

Two new benzofuran derivatives from the fungus *Stereum* sp. YMF1.1684

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ABSTRACT

A basidiomycete fungus, *Stereum* sp. YMF1.1684, was studied for its ability to produce new compounds on nutrient-enriched cultivation media. One new benzofuran, 2-hydroxy-1-((S)-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-yl)propan-1-one, named as phenostereum A (**1**) and one novel dimer benzofuran, named as phenostereum B (**2**) were isolated from EtOAc extract of the culture broth of the fungus *Stereum* sp. YMF1.1684. The structures were elucidated using spectroscopic data from 1D, 2D NMR and HRESIMS experiments.

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1. Introduction

Fungi play an important role in the sources of new natural products with specialized biological activities (Schulz et al., 2002). The increasing number of new compounds impressively demonstrated that fungus' potential producing a high proportion of unknown natural products is far from being exploited. In previous investigations, a series of interesting new compounds have been isolated from the fungal genus *Stereum*, including acetylenic aromatics (Dubin et al., 2000), sesquiterpenes (Yun et al., 2002a), phenolic compounds (Omolo et al., 2002), chromene, and aromatic-aldehyde derivatives (Yun et al., 2002b). In recent years, our ongoing search for new bioactive fungal metabolites from the basidiomycete genera, especially in the genus *Stereum*, not only has sesquiterpenes, such as cadinane sesquiterpenoids and stereumane-type sesquiterpenes (Li et al., 2008, 2011; Liu et al., 2010), but also has aromatic compounds (Li et al., 2006). *Stereum* belong to the Stereaceae family (Basidiomycetes), which have predominantly tropical or subtropical distribution and are often found associated with woody debris, rotting trunks and sometimes on buried dead wood (Reid, 1965). In China, this fungus is widespread, although it has not been chemically investigated for the production of secondary metabolites comprehensively. In the course of biochemical studies on *Stereum* which was collected in Xishuangbanna, Yunnan Province, we investigated the constituents of *Stereum* sp. YMF1.1684, which led to the isolation of two

new benzofuran substances (**1–2**). This paper deals with the structural elucidation of compounds **1** and **2**.

2. Results and discussion

Phenostereum A (**1**) was obtained as colorless powder. It had molecular formula $C_{14}H_{16}O_3$, as deduced from HRESIMS (m/z 255.0993 $[M+Na]^+$), with 7 degrees of unsaturation. In accordance with the molecular formula, 14 carbon resonances were resolved in the ^{13}C NMR spectrum (Table 1), and were further classified by DEPT experiment into the categories of 2 methyls, 2 methylenes (1 olefinic one), 5 methines, and 5 quaternary carbons. In addition, two methyls (δ_H 1.75 (3H, s) and δ_H 1.43 (3H, d, $J = 7.0$ Hz) distinguished by analysis of the 1H NMR data (Table 1). Structure of **1** was established by detailed HMQC and HMBC experiments. The HMBC data showed correlations between H-2 (δ_H 5.29) and the carbons at δ_C 34.0 (C-3), 128.1 (C-3a) and 165.0 (C-7a), between H-3 (δ_H 3.40 and 3.09) and the carbons at δ_C 87.3 (C-2), 128.1 (C-3a) and 165.0 (C-7a), between H-4 (δ_H 7.79) and the carbons at δ_C 34.0 (C-3), 131.1 (C-6) and 165.0 (C-7a), between H-7 (δ_H 6.85) and the carbons at δ_C 126.4 (C-5), 128.1 (C-3a) and 165.0 (C-7a), which suggested the 2,3-dihydrobenzofuran part unit. The other three substituent groups were assigned by following correlations: one prop-2-en-yl group was placed at C-2 (δ_C 87.3) on the basis of HMBC correlations from H-9 (δ_H 5.08 and 4.93) to C-8 (δ_C 143.2), C-2 (δ_C 87.3) and C-10 (δ_C 17.3), the 2-hydroxy-propan-1-one group was assigned at C-5 (δ_C 126.4) on the basis of HMBC correlations from H-12 (δ_H 5.06) to C-11 (δ_C 200.7) and C-13 (δ_C 23.0), and from H-4 (δ_H 7.79) and H-6 (δ_H 7.76) to C-11 (δ_C 200.7). H-2 might be placed in an axial position, since H-2 only has a correlation with

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Table 1
The NMR data of compound **1**.

Position	¹ H	¹³ C	HMBC	COSY
2	5.29 (1H, m)	87.3	3, 3a, 7a, 8, 9, 10	H-3
3	3.40 (1H, m)	34.0	2, 3a, 8, 7a	H-2
	3.09 (1H, m)		2, 3a, 8, 7a	H-2
3a	–	128.1	–	–
4	7.79 (1H, s)	126.2	3, 6, 7a, 11	–
5	–	126.4	–	–
6	7.76 (1H, d, 8.4)	131.1	4, 5, 7a, 11	H-7
7	6.85 (1H, d, 8.4)	109.5	5, 3a, 7a	H-6
7a	–	165.0	–	–
8	–	143.2	–	–
9	5.08 (1H, s)	113.1	2, 8, 10	–
	4.93 (1H, s)		2, 8, 10	–
10	1.75 (3H, s)	17.3	2, 8, 9	–
11	–	200.7	–	–
12	5.06 (2H, m)	69.0	11, 13	H-13
13	1.43 (3H, d, 7.0)	23.0	11, 12	H-12

H-3B in its ROESY spectrum. So the structure of **1** elucidated as 2-hydroxy-1-((S)-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-yl)propan-1-one, and named as phenostereum A (Fig. 1).

Compound **2** had molecular formula C₂₂H₂₂O₄, as deduced from HRESIMS (*m/z* 373.1421 [M+Na]⁺), with 12 degrees of unsaturation. According to the ¹³C NMR spectrum, compound **2** possess 16 olefinic carbons, and were further classified by DEPT experiment into the categories of two benzene ring and two double bonds. Structure of **2** was established by detailed HMQC and HMBC experiments (Table 2). The HMBC data showed correlations between H-11 (δ_H 1.70, s) and the carbons at δ_C 144.2 (C-9), 112.4 (C-10) and 86.0 (C-2), between H-10 (δ_H 4.83 and 5.01) and the carbons at δ_C 17.4 (C-11) and 86.0 (C-2), between H-2 (δ_H 5.09) and the carbons at δ_C 17.4 (C-11), 35.3 (C-3), 154.0 (W) (C-8), between H-3 (δ_H 2.96 and 3.24) and the carbons at δ_C 86.0 (C-2), 128.0 (C-3a) and 144.2 (C-9), together with other correlations to establish 2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-ol part unit. The other part is also a benzene ring with three substituent groups according to the ¹³C and DEPT spectra (151.9 (s), 152.3 (s), 115.0 (d), 138.2 (s), 122.0 (d), 113.8 (d)). One of substituent group was 2-(hydroxymethyl)but-2-en group, which was placed at C-4' (δ_C 138.2) on the basis of HMBC correlations from H-5' (δ_H 6.86) to C-7' (δ_C 31.0), C-4' (δ_C 138.2) and C-1' (δ_C 151.9), from H-7' (δ_H 3.26, s) to C-3' (δ_C 115.0), C-4' (δ_C 138.2), C-2' (δ_C 152.3), and C-8' (δ_C 134.2), and from H-10' (δ_H 1.46) to C-11' (δ_C 75.0), C-9' (δ_C 120.3) and C-8' (δ_C 134.2), which establish the 4-(2-(hydroxymethyl)but-2-en-1-yl)benzene-1,2-diol part unit, and then correlation between H-11' and C-2' (δ_C 152.3) suggested that C-11' and C-2' were connected by oxo bridge. The two part units were connected between C-5 and C-1' by peroxo bridge, and named as phenostereum B (Fig. 1).

In addition, the antibacterial and antitumor experiments showed that these compounds have no inhibitive activity against tested microbiology and cell lines.

The discovery of novel secondary metabolites is a goal of both pharmaceutical and natural product chemists. Microorganisms are well established sources for such substances. There has been evidence that the production of secondary metabolites by an organism is not random, but seems to be correlated with the

Table 2
The NMR data of compound **2**.

Position	¹ H	¹³ C	HMBC
2	5.09 (1H, t, <i>J</i> =8.6 Hz)	86.0, d	11, 3, 8 (W)
3	3.24 (1H, dd, <i>J</i> =8.6, 15.8 Hz)	35.3, t	2, 3a, 4, 8, 9
	2.96 (1H, dd, <i>J</i> =8.6, 15.8 Hz)		2, 3a, 4, 8, 9
3a	–	128.0, s	–
4	6.61 (brs)	112.4, d	3, 6, 8
5	–	149.7, s	–
6	6.51–6.54 (3H, m, overlap)	114.4, d	–
7	6.59 (1H, d, <i>J</i> =8.4 Hz)	109.3, d	3a, 5
8	–	154.0, s	–
9	–	144.2, s	–
10	5.01 (1H, s)	112.3, t	2, 11
	4.83 (1H, s)		2, 11
11	1.70 (3H, s)	17.4, q	2, 9, 10
1'	–	151.9, s	–
2'	–	152.3, s	–
3'	6.51–6.54 (3H, m, overlap)	115.0, d	–
4'	–	138.2, s	–
5'	6.86 (1H, d, <i>J</i> =8.5 Hz)	122.0, d	–7' (W), 4', 1'
6'	6.51–6.54 (3H, m, overlap)	113.8, d	–
7'	3.26 (2H, s)	31.0, t	2', 3', 4', 5', 8'
8'	–	134.2, s	–
9'	5.52 (1H, brs)	120.3, d	4', 7', 10', 11'
10'	1.46 (3H, d, <i>J</i> =4.8 Hz)	20.3, q	8', 9', 11'
11'	4.30 (2H, s)	75.0, t	2', 7', 8', 9', 10'

ecological niche (Gloer, 1995). Fungi have proved to be a rich source of multiple ecological habitats, which live in complex ecosystems where they compete and communicate with other organisms (Losada et al., 2009). The evolution of these metabolites most likely accomplished because microorganisms used them as chemical signals for communication, to defend the habitat or to inhibit the growth of competitors. The genus of *Stereum* is often found associated with woody debris, rotting trunks and sometimes on buried dead wood. So, we need a better understanding of the relationship of these benzofuran metabolites and the microorganisms which are the producer of those secondary metabolisms.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Jasco DIP-370 digital polarimeter. NMR spectra were obtained with Bruker AVANCE III-600 spectrometer with TMS as internal standard. ESIMS and HRESIMS were recorded on Finnigan LCQ-Advantage and VG Auto-Spec-3000 mass spectrometer respectively. Column chromatography (CC) was performed on silica gel G (200–300 mesh, Qingdao Marine Chemical Factory, China) and Sephadex LH-20 (Amersham Pharmacia, Sweden).

3.2. Fungal material

The fungus used in this study was collected in Xishuangbanna, Yunnan Province, PR China. The mycelium of the basidiomycete was separated from its fruiting body and deposited in Laboratory for Conservation and Utilization of Bio-resources, Yunnan University,

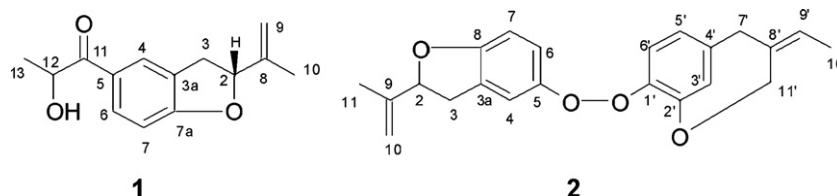


Fig. 1. The structures of compounds **1** and **2**.

Yunnan Province, China (culture collection number YMF1.1684) and subcultured on PDA (potato 200 g, glucose 20 g, agar 18 g, and water 1000 mL) at 28 °C for a period of 10 days. The mycelium-containing agar was then cut into pieces (1 cm × 1 cm), and inoculated into 250 mL Erlenmeyer flasks, each containing 50 mL of potato dextrose broth (PDB: potato 200 g, glucose 20 g, and water 1000 mL) (10 pieces for each flask). After incubation at 28 °C for 10 days on a rotary shaker (180 rpm), each primary culture was transferred into a 500 mL Erlenmeyer flasks containing 150 mL of the same liquid medium (PDB), and incubated at 28 °C for 10 days on a rotary shaker (180 rpm).

3.3. Extraction and isolation

The EtOAc extract (3.6 g) of the culture broth (15 L) was subjected to Silica Gel G CC (petroleum ether/acetone 20:1–2:1) to yield fractions A1–A5. Fraction A1 was purified further by Silica Gel G CC (200–300 mesh; petroleum ether/acetone 20:1 and 17:1) to give fraction A1-1 and fraction A1-2, respectively. Fraction A1-1 was subjected to Silica Gel G CC (petroleum ether/EtOAc 80:1) to and then repeatedly purified by Sephadex LH-20 CC eluted with acetone to furnish compound **2** (6 mg). Fraction A1-2 was applied to a Silica Gel G CC (petroleum ether/EtOAc 30:1) and then purified by Sephadex LH-20 CC eluted with acetone to furnish compound **1** (5 mg).

3.3.1. *Phenostereum A*

Colorless powder; $[\alpha]_D^{16} +5.2$ (CHCl₃, c 0.15); NMR data see Table 1; ESIMS m/z : 233 [M+H]⁺, 255 [M+Na]⁺; HRESIMS m/z : 255.0993 [M+Na]⁺, calcd. for C₁₄H₁₆O₃Na, 255.0997.

3.3.2. *Phenostereum B*

Colorless powder; $[\alpha]_D^{16} -10.4$ (CHCl₃, c 0.14); NMR data see Table 2; ESIMS m/z : 351 [M+H]⁺, 373 [M+Na]⁺; HRESIMS m/z : 373.1421 [M+Na]⁺, calcd. for C₂₂H₂₂O₄Na, 373.1415.

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