

ISOLATION, ^1H , ^{13}C NMR ASSIGNMENTS, AND CRYSTAL STRUCTURE OF CHRODRIMANIN B FROM A MARINE FUNGUS *Aspergillus* sp.

Mei-Yan Wei,^{1,2} Guang-Ying Chen,^{1*} Yu Wang,³ Xiu-Li Zhang,³
Chang-Yun Wang,^{3*} and Chang-Lun Shao^{3*}

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A terrestrial fungal metabolite, chrodrimanin B (1), has been isolated for the first time from the fermentation broth of a marine-derived fungus Aspergillus sp. collected from the South China Sea. Detailed assignments for the proton and carbon of 1 have been unambiguously elucidated by combined spectroscopic methods including 1D and 2D NMR spectral data, and the relative configurations were also confirmed by single-crystal X-ray data.

Keywords: marine-derived fungus, chrodrimanin, structure elucidation, X-ray.

Marine-derived fungi have been recognized as a potential source of structurally novel and biologically potent metabolites, and a growing number of marine fungi have been reported to produce novel bioactive secondary metabolites [1–3]. As part of our continuous investigation into new bioactive natural products from marine fungi collected from the South China Sea [4–8], one terrestrial fungal metabolite, chrodrimanin B (**1**) [9], together with five new phenolic bisabolane-type sesquiterpenoids [10], were isolated from the fermentation broth of a marine-derived fungus *Aspergillus* sp. This is the first report of the isolation of chrodrimanin B (**1**) from a marine organism. Herein, we report the isolation, structure elucidation, complete NMR assignments, relative stereochemistries, and single-crystal X-ray data of **1**.

Compound **1** was isolated as a colorless solid. The molecular formula of **1** was established as $\text{C}_{27}\text{H}_{32}\text{O}_8$ (12 degrees of unsaturation) from the results of EI-MS and NMR data. The IR absorption bands indicated the existence of hydroxyl (3535 cm^{-1}) and ester ($1753, 1672\text{ cm}^{-1}$) groups. In the ^1H NMR spectrum (Table 1), compound **1** has two exchangeable hydroxyl groups at δ 11.05 and 2.74, one aromatic proton signal at δ 6.50 (s), a pair of *cis* olefinic protons at δ 7.15 (d, $J = 10.2\text{ Hz}$) and 5.97 (d, $J = 10.2\text{ Hz}$), three oxygenated methine protons at δ 6.16 (d, $J = 1.8\text{ Hz}$), 4.71 (qd, $J = 6.6, 1.8\text{ Hz}$), and 4.16 (dd, $J = 8.4, 2.4\text{ Hz}$), and six methyl groups at δ 2.16 (s), 1.48 (d, 6.6), 1.35 (s), 1.27 (s), 1.16 (s), and 1.15 (s).

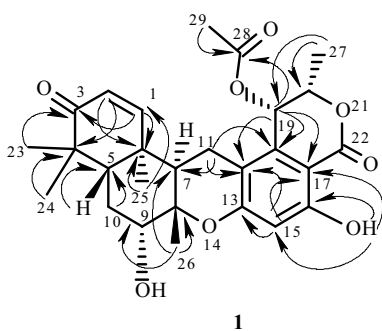


Fig. 1

Fig. 1. The chemical structure and key HMBC correlations of compound **1**.

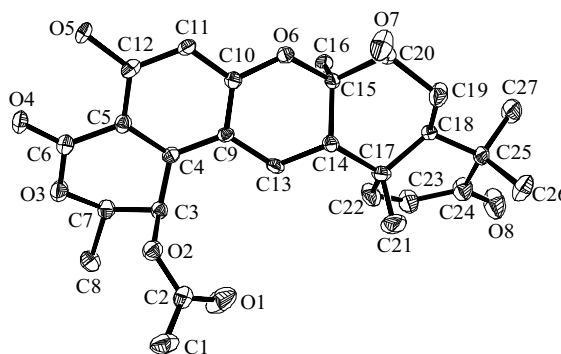


Fig. 2

Fig. 2. Crystal structure of compound **1**.

1) Key Laboratory of Tropical Medicinal Plant Chemistry of the Ministry of Education, Hainan Normal University, Haikou, 571158, P. R. China, e-mail: chgying123@163.com; 2) School of Pharmacy, Guangdong Medical College, Dongguan, 523808, P. R. China; 3) School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, P. R. China, e-mails: changyun@ouc.edu.cn; shaochanglun@163.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 4, pp. 506–508, July–August, 2011. Original article submitted May 30, 2010.

TABLE 1. NMR Data (CDCl₃, δ, J/Hz) of **1**^a

C atom	δ _C , mult.	δ _H	HMBC	C atom	δ _C , mult.	δ _H	HMBC
1	156.2 (CH)	7.15 (d, J = 10.2)	C-3, C-6	16	162.4 (C)	–	
2	127.7 (CH)	5.97 (d, J = 10.2)	C-4, C-6	17	102.4 (C)	–	
3	203.8 (C)	–		18	136.0 (C)	–	
4	44.8 (C)	–		19	64.2 (CH)	6.16 (d, J = 1.8)	C-12, C-17, C-18, C-28
5	42.5 (CH)	2.17 (dd, J = 14.4, 4.8)	C-25	20	76.2 (CH)	4.71 (qd, J = 6.6, 1.8)	C-27
6	38.6 (C)	–		22	168.9 (C)	–	C-3, C-4, C-24
7	42.0 (CH)	2.26 (dd, J = 14.4, 5.4)	C-5, C-6, C-8	23	21.4 (CH ₃)	1.15 s	C-4, C-5, C-23
8	79.8 (C)	–		24	27.5 (CH ₃)	1.16 s	C-1, C-6, C-7
9	72.0 (CH)	4.16 (dd, J = 8.4, 2.4)		25	27.7 (CH ₃)	1.35 s	C-7, C-8, C-9
10	26.7 (CH ₂)	2.24 (ddd, J = 14.4, 8.4, 4.8) 1.85 (td, J = 14.4, 2.4)	C-5	26	21.5 (CH ₃)	1.27 s	C-19, C-20
11	21.6 (CH ₂)	2.95 (dd, J = 15.6, 5.4) 2.61 (dd, J = 15.6, 14.4)	C-7, C-12, C-13, C-18	27	16.5 (CH ₃)	1.48 (d, J = 6.6)	
12	112.4 (C)	–		28	170.7 (C)	–	
13	159.7 (C)	–		29	20.8 (CH ₃)	2.16 s	C-28
15	106.4 (CH)	6.50 s	C-12, C-13, C-16, C-17	9-OH		2.74 s	
				16-OH		11.05 s	C-15, C-16, C-17

^aMeasured at 600 MHz (¹H) and 150 MHz (¹³C).

The ¹³C NMR spectrum (Table 1) displayed 27 carbon signals, including those assigned to one α,β-unsaturated ketone carboxyl (δ 203.8), two ester carboxyls (δ 170.7, 168.9), eight olefinic carbons, and four oxygenated carbons (δ 79.8, 76.2, 72.0, 64.2). With 12 degrees of unsaturation accounted for by the molecular formula, the structure of **1** was suggested to contain five rings, in association with eight olefinic carbons and three carboxyl groups. Detailed assignments of the carbon and proton signals were unambiguously accomplished by a comprehensive analysis of 2D NMR spectral data (Table 1). The HMBC correlations from the hydrogen-bonded hydroxyl proton (δ_H 11.05) to C-15, C-16, and C-17, from H-19 to C-12, C-17, and C-18, together with the correlations between H-19 and H-20 and H-27 in the ¹H–¹H COSY spectrum revealed the presence of an isocoumarin moiety in compound **1** (Fig. 1). A series of HMBC correlations from H-1 to C-3 and C-6, from H-2 to C-4 and C-6, from H₃-23 and H₃-24 to C-3, C-4, and C-5, from H₃-25 to C-1, C-6, and C-7, from H₃-26 to C-7, C-8, and C-9, together with the ¹H–¹H COSY correlations from H-5 to H-10 and H-9, confirmed the presence of a sesquiterpenoid moiety. Finally, the connection between C-11 and C-12 was established based on the HMBC correlations from H₂-11 to C-7, C-12, and C-18. Crystals of **1** suitable for X-ray diffraction were obtained by slow evaporation of solution of **1** in methanol–EtOAc (1:1). The structure of **1** was further confirmed by a single-crystal X-ray analysis (Fig. 2, CCDC No. 680923), and the relative configurations of **1** could be determined as 5*S**, 6*R**, 7*R**, 8*S**, 9*R**, 19*S**, and 20*S**.

Surprisingly, although the structure type of chrodriamanin is relatively rare, chrodriamanin B (**1**) has been reported, but only in a patent [9], as a secondary metabolite of a fungal strain of the genus *Penicillium*. It is reported to have insecticidal and insect-repelling effects. This is the first report of the isolation of chrodriamanin B (**1**) from a marine organism. In our preliminary assay, compound **1** did not exhibit *in vitro* cytotoxicity toward A-549 human lung carcinoma and MCF-7 human breast adenocarcinoma cell lines at the concentration of 100.0 μg/mL.

EXPERIMENTAL

¹H and ¹³C NMR spectra were recorded on a JEOL Eclips-600 spectrometer. EI-MS were measured on a Thermo DSQ EI-mass spectrometer. IR spectra were measured on a Bruker VECTOR 22 spectrophotometer. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh), octadecylsilyl silica gel (Unicorn; 45–60 μm), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G₆₀, F₂₅₄) were used for thin-layer chromatography (TLC).

Fungus Material and Culture Conditions. The marine-derived fungus *Aspergillus* sp. was isolated from a piece of tissue from the inner part of the gorgonian *D. gemmacea* (GX-WZ-20080034), which was collected from the South China Sea in September, 2008. The strain was deposited in the Key Laboratory of Marine Drugs, the Ministry of Education of China,

School of Medicine and Pharmacy, Ocean University of China, Qingdao, P. R. China, with the access code ZJ-2008001. Thirty liters of the fungal strain was cultivated in liquid medium (10.0 g of glucose, 2.0 g of yeast extract, 2.0 g of peptone in 1 L of seawater, in a 1.0 L Erlenmeyer flasks each containing 400 mL of culture broth) at 27°C without shaking for 30 days.

Extraction and Separation of Metabolites. The fungal cultures were filtered through cheesecloth, and the filtrate (30.0 L) was extracted with EtOAc (2 × 30.0 L). The organic extracts were concentrated *in vacuo* to yield a yellow oily residue (2.50 g). This extract was chromatographed on a silica gel column using a stepwise gradient of petroleum ether–EtOAc to afford eight fractions (Fractions 1–8). Fraction 2 (0.35 g) was isolated by column chromatography on silica gel eluted with petroleum ether–EtOAc (8:2) and then subjected to Sephadex LH-20 eluted with mixtures of petroleum ether–CHCl₃–MeOH (2:1:1) to obtain compound **1** (11.2 mg).

Chrodrimanin B (1): colorless crystals. ¹H, ¹³CNMR, see Table 1. IR (KBr, ν_{\max}): 3535, 2942, 1753, 1672, 1479, 1374, 1220, 1172, 1033, 981, 905 cm⁻¹. Mass spectrum (EI-MS⁺ m/z , I_{rel} %): 484 (11) [M]⁺, 424 (52), 409 (100), 406 (12), 391 (49), 270 (56), 205 (24).

The crystals are orthorhombic, space group P212121 with $a = 10.7484$ (15) Å, $b = 13.1269$ (18) Å, $c = 16.739$ (2) Å, $\alpha = \beta = \gamma = 90.00^\circ$, C₂₇H₃₂O₈, $M_r = 484.53$, $V = 2361.7$ (6) Å³, $Z = 4$, $D_c = 1.363$ g/cm³, $F(000) = 1032$, $\mu = 0.100$ mm⁻¹, final $R = 0.0431$ and $wR = 0.1018$ for 3484 observed reflections ($I > 2\sigma(I)$). The crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre (CCDC No.680923).

Cytotoxic Activity. The compound was tested *in vitro* against A-549 and MCF-7 cell lines. Both cell lines were maintained in RPMI 1640 medium (Gibco) containing 10% FBS (Gibco), 2 mg/mL sodium bicarbonate, 100 µg/mL penicillin sodium salt, and 100 µg/mL streptomycin sulfate. Cells were grown to 70% confluence, trypsinized with 0.05% trypsin-2 mM EDTA, and plated for experimental use. In all experiments, the cells were grown in RPMI-1640 medium with 10% FBS for 24 h prior to treatment. The compound was dissolved in DMSO at a concentration of 100 mM, then diluted in tissue culture medium and filtered before use. The cells (1.0×10^4) were seeded in 96-well tissue culture plates, treated with the tested compound or vehicle (0.1% DMSO) at various concentrations, and incubated for 48 h followed by MTT assay at 570 nm. The IC₅₀ values of the tested compound against different cell lines were obtained from the concentration–effect curves. Each experiment was repeated at least three times, and the combined data were analyzed using the Student's paired test.

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