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CYTOCHALASINS FROM A *DALDINIA* SP. OF FUNGUS

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Key Word Index—*Daldinia* sp.; fungus; Ascomycetes; Xylariaceae; 10-phenyl-(11)-cytochalasans; 10-phenyl-22-oxa-(12)-cytochalasan; cytochalasins; hydroperoxide.

Abstract—Eleven new cytochalasins have been isolated from an unidentified *Daldinia* sp. of fungus. These were identified as 10 10-phenyl-(11)-cytochalasans and one 10-phenyl-22-oxa-(12)-cytochalasan. The latter metabolite is the first report of a 22-oxa-(12)-cytochalasan. Their structures were established by spectroscopic methods, in particular by high-field NMR spectroscopy.

INTRODUCTION

We have previously reported on the isolation and structure elucidation of five new 10-phenyl-(11)-cytochalasans (**1**–**5**) from an unidentified *Daldinia* sp. of fungus belonging to the Xylariaceae [1]. From the organic extract of the same species, we have now isolated a further 11 cytochalasins (**6**–**16**). Ten of these belong to the 10-phenyl-(11) class of cytochalasans (**6**–**15**) while the other is a 10-phenyl-22-oxa-(12)-cytochalasan (**16**). This latter compound is the first in a new class of cytochalasans. In this paper, we report on the isolation and characterization of these 11 new cytochalasins (**6**–**16**).

RESULTS AND DISCUSSION

Chromatography on silica gel and preparative HPLC of the ethyl acetate extract of the *Daldinia* sp. yielded a further 11 new cytochalasins (**6**–**16**). Their structures were established by detailed analysis of the NMR spectra, which included phase-sensitive DQF-COSY [2], HMQC [3], HMBC [4] and NOESY [5] experiments, and also by comparison with the NMR data for cytochalasins **1**–**5** [1].

The HRMS of cytochalasin **6** gave the molecular formula $C_{28}H_{35}NO_3$ ($[M]^+$ at m/z 433.2621). The intense fragments at m/z 342 and 91 in its MS suggested the presence of a benzyl group and its infrared spectrum showed the presence of hydroxyl, amidic and enone groups. The ^1H (Table 1) and ^{13}C (Table 2) NMR data are very similar to those of cytochalasin **1** [1] except for the absence of an oxygenated quaternary carbon with a tertiary methyl attached and the presence of a methine [δ_H 2.63 (*m*); δ_C 34.3] with a secondary methyl attached [δ_H 1.14 (*d*, $J = 7.1$ Hz); δ_C 17.6]. Moreover, in the ^1H NMR spectrum both H-19 [δ_H 6.52 (*dd*, $J = 16.1$, 6.8 Hz)] and H-20 [δ_H 7.05 (*dd*, $J = 16.1$, 1.5 Hz)] appear as double doublets. This evidence reveals cytochalasin **6** as (11)-cytochalasa-6(12),13,19-triene-1,21-dione-16,18-dimethyl-7-hydroxy-10-phenyl-(7*S**,13*E*,16*S**,18*R**,19*E*).

Cytochalasin **7** has the molecular formula $C_{28}H_{37}NO_3$ ($[M]^+$ at m/z 435.2772) corresponding to the dihydro derivative of **6**. Its NMR data (Tables 1 and 2) contained signals for two methylene groups [δ_H 1.42 (*m*), 1.33 (*m*); δ_C 27.1 (*t*) and δ_H 3.56 (*br dd*, $J = 17.0$, 8.0 Hz); 1.78 (*m*); δ_C 37.0 (*t*)] and a saturated ketone (δ_C 211.2) instead of the proton and carbon signals belonging to the enone moiety in **6**. Furthermore, the infrared absorption band attributable to the enone group of **6** was no longer present in the spectrum of **7**: The above spectral data indicates that **7** is the 19,20-dihydro derivative of **6**. The structure of **7** is therefore represented as (11)-cytochalasa-6(12),13-diene-1,21-dione-16,18-dimethyl-7-hydroxy-10-phenyl-(7*S**,13*E*,16*S**,18*S**).

The HRMS of cytochalasin **8** showed an $[M]^+$ at m/z 451.2721, indicating a molecular formula of $C_{28}H_{37}NO_4$. The NMR spectra of **8** are similar to cytochalasin **7** (Tables 1 and 2). The differences can be explained by the presence of a secondary alcohol [δ_H 3.49 (*br t*, $J = 7.3$ Hz); δ_C 70.3 (*d*)] in **8** relative to **7** and the absence of a methylene carbon. Thus **8** is (11)-cytochalasa-6(12),13-diene-1,21-dione-7,19-dihydroxy-16,18-dimethyl-10-phenyl-(7*S**,13*E*,16*S**,18*R**,19*R**).

Cytochalasin **9** has the molecular formula $C_{28}H_{35}NO_3$ ($[M]^+$ at m/z 433.2610). Comparison of the ^1H (Table 1) and ^{13}C (Table 2) NMR data of **9** with cytochalasin **1** [1] showed similarities. It is clear that **1** and **9** have the same macrocyclic ring, but **9** has a modified six-membered ring relative to **1**. This dissimilarity is explained by a trisubstituted double bond [δ_H 5.46 (*m*); δ_C 140.2 (*s*), 125.5 (*d*): δ_H 1.75 (*br s*); δ_C 20.0 (*q*)] in **9** relative to **1** instead of a secondary alcohol and

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Table 1. ^{1}H NMR data for cytochalasins **6**–**11**

H	6	7	8	9	10	11
2(NH)	5.47 (*br s*)	5.45 (*br s*)	5.47 (*br s*)	5.62 (*br s*)	5.87 (*br s*)	5.56 (*br s*)
3	3.30 (*m*)	3.30 (*m*)	3.33 (*m*)	3.25 (*m*)	3.44 (*m*)	3.25 (*m*)
4	3.33 (*dd*, 5.8, 2.5)	2.99 (*dd*, 6.0, 2.4)	3.05 (*dd*, 5.6, 2.7)	3.13 (*dd*, 5.3, 3.3)	3.15 (*dd*, 5.9, 2.9)	3.19 (*dd*, 5.6, 3.2)
5	2.79 (*qd*, 6.6, 5.8)	2.84 (*qd*, 6.8, 6.0)	2.83 (*qd*, 6.6, 5.6)	2.45 (*qd*, 7.1, 5.3)	2.50 (*m*)	2.44 (*qd*, 7.3, 5.6)
7	4.10 (*br d*, 10.0)	4.10 (*br d*, 10.0)	4.08 (*br d*, 10.0)	5.46 (*m*)	5.69 (*m*)	5.46 (*m*)
8	2.43 (*dd*, 10.0, 10.0)	2.47 (*dd*, 10.0, 10.0)	2.43 (*dd*, 10.0, 10.0)	2.58 (*m*)	2.63 (*br dd*, 10.0, 3.0)	2.57 (*m*)
10a	2.70 (*dd*, 13.4, 5.4)	2.65 (*dd*, 13.4, 5.1)	2.73 (*dd*, 13.7, 4.9)	2.79 (*dd*, 13.4, 4.6)	2.73 (*dd*, 13.4, 5.4)	2.78 (*dd*, 13.4, 4.9)
10b	2.50 (*dd*, 13.4, 9.0)	2.45 (*dd*, 13.4, 8.3)	2.45 (*dd*, 13.7, 7.8)	2.48 (*dd*, 13.4, 9.0)	2.52 (*dd*, 13.4, 8.5)	2.48 (*dd*, 13.4, 9.0)
11-Me	1.10 (*d*, 6.6)	1.01 (*d*, 6.8)	1.07 (*d*, 6.6)	1.18 (*d*, 7.1)	1.20 (*d*, 7.1)	1.17 (*d*, 7.3)
12	5.28 (*br s*)*Z*	5.26 (*br s*)*Z*	5.30 (*br s*)*Z*	1.75 (*br s*), Me	4.10 (*br d*, 13.2)	1.74 (*br s*), Me
	5.09 (*br s*)*E*	5.08 (*br s*)*E*	5.11 (*br s*)*E*		4.07 (*br d*, 13.2)	
13	5.85 (*ddd*, 15.4, 10.0, 1.6)	6.05 (*ddd*, 15.4, 10.0, 1.5)	6.04 (*ddd*, 15.4, 10.0, 1.2)	5.90 (*ddd*, 15.4, 10.0, 1.6)	5.90 (*ddd*, 15.6, 10.0, 1.4)	5.93 (*br dd*, 15.4, 10.0)
14	5.19 (*ddd*, 15.4, 11.0, 4.8)	5.40 (*ddd*, 15.4, 11.0, 4.4)	5.37 (*ddd*, 15.4, 11.0, 4.9)	5.05 (*ddd*, 15.4, 11.2, 4.4)	5.07 (*ddd*, 15.6, 11.0, 4.4)	4.98 (*ddd*, 15.4, 8.8, 6.8)
15*R*	2.02 (*m*)	2.04 (*m*)	2.06 (*m*)	2.02 (*m*)	2.02 (*m*)	1.93 (*m*, 2H)
15*S*	1.82 (*br q*, 11.0)	1.78 (*br q*, 11.0)	1.79 (*br q*, 11.0)	1.82 (*br q*, 11.2)	1.81 (*br q*, 11.0)	
16	1.45 (*m*)	1.42 (*m*)	1.51 (*m*)	1.32 (*m*)	1.34 (*m*)	1.54 (*m*)
17*S*	1.94 (*br dt*, 13.9, 4.1)	1.17 (*dt*, 14.5, 4.5)	1.65 (*m*)	1.97 (*br dd*, 13.1, 3.0)	1.95 (*br dd*, 13.2, 2.8)	
17*R*	1.54 (*dt*, 13.9, 3.4)	1.68 (*br d*, 14.5)	1.27 (*dt*, 13.5, 4.7)	1.63 (*dd*, 13.1, 3.8)	1.64 (*dd*, 13.2, 3.9)	3.88 (*m*)
18	2.63 (*m*)	1.56 (*m*)	1.63 (*m*)			2.67 (*m*)
19	6.52 (*dd*, 16.1, 6.8)	1.33 (*m*) *S*		6.56 (*d*, 15.9)	6.58 (*d*, 15.6)	6.19 (*dd*, 16.1, 7.2)
		1.42 (*m*)*R*	3.49 (*br t*, 7.3)*R*			
20	7.05 (*dd*, 16.1, 1.5)	3.56 (*br dd*, 17.0, 8.0) *S*	4.18 (*d*, 17.8) *S*	7.31 (*d*, 15.9)	7.23 (*d*, 15.6)	7.16 (*dd*, 16.1, 1.5)
		1.78 (*m*)*R*	1.61 (*dd*, 17.8, 7.3)*R*			
22-Me	1.03 (*d*, 7.1)	0.97 (*d*, 6.8)	1.00 (*d*, 7.5)	1.05 (*d*, 7.1)	1.05 (*d*, 7.1)	1.00 (*d*, 7.1)
23-Me	1.14 (*d*, 7.1)	0.89 (*d*, 7.1)	1.01 (*d*, 7.5)	1.39 (*s*)	1.39 (*s*)	1.12 (*d*, 6.8)
2′,6′	7.13 (*m*)	7.08 (*m*)	7.09 (*m*)	7.12 (*m*)	7.12 (*m*)	7.12 (*m*)
3′,5′	7.31 (*m*)	7.30 (*m*)	7.30 (*m*)	7.30 (*m*)	7.29 (*m*)	7.30 (*m*)
4′	7.23 (*m*)	7.23 (*m*)	7.26 (*m*)	7.23 (*m*)	7.22 (*m*)	7.23 (*m*)
OH	1.78 (*br s*)	1.72 (*br s*)	2.71 (*br s*)	2.23 (*br s*)	2.62 (*br s*)	2.02 (*br s*)
			1.73 (*br s*)		1.86 (*br s*)	

Numbers in parentheses are coupling constants (*J*) in Hz.
Assignments confirmed by two-dimensional experiments (phase-sensitive DQF-COSY, HMQC, HMBC and NOESY).

Table 2. ^{13}C NMR data for cytochalasins **6**–**11**

C	**6**	**7**	**8**	**9**	**10**	**11**
1	173.4 *s*	174.0 *s*	173.1 *s*	174.1 *s*	174.3 *s*	174.4 *s*
3	53.1 *d*	52.6 *d*	52.5 *d*	54.9 *d*	54.8 *d*	54.8 *d*
4	44.9 *d*	46.4 *d*	*45.2 *d*	49.0 *d*	48.6 *d*	48.6 *d*
5	31.7 *d*	31.8 *d*	31.9 *d*	34.9 *d*	34.0 *d*	34.8 *d*
6	148.6 *s*	148.6 *s*	148.3 *s*	140.2 *s*	143.2 *s*	140.1 *s*
7	71.6 *d*	71.5 *d*	71.3 *d*	125.5 *d*	128.5 *d*	125.6 *d*
8	51.8 *d*	51.3 *d*	51.5 *d*	50.0 *d*	49.3 *d*	49.9 *d*
9	63.4 *s*	64.0 *s*	63.9 *s*	68.4 *s*	68.7 *s*	68.8 *s*
10	44.2 *t*	44.0 *t*	43.7 *t*	45.1 *t*	44.9 *t*	45.1 *t*
11	13.2 *q*	13.0 *q*	13.2 *q*	13.5 *q*	12.2 *q*	13.4 *q*
12	114.0 *t*	114.3 *t*	114.5 *t*	20.0 *q*	63.2 *t*	19.9 *q*
13	127.0 *d*	127.5 *d*	128.2 *d*	128.5 *d*	128.2 *d*	128.5 *d*
14	138.6 *d*	136.9 *d*	136.8 *d*	135.0 *d*	135.2 *d*	135.0 *d*
15	42.9 *t*	42.6 *t*	43.0 *t*	42.9 *t*	43.0 *t*	40.9 *t*
16	28.7 *d*	*27.2 *d*	27.8 *d*	29.3 *d*	29.2 *d*	33.3 *d*
17	46.7 *t*	37.9 *t*	38.3 *d*	54.6 *t*	54.7 *t*	79.4 *d*
18	34.3 *d*	31.0 *d*	37.0 *d*	73.1 *s*	73.1 *s*	43.2 *d*
19	154.5 *d*	*27.1 *t*	70.3 *d*	152.3 *d*	153.1 *d*	148.6 *d*
20	132.2 *d*	37.0 *t*	*45.3 *t*	132.1 *d*	131.7 *d*	133.6 *d*
21	196.5 *s*	211.2 *s*	212.1 *s*	200.0 *s*	199.6 *s*	197.8 *s*
22	26.2 *q*	25.7 *q*	26.1 *q*	25.7 *q*	25.7 *q*	17.0 *q*
23	17.6 *q*	20.4 *q*	14.2 *q*	25.6 *q*	25.4 *q*	11.6 *q*
1′	137.3 *s*	136.7 *s*	136.4 *s*	137.3 *s*	137.1 *s*	137.3 *s*
2′(2C)	129.2 *d*	129.4 *d*	129.6 *d*	129.1 *d*	129.3 *d*	129.1 *d*
3′(2C)	128.8 *d*	128.8 *d*	128.8 *d*	128.8 *d*	128.8 *d*	128.8 *d*
4′	126.9 *d*	127.0 *d*	127.1 *d*	126.9 *d*	126.9 *d*	126.9 *d*

*Values may be interchanged.
Assignments confirmed by two-dimensional experiments (HMQC and HMBC).

exomethylene groups. Therefore, cytochalasin **9** was formulated as (11)-cytochalasa-6,13,19-triene-1,21-dione-18-hydroxy-16,18-dimethyl-10-phenyl-(6*Z*,13*E*,16*S**, 18*S**,19*E*).

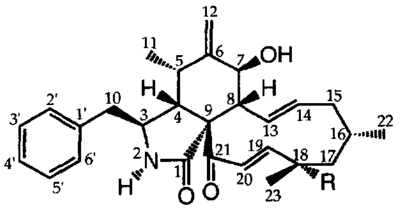
Cytochalasin **10** has the molecular formula $C_{28}H_{35}NO_4$ ($[M]^+$ at *m/z* 449.2565) and it is obvious from its NMR spectra (Tables 1 and 2) that it is the C-12 primary alcohol derivative [δ_H 4.10 (*br d*, $J = 13.2$ Hz); 4.07 (*br d*, $J = 13.2$ Hz): δ_C 63.2 (*t*)] of cytochalasin **9**. Thus, cytochalasin **10** is (11)-cytochalasa-6,13,19-triene-1,21-dione-12,18-dihydroxy-16,18-dimethyl-10-phenyl-(6*E*,13*E*,16*S**,18*S**,19*E*).

The molecular formula of cytochalasin **11** is $C_{28}H_{35}NO_3$, as determined by HRMS ($[M]^+$ at *m/z* 433.2622). The NMR data (Tables 1 and 2) for **11** reveal that it has the same six-membered ring as cytochalasin **9**, but the macrocyclic ring is a little different from the cytochalasins isolated to date. The difference can be clarified by a C-17 secondary alcohol [δ_H 3.88 (*m*); δ_C 79.4] in **11** relative to the macrocyclic ring system of cytochalasin **6**. The NOEs observed between (i) H-17 and H-20 and (ii) H-17 and H_2-15, indicate the configuration at C-17 is *R*. Cytochalasin **11** is therefore (11)-cytochalasa-6,13,19-triene-1,21-dione-17-hydroxy-16,18-dimethyl-10-phenyl-(6*Z*,13*E*,16*S**,17*R**,18*S**,19*E*).

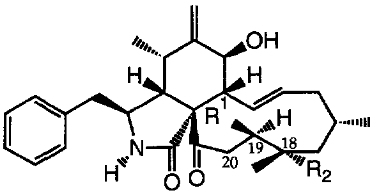
The NMR data (Tables 3 and 4) of cytochalasin **12**, $C_{28}H_{35}NO_4$ ($[M]^+$ at *m/z* 449.2567), show that it has the same macrocyclic ring as **1** [1], but differs from **1** by the presence of a trisubstituted epoxide [δ_H 2.91 (*d*, $J = 5.9$ Hz); δ_C 62.2 (*d*), 57.7 (*s*)] and a tertiary methyl (δ_H 1.22 (*s*, 3H); δ_C 19.5 (*q*)] in the six-membered ring instead of a secondary alcohol and exomethylene groups. The NOEs observed between (i) H-7 and H_3-12, (ii) H-7 and H-13 and (iii) H-3 and H_3-12 indicate the configuration at C-7 is *S* (*cis*-6β,7β-epoxide). Thus, cytochalasin **12** is (11)-cytochalasa-13,19-diene-1,21-dione-6,7-epoxy-18-hydroxy-16,18-dimethyl-10-phenyl-(7*S**,13*E*,16*S**, 18*S**,19*E*).

When comparing the ^{1}H (Table 3) and ^{13}C (Table 4) NMR data of cytochalasin **13**, $C_{28}H_{37}NO_5$ ($[M]^+$ at *m/z* 467.2663), with cytochalasins **3** [1] and **12** (Tables 3 and 4) it is clear that **13** is a 10-phenyl-(11)-cytochalasan containing the macrocyclic ring of **3** and the six membered ring of **12**. Cytochalasin **13** is therefore (11)-cytochalas-13-ene-1,21-dione-6,7-epoxy-18,19-dihydroxy-16,18-dimethyl-10-phenyl-(7*S**,13*E*,16*S**,18*S**, 19*R**).

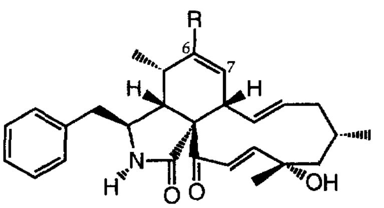
Analysis of the NMR spectra (Tables 3 and 4) for cytochalasin **14**, $C_{28}H_{33}NO_5$ ($[M]^+$ at *m/z* 463.2364), and **1** [1] indicates that these are 10-phenyl-(11)-cytochalasans with similar structures. The most significant difference is that the ^{13}C NMR spectrum (Table 4) of cytochalasin **14** contains one more carbonyl carbon (δ_C 209.9 (*s*)) and one less methylene carbon relative to the



R
1 OH
6 H



	R^1	R^2		R^1	R^2
2	H	OH	**5**	OAc	OH
3	OH	OH	**7**	H	H
4	OMe	OH	**8**	OH	H



R
9 Me
10 CH_2OH

^{13}C NMR spectrum of **1**. From a more detailed analysis of the one- and two-dimensional NMR spectra of **14** it is clear that it contains a carbonyl group at C-17. Thus, cytochalasin **14** is (11)-cytochalasa-6(12),13,19-triene-1,17,21-trione-7,18-dihydroxy-16,18-dimethyl-10-phenyl-(7*S**,13*E*,16*S**,18*R**,19*E*).

Cytochalasin **15** has the molecular formula $C_{28}H_{35}NO_5$ ($[M]^+$ at *m/z* 465.2514). Comparison of the NMR data (Tables 1–4) shows that **15** is a 10-phenyl-(11)-cytochalasan with the same macrocyclic ring as **11**, but with a different six-membered ring from the cytochalasins isolated to date. The NMR spectra show that this ring contains a tetrasubstituted double bond (δ_C 130.9, 128.3), two vinyl methyl groups [δ_C 17.3, 14.1; δ_H 1.73, 1.45 (both *br s*)] and an oxygenated methine [δ_C 83.3; δ_H 4.32 (*br d*, $J = 10.0$ Hz)]. The six-membered ring carbon skeleton shown for structure **15** was suggested by this data and confirmed by two-dimensional experiments. The 7-OH derivative of cytochalasin **15** was ruled out because when the ^{13}C NMR data for **15** (Table 4) were compared with cytochalasans [6–8] containing the same six-membered ring part structure, they revealed that C-7 in cytochalasin **15** (δ_C 82.0 in DMSO-d_6 at 200 MHz) is substantially more deshielded (*ca* 14 ppm) than expected for an allylic alcohol and is more characteristic of an allylic carbon bearing a hydroperoxy group [9–11]. This is supported by the signal at δ_H 7.36 (*s*) as such low field signals are characteristic of a hydroperoxy group [12, 13]. Moreover, the HRMS gave ions at *m/z* 449.2559 (6%) for $C_{28}H_{35}NO_4$ and 431.2456 (5%) for $C_{28}H_{33}NO_3$ which reflect fragmentations of $[M - O]^+$ and $[M - H_2O_2]^+$, respectively, and support the presence of a hydroperoxy group [9, 12]. This evidence reveals cytochalasin **15** as (11)-cytochalasa-5,13-19-triene-1,21-dione-7-hydroperoxy-17-hydroxy-16,18-dimethyl-10-phenyl-(5*Z*,7*S**,13*E*,16*S**,17*R**,18*S**,19*E*). This is the first report of a cytochalasan hydroperoxide. However, cytochalasin U, isolated from *Phoma exigua* var. *heteromorpha*, has a peroxyester group [14].

Cytochalasin **16** has the molecular formula $C_{28}H_{35}NO_5$ ($[M]^+$ at *m/z* 465.2513), as determined by HRMS. The ^1H and ^{13}C NMR data (Tables 3 and 4) are very similar to those for cytochalasin **1** [1]. The differences in NMR data can be explained by the enone system in **1** being changed to an α,β-unsaturated ester in **16** [δ_H 7.23 (*d*, $J = 15.9$ Hz); 5.75 (*d*, $J = 15.9$ Hz). δ_C 166.9 (*s*), 159.0, 118.9 (both *d*)], thus increasing the size of the macrocyclic ring in **16** to 12 atoms. This is supported by the additional oxygen in the molecular formula of **16** relative to **1** and also the large downfield shift of C-9 (20.3 ppm) in **16** relative to **1**. Thus, cytochalasin **16** is 22-oxa-(12)-cytochalasa-6(12),13,19-triene-1,21-dione-7,18-dihydroxy-16,18-dimethyl-10-phenyl-(7*S**,13*E*,16*S**,18*S**,19*E*). This is the first report of a 22-oxa-(12)-cytochalasan.

NOESY experiments on cytochalasins **6**–**16** reveal that they have the same relative stereochemistry and the same conformation for the macrocyclic ring as cytochalasins **1**–**5**, even cytochalasin **16** with 12 atoms in the macrocyclic ring. These results further support the deduction made by Buchanan *et al.* [1] for cytochalasins **1**–**5**: "despite the large size of the macrocyclic ring and different functionalities the macrocyclic framework is a stable, relatively rigid structural unit and is not as flexible as might be expected."

The cytochalasins isolated from this fungal source, which possess a C-20 methylene group (**2**–**5**, **7**, **8**, **13**), have their H-20*S* proton in an unusually downfield-shifted position. This is because the H-20*S* proton is lying in the plane of the adjacent carbonyl groups π-system and thus experiences deshielding by the anisotropic effect.

EXPERIMENTAL

General. TLC and PLC: Merck precoated silica gel 60 F_{254} and visualized under UV light (254 nm) and by spraying with 30% H_2SO_4 and heating. R_f values refer to EtOAc as eluent. Flash CC: silica gel 60 (40–63 μm); HPLC: Chemcosorb 5Si-U 10 × 250 mm (B); NMR ^1H, 600 MHz, ^{13}C, 150 MHz: $CDCl_3$ soln relative to TMS at δ_H 0 and $CDCl_3$ at δ_C 77.0. Multiplicities were determined by DEPT experiments and/or by an HMBC experiment. IR: $CHCl_3$; $[\alpha]_D$: $CHCl_3$ or MeOH soln; EIMS: 70 eV.

Fungus. Daldinia sp. was collected at two separate sites in Tokushima, both samples were found growing on the same host plant, *Quercus acutissima*. One sample was

Table 3. ^{1}H NMR data for cytochalasins **12–16**

H	**12**	**13**	**14**	**15***	**16**
2(NH)	5.86 (*br s*)	5.99 (*br s*)	5.41 (*br s*)	5.97 (*br s*)	5.78 (*br s*)
3	3.63 (*m*)	3.66 (*m*)	3.29 (*m*)	3.42 (*br t*, 7.6)	3.31 (*m*)
4	3.20 (*br d*, 6.2)	3.01 (*dd*, 6.2, 1.5)	3.24 (*dd*, 5.6, 2.4)	3.59 (*br s*)	3.04 (*dd*, 5.4, 2.7)
5	1.78 (*qd*, 7.1, 6.2)	1.79 (*qd*, 7.3, 6.2)	2.77 (*qd*, 6.6, 5.6)		3.27 (*qd*, 6.6, 5.4)
7	2.91 (*d*, 5.9)	2.91 (*d*, 5.9)	4.06 (*br d*, 10.0)	4.32 (*br d*, 10.0)	3.93 (*br d*, 10.3)
8	2.12 (*dd*, 9.5, 5.9)	2.13 (*dd*, 9.8, 5.9)	2.40 (*dd*, 10.0, 10.0)	2.70 (*dd*, 10.0, 10.0)	3.32 (*dd*, 10.3, 10.3)
10a	2.60 (*dd*, 13.4, 6.6)	2.62 (*dd*, 13.7, 5.9)	2.66 (*dd*, 13.4, 5.4)	2.64 (*dd*, 13.3, 7.6)	2.85 (*dd*, 13.8, 5.6)
10b	2.56 (*dd*, 13.4, 7.6)	2.52 (*dd*, 13.7, 7.1)	2.46 (*dd*, 13.4, 8.8)	2.61 (*dd*, 13.3, 7.6)	2.82 (*dd*, 13.8, 8.5)
11-Me	0.96 (*d*, 7.1)	1.02 (*d*, 7.3)	1.00 (*d*, 6.6)	1.45 (*br s*)	1.03 (*d*, 6.6)
12	1.22 (*s*),Me	1.22 (*s*),Me	5.25 (*br s*)*Z*	1.73 (*br s*),Me	5.33 (*br s*)*Z*
			5.08 (*br s*)*E*		5.13 (*br s*)*E*
13	5.96 (*ddd*, 15.6, 9.5, 1.2)	6.22 (*ddd*, 15.4, 9.8, 1.2)	5.80 (*ddd*, 15.4, 10.0, 1.4)	6.00 (*br dd*, 15.4, 10.0)	5.92 (*ddd*, 15.4, 10.3, 2.0)
14	5.07 (*ddd*, 15.6, 10.7, 4.6)	5.20 (*ddd*, 15.4, 11.0, 4.4)	5.19 (*ddd*, 15.4, 11.0, 4.6)	5.09 (*ddd*, 15.4, 9.0, 6.5)	5.40 (*ddd*, 15.4, 11.1, 3.7)
15*R*	2.04 (*m*)	2.05 (*m*)	2.08 (*m*)	1.98 (*m*, 2H)	2.16 (*m*)
15*S*	1.84 (*br q*, 10.7)	1.83 (*br q*, 11.0)	2.58 (*br q*, 11.0)		2.02 (*ddd*, 14.4, 11.1, 9.0)
16	1.31 (*m*)	1.26 (*m*)	2.70 (*ddd*, 11.0, 6.8, 1.8)	1.54 (*m*)	1.33 (*m*)
17*S*	1.98 (*br dd*, 13.0, 2.2)	1.82 (*br d*, 12.8)			1.78 (*dd*, 13.9, 5.6)
17*R*	1.65 (*dd*, 13.0, 3.9)	1.26 (*m*)		3.81 (*m*)	1.68 (*dd*, 13.9, 2.2)
18				2.73 (*m*)	
19	6.62 (*d*, 15.9)		6.36 (*d*, 15.6)	6.40 (*dd*, 16.0, 7.4)	7.23 (*d*, 15.9)
		3.67 (*d*, 7.8)*R*			
20	7.23 (*d*, 15.9)	4.22 (*d*, 18.8)*S*	6.97 (*d*, 15.6)	6.88 (*dd*, 16.0, 1.3)	5.75 (*d*, 15.9)
		1.80 (*dd*, 18.8, 7.8)*R*			
22-Me	1.06 (*d*, 7.1)	1.05 (*d*, 6.6)	1.22 (*d*, 6.8)	1.02 (*d*, 7.1)	1.05 (*d*, 7.1), 23-Me
23-Me	1.41 (*s*)	1.29 (*s*)	1.62 (*s*)	1.16 (*d*, 6.8)	1.35 (*s*), 24-Me
2′,6′	7.14 (*m*)	7.10 (*m*)	7.11 (*m*)	7.18 (*m*)	7.17 (*m*)
3′,5′	7.32 (*m*)	7.32 (*m*)	7.30 (*m*)	7.33 (*m*)	7.32 (*m*)
4′	7.25 (*m*)	7.27 (*m*)	7.23 (*m*)	7.25 (*m*)	7.25 (*m*)
OH	1.64 (*br s*)	2.66 (*br s*)	4.71 (*s*)		
		1.71 (*br s*)	1.65 (*br d*, 2.5)		
OOH				7.36 (*s*)	

Numbers in parentheses are coupling constants (*J*) in Hz.
Assignments confirmed by two-dimensional experiments (phase-sensitive DQF-COSY, HMQC, HMBC and NOESY).
*$CDCl_3$ included three drops of CD_3OD.

Table 4. ^{13}C NMR data for cytochalasins **12**–**16**

C	**12**	**13**	**14**	**15***	**16**
1	174.3 *s*	173.9 *s*	172.6 *s*	174.7 *s*	171.4 *s*
3	53.6 *d*	53.1 *d*	53.0 *d*	59.0 *d*	53.7 *d*
4	46.9 *d*	46.8 *d*	45.1 *d*	47.4 *d*	49.6 *d*
5	36.1 *d*	36.3 *d*	31.6 *d*	128.3 *s*	31.5 *d*
6	57.7 *s*	57.5 *s*	148.4 *s*	130.9 *s*	148.4 *s*
7	62.2 *d*	61.8 *d*	71.4 *d*	83.3 *d*†	69.8 *d*
8	50.1 *d*	49.9 *d*	51.7 *d*	47.6 *d*	49.9 *d*
9	65.5 *s*	65.8 *s*	64.1 *s*	63.3 *s*	83.8 *s*
10	44.9 *t*	44.4 *t*	44.2 *t*	42.6 *t*	44.5 *t*
11	12.5 *q*	12.5 *q*	13.0 *q*	17.3 *q*	13.8 *q*
12	19.5 *q*	19.5 *q*	114.4 *t*	14.1 *q*	114.9 *t*
13	127.5 *d*	128.9 *d*	129.8 *d*	128.6 *d*	124.4 *d*
14	135.6 *d*	133.9 *d*	134.9 *d*	135.0 *d*	140.3 *d*
15	43.0 *t*	42.9 *t*	38.3 *t*	41.3 *t*	44.0 *t*
16	29.0 *d*	29.7 *d*	42.9 *d*	33.0 *d*	30.0 *d*
17	54.9 *t*	45.1 *t*	209.9 *s*	79.4 *d*	53.6 *t*
18	73.1 *s*	75.4 *s*	78.6 *s*	43.0 *d*	72.8 *s*
19	154.1 *d*	71.2 *d*	143.3 *d*	152.1 *d*	159.0 *d*
20	131.4 *d*	43.3 *t*	134.3 *d*	132.0 *d*	118.9 *d*
21	198.1 *s*	212.0 *s*	197.4 *s*	196.4 *s*	166.9 *s*
22	25.6 *q*	25.6 *q*	19.8 *q*	17.1 *q*	27.1 *q* (C-23)
23	25.8 *q*	23.1 *q*	23.5 *q*	11.4 *q*	22.3 *q* (C-24)
1′	136.7 *s*	136.1 *s*	137.0 *s*	137.1 *s*	137.3 *s*
2′(2C)	129.3 *d*	129.7 *d*	129.2 *d*	129.2 *d*	129.1 *d*
3′(2C)	128.9 *d*	128.9 *d*	128.9 *d*	128.7 *d*	128.9 *d*
4′	127.1 *d*	127.2 *d*	127.0 *d*	126.9 *d*	127.0 *d*

Assignments confirmed by two-dimensional experiments (HMQC and HMBC).
*$CDCl_3$ included three drops of CD_3OD.
†δ_C 82.0 in DMSO-d_6 at 200 MHz.

collected in June 1992 (259 g) and the other sample in June 1993 (515 g). The *Daldinia* sp. remains unidentified (the identity of this fungus was very difficult to determine, but it could very tentatively be identified as *D. vernicosa*). A voucher specimen is deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. The fresh material (259 g and 515 g) was extracted with EtOAc to yield 11.43 g and 22.80 g of crude extract, respectively. Based on TLC and ^{1}H NMR, the two extracts were combined (34.23 g) and then chromatographed by flash CC over silica gel using an *n*-hexane–EtOAc gradient. The most polar fractions eluted were further chromatographed by flash CC and PLC and final purification by HPLC to give cytochalasins **6** (37 mg, R_f 0.61), **7** (4 mg, R_f 0.54), **8** (4 mg, R_f 0.48), **9** (37 mg, R_f 0.48), **10** (7 mg, R_f 0.17), **11** (34 mg, R_f 0.53), **12** (5 mg, R_f 0.27), **13** (9 mg, R_f 0.21), **14** (2 mg, R_f 0.44), **15** (2 mg, R_f 0.46) and **16** (6 mg, R_f 0.25).

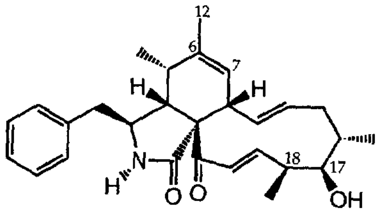
(11)-*Cytochalasa*-6(12),13,19-*triene*-1,21-*dione*-16,18-*dimethyl*-7-*hydroxy*-10-*phenyl*-(7*S**,13*E*,16*S**,18*R**,19*E*) (**6**). Amorphous solid, $[\alpha]_D$ −16.1° ($CHCl_3$; *c* 0.31). HRMS: *m/z* 433.2621 $[M]^+$ calcd for $C_{28}H_{35}NO_3$: 433.2617; EIMS *m/z* (rel. int.): 433 $[M]^+$ (33), 405 (5), 342 (100), 324 (19), 314 (23), 296 (16), 200 (7), 178 (20), 91 (45); IR ν_{max} cm^{-1}: 3250 (NH, OH), 1688 (C=O), 1620; ^{1}H and ^{13}C NMR: Tables 1 and 2.

(11)-*Cytochalasa*-6(12),13-*diene*-1,21-*dione*-16,18-*dimethyl*-7-*hydroxy*-10-*phenyl*-(7*S**,13*E*,16*S**,18*S**) (**7**). Amorphous solid, $[\alpha]_D$ +66.5° (MeOH; *c* 0.18). HRMS: *m/z* 435.2772 $[M]^+$ calcd for $C_{28}H_{37}NO_3$: 435.2774; EIMS *m/z* (rel. int.): 435 $[M]^+$ (40), 417 (13), 407 (15), 344 (100), 326 (66), 260 (16), 91 (42); IR ν_{max} cm^{-1}: 3290 (NH, OH), 1690 (C=O); ^{1}H and ^{13}C NMR: Tables 1 and 2.

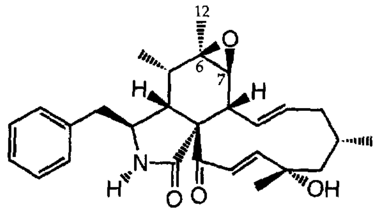
(11)-*Cytochalasa*-6(12),13-*diene*-1,21-*dione*-7,19-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*-(7*S**,13*E*,16*S**,18*R**,19*R**) (**8**). Amorphous solid, $[\alpha]_D$ +70.3° (MeOH; *c* 0.13). HRMS: *m/z* 451.2721 $[M]^+$ calcd for $C_{28}H_{37}NO_4$: 451.2723; EIMS *m/z* (rel. int.): 451 $[M]^+$ (28), 433 (44), 360 (95), 342 (100), 324 (44), 300 (41), 216 (62), 174 (38), 91 (92); IR ν_{max} cm^{-1}: 3340 (NH, OH), 1698 (C=O); ^{1}H and ^{13}C NMR: Tables 1 and 2.

(11)-*Cytochalasa*-6,13,19-*triene*-1,21-*dione*-18-*hydroxy*-16,18-*dimethyl*-10-*phenyl*-(6*Z*,13*E*,16*S**,18*S**,19*E*) (**9**). Amorphous solid, $[\alpha]_D$ −98.4° ($CHCl_3$; *c* 0.34). HRMS: *m/z* 433.2610 $[M]^+$ calcd for $C_{28}H_{35}NO_3$: 433.2617; EIMS *m/z* (rel. int.): 433 $[M]^+$ (34), 416 (56), 397 (24), 342 (39), 324 (46), 270 (23), 200 (94), 173 (40), 148 (56), 133 (43), 91 (100); IR ν_{max} cm^{-1}: 3340 (NH, OH), 1688 (C=O), 1622; ^{1}H and ^{13}C NMR: Tables 1 and 2.

(11)-*Cytochalasa*-6,13,19-triene-1,21-dione-12,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*-(6*E*,13*E*,16*S**,18*S**,19*E*) (**10**). Amorphous solid, $[\alpha]_D$ −70.4° ($CHCl_3$;



11



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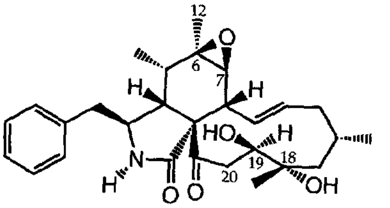
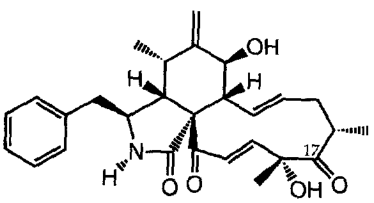


13



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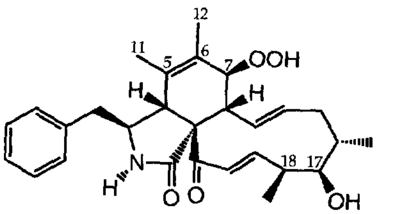
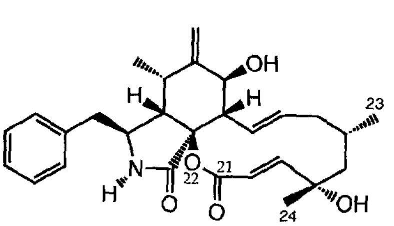


15



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c 0.31). HRMS: *m/z* 449.2565 $[M]^+$ calcd for $C_{28}H_{35}NO_4$: 449.2566; EIMS *m/z* (rel. int.): 449 $[M]^+$ (21), 431 (57), 413 (48), 395 (21), 358 (18), 340 (22), 322 (18), 304 (15), 213 (32), 200 (96), 173 (100), 91 (80); IR ν_{max} cm^{-1}: 3372 (NH, OH), 1690 (C=O), 1622; ^{1}H and ^{13}C NMR: Tables 1 and 2.

(11)-*Cytochalasa*-6,13,19-*triene*-1,21-*dione*-17-*hydroxy*-16,18-*dimethyl*-10-*phenyl*-(6*Z*,13*E*,16*S**,17*R**,18*S**,19*E*) (**11**). Amorphous solid, $[\alpha]_D$ −126.6° ($CHCl_3$; *c* 0.54). HRMS: *m/z* 433.2622 $[M]^+$ calcd for $C_{28}H_{35}NO_3$: 433.2617; EIMS *m/z* (rel. int.): 433 $[M]^+$ (52), 415 (37), 397 (20), 346 (14), 255 (37), 200 (100), 173 (22), 91 (52); IR ν_{max} cm^{-1}: 3300 (NH, OH), 1682 (C=O), 1616; ^{1}H and ^{13}C NMR: Tables 1 and 2.

(11)-*Cytochalasa*-13,19-*diene*-1,21-*dione*-6,7-*epoxy*-18-*hydroxy*-16,18-*dimethyl*-10-*phenyl*-(7*S**,13*E*,16*S**,18*S**,19*E*) (**12**). Amorphous solid, $[\alpha]_D$ −57.7° ($CHCl_3$; *c* 0.23). HRMS: *m/z* 449.2567 $[M]^+$ calcd for $C_{28}H_{35}NO_4$: 449.2567; EIMS *m/z* (rel. int.): 449 $[M]^+$ (68), 432 (92), 358 (67), 340 (60), 312 (32), 270 (33), 91 (100), 43 (45); IR ν_{max} cm^{-1}: 3320 (NH, OH), 1690 (C=O); ^{1}H and ^{13}C NMR: Tables 3 and 4.

(11)-*Cytochalas*-13-*ene*-1,21-*dione*-6,7-*epoxy*-18,19-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*-(7*S**,13*E*,16*S**,18*S**,19*R**) (**13**). Amorphous solid, $[\alpha]_D$ −49.0° ($CHCl_3$; *c* 0.35). HRMS: *m/z* 467.2663 $[M]^+$ calcd for $C_{28}H_{37}NO_5$: 467.2672; EIMS *m/z* (rel. int.): 467 $[M]^+$ (3), 449 (52), 432 (100), 358 (59), 340 (74), 91 (87); IR ν_{max} cm^{-1}: 3350 (NH, OH), 1692 (C=O); ^{1}H and ^{13}C NMR: Tables 3 and 4.

(11)-*Cytochalasa*-6(12),13,19-*triene*-1,17,21-*trione*-7,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*-(7*S**,13*E*,16*S**,18*R**,19*E*) (**14**). Amorphous solid, $[\alpha]_D$ −38.1° ($CHCl_3$; *c* 0.05). HRMS: *m/z* 463.2364 $[M]^+$ calcd for $C_{28}H_{33}NO_5$: 463.2359; EIMS *m/z* (rel. int.): 463 $[M]^+$ (14), 445 (7), 420 (11), 402 (25), 372 (33), 347 (28), 254 (32), 172 (33), 91 (100); IR ν_{max} cm^{-1}: 3360 (NH, OH), 1686 (C=O), 1618; ^{1}H and ^{13}C NMR: Tables 3 and 4.

(11)-*Cytochalasa*-5,13,19-*triene*-1,21-*dione*-7-*hydroperoxy*-17-*hydroxy*-16,18-*dimethyl*-10-*phenyl*-(5*Z**,7*S**,13*E*,16*S**,17*R**,18*S**,19*E*) (**15**). Amorphous solid, $[\alpha]_D$ +16.9° (MeOH; *c* 0.09). HRMS: *m/z* 465.2514 $[M]^+$ calcd for $C_{28}H_{35}NO_5$: 465.2516, 449.2559 $[M-O]^+$ calcd for $C_{28}H_{35}NO_4$: 449.2556, 431.2456 $[M-H_2O_2]^+$ calcd for $C_{28}H_{33}NO_3$: 431.2460; EIMS *m/z* (rel. int.): 465 $[M]^+$ (3), 449 (6), 448 (4), 447 (9), 433 (3), 432 (4), 431 (5), 358 (26), 252 (24), 91 (100); IR ν_{max} cm^{-1}: 3347 (NH, OH), 1686 (C=O); ^{1}H and ^{13}C NMR: Tables 3 and 4.

22-*Oxa*-(12)-*cytochalasa*-6(12),13,19-*triene*-1,21-*dione*-7,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*-(7*S**,13*E*,16*S**,18*S**,19*E*) (**16**). Amorphous solid, $[\alpha]_D$ +67.8° (MeOH; *c* 0.27). HRMS: *m/z* 465.2513 $[M]^+$ calcd for $C_{28}H_{35}NO_5$: 465.2515; EIMS *m/z* (rel. int.): 465 $[M]^+$ (7), 447 (22), 405 (25), 374 (100), 356 (33), 338 (46), 91 (50); IR ν_{max} cm^{-1}: 3360 (NH, OH), 1705 (C=O); ^{1}H and ^{13}C NMR: Tables 3 and 4.

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