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A 10-PHENYL-[11]-CYTOCHALASAN FROM A SPECIES OF *DALDINIA*

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Key Word Index—*Daldinia* sp.; fungus; Ascomycetes; Xylariaceae; 10-phenyl-[11]-cytochalasans; cytochalasins; naphthalene derivative; δ-lactone; X-ray crystallographic analysis.

Abstract—The structure of [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**), a 10-phenyl-[11]-cytochalasan isolated from an unidentified species of *Daldinia* was unambiguously confirmed by X-ray crystallographic analysis. A new naphthalene derivative was also isolated, together with a δ-lactone. The structures of these two compounds were established by spectroscopic methods.

INTRODUCTION

We have already reported the isolation and structural elucidation of 15 new 10-phenyl-[11]-cytochalasans and a 10-phenyl-22-oxa-[12]-cytochalasan from an unidentified species of the fungus, *Daldinia* [1, 2]. Previous studies on *D. concentrica* reported the isolation of naphthalene, binaphthyl, benzophenone and azaphilone derivatives [3–5].

The crystal structure provides accurate information on the relative stereochemistry and conformation of the multiple fused ring system in the cytochalasan class of fungal metabolites. However, for the macrocyclic ring there is little knowledge of the possible conformational orientations. The cytochalasans display a wide range of biological activities [6–8], and, thus, have great potential in cell biology and medicine. Therefore, precise information on the structure and conformation of these compounds is important in order to understand their chemical and biological activity. In the present paper, we report the X-ray crystal structure for one of the 10-phenyl-[11]-cytochalasans (**1**) and also the isolation and characterization of a new naphthalene derivative (**2**) and a δ-lactone (**3**) not isolated previously from a natural source.

RESULTS AND DISCUSSION

Chromatography on silica gel and preparative HPLC of the ethyl acetate extract of the *Daldinia* species yielded the 16 new cytochalasins already described [1, 2], a new naphthalene derivative (**2**) and a δ-lactone (**3**).

The isolation and characterization of **1**, [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**), was previously reported by us [2]. However, at this time we were unable to produce a suitable crystal for X-ray analysis of **1** or indeed any of the other 15 cytochalasins we previously isolated. Now we have obtained suitable single crystals of **1** and X-ray crystallographic analysis unequivocally confirmed the complete structure and relative stereochemistry of this compound. Furthermore, the crystal structure confirms the conformation of the 11-membered ring which we previously established from NMR experiments [1, 2]. Thus, the X-ray analysis is in agreement with the structure, relative stereochemistry and conformation we concluded from NMR results. An ORTEP drawing of **1** is shown in Fig. 1 and the crystal data is given in the Experimental.

Even though the absolute stereochemistry of **1** was not determined by X-ray analysis, the stereochemistry of the perhydroisoindol-1-one nucleus is in accord with those of other cytochalasans, in particular, cytochalasin E (Ag^+ adduct) [9], cytochalasin D′ (*p*-bromobenzoate ester of cytochalasin D) [10] and cytochalasin B (Ag^+ adduct) [11], whose absolute stereochemistries have been determined by X-ray analysis (the enantiomer chosen for **1** was by analogy with these compounds). The X-ray crystal structures of two other 10-phenyl-[11]-cytochalasans have also been reported, cytochalasin D′ [10] and cytochalasin H [6]. Comparison of the ORTEP diagrams for cytochalasins D′ and H with that of **1** isolated from the present extract shows a good similarity in the conformations of their macrocyclic rings, thus giving further support to the deduction that, regardless of the different functionalities and in the

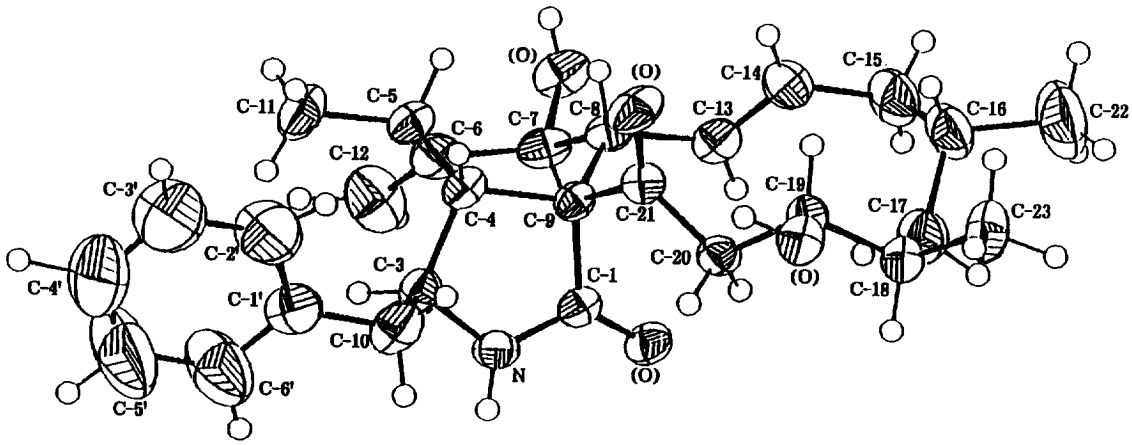


Fig. 1. ORTEP drawing of compound **1**.

results and crystal data predict the same conformation, indicating that the ring framework of these molecules maintains the same solid-state conformation in solution.

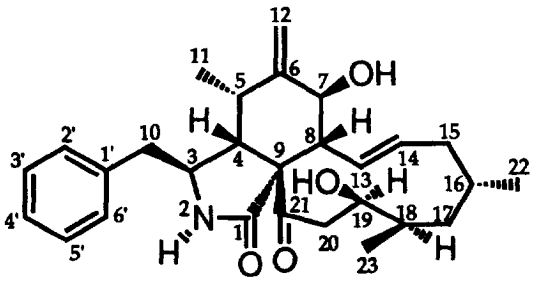
Compound **2** had the molecular formula $C_{11}H_{12}O_4$ ($[M]^+$ at *m/z* 208.0736). The IR spectrum showed the presence of hydroxyl (ν_{max} 3451 cm^{-1}) and ketonic carbonyl (ν_{max} 1640 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed resonances for an aromatic hydroxyl proton [δ_H 11.88 (*s*)], two aromatic protons which are *ortho*-related [δ_H 7.16 (*d*, $J = 9.0$ Hz); 6.92 (*d*, $J = 9.0$ Hz)], an aromatic methoxyl [δ_H 3.88(*s*)], an allylic oxygenated methine [δ_H 5.27 (*t*, $J = 4.2$ Hz)] and another hydroxyl proton [δ_H 2.80 (*br s*)]. The ^{13}C NMR spectrum (Table 1) indicated the presence of 11 carbons: one unsaturated carbonyl [δ_C 204.9 (*s*)], six aromatic carbons [δ_C 156.1, 148.9, 131.3, 115.5 (all *s*), 120.7 and 117.8 (both *d*)], one oxygenated carbon [δ_C 62.0 (*d*)]; one methoxyl carbon (δ_C 56.5) and two methylenes (δ_C 33.5 and 28.9). The above information, together with the results of HMQC [12] and HMBC [13] experiments (Table 1) reveals the structure depicted for **2**.

The orientation of the two rings in **2** was derived from the C-1 chemical shift [δ_C 204.9 (*s*)], which is more deshielded than expected (8–10 ppm) [14]. This can be explained by hydrogen-bonding with the aromatic hydroxyl, causing deshielding of the carbonyl carbon. Also, the very low field chemical shift of the aromatic hydroxyl [δ_H 11.88 (*s*)] indicates that it has hydrogen-bonding. The aromatic ring of **2** is planar while the other six-membered ring has a half-boat conformation, in which C-4, C-4a, C-8a, C-1 and C-2 are approximately in one plane, while C-3 is out of the plane. The unusually low field chemical shift of H-4 and its coupling pattern [δ_H 5.27 (*t*, $J = 4.2$ Hz)] indicate that the C-4 hydroxyl is equatorial on the six-membered ring. From the above data, **2** was identified as (4*R**)-4,8-dihydroxy-3-hydro-5-methoxy-1-naphthalenone; its absolute stereochemistry was not determined.

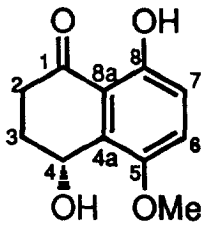
Also isolated from the fungal extract was a δ-lactone, (4*R*,6*R*)-4-hydroxy-6-methyltetrahydropyran-2-one (**3**). As far as we know this compound has not previously been isolated from a natural source. How-

Table 1. NMR data for compound **2**

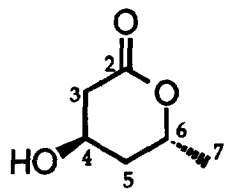
Site	^{13}C	^1H	Long-range $^{13}C/^1H$ correlations observed in the HMBC spectrum (correlated C)
1	204.9 *s*		
8	156.1 *s*		
5	148.9 *s*		
4a	131.3 *s*		
6	120.7 *d*	7.16 (*d*, $J = 9.0$ Hz)	4a, 5, 8
7	117.8 *d*	6.92 (*d*, $J = 9.0$ Hz)	5, 8, 8a
8a	115.5 *s*		
4	62.0 *d*	5.27 (*t*, $J = 4.2$ Hz)	
OMe	56.5 *q*	3.88 (*s*)	5
2	33.5 *t*	3.06 (*ddd*, $J = 17.0, 11.1, 5.7$ Hz) 2.58 (*dt*, $J = 17.0, 4.8$ Hz)	1, 3, 4
3	28.9 *t*	2.28 (*m*, 2H)	



(1)



(2)



(3)

ever, it has been synthesized before and was identified by comparison of its physical and spectroscopic data with authentic samples [15, 16].

EXPERIMENTAL

General. TLC: 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 mm). HPLC: Chemcosorb 5Si–U 10 × 250 mm (B). NMR spectra (^{1}H, 600 and 400 MHz; ^{13}C, 150 and 50 MHz): $CDCl_3$ soln relative to TMS at δ_H 0 and $CDCl_3$ at δ_C 77.0. Multiplicities were determined by DEPT and/or by HMQC. IR spectra were measured in $CHCl_3$ soln and UV spectra in EtOH soln $[\alpha]_D$ were measured in $CHCl_3$ or MeOH soln. EIMS were measured at 70 ev.

Fungus. The species of *Daldinia* was collected at two separate sites in Tokushima, both samples being found growing on the same host plant, *Quercus acutissima*. One sample was collected in June 1992 (259 g), the other in June 1993 (515 g). The *Daldinia* sp. deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. Fresh material (259 and 515 g) was extracted with EtOAc to yield 11.43 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 subjected to flash CC on silica gel using an *n*-hexane – EtOAc gradient. The most polar frs eluted were further subjected to flash CC and prep. TLC and final purification by HPLC to give **1** (4 mg, R_f 0.48), **2** (3 mg, R_f 0.53) and **3** (70 mg, R_f 0.28).

[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**) (**1**). Crystals from EtOAc, mp 196–199°. Crystal data: $C_{28}H_{37}NO_4$, M_r 451, monoclinic crystal system, space group $P2_1$ with a = 14.914(2) Å, b = 8.716(2) Å, c = 10.047(2) Å, V = 1260.8(4) Å^3, Z = 2, D_{obs} = 1.10 g cm^{-3}, D_{calc} = 1.09 g cm^{-3}, Cu K_α radiation (λ = 1.54178, μ = 9.76 cm^{-1}). Diffraction measurements were made on a Mac Science MXC 18 diffractometer using Cu K_α radiation. Of 2435 reflections, 2222 were unique. The structure was solved by SHELXS-86 and refined by full matrix least squares. The function $S[w(|F_o|^2 - |F_c|^2)^2]$ was minimized, in which $w = 1.0/[s|F_o|^2 + 0.0004|F_o|^2]$. The reflections used were 2132 and the number of variables was 409. Final R = 0.043, R_w = 0.051, S = 1.45. The maximum negative and positive peaks in the final difference map were −0.23 and 0.18 eÅ^{-3}, respectively.

(4*R**)-4,8-*Dihydroxy*-3-*hydro*-5-*methoxy*-1-*naphthalenone* (**2**). Amorphous solid. $[\alpha]_D$ + 16.7° ($CHCl_3$, *c* 0.12). HRMS: *m/z* 208.0736 [M]$^+$calc. for $C_{11}H_{12}O_4$: 208.0736. EIMS *m/z* (rel. int.): 208 [M]$^+$ (100), 190 (19), 175 (30), 165 (9), 151 (21), 147 (14), 137 (15), 55 (7). UV λ_{max} (EtOH) nm (log ε): 210 (3.99), 232 (4.03), 262 (3.69), 367 (3.47). IR ν_{max} cm^{-1}: 3451 (OH); 1640 (C=O). ^{1}H and ^{13}C NMR in Table 1.

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