Two New Compounds from the Ascomycete *Daldinia concentrica*

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Two new compounds, 1-isopropyl-2,7-dimethylnaphthalene (**1**) and 21-(acetyloxy)-6,13,14-trihydroxy-16,18-dimethyl-10-phenyl[11]cytochalasa-7,19-dien-1-one (**2**), were isolated from the fruiting bodies of *Daldinia concentrica*. The structures were established by spectroscopic methods.

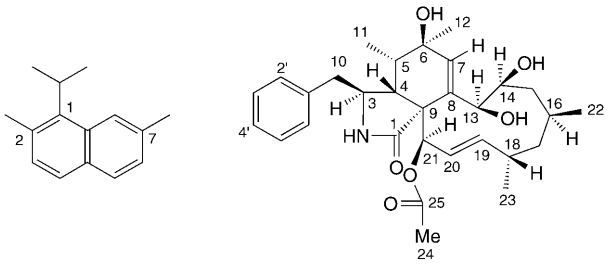
Introduction. – *Daldinia concentrica* has aroused a great deal of scientific interest because of its unique secondary metabolites. *Allport* and *Bu'Lock* studied European and American *Daldinia* sp. in 1958 and 1960 [1][2], which resulted in the identification of characteristic metabolites in their stromata and cultures. Some of these compounds have antimicrobial and nematocidal activities [3]. During recent studies on *Daldinia* sp., more than 20 new metabolites have been discovered, including benzoquinones [4], a binaphthyl and benzophenones [5], cytochalasins [6–8], a daldiniapyrone and daldinones [9], heptenetriols [10], triterpenoids [9][11][12], and derivatives of azaphilone [13], of which some show a range of biological activities. A benzofuran lactone has recently been isolated that has been found to be active against HIV-1 *in vitro* [14]. More recently, we reported two aromatic steroids from *Daldinia concentrica* and proposed that the origin of these compounds is derived from the transformation undergone by their precursor due to microbial action. These two compounds are recognized to be the long-sought, biological precursor steroids for organic matter in Earth's subsurface [15].

In the present study, the structures of the new alkylnaphthalene **1** and the new cytochalasin **2** isolated from *Daldinia concentrica* are reported. Alkylnaphthalenes have been isolated from some plants [16–18] and some geological samples [19][20]. Their occurrence plays an important role in the study of the sedimentary process, and can also be used as a maturity parameter for some sediments and crude oils. Cytochalasins are a group of fungal secondary metabolites identified from diverse fungal sources which have a wide range of biological activities [21–23][6–8] but are best known for their various effects on mammalian [24]. The most unusual of their properties is their ability to cause cells to extrude their nuclei, leading to the formation of nuclei-free cells. At lower concentrations, they interfere with cell diversion by preventing cytoplasmic division leading to binuclear or polynuclear cells and also inhibit cell movement [6].

Results and Discussion. – Compound **1** was obtained as colorless oil. The molecular formula of **1** was deduced to be $C_{15}H_{18}$ on the basis of a molecular-ion peak at *m/z* 198 in a FAB-MS (positive mode) and its ^{13}C-NMR spectrum (*Table 1*). Further spectral data allowed to elucidate the structure of compound **1** as 1-isopropyl-2,7-dimethylnaphthalene (*Fig. 1*).

Table 1. *^{1}H- and ^{13}C-NMR Spectral Data* ($CDCl_3$) *of* **1**. δ in ppm, *J* in Hz.

	δ(C) (DEPT)	δ(H)	^{1}H,^{1}H-COSY	^{13}C,^{1}H-HMBC (selected)
C(1)	142.1 (*s*)			H–C(3), H–C(8), *Me*–C(2), *Me*$_2$CH
C(2)	131.8 (*s*)			*Me*–C(2), H–C(4)
H–C(3)	125.6 (*d*)	7.18 (*d*, *J* = 7.3)	H–C(4)	*Me*–C(2)
H–C(4)	121.4 (*d*)	7.25 (*d*, *J* = 7.3)	H–C(3)	
H–C(5)	124.8 (*d*)	7.87 (*d*, *J* = 8.8)	H–C(6)	
H–C(6)	127.2 (*d*)	7.30 (*d*, *J* = 8.8)	H–C(5), H–C(8)	Me–C(7)
C(7)	134.7 (*s*)			H–C(5), *Me*–C(7)
H–C(8)	122.9 (*d*)	7.90 (*s*)	H–C(6)	H–C(6), *Me*–C(7)
C(8a)	131.1 (*s*)			
C(4a)	131.5 (*s*)			H–C(3), H–C(6)
Me–C(2)	19.4 (*q*)	2.61 (*s*)		H–C(3)
Me–C(7)	22.0 (*q*)	2.52 (*s*)		H–C(8)
Me$_2$*CH*	28.2 (*d*)	3.70 (*m*)	*Me*$_2$CH	*Me*$_2$CH
Me$_2$CH	23.6 (*q*)	1.36 (*d*, *J* = 6.8)	Me$_2$*CH*	Me$_2$*CH*



1 **2** [1])

Fig. 1. *Structures of compounds* **1** *and* **2**

The ^{13}C-NMR spectrum of **1** showed fifteen signals (4 Me, 6 CH, 5 C) including ten aromatic C-atoms. Considering the seven degrees of unsaturation, a naphthalene skeleton was deduced. In the ^{1}H-NMR spectrum, a *d* was observed at δ 1.36 (6 H), suggesting the existence of an ^{i}Pr group. Thus, compound **1** was a naphthalene substituted by 2 Me and 1 ^{i}Pr groups. Their location was determined by 2D-NMR. In the ^{1}H,^{1}H-COSY, the cross-peaks H–C(5)/H–C(6), H–C(6)/H–C(8), H–C(3)/H–C(4) allowed to place H–C(5), H–C(6), H–C(8) at one ring and H–C(3), and H–C(4) at the other ring of the naphthalene structure (*Table 1*). In the ^{1}H,^{13}C-HMBC spectrum, the correlations H–C(3), H–C(8), *Me*–C(2), and *Me*$_2$CH/C(1), *Me*–C(2)/C(3), and *Me*–C(7)/C(6) and C(8) were observed, suggesting the position of an ^{i}Pr group at C(1) and of the Me groups at C(2) and C(7).

[1]) Trivial atom numbering; for the systematic name, see *Exper. Part.*

Table 2. *^{1}H- and ^{13}C-NMR Spectral Data* (CD_3OD) *of* **2**[1]). δ in ppm, J in Hz.

	δ(C) (DEPT)	δ(H)	^{1}H,^{1}H-COSY	^{13}C,^{1}H-HMBC (selected)
C(1)	178.5 (*s*)			H–C(3), H–C(21)
H–C(3)	55.6 (*d*)	3.86 (*m*)	H–C(4), CH_2(10)	H–C(5)
H–C(4)	45.1 (*d*)	2.95 (*t*, *J* = 6.0)	H–C(3), H–C(5)	CH_2(10), Me(11), H–C(21)
H–C(5)	39.7 (*d*)	2.09 (*m*)	H–C(4), Me(11)	H–C(3), H–C(7), Me(12)
C(6)	70.7 (*s*)			H–C(4), Me(11)
H–C(7)	142.2 (*d*)	6.04 (*s*)		Me(12), H–C(13)
C(8)	135.9 (*s*)			H–C(4), H–C(14)
C(9)	55.4 (*s*)			H–C(7), H–C(13), H–C(20)
CH_2(10)	45.4 (*t*)	3.10 (*dd*, *J* = 4.5, 13.6), 2.81 (*dd*, *J* = 8.1, 13.6)	H–C(3)	H–C(4), H–C(2′,6′)
Me(11)	12.7 (*q*)	1.09 (*d*, *J* = 7.2)	H–C(5)	H–C(4)
Me(12)	23.7 (*q*)	1.19 (*s*)		H–C(5), H–C(7)
H–C(13)	74.1 (*d*)	4.02 (*d*, *J* = 8.8)	H–C(14)	H–C(7), CH_2(15)
H–C(14)	71.4 (*d*)	3.71 (br. *t*, *J* = 8.8)	H–C(13), CH_2(15)	
CH_2(15)	46.8 (*t*)	1.58 (*dd*, *J* = 9.6, 13.8), 1.16 (*m*)	H–C(14), H–C(16)	H–C(13), CH_2(17)
H–C(16)	29.7 (*d*)	1.28 (*m*)	CH_2(15), CH_2(17), Me(22)	H–C(14)
CH_2(17)	46.4 (*t*)	1.40 (*dd*, *J* = 6.1, 13.5), 0.84 (*ddd*, *J* = 6.4, 8.8, 13.5)	H–(16), H–C(18)	CH_2(15), H–C(19)
H–C(18)	40.9 (*d*)	2.19 (*m*)	CH_2(17), H–C(19), Me(23)	H–C(20)
H–C(19)	147.6 (*d*)	5.84 (*dd*, *J* = 10.6, 14.8)	H–C(18), H–C(20)	CH_2(17), H–C(21)
H–C(20)	123.6 (*d*)	5.32 (*dd*, *J* = 10.2, 14.8)	H–C(19), H–C(21)	H–C(18)
H–C(21)	76.7 (*d*)	5.89 (*d*, *J* = 10.2)	H–C(20)	H–C(4), H–C(19)
Me(22)	22.0 (*q*)	0.93 (*d*, *J* = 6.6)		CH_2(15), CH_2(17)
Me(23)	21.0 (*q*)	0.96 (*d*, *J* = 6.5)	H–C(18)	CH_2(17), H–C(19)
Me(24)	21.5 (*q*)	2.05 (*s*)		
C(25)	171.3 (*s*)			H–C(21)
C(1′)	139.1 (*s*)			H–C(3), CH_2(10), H–C(3′,5′)
H–C(2′,6′)	130.7 (*d*)	7.26 (*d*, *J* = 7.1)		CH_2(10)
4-C(3′,5′)	129.9 (*d*)	7.34 (*t*, *J* = 7.5)		
H–C(4′)	128.0 (*d*)	7.25 (*t*, *J* = 7.3)		

The molecular formula of compound **2** was determined to be $C_{30}H_{41}NO_6$ on the basis of the HR-TOF-MS (*m*/*z* 534.2818 ($[M+Na]^+$)). The structure of a [11]cytochalasadienone for **2** (see *Fig. 1*) was established by its spectral data (*Table 2*) and comparison with literature data.

The ^{13}C-NMR spectrum of **2** (CD_3OD) showed 28 resolved peaks corresponding to 28 C-atoms (*Table 2*), which were classified into 5 Me, 3 CH_2, 4 CH, 4 NCH or OCH, 6 sp^2 CH, 2 sp^3 C, 2 sp^2 C, and 2 C=O groups by analysis of the DEPT spectra. The ^{1}H-NMR spectrum of **2** displayed 37 proton signals (*Table 2*). Deduced from the molecular formula, the presence of 2 OH groups, one amino group, and

one Ac group was suggested. The connectivity of the proton and C-atoms was established by the $^{1}H,^{13}C$-HMQC spectrum. Analysis of the $^{1}H,^{1}H$-COSY plot revealed the five partial structures **I**–**V** (*Fig. 2*). $^{13}C,^{1}H$ Long-range couplings (^{3}J) observed in the $^{1}H,^{13}C$-HMBC experiment gave the following evidence: *1*) The cross-peaks from H–C(3) (δ 3.86) to C–C(1′) (δ 139.1), from CH_2–C(10) (δ 3.10, 2.81) to C(2′,6′) (δ 130.7), from H–C(3′,5′) (δ 7.34) to C–C(1′) showed the presence of a benzyl moiety connecting with partial structure **I**[1]). *2*) The cross-peaks from H–C(4) (δ 2.95) to C(6) (δ 70.7), C(8) (δ 135.9), C(10) (δ 45.4), C(11) (δ 12.7), and C(21) (δ 76.7), from H–C(5) (δ 2.09) to C(3) (δ 55.6) and C(12) (δ 23.7), from H–C(7) (δ 6.04) to C(5) (δ 39.7), C(9) (δ 55.4), and C(12), from Me(11) (δ 1.09) to C(4) (δ 45.1), and from Me(12) (δ 1.19) to C(5) (δ 39.7) and C(7) (δ 142.2) indicated a 4,5,6,8,9-pentasubstituted[1]) cyclohexene containing the partial structure **I**, **III**, and **IV**. *3*) The long-range couplings from H–C(3) and H–C(4) to C(1) (δ 178.5) suggested that a pyrrole ring was attached to the cyclohexane ring. *4*) The long-range couplings from H–C(7) to C(13) (δ 74.1), from H–C(13) (δ 4.02) to C(7) and C(9), and from H–C(14) (δ 3.71) to C(8) suggested that partial structure **II** was attached to the cyclohexene ring. *5*) The long-range couplings from H–C(13) to C(15) (δ 46.8), from H–C(14) to C(16) (δ 29.7), from CH_2(15) (δ 1.58, 1.16) to C(13), C(17) (δ 46.4), and C(22) (δ 22.0), from CH_2(17) (δ 1.40, 0.84) to C(15), C(19) (δ 147.6), C(22), and C(23) (21.0), from H–C(18) (δ 2.19) to C(20) (δ 123.6), from H–C(19) (δ 5.84) to C(17), C(21), and C(23), from H–C(20) (δ 5.32) to C(9) and C(18) (δ 40.9), from H–C(21) to C(1) (δ 178.5), C(4), C(19), and C(25) (δ 171.3) suggested the connection of partial structures **I**–**V** and the pyrrole ring. The J(H,H) was 14.8 Hz between H–C(19) (δ 5.84) and H–C(20) (δ 5.32), indicating that the olefin moiety has the (*E*)-configuration. The δ(C) 74.1, 71.4, and 76.7 supported the presence of 2 OH and 1 Ac group. Finally, the cytochalasan skeleton was suggested because of the degree of unsaturation and the molecular formula of **2**.

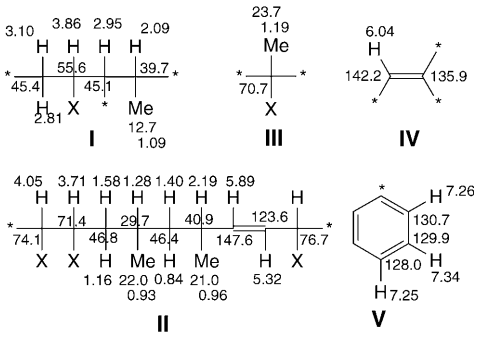


Fig. 2. *Partial structures* **I** *to* **V** *of compound* **2**

The relative configuration of **2** was established by NOESY experiments (*Table 3*). The NOEs H–C(4)/CH_2(10) and H–C(3)/Me(11), indicated the β-configurations for H–C(4) and H–C(5), and an α-configuration for H–C(3) [25][26]. No NOEs between Me(12) and H–C(4) and H–C(5) revealed an α configuration for Me(12) [27]. H–C(7) displayed NOEs to Me(12), H–C(13), and H–C(14), and

Table 3. *NOEs of compound* **2**

H	NOE at H	H	NOE at H
H–C(3)	CH_2(10), Me(11)	H–C(19)	CH_2(15), Me(23)
H–C(4)	H–C(5), CH_2(10), Me(11), H–C(20)	H–C(20)	H–C(4), H–C(18)
H–C(5)	H–C(4)	H–C(21)	H–C(13), H–C(14)
H–C(7)	Me(12) H–C(13), H–C(14)	Me(22)	CH_2(15), CH_2(17), H–C(18)
H–C(13)	H–C(7), CH_2(15), CH_2(17), H–C(21)	Me(23)	H–C(16), CH_2(17), H–C(19)
H–C(14)	H–C(7), CH_2(15), CH_2(17), H–C(21)		

H–C(21) displayed NOEs to H–C(13) and H–C(14), indicating that H–C(13), H–C(14), and H–C(21) were on the α side of the 11-membered ring. On the other hand, NOEs from H–C(4)/H–C(20), H–C(20)/H–C(18), H–C(18)/Me(22), and Me(23)/H–C(16) required H–C(18), H–C(20), and Me(22) to be on the β side of the ring [25][26].

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Experimental Part

General. CC = Column chromatography. M.p.: *XRC-1* apparatus (Sichuan University, Sichuan, People's Republic of China). Optical rotation; *Horiba-SEPA-300* automatic polarimeter (*Horiba*, Tokyo, Japan). UV Spectra: *Shimadzu-UV2401PC* spectrophotometer (*Shimadzu Corporation*, Kyoto, Japan); λ_{max} in nm. IR Spectra: *Bruker Tensor-27* spectrophotometer (*Bruker*, Karlsruhe, Germany); in cm^{-1}. 1D and 2D NMR Spectra: *Bruker DRX-500* NMR instruments (*Bruker*, Karlsruhe, Germany); at 500 MHz for ^1H and 125 MHz for ^{13}C; δ in ppm rel. to $SiMe_4$ as an internal standard, coupling constants *J* in Hz. Mass Spectra: *VG-Autospec-3000* mass spectrometer (*VG*, Manchester, England) and *API Qstar Pulsar* (*Applied Biosystems*, Foster City, USA); in *m*/*z* (rel.%).

Mushroom Material. Fruiting bodies of *Daldinia concentrica* were collected in Laojunshan, Yunnan, P. R. China, in 2003. The voucher specimen was deposited at the herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and Isolation. Dried and powdered fruiting bodies (11.5 kg, dry weight) were extracted with $CHCl_3$ (4 × 50 l) at r.t. The extract (340 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 100:0, 95:5, 9:1 (*v*/*v*)): *Fractions 1–21*. *Fr. 1* (eluted with $CHCl_3$; 1 g) was separated by CC (silica gel, petroleum ether/acetone 100:0, 98:2, 95:5, 9:1, 8:2): *Fr. 1.1–1.22*. *Fr. 1.4* (eluted with petroleum ether) was purified by prep. TLC (petroleum ether/hexane 45:1): **1** (41.0 mg). *Fr. 10* (eluted with $CHCl_3$; 0.6 g) was separated by CC (silica gel, $CHCl_3$/MeOH 100:0, 95:5, 9:1). *Fr. 10.1–10.7*. *Fr. 10.5* (eluted with $CHCl_3$/MeOH 95:5; 0.1 g) was purified by CC (*Sephadex LH-20*): **2** (11.2 mg).

1-Isopropyl-2,7-dimethylnaphthalene (**1**): Colorless oil. UV ($CHCl_3$): 240, 292. IR: 3064, 3008, 2962, 2925, 2868, 1626, 1601, 1460, 1442, 1382, 1362, 831, 807, 778. NMR: see *Table 1*. FAB-MS (pos.): 198 (60, M^+).

21-(Acetyloxy)-6,13,14-trihydroxy-16,18-dimethyl-10-phenyl[11]cytochalasa-7,19-dien-1-one (= *(3*S*, 3a*R*,4*S*,5*R*,7*R*,8*S*,10*S*,12*S*,15*R*,15a*R*)-15-(Acetyloxy)-2,3,3a,4,5,7,8,9,10,11,12,15-dodecahydro-5,7,8-trihydroxy-4,5,10,12-tetramethyl-3-(phenylmethyl)-1*H*-cycloundec[d]isoindol-1-one*; **2**): Colorless needles. M.p. 197–199°. $[\alpha]_D^{24.4} = +51.17$ (MeOH, $c = 1.3$). UV (MeOH): 204. IR: 3426, 3028, 2953, 2926, 1743, 1687, 1496, 1453, 1371, 1234, 1102, 1016, 956, 702. NMR: see *Table 2*. FAB-MS (neg.): 510 (100, $[M-H]^-$). HR-TOF-MS: 534.2818 ($[M+Na]^+$, $C_{30}H_{41}NNaO_6^+$; calc. 534.2831).

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