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Chemical Constituents of the Ascomycete *Daldinia concentrica*

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Four compounds, daldinone A (**1**), daldinone B (**2**), daldiniapyrone (4-methoxy-5-carbomethoxy-6-pentyl-2*H*-pyran-2-one, **3**), and daldinialanone (22*R*-hydroxylanosta-7,9(11),24-trien-3-one, **4**), were isolated from an ethyl acetate extract of fruit bodies of *Daldinia concentrica* collected in Europe. In addition, 11 known compounds, 4:5:4′:5′-tetrahydroxy-1:1′-binaphthyl (**5**), 3,4,5-trihydroxy-1-tetralone (**6**), (+)-orthosporin (**7**), curuilignan D (**8**), (*22E*)-cholesta-4,6,8(14),22-tetraen-3-one (**9**), 3*β*,22-dihydroxylanosta-7,9(11),24-triene (**10**), concentricol (**11**), concentricol B (**12**), concentricol C (**13**), concentricol D (**14**), and phenochalasin B (**15**), were obtained. The structures of the new compounds were elucidated by 2D NMR, MS, IR, and UV spectra and by X-ray crystallographic analysis. The absolute configurations of **1** and **4** were determined by CD spectroscopy and the modified Mosher's method, respectively. The chemotaxonomic relevance of the compounds obtained in this investigation is discussed.

The chemical constituents of the Ascomycete *Daldinia concentria* (Bolt.: Fr.) Ces & De Not. (Xylariaceae) were first investigated by Allport and Bu'Lock in 1958, who reported 4:5:4′:5′-tetrahydroxy-1:1′-binaphthyl and dihydroxyperylene quinone from its fruit bodies,[1] and in 1960 2,6-dihydroxybutyrophenone, 8-methoxy-1-naphthol, and 2-hydroxy-5-methylchromone from mycelial cultures of this fungus.[2] Later, our group reported the isolation of novel binaphthyl benzophenone derivatives named daldinals,[3] three new azaphilone derivatives named daldinins A−C,[4] and ultimately 16 10-phenyl-[11]-cytochalasans from two Japanese *Daldinia* species (which were identified as *D. chidiae* and *D. eschscholzii*, respectively).[5−8] Recently, we studied the chemical constituents of the European *D. concentrica* and isolated four novel squalene-type triterpenes named concentricol[8] and concentricols B, C, and D.[9]

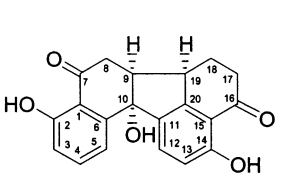
Previous work has indicated that their secondary metabolite constituents are of great chemotaxonomic significance in *Daldinia* and other genera of the Xylariaceae.[8,10,11] During previous studies based on HPLC profiling and other methods, several yet unknown metabolites were detected in the crude extracts, whose chemotaxonomic significance remained to be clarified. Recently, we encountered rather large amounts of the fruit bodies of *D. concentrica*, which enabled us to continue our studies on the chemical constituents of its EtOAc extract.

We wish to report here on the isolation and characterization of four new compounds, daldinone A (**1**), daldinone B (**2**), daldiniapyrone (**3**), and daldinialanone (**4**), along with 11 known compounds.

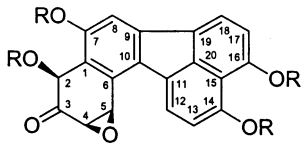
Results and Discussion

The EtOAc extract of the fruit bodies of *Daldinia concentrica* was subjected repeatedly to silica gel and Sephadex LH-20 column chromatography, followed by preparative HPLC to yield 15 compounds (**1**−**15**).

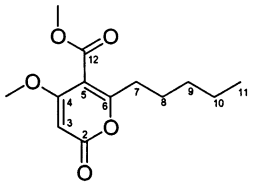
Daldinone A (**1**) was obtained as an oil, and its molecular formula was found to be $C_{20}H_{16}O_5$ by HREIMS (M^+, *m/z* 336.0982). The IR spectrum indicated absorption bands for hydroxyl (3356 cm^{-1}) and conjugated carbonyl (1644 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** (Table 1) showed five aromatic protons, three methylene groups, two methine groups, and two phenolic protons at low field (*δ* 11.0 and 12.4), indicating hydrogen bonding with the ketones. The ^{13}C NMR spectrum (Table 1) exhibited 20 carbon signals including two ketones, two phenols, and one tertiary alcohol (*δ* 80.0). The NMR data of **1** were similar to those of truncatone[12] and hortein.[13] The $^1H-^1H$ COSY spectrum of **1** showed correlations between H-8 and H-9, H-9 and H-19, H-19 and H-18, and H-18 and H-17 (Figure 1), indicating the partial structure [$-CH_2-CH-CH-CH_2-CH_2-$] in **1**. In addition, its HMBC spectrum showed correlations between C-10/H-5, H-8, H-9, and H-19. Therefore, the hydroxyl group could be located at C-10. The relative configurations were assigned by analysis of the NOESY data for **1**. NOE correlations were observed between H-5 and H-12, H-9 and H-19, and H-8*β* and H-18*β*, indicating that the configurations of both H-9 and H-19 were *cis*. Furthermore, the CD spectrum showed a negative first (255 nm: $\Delta\epsilon$ −23.9) and a positive second Cotton effect (229 nm: $\Delta\epsilon$ + 43.1), confirming that the configurations



1



2 R=H
16 R=Ac



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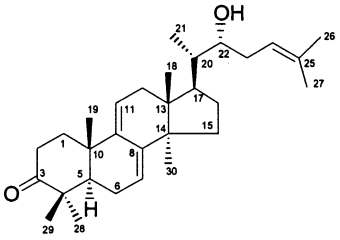

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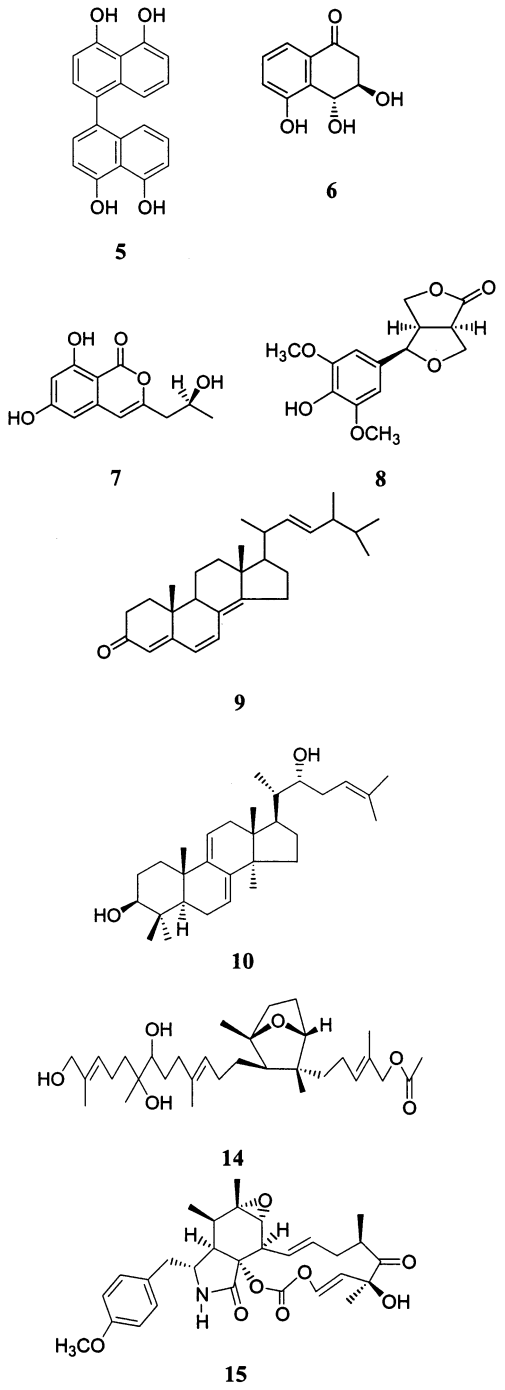

of all carbon centers C-9, C-10, and C-19 were α.[14] Therefore, compound **1** was determined to be 2,10,14-trihydroxy-9,19:10,11-bis-7,16-tetralone, a new natural product, for which the trivial name daldinone A was proposed.

Daldinone B (**2**) was obtained as a grayish powder, the molecular formula of which was found to be $C_{20}H_{12}O_6$ by HRFABMS ([M + H]$^+$, *m/z* 349.0691). The IR spectrum of **2** showed absorption bands at 3440 cm^{-1} (OH), 1676 cm^{-1} (C═O), and 1608 cm^{-1} (C═C). The ^{1}H NMR spectrum of **2** (Table 1) showed signals for five aromatic protons, one secondary alcohol (δ 5.75), and two protons bearing an oxirane ring (δ 3.81 and 4.00). Its ^{13}C NMR spectrum (Table 1) exhibited 20 carbon signals including one ketone and three phenol carbons. The proposed structure of **2** was proven partly from the ^{1}H−^{1}H COSY spectrum, which showed ^{1}H−^{1}H correlations between H-2 and H-4, H-4 and H-5 (Figure 2), and partly by the HMBC spectrum, which revealed long-range correlations between H-2/C-1, C-3, C-4, C-5, and C-6; H-4/C-1, C-2, and C-5; and H-5/C-3, C-4, and C-6.[15] In addition, an NOE correlation between H-5 and H-12 was observed, and the singlet signal of H-8 showed HMBC correlations with C-1, C-7, C-9, and C-10. Determination of the stereochemistry of the carbon centers C-2, C-4, and C-5 was accomplished by a NOESY experiment, which revealed NOE correlations between H-2 and H-4, and H-4 and H-5, pointing toward a *cis* or α-face configuration.

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** ($CDCl_3$) and **2** (CD_3OD)

	compound **1**		compound **2**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		115.3 s		126.0 s
2		161.8 s	5.75 dd (0.8, 2.2)	62.7 d
3	6.91 dd (1.1, 8.2)	117.6 d		198.5 s
4	7.51 dd (8.0, 8.2)	137.6 d	4.00 dd (2.2, 4.1)	57.1 d
5	7.15 dd (1.1, 8.0)	119.6 d	3.81 dd (0.8, 4.1)	55.7 d
6		143.0 s		127.4 s
7		202.7 s		155.7 s
8α	3.30 dd (5.8, 17.6)	35.7 t	7.60 s	113.0 d
8β	2.93 dd (2.2, 17.6)			
9	2.66 ddd (2.2, 5.8, 10.4)	55.9 d		127.3 s
10		80.0 s		130.1 s
11		148.8 s		127.9 s
12	7.82 d (8.2)	131.1 d	8.46 d (8.0)	129.1 d
13	6.92 dd (1.1, 8.2)	116.9 d	6.82 d (8.0)	110.6 d
14		161.0 s		156.4 s
15		114.1 s		112.6 s
16		203.0 s		157.5 s
17α	2.74 ddd (2.8, 4.9, 17.6)	38.0 t	6.86 d (7.4)	110.4 d
17β	2.58 dd (4.6, 17.9)			
18α	1.81 dd (3.9, 17.6)	28.3 t	7.81 d (7.4)	123.1 d
18β	2.44 ddd (2.5, 4.6, 17.6)			
19	2.80 dd (4.7, 10.4)	41.6 d		143.2 s
20		136.8 s		137.1 s
HO-2	12.4 s			
HO-14	11.0 s			

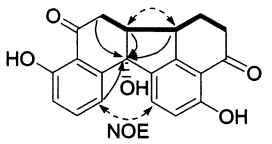

Figure 1. Important ^{1}H−^{1}H correlations (bold line), long-range ^{1}H−^{13}C correlations (arrows), and NOESY correlations of **1**.

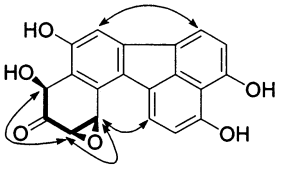

Figure 2. Important ^{1}H−^{1}H correlations (bold line) and NOESY correlations (arrows) of **2**.

Acetylation of compound **2** with acetic anhydride in pyridine afforded daldinone B tetraacetate (**16**), the molecular formula of which was established as $C_{28}H_{20}O_{10}$ by HRFABMS (M$^+$, *m/z* 516.1046). The IR spectrum of **16** was similar to that of **2** except for the disappearance of the signals corresponding to hydroxyl groups and the presence of absorption bands of acetyl groups at 1767 cm^{-1}. The ^{1}H NMR spectra of **16** showed signals for four acetyl groups, one of which gave resonances at higher field (δ 2.08) due to linkage with an aliphatic carbon; three other acetoxyl

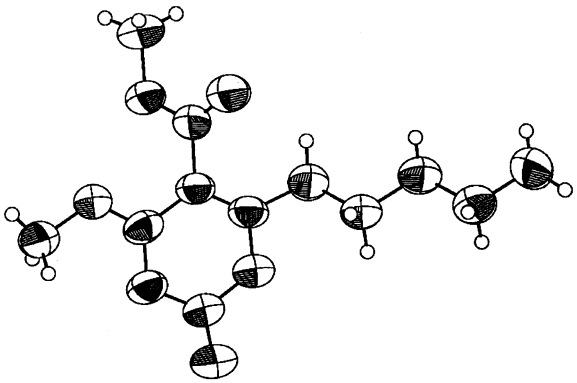

Figure 3. ORTEP drawing of **3**.

groups located at the benzene ring gave resonances at lower field (δ 2.40, 2.44, and 2.44). The spectral data of compound **16** indicated the presence of four hydroxyl groups in **2**, which was determined to be 14,16-dihydroxynaphthyl-9,-19:10,11-4,5-epoxy, 2,7-dihydroxy-3-tetralone.

Daldiniapyrone (**3**) was isolated as colorless needles with a molecular formula of $C_{13}H_8O_5$ (M^+, *m*/*z* 254.1152), which was established by HREIMS and ^{13}C NMR data. Its IR and UV spectra showed absorption bands at 1751 cm^{-1} (C═O) and 1645 cm^{-1} (C═C) and absorption maxima at 282 and 243 nm, respectively. The ^1H NMR spectrum of **3** showed an olefinic proton, two methoxyl groups, four methylenes, and one methyl group. The ^{13}C NMR spectrum of **3** revealed the signals of two ester carbonyls (δ 165.2 and 165.6), four olefinic carbons (δ 88.4, 110.4, 167.6, and 170.2), and two methoxyl groups. The $^1H-^1H$ COSY spectrum indicated $^1H-^1H$ correlations between H-11/H-10, H-10/H-9 and H-11, H-9/H-8 and H-10, and H-8/H-7 and H-9, suggesting that the partial structure from C-7 to C-11 was a *n*-pentyl group linked at C-6 due to the HMBC correlation between H-7 and C-6.[16] One methoxyl group was located at C-4 due to the HMBC correlation with C-4. The other methoxyl group showed a strong correlation with C-12 and a weak (four-bond) correlation with C-5 in the HMBC spectrum, indicating the presence of a carbomethoxyl group at C-5. Finally, the structure of **3** was determined by X-ray crystallographic analysis (Figure 3) as 4-methoxy-5-carbomethoxy-6-pentyl-2*H*-pyran-2-one. Although this compound, for which we propose the trivial name daldiniapyrone, constitutes an unprecedented metabolite, there are numerous examples of pyran metabolites from fungi[17] in the literature, including species in the Xylariaceae. For instance, γ-pyrone-3-acetic acid has been reported from a *Xylaria* species.[18]

Daldinialanone (**4**) was obtained as colorless needles. Its molecular formula was deduced as $C_{30}H_{46}O_2$ by HREIMS (M^+, *m*/*z* 438.3488). The IR spectrum of **4** showed absorption bands at 3556, 1709, and 1605 cm^{-1}, indicating the presence of OH, C═O, and C═C functional groups, respectively. The ^1H NMR spectrum of **4** (Table 2) showed five tertiary methyl groups, one secondary methyl group, two vinylic methyls, and three olefinic protons. The ^{13}C NMR spectrum of **4** (Table 2) revealed resonances for 30 carbons including one ketone and six olefinic carbons (Table 2). The ^1H and ^{13}C NMR spectral data of **4** were very similar to those of 21-hydroxylanosta-7,9(11),24-trien-3-one,[19] suggesting the presence of a lanosta-7,9(11),24-triene partial structure except for the chemical shifts of the side chain at C-20 through C-27. The NMR data of the partial structure at C-20 though C-27 were very similar to those of 3β,22-dihydroxylanosta-7,9(11),24-triene.[20]

Table 2. ^1H and ^{13}C NMR Data for Compounds **4** and **10** in $CDCl_3$

	compound **4**		compound **10**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	2.29 ddd (3.3, 5.8, 13.2)	36.6 t	2.00 dd (3.6, 9.9)	35.7 t
	1.76 (m)		1.47 m	
2	3.35 ddd (3.3, 4.7, 14.8)	34.8 t	1.72 m	27.8 t
	2.78 dt (5.8, 14.8)		1.66 m	
3		216.7 s	3.25 (dd, 4.1, 11.5)	78.9d
4		47.4 s		38.7 s
5	1.54 dd (3.9, 12.1)	50.7 d	1.10 dd (4.4, 11.3)	49.1 d
6	2.67 m	23.6 t	2.10 m	23.0 t
	2.20 dd (5.2, 12.1)		2.06 m	
7	5.52 d (6.6)	120.1 d	5.49 d (6.3)	120.4 d
8		142.2 s		142.2 s
9		144.5 s		145.9 s
10		37.2 s		37.4 s
11	5.40 d (6.3)	117.2 d	5.33 d (6.6)	116.2 d
12	2.24 brd (17.9)	37.7 t	2.22 brd (17.0)	37.8 t
	2.12 dd (6.3, 17.9)		2.12 dd (4.1, 17.0)	
13		44.1 s		44.1 s
14		49.8 s		50.0 s
15	1.68 m	31.6 t	1.65 m	31.6 t
	1.43 m		1.40 m	
16	1.89 m	26.9 t	1.88 m	26.9 t
	1.46 m		1.42 m	
17	1.65 m	47.7 d	1.64 m	47.7 d
18	0.62 s	15.6 q	0.60 s	15.6 q
19	1.20 s	22.0 q	1.01 s	22.7 q
20	1.81 ddd (3.3, 6.6, 14.3)	41.4 d	1.79 m	41.4 d
21	0.95 d (6.6)	12.4 q	0.94 d (6.6)	12.4 q
22	3.68 dt (3.9, 7.8)	73.3 d	3.68 dd (3.6, 12.4)	73.0 d
23	2.05 m	29.1 t	2.06 m	29.1 t
	1.31 m		1.31 m	
24	5.19 dt (1.4, 8.0)	121.2 d	5.19 m	121.3 d
25		135.2 s		135.2 s
26	1.66 s	18.0 q	1.66 s	18.0 q
27	1.75 s	26.0 q	1.75 s	26.0 q
28	1.13 s	22.4 q	0.88 s	15.8 q
29	1.09 s	25.3 q	0.98 s	28.1 q
30	0.88 s	25.5 q	0.88 s	25.6 q

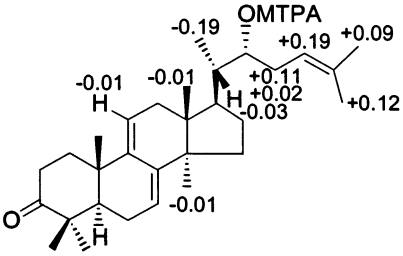

Figure 4. $\Delta\delta$ values [$\delta(-) - \delta(+)$] for the MTPA esters of compound **4**.

The absolute configuration of compound **4** at C-22 was determined by the modified Mosher's method.[21] Examination of the $\Delta\delta$ values [$\delta_{(-)} - \delta_{(+)}$] for (−)-MTPA ester **4a** and the (+)-MTPA ester **4b** (Figure 4) enabled assignment of the absolute configuration at C-22 to be *R*.[22,23] On the basis of the aforementioned data, daldinialanone (**4**) was identified as 22*R*-hydroxylanosta-7,9(11),24-trien-3-one.

The binaphthyl **5** is present in all *Daldinia* species so far examined[1] and is also a major metabolite of some species belonging to other Xylariaceous genera such as *Hypoxylon* and *Nemania*.[11] Daldinones A (**1**) and B (**2**) are probably both derived from the binaphthyl **5**. The tetralone **6**[24] was also previously encountered in another Xylariaceous fungus, *Hypoxylon mammatum* (which is actually an invalid synonym for *Entoleuca mammata* sensu Ju and Rogers).[11]

The isolation of orthosporin (**7**), which has already been encountered from a Xylariaceae species and several other fungi such as *Drechslera siccans*[25] and *Rhynchosporium orthosporum*,[26] is interesting insofar as it is the first record of an isocoumarin derivative from the fruit bodies of species in the Xylariaceae. Structurally related mellein derivatives of the dihydroisocoumarin type are fairly common in other genera of the Xylariaceae such as *Biscogniauxia* and *Hypoxylon*,[10,11] but such compounds are lacking in cultures of *Daldinia*.

Curuilignan D (**8**) was previously obtained only from the higher plant *Artemisia caruifolia*[27] and is herein reported as a fungal constituent for the first time. During our studies, the compound was obtained as a minor metabolite, but as revealed by TLC and analytical HPLC, it was also present in the crude extract prepared from the outermost section of the fruit bodies of *D. concentrica*. Therefore, the compound either constitutes a true fungal metabolite or is accumulated into the fungal fruit bodies from the plant substrate. Furthermore, the compound also bears a structural resemblance to xylobovide, a metabolite from cultures of another Xylariaceae species, *Xylaria obovata*.[28]

Compounds **4**, (*22E*)-cholesta-4,6,8(14),22-tetraen-3-one (**9**),[29] and 3β,22-dihydroxylanosta-7,9(11),24-triene (**10**)[20] belong to a class of fairly common fungal metabolites. Still, it is noteworthy that daldinialanone (**4**) has not been reported before from any other fungal species.

A major metabolite of this fungus had previously been reported to be concentricol (**11**),[8] and the isolation and characterization of concentricols B (**12**), C (**13**), and D (**14**), which coeluted with the metabolites treated herein as novel natural products, is reported elsewhere.[9]

Phenochalasin B (**15**) has been obtained previously as a metabolite with anticancer activity from cultures of a *Libertella* species[30] and the culture broth of a *Phomopsis* species.[31] Even though cytochalasins are widespread in Xylariaceae cultures,[10,11] they have been rarely encountered in the stromata (i.e., fruit bodies) of these fungi. The occurrence of cytochalasins in *D. concentrica* also further confirms that this species and the pantropical *D. eschscholzii* are related, as already deduced from their morphological similarities.[11] Out of more than 500 collections and 18 species of *Daldinia* we have so far examined, only some stromata of *D. eschscholzii* collected in Japan[5−8] have proven to contain extraordinary amounts of cytochalasins, while these compounds were only tentatively identified by HPLC-MS of crude extracts in other collections of *D. eschscholzii* and in *D. concentrica*.[8,11]

In summary, it is surprising that the fruit bodies of three different *Daldinia* species studied by us in the past differ significantly in their secondary metabolism. While two Japanese collections were found to be extremely rich in various polyketides (*D. childiae*)[3,4] or cytochalasins (*D. eschscholzii*),[5−8] respectively, the European *D. concentrica* yielded concentricols (**11**−**14**) in addition to metabolites of both the aforementioned types.[8,9] However, apart from the omnipresent binaphthyl derivatives, none of the three species investigated yielded the same major metabolites, and the main components of *D. childiae* were not even encountered in the fruit body extracts of *D. concentrica*.[11,12]

It remains to be seen whether these or related compounds are encountered in other species within the genus *Daldinia*, and HPLC profiling studies[8] using substances characterized in the present study as standards are presently under way.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-1000 polarimeter with MeOH or $CHCl_3$ as solvent. UV spectra were obtained on a Shimadzu UV-1650PC instrument in MeOH. CD spectra were measured on a JASCO J-725 spectrometer in EtOH. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C), using either $CDCl_3$ or CD_3OD as solvent. Chemical shifts are given relative to TMS (δ 0.00) as internal standard (^1H) and δ 77.0 (ppm) from $CDCl_3$ and δ 49.0 (ppm) from CD_3OD as standards (^{13}C). Mass spectra including high-resolution mass spectra were recorded on a JEOL JMS AX-500 spectrometer. X-ray reflection data were measured on a DIP Image diffractometer using Mo Kα radiation ($\lambda = 0.71073$ Å). Preparative medium-pressure liquid chromatography (MPLC) was performed with a Work-21 pump (Lab-Quatec Co., Ltd, Japan) and a Lobar column (Merck). Preparative HPLC was performed on a Shimadzu liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II or 5 SL-II column. Column chromatography was carried out on silica gel 60 (0.2−0.5 mm, 0.04−0.063 mm, Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech, $CHCl_3$−MeOH, 1:1).

Fungal Material. Fruit bodies of *Daldinia concentrica* were collected from trunks of *Fraxinus excelsior* in the Neandertal near Haan-Gruiten, North Rhine Westphalia, Germany, in April 2000 and June 2001 and identified by M.S., J. D. Rogers, and H. Wollweber. Voucher specimens are deposited at WSP (Herbarium of Washington State University, Pullman, WA), at Fuhlrott Museum, Wuppertal, Germany (No. Ww 3912), and at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan (No. VN02002).

Extraction and Isolation. The EtOAc extract (8.49 g) of fruit bodies of *D. concentrica* was divided into 10 fractions by column chromatography on silica gel (gradient of hexane−EtOAc and then EtOAc−MeOH). Fraction 2 (777.8 mg) was rechromatographed on SiO_2 (hexane−EtOAc, 3:1) to give compound **4** (239.2 mg) and a mixture (181.4 mg) that was separated by reversed-phase preparative HPLC (MeOH) to afford compound **9** (60.2 mg). Fraction 3 (585.8 mg) was rechromatographed by SiO_2 ($CHCl_3$−MeOH gradient) and then isolated by preparative HPLC (hexane−EtOAc, 3:1) to give compound **10** (39.4 mg). Fraction 4 (232.1 mg) was chromatographed on Sephadex LH-20 and then purified by MPLC (RP-18, CH_3CN-H_2O,7:3) to afford compound **3** (45.3 mg). Fraction 5 (387.4 mg) was chromatographed on Sephadex LH-20 to give two fractions. Fraction 5-1 was purified by reversed-phase preparative HPLC (CH_3CN-H_2O, 6:4) to give compound **1** (5.0 mg). Fraction 5-2 was found to contain compound **5** (172.2 mg) in the pure state. Fraction 6 (197.6 mg) was isolated by Sephadex LH-20 column chromatography, followed by reversed-phase preparative HPLC (MeOH−H_2O, 4:6), to give compound **8** (6.2 mg). Fraction 7 (323.0 mg) was purified by reversed-phase preparative HPLC (MeOH−H_2O, 6:4) to afford compound **7** (22.9 mg). Fraction 8 (1034.6 mg) was chromatographed on Sephadex LH-20 and divided into three fractions. Fraction 8-1 (835.4 mg) was purified by SiO_2 column chromatography ($CHCl_3$−MeOH−H_2O, 25:2:0.1) to afford compound **15** (25.2 mg) and two mixtures that were separated by reversed-phase preparative HPLC (CH_3CN-H_2O, 7:3) to give compound **14** (2.3 mg) from an intermediate fraction; another fraction from this separation was further purified by preparative HPLC (EtOAc) to yield compound **13** (28.6 mg). Compound **6** (11.6 mg) was isolated from fraction 8-2 by SiO_2 column chromatography ($CHCl_3$−MeOH−H_2O, 25:2.5:0.15). Fraction 8-3 was found to contain compound **2** (15.2 mg) in the pure state. Both fractions 9 (872.1 mg) and 10 (1573.0 mg) were chromatographed on Sephadex-LH 20 and then preparative MPLC ($CHCl_3$−MeOH−H_2O, 6.5:1.5:1, lower phase) to give compounds **12** (95.9 mg) and **11** (781.2 mg), respectively.

Daldinone A (1): oil; $[\alpha]_{20}^{D}$ −290.1° (*c* 0.77, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.3), 259 (4.0), 335 (3.7) nm; CD (EtOH) λ_{ext} nm ($\Delta\epsilon$) 255 (−23.9), 229 (+43.1); IR (KBr) ν_{max} 3356, 1644 cm^{-1}; ^1H and ^{13}C NMR, Table 1; EIMS *m/z* 336 $[M]^+$ (100), 319 (50), 294 (14), 215 (44), 173 (6), 121 (8); HREIMS *m/z* 336.0982 (calcd for $C_{20}H_{16}O_5$, 336.0998).

Daldinone B (2): grayish powder; $[\alpha]_{20}^{D}$ +300.9° (*c* 0.89, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.3), 244 (3.3), 346 (4.0) nm; CD (EtOH) λ_{ext} nm ($\Delta\epsilon$) 313 (−2.18), 258 (+2.45), 245 (−2.18); IR (KBr) ν_{max} 3440, 1676, 1609 cm^{-1}; ^1H and ^{13}C NMR, Table 1; FABMS *m/z* 349 $[M + H]^+$; HRFABMS *m/z* 349.0691 (calcd for $C_{20}H_{13}O_6$, 349.0712).

Acetylation of Daldinone B (16). Daldinone B (**2**) (3.8 mg) in pyridine (1 mL) was acetylated with acetic anhydride (1 mL), and workup as usual afforded a tetraacetate (**16**) (3.0 mg) as an oil: $[\alpha]_{20}^{D}$ −387.3° (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.6), 238 (4.6), 319 (4.4) nm; IR (KBr) ν_{max} 1767, 1697, 1598 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.72 (1H, d, *J* = 8.0 Hz, H-12), 7.94 (1H, d, *J* = 7.7 Hz, H-18), 7.88 (1H, s, H-8), 7.34 (1H, d, *J* = 8.0 Hz, H-13), 7.33 (1H, d, *J* = 7.7 Hz, H-17), 6.91 (1H, d, *J* = 2.6 Hz, H-2), 3.97 (1H, dd, *J* = 2.6, 4.0 Hz, H-4), 3.91 (1H, dd, *J* = 0.7, 4.0 Hz, H-5), 2.44 (6H, s, *CH*$_3$-CO-14 and *CH*$_3$CO-16), 2.40 (3H, s, *CH*$_3$CO-7), 2.08 (3H, s, *CH*$_3$CO-2); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 195.0 (s, C-3), 170.0 (s, CH_3*CO*-2), 169.2 (s, CH_3*CO*-14 and 16), 169.1 (s, CH_3*CO*-7), 148.5 (s, C-7), 146.8 (s, C-16), 146.5 (s, C-14), 142.7 (s, C-19), 136.1 (s, C-20), 135.2 (s, C-9), 132.7 (s, C-11), 132.3 (s, C-10), 128.5 (d, C-12), 128.3 (s, C-6), 126.7 (s, C-1), 122.5 (d, C-13), 121.6 (d, C-17 and C-18), 120.3 (d, C-8), 118.1 (s, C-15), 63.0 (d, C-2), 54.1 (d, C-5), 52.9 (d, C-4), 21.3 (q, *CH*$_3$CO-14 and 16), 20.9 (q, *CH*$_3$CO-7), 20.7 (q, *CH*$_3$CO-2); FABMS *m/z* 516 $[M]^+$; HRFABMS *m/z* 516.1046 (calcd for $C_{28}H_{20}O_{10}$, 516.1056).

4-Methoxy-5-carbomethoxy-6-pentyl-2*H*-pyran-2-one (3): colorless needles (hexane); mp 56−58 °C; UV (MeOH) λ_{max} (log ϵ) 209 (5.5), 243 (4.8), 282 (4.9) nm; IR (KBr) ν_{max} 1751, 1645, 1568 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 5.62 (1H, s, H-3), 3.88 (3H, s, OMe-4), 3.84 (3H, s, OMe-12), 2.56 (2H, dd, *J* = 7.4, 7.7 Hz, H-7), 1.68 (2H, m, H-8), 1.34 (2H, m, H-10), 1.33 (2H, m, H-9), 0.91 (3H, t, *J* = 6.9 Hz, H-11); ^{13}C NMR (CD_3OD, 150 MHz) δ 170.2 (s, C-4), 167.6 (s, C-5), 165.6 (s, C-12), 165.2 (s, C-2), 110.4 (s, C-6), 88.4 (d, C-3), 57.5 (q, OMe-4), 53.2 (q, OMe-12), 32.9 (t, C-7), 32.2 (t, C-9), 28.0 (t, C-8), 23.3 (t, C-10), 14.2 (q, C-11); EIMS *m/z* 254 $[M]^+$ (52), 223 (29), 211 (11), 198 (72), 183 (100), 166 (92), 156 (32), 128 (23), 125 (16), 66 (11), 43 (13); HREIMS *m/z* 254.1152 (calcd for $C_{13}H_{18}O_5$, 254.1154).

Crystal Data for 3. Data collection: DIP Image plate. Cell refinement: Scalepack (HKL). Data reduction: maXus.[32] Program used to solve structure: maXus.[32] Refinement: on F^2 full matrix least-squares. Diffractometer: DIP Image plate. $C_{13}H_{18}O_5$, MW 254, monoclinic, $P2_1/c$, a = 18.166(10) Å, b = 4.303(3) Å, c = 18.406 Å, α = 90.00°, β = 109.75(2)°, γ = 90.00°, V = 1354.1(13) Å^3, Z = 4, Mo Kα radiation, λ = 0.71073 Å, μ = 0.09 mm^{-1}, 815 reflections, 173 parameters; only coordinates of H atoms refined, R = 0.085, R_w = 0.423, S = 1.206.

22*R*-Hydroxylanosta-7,9(11),24-trien-3-one (4): colorless needles (MeOH); mp 145−148 °C; $[\alpha]_{20}^{D}$ +40.5° (*c* 1.48, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 236 (4.3), 243 (4.3), 251 (4.2), 279 (2.9), 342 (2.8) nm; IR (KBr) ν_{max} 3556, 1709, 1605 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) (Table 2); EIMS *m/z* 438 $[M]^+$ (43), 426 (15), 369 (40), 368 (100), 280 (25), 257 (29), 157 (12), 124 (22), 121 (15), 81 (59), 69 (46), 67 (54), 55 (42) 41 (26); HREIMS *m/z* 438.3488 (calcd for $C_{30}H_{46}O_2$, 438.3498).

Preparation of (+)-MTPA Ester of Compound 4 (4b). To a solution of compound **4** (10.6 mg) in dry CH_2Cl_2 (3 mL) were added (+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) (80 mg), dicyclohexylcarbodiimide (DCC) (85 mg), and (dimethylamino)pyridine (DMAP) (60 mg), and the mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated in vacuo, and then the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with brine, 1 N HCl, 5% $NaHCO_3$, and brine successively and dried over $MgSO_4$. The filtrate was evaporated in vacuo to give a crude oil (119.0 mg), which was purified by silica gel column chromatography (hexane−EtOAc, 5:1) to afford the (+)-MTPA ester (**4b**) (12.7 mg) as an oil: IR (KBr) ν_{max} 1741, 1709, 1601 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.54 (1H, d, *J* = 6.6 Hz, H-7), 5.39(1H, d, *J* = 6.3 Hz, H-11), 4.93 (1H, t, *J* = 1.4 Hz, H-24), 2.20 (1H, m, H-23), 2.06 (1H, m, H-23), 1.91 (1H, m, H-20), 1.59 (3H, s, H-27), 1.50 (3H, s, H-26), 1.21 (3H, s, H-19), 1.14 (3H, s, H-28), 1.09 (3H, s, H-29), 0.97 (3H, d, *J* = 6.6 Hz, H-21), 0.88 (3H, s, H-30), 0.61 (3H, s, H-18); FABMS *m/z* 654 $[M]^+$; HRFABMS *m/z* 654.3888 (calcd for $C_{40}H_{53}O_4F_3$, 654.3896).

Preparation of (−)-MTPA Ester of Compound 4 (4a). To a solution of compound **4** (15.0 mg) in dry CH_2Cl_2 (3 mL) were added (−)-MTPA (109.3 mg), DCC (117.6 mg), and DMAP (75.6 mg), and the mixture was stirred at room temperature overnight. The reaction mixture was treated as described above to give the crude oil (133 mg), which was purified by SiO_2 column chromatography (hexane−EtOAc, 4:1) to give the (−)-MTPA ester (**4a**) (16.7 mg) as an oil: IR (KBr) ν_{max} 1741, 1709, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.54 (3H, d, *J* = 6.6 Hz, H-7), 5.37 (1H, d, *J* = 6.0 Hz, H-11), 5.12 (1H, td, *J* = 3.0, 10.4 Hz, H-24), 2.22 (1H, m, H-23), 2.18 (1H, m, H-23), 1.88 (1H, m, H-20), 1.70 (3H, s, H-27), 1.59 (3H, s, H-26), 1.21 (3H, s, H-19), 1.14 (3H, s, H-28), 1.09 (3H, s, H-29), 0.87 (3H, s, H-30), 0.77 (3H, d, *J* = 6.6 Hz, H-21), 0.60 (3H, s, H-18); FABMS *m/z* 654 $[M]^+$; HRFABMS *m/z* 654.3882 (calcd for $C_{40}H_{53}O_4F_3$, 654.3896).

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