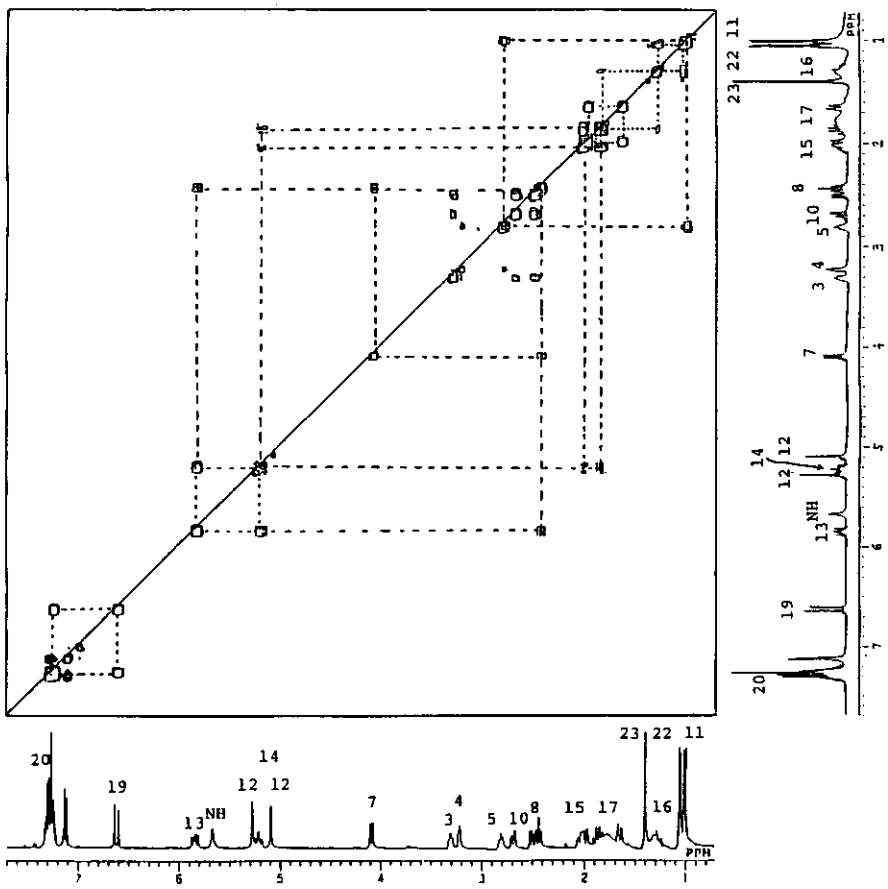


113

Figure 17. New cytochalasins iolated from the fungus *Daldinia concentrica*



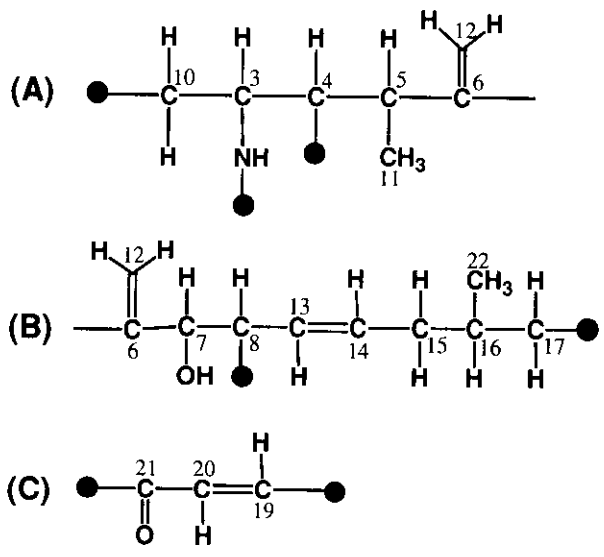


Figure 17. 400 MHz ^{1}H-^{1}H-COSY spectrum of cytochalasin (**99**)

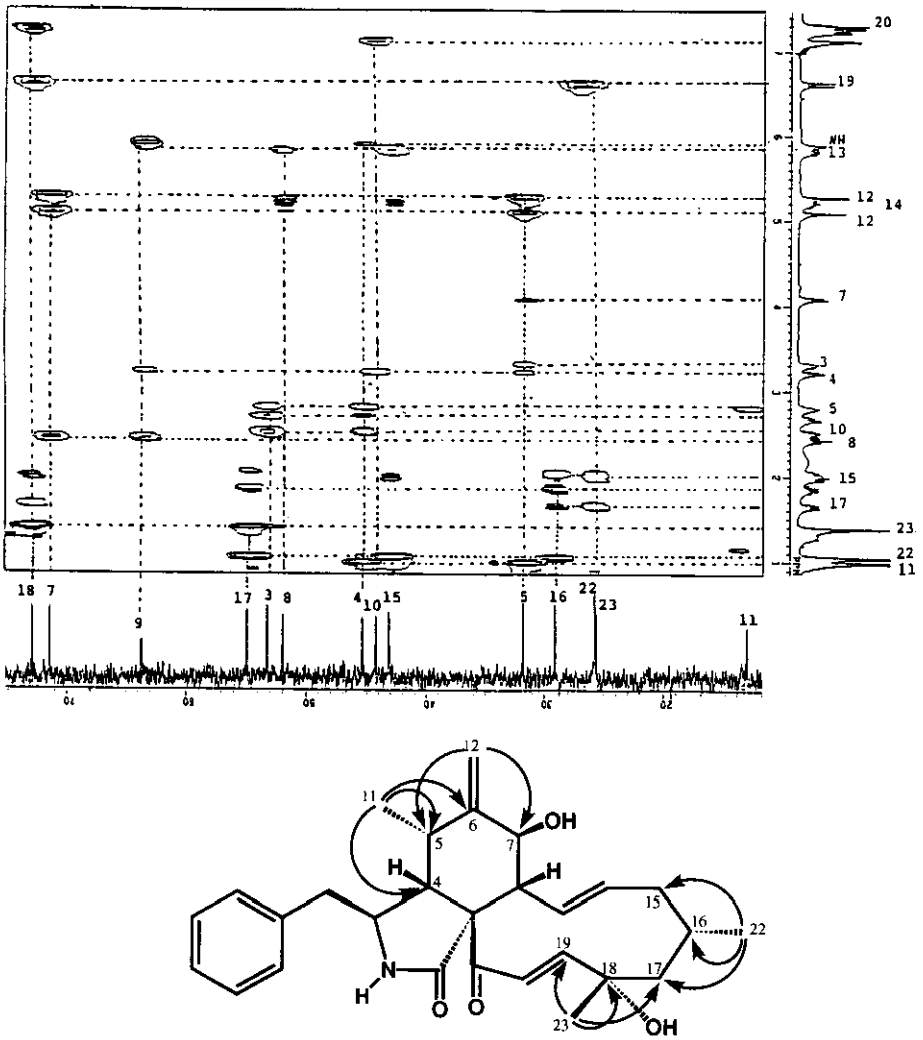


Figure 18. 400 MHz HMBC spectrum of cytochalasin (**99**)

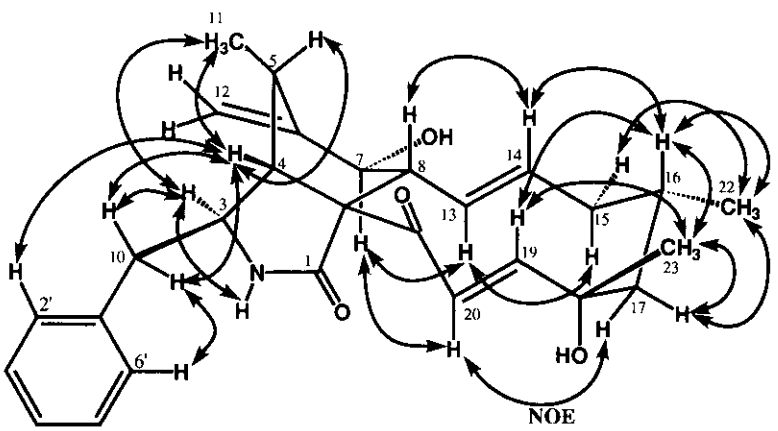
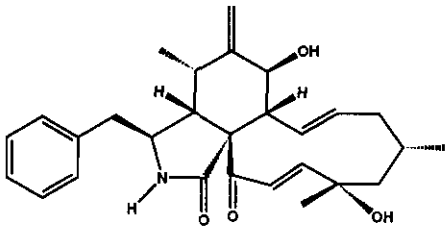


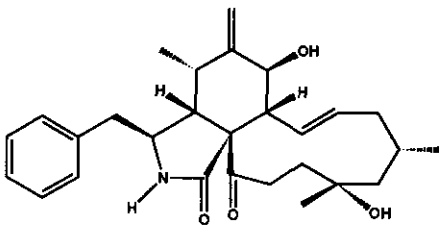
Figure 19. 600 MHz NOESY spectrum of cytochalasin (**99**)

The cytotoxicity testing of five cytochalasins (**99** and **101~104**) on the KB cell line was investigated as shown in Figure 21. Cytochalasin (**99**) inhibited KB cell completely at a concentration of 10^{-5} M, Cytochalasins (**101~104**) inhibited KB cell almost completely at a concentration of 10^{-4} M, respectively. The plant growth inhibitory activity of cytochalasin (**99**) was tested against rice seedling as shown in Figure 22. Compound (**99**) completely inhibited germination of the root of rice in husk at 100 ppm. The 50 % growth inhibition of **99** against roots was 10 ppm.



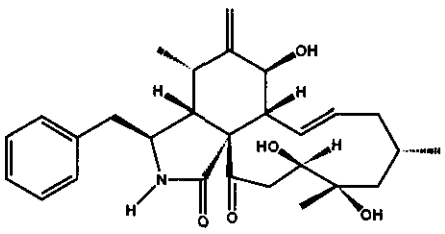
99

Concentration	% of Inhibition
10^{-6} M	58 %
10^{-5} M	96 %



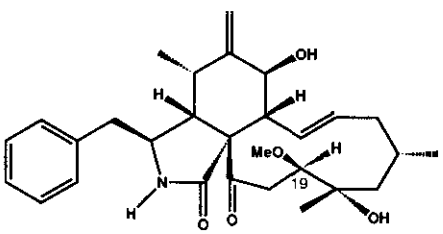
101

10^{-5} M	50 %
10^{-4} M	84 %



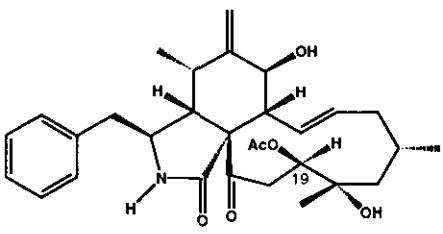
102

10^{-5} M	44 %
10^{-4} M	89 %



103

10^{-5} M	50 %
10^{-4} M	84 %



104

10^{-5} M	28 %
10^{-4} M	96 %

Figure 21. Cytotoxic activity (KB cell line) of cytochalasins (**99**) and (**101~104**)

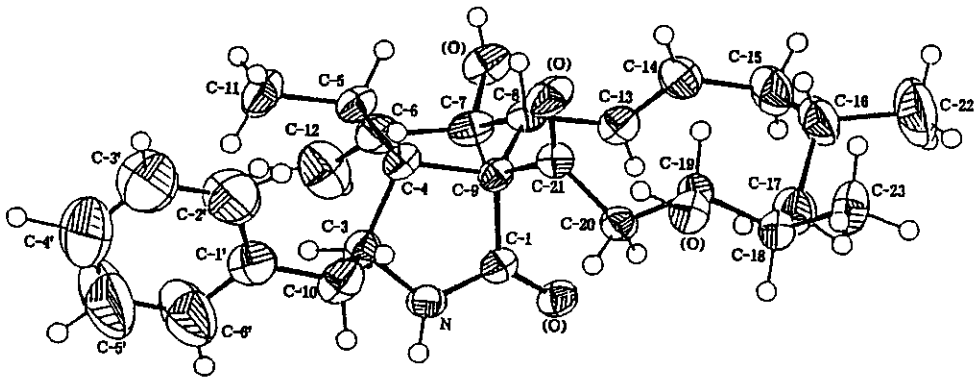


Figure 20. ORTEP drawing of cytochalasin (**106**)

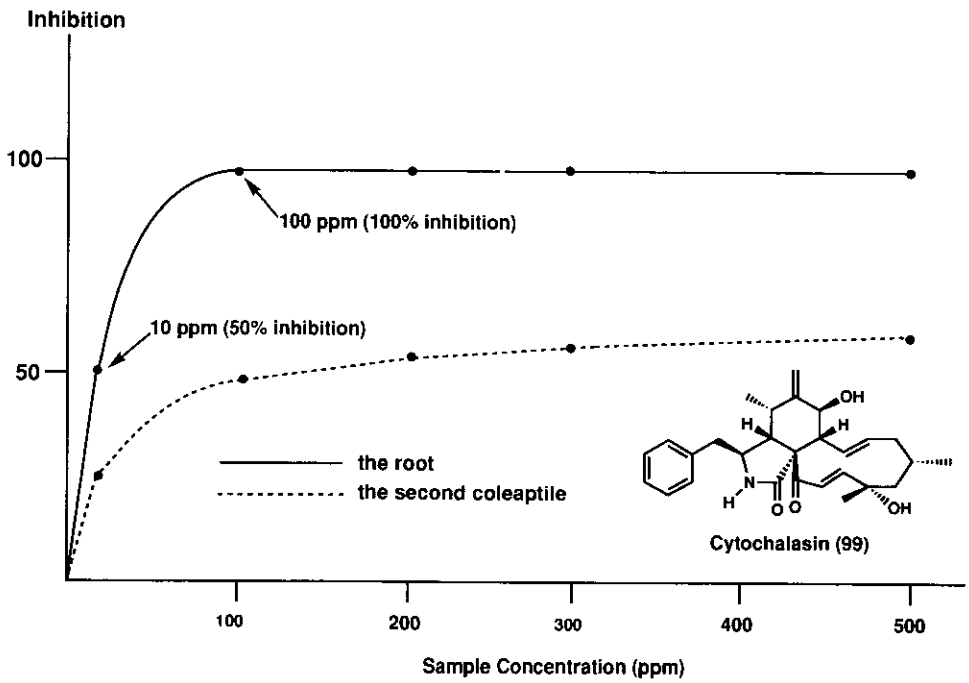
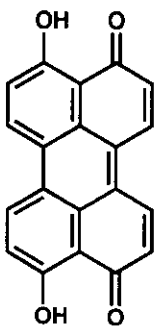


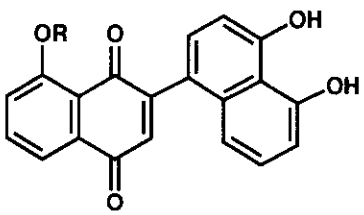
Figure 22. Plant-growth inhibitory activity of cytochalasin (**99**) against rice seedling

7. A Novel Aromatic Compound of the Ascomycetes Fungus *Hypoxylon truncatum*

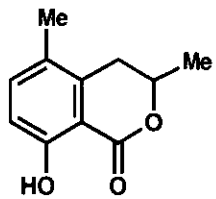
The *Hypoxylon* species produced pigments showing purple, dark red or black colors. Hypoxylone (**116**) have been isolated as the main pigment of the dark purple carpophores of *Hypoxylon sclerophaeum* collected in France.[46] The 4,9-dihyroxyperylene-3, 10-quinone (**73**) is also present in the black sporophores of *Bulgaria inquinans*, *Daldinia concentrica* and *Hypoxylon fuscum* accompanied by large quantities of black polymeric material indicating the close relationship of *Bulgaria* and *Daldinia* with *Hypoxylon* in the Xylariaceae. The fruit bodies of the European fungus, *Hypoxylon truncatum* have been shown to contain 3-methyl-3,4-dihydroisocoumarin, 5-methylmellein (**117**). We investigated the chemical constituents of the ethyl acetate extract of the Japanese species of *H. truncatum*, and isolated a novel perylenequinone, truncatone (**118**).[55]



73



116

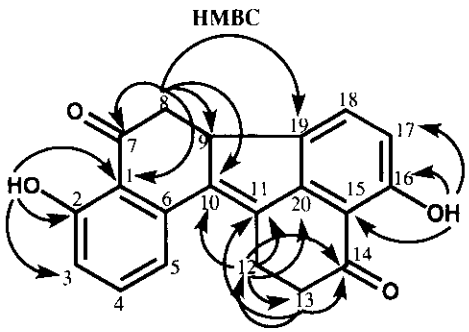


117

Dry *H. truncatum* (173 g), collected in Tokushima was extracted with ethyl acetate for 1 week. The extract (16.6 g) was subjeted repeatedly to column chromatography on silica gel (n-hexane-AcOEt and $CHCl_3$-AcOEt gradients) to afford truncatone (**118**, 210 mg) as a yellow solid and the binaphtyl (**74**, 10 mg) as a dark red solid, respectively. Compound (**118**) have the molecular formula $C_{20}H_{14}O_4$, and showed a specific rotation of $[\alpha]_D$ -162° ($CHCl_3$). The IR, UV, ^1H and ^{13}C NMR ($CDCl_3$) spectra of **111** indicated the presence of two phenolic hydroxyl [3225 cm^{-1}; δ_H 10.6, 12.6 (each 1H, s)], two conjugated ketone [1649 cm^{-1}; λ_{max} nm (log ε) : 289 (4.64), 304 (4.64); δ_C 201.5, 204.0 (each *s*)]. The CD spectrum of **118** showed the negative first (281 nm: Δε -17.28) and the positive second Cotton effects (260 nm: Δε +19.41). Methylation (MeI / K_2CO_3 / acetone) gave trimethyl ether (**119**) ($[\alpha]_D$ ±0°) showing a racemate. The relative structure of **118** was deduced from careful analysis of HMBC (Figure 23) of **118** and NOESY (Figure 24)

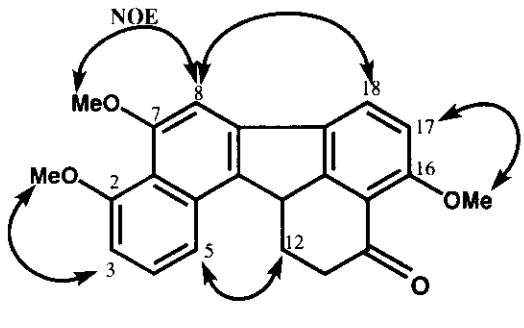
of **119**.

Truncatone (**118**) might be biosynthesized from binaphtyl (**74**), whch was isolated from the same fungus, as shown in Scheme 8.



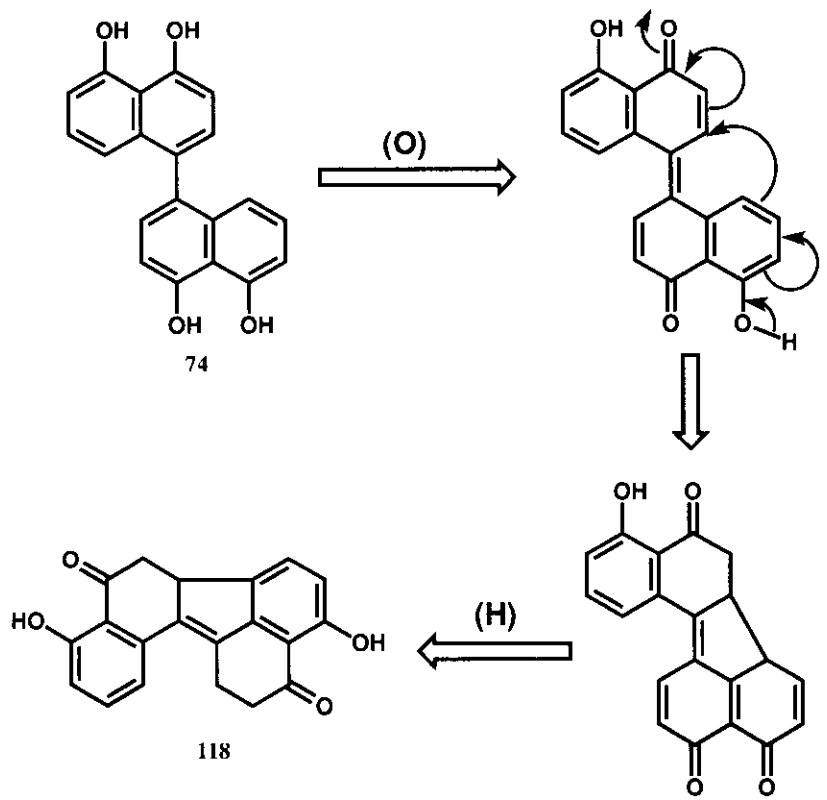
118

Figure 23. HMBC correlations of **118**



119

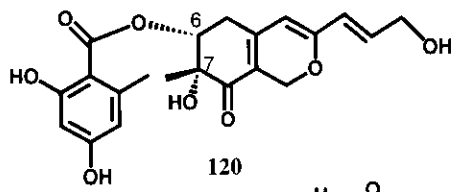
Figure 24. NOESY correlations of **119**



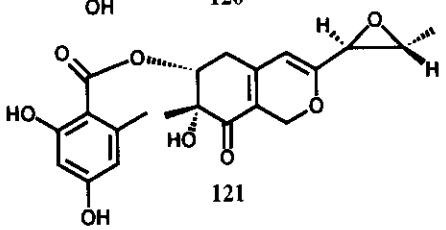
Scheme 8. Possible biogenetic pathway of truncatone (**118**)

8. Three Novel Azaphilones of the Ascomycetes Fungus *Entonaema spdendens*

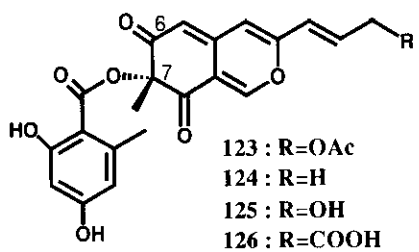
Entonaema spdendens is very rare inedible fungus belonging to the Xylariaceae, and the inside is filled with a reddish yellow jellied liquid. We investigated the chemical constituents of the EtOAc extract (2.80 g) of fresh *E. spdendens* (282 g), and isolated three novel azaphilones, entonaemin A (**120**, 309 mg), B (**121**, 29 mg), and C (**122**, 84 mg) as a reddish yellow liquid with a known azaphilone, (+)-mitorubrinol acetate (**123**), which was isolated from *Hypoxylon fragiforme* with (+)-mitorubrin (**124**), (+)-mitorubrinol (**125**), and mitorubrinic acid (**126**) by Steglich *et al.*[56]



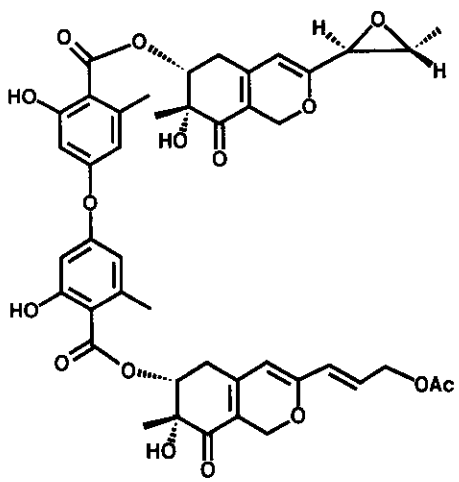
120



121



123 : R=OAc
124 : R=H
125 : R=OH
126 : R=COOH



122

The IR, UV, ^1H and ^{13}C NMR ($CDCl_3$) spectra of entonaemin A (**120**), $C_{21}H_{22}O_8$ (HRMS: *m/z* 402.1322) showed the presence of two phenolic hydroxyl [3391 cm^{-1}; δ_H 9.5, 11.5 (each 1H, *s*)], a conjugated carbonyl [1645 cm^{-1}; λ_{max} nm (log ε) : 289 (4.64), 304 (4.64); δ_C 196.2 (*s*)], tertiary hydroxyl [δ_C 74.9 (*s*)], primary hydroxyl [δ_H 4.25 (2H, *dd*, J=2.2, 4.4 Hz); δ_C 62.2 (*t*)], and 1,2,3, 5-substituted aromatic [δ_H 6.21, 6.22 (each, *d*, J=2.4 Hz)] groups. The orsellinic acid (**127**) obtained by reduction ($NaBH_4$/MeOH) of **120** was allowed to react with MeI and K_2CO_3 to give the methyl ether (**128**), which was identical with the methyl ether (**128**) obtained by the methylation of orsellinic acid methyl ester (**129**) isolated from the liverwort *Blasia pusilla*[57] as shown in

Scheme 8. The relative structure of **120** was deduced from careful analysis of HMBC (Figure 23) and NOESY (Figure 24) of **120**, and the position of ester linkage of orsellinic acid was determined to be C-6 postion of azaphilone.

The structures of entonaemins B (**121**) and C (**122**) was deduced from careful analysis of the 2D NMR spectra including HMBC and NOESY, and a comparison with the spectral data with those of entonaemin A (**120**).

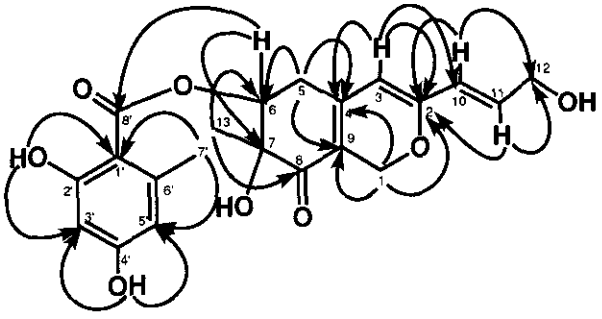


Figure 23. HMBC correlations of entonaemin A (**120**)

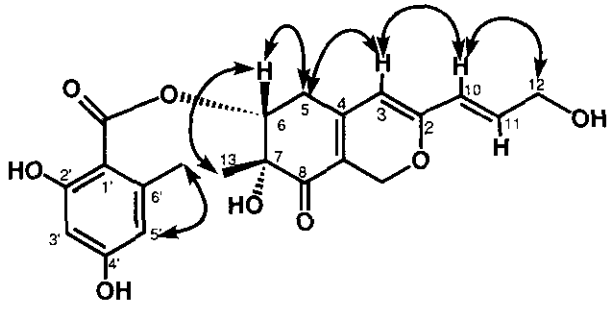
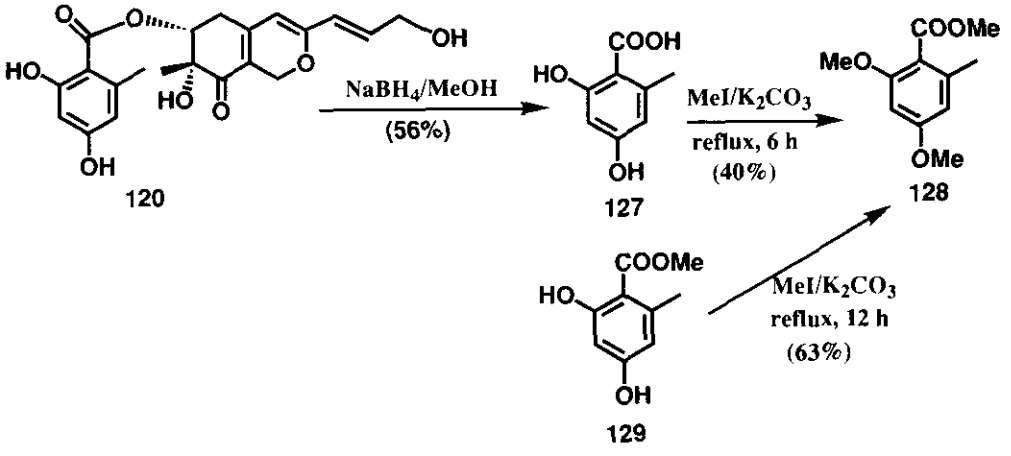


Figure 24. NOESY correlations of entonaemin A (**120**)



Scheme 9.

9. Aromatic Compounds of the Ascomycetes Fungus *Ascoclavulia sakaii*

Inedible fungus *Ascoclavulia sakaii* belonging to the Leotioideae is rare in Japan. Fractionation of EtOAc extract (5.78 g) of fresh *A. sakai* (343 g) resulted in the isolation of a new depsidone, ascoclavulinic acid A (**130**, 896 mg), a new depside, ascoclavulinic acid B (**131**, 366 mg), and a novel nitroso compound, ascoclavin (**132**, 53 mg).[55] The structures of ascoclavulinic acids A (**130**) and B (**131**) were deduced from careful analysis of HMBC and NOESY of **133** and **134** obtained by methylation (MeI / K_2CO_3) of **130** and **131**, respectively. The structure of ascoclavin (**132**) was established by X-Ray crystallography of a dimethyl ether (**135**) of **132** as shown in Figure 25. Depsidones such as colensoic acid (**136**) and depsides such as perlatolic acid (**137**) are chracteristic markers of lichens (symbiotic organisms of fungi and algae), and show interesting biological activities such as antibiotic, antitumor, and anti-HIV-I.[58] The Ascomycetes fungus *A. sakaii* was closely related to lichens, because depsides and depsidones were isolated from both species. The nitrosoimine compound such as ascoclavin (**132**) is very rare example in nature.

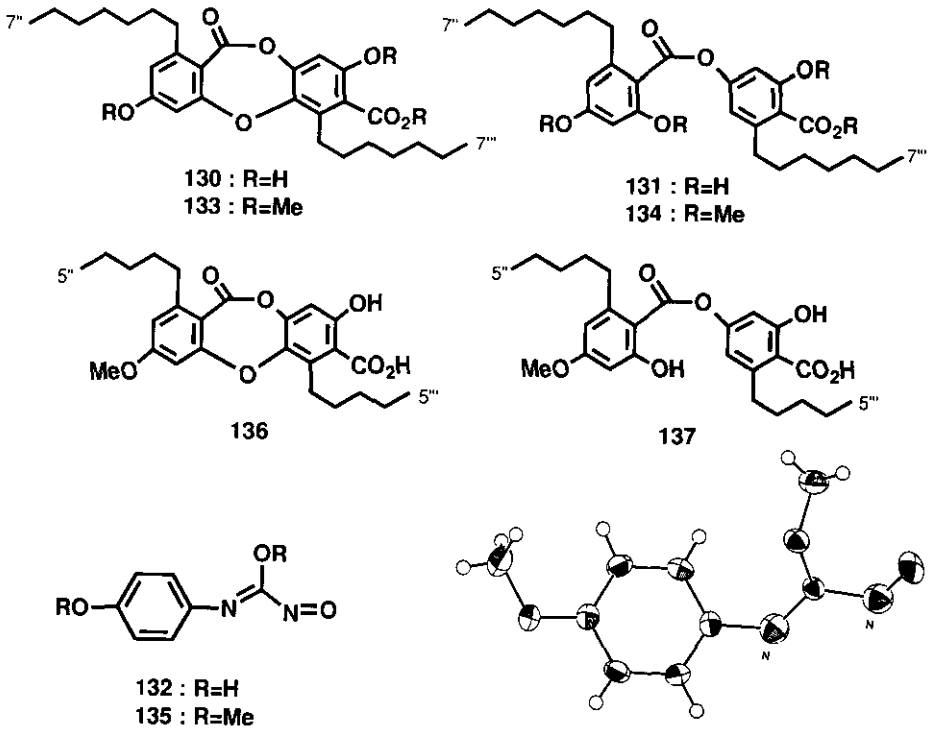


Figure 25. ORTEP drawing of **135**

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