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NOTE

A new stachybotrin congener from a soil fungus *Stachybotrys parvispora* strain HS-FG-843

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Fungi have been regarded as an attractive source for discovering new bioactive compounds.[1,2] Several bioactive metabolites from fungi, such as penicillins, cephalosporins, mevastatin and lovastatin, have been the important products of pharmaceutical industry.[3] With the aim of searching for bioactive metabolites from fungi, our studies of fungi imperfeci from a variety of ecological groups have resulted in the discovery of several kinds of new natural products with antitumor activity.[4–6] As part of our continuous screening for more active secondary metabolites from soil-derived fungi imperfeci, a new stachybotrin congener, named stachybotrin G (**1**) and a known metabolite, stachybotrin (**2**) were obtained from *Stachybotrys parvispora* strain HS-FG-843. Here, the report details the fermentation, isolation, structure elucidation and bioactivity of the new compound.

The *S. parvispora* strain HS-FG-843 was isolated from a soil sample collected from the peak of a bamboo forest of Hunan province, China. It was provided and identified as *S. parvispora* by Professor Tianyu Zhang at the Shandong Agricultural University, China. The strain HS-FG-843 has been deposited in the Pharmaceutical Research Culture Collection, Zhejiang Hisun Group Co., Ltd with accession No. HS-FG-843.

The strain was grown and maintained on potato dextrose agar slant and incubated for 6–7 days at 24 °C. The stock culture was transferred into 1 l Erlenmeyer flasks containing 250 ml of the seed medium and incubated at 24 °C for 24 h, shaken at 150 r.p.m. Then, 1 l of the culture was transferred into a 50-l fermentor containing 30 l of producing medium consisting of peptone 0.5%, potato starch 0.5%, yeast extract 0.2%, NaCl 0.4%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.2% (pH 6.2–6.4). The fermentation was carried out at 24 °C for 7 days stirred at 100 r.p.m. with an aeration rate of 900 l of air per hour.

The final 30 l of broth from 50 l fermentor was filtered and the resulting cake was washed with water (3 l) and subsequently extracted with MeOH (3 l). The supernatant and the wash water were subjected to a Diaion HP-20 resin (Mitsubishi Chemical Co., Ltd., Tokyo, Japan) column eluting with 95% EtOH (5 l). The MeOH extract and the EtOH eluents were evaporated under reduced pressure to a volume of 1 l at 50 °C and the resulting concentrate was extracted three times using an equal volume of EtOAc. The combined EtOAc phase was concentrated under reduced pressure to yield a mixture (15 g). The mixture was subjected to a Sephadex LH-20 gel (GE Healthcare, Glies, UK) column eluted with $CHCl_3$/MeOH (1 : 1, v/v) and detected by TLC to give four fractions (Fr.1 to Fr.4). The Fr.2 was chromatographed on a silica gel (Qingdao Haiyang Chemical Group, Qingdao, Shandong, China; 100–200 mesh) column and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0–50:50, v/v) to obtain three fractions Fr.2-1 to Fr.2-3 based on the TLC profiles. The Fr.2-2 was subjected to another silica gel column eluted with $CHCl_3$/MeOH (90:10–70:30, v/v) to give three fractions (Fr.2-2-1, Fr.2-2-2 and Fr.2-2-3). Fr.2-2-3 was further isolated by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5μm, 250 × 9.4 mm i.d; 1.5 ml min^{-1}; 220 nm; Agilent, Palo Alto, CA, USA) eluting with MeOH/H_2O (78:22, v/v) to obtain compound **1** (t_R 23.4 min, 3.6 mg). The Fr.2-2-1 was purified by semi-preparative HPLC eluting with MeOH/H_2O (74:26) to yield stachybotrin (**2**) (t_R 23.2 min, 23 mg). ^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany). The ESIMS and high-resolution electrospray ionization MS (HRESIMS) spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Milford, MA, USA).

Compound **1** was isolated as a white amorphous powder with $[\alpha]_D^{25}+50$ (*c* 0.036, EtOH) and UV (EtOH) λ_{max} nm (log ε): 338 nm (4.89), 285 nm (4.95), 228 nm (5.08). Its molecular formula was determined as $C_{28}H_{38}N_2O_5$ according to HRESIMS (found *m/z* 483.2846 $[M+H]^+$, calcd 483.2853). The IR spectrum of **1** showed absorption bands at 3414 cm^{-1} (OH), 1650 (C = O), 1610 cm^{-1} (C = O) in the functional group region. ^{1}H NMR spectrum of **1** showed an aromatic proton singlet at δ_H 6.93 (1H, s), three tertiary methyl signals at δ_H 0.85 (3H, s), 0.96 (3H, s) and 0.98 (3H, s), one aliphatic methyl doublet at δ_H 0.69 (3H, d, $J = 6.5$ Hz). Its ^{13}C NMR and DEPT spectra displayed 28 carbon resonances, including two amide or ester carbonyl carbons at δ_C 169.4 (s, 2C), one sp^2 methine,

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five sp^2 quaternary carbons (two oxygenated), three sp^3 quaternary carbons at δ_C 98.6 (s), 42.2 (s) and 37.6 (s), four sp^3 methines at δ_C 75.7 (d), 52.3 (d), 39.9 (d) and 37.2 (d), and four methyls in addition to nine methylenes.

Table 1 ^{1}H and ^{13}C NMR data for compounds 1 (in $CDCl_3$) and 2 (in DMSO-d_6) (δ, p.p.m)

	δ_H (J in Hz)		δ_C	
Position	*1*	*2*	*1*	*2*
1	1.00 m	0.90 m	24.0 (t)	24.3 (t)
	1.73 m	1.73 m	—	—
2	1.50 m	1.40 m	26.4 (t)	25.4 (t)
	1.87 m	1.81 m	—	—
3	3.39 m	3.17 br s	75.7 (d)	73.9 (d)
4	—	—	37.6 (s)	37.8 (s)
5	2.03 m	2.01 br d (9.2)	39.9 (d)	39.6 (d)
6	1.42 m	1.37 m	20.9 (t)	20.9 (t)
	1.57 m	1.45 m	—	—
7	1.44 m	1.37 m	31.1 (t)	31.3 (t)
	1.53 m	1.52 m	—	—
8	1.75 m	1.78 m	37.2 (d)	36.9 (d)
9	—	—	98.6 (s)	98.2 (s)
10	—	—	42.2 (s)	42.3 (s)
11	2.82 d (16.8)	2.74 d (16.9)	31.8 (t)	32.2 (t)
	3.19 d (16.8)	3.10 d (16.9)	—	—
12	—	—	117.3 (s)	116.8 (s)
13	—	—	152.7 (s)	154.3 (s)
14	6.93 s	6.54 s	102.3 (d)	101.3 (d)
15	—	113.7 (s)	112.5 (s)	
16	—	—	133.7 (s)	134.6 (s)
17	—	—	156.2 (s)	156.2 (s)
18	—	—	169.4 (s)	168.0 (s)
19	4.28 d (16.1)	4.29 d (16.7)	45.6 (t)	48.2 (t)
	4.39 d (16.1)	4.35 d (16.7)	—	—
20	0.69 d (6.5)	0.64 d (6.2)	15.6 (q)	15.9 (q)
21	0.96 s	0.94 s	16.0 (q)	16.3 (q)
22	0.85 s	0.79 s	22.4 (q)	22.8 (q)
23	0.98 s	0.87 s	28.3 (q)	29.1 (q)
24	4.83 dd (11.4, 6.5)	3.59 m	52.3 (d)	59.8 (t)
25	2.05 m	3.52 m	25.0 (t)	45.1 (t)
	2.15 m	—	—	—
26	2.03 m	—	22.1 (t)	—
27	3.39 m	—	42.4 (t)	—
28	—	—	169.4 (s)	—

The proton signal at δ_H 6.93 (1H, s) in the ^{1}H NMR spectrum and the carbon resonances at δ_C 156.2 (s), 152.7 (s), 133.7 (s), 117.3 (s) and 102.3 (s) revealed the presence of a penta-substituted benzene moiety in **1.** Two protons of an AB system at δ_H 2.82 (d, J = 16.8 Hz) and 3.19 (d, J = 16.8 Hz), and the similar two protons of an AB system at δ_H 4.28 (d, J = 16.1 Hz) and 4.39 (d, J = 16.11 Hz) observed in ^{1}H NMR spectrum in connection with HMQC spectrum were assigned to the protons of two isolated methylene groups. Comparison of the NMR data (Table 1) of **1** with those of stachybotrin (**2**) isolated from this strain and stachybotrins D-F obtained from the sponge-derived fungus *Stachybotrys chartarum* MXH-X73[7] suggested that **1** belonged to the stachybotrin series containing a combination of a spirobenzofuran system with a sesquiterpenoid drimane nucleus.

Further detailed analysis of the NMR data revealed that the differences between **1** and stachybotrin (**2**) were that the isolated C-24 and C-25 methylene groups in **2** were replaced by the five carbon signals at δ_C 169.4 (s), 52.3 (d), 42.4 (t), 25.0 (t), and 22.1 (t). Considering the molecular formula $C_{28}H_{38}N_2O_5$ of **1**, the above five carbon signals were assigned to a six-membered lactam subunit as shown in Figure 1. This result was supported by the ^{1}H NMR and ^{13}C NMR chemical shifts of C-24 (δ_H 4.83, δ_C 52.3) and C-27 (δ_H 3.39, δ_C 42.4) and the HMBC correlation from δ_H 3.39 to δ_C 169.4. The connection of C-24 with C-18 and C-19 through a nitrogen atom was confirmed by the HMBC correlations from δ_H 4.83 to δ_C 169.4 (C-19) and from δ_H 4.39, 4.28 to δ_C 52.3 (C-24). The signal of H-24 was observed in ^{1}H NMR spectrum of **1** at δ_H 4.83 (dd, J = 11.4, 6.5 Hz) and those constants showed the axial orientation of H-24. Biogenetically, the relative configuration of **1** was assigned by analogy with **2** and stachybotrins D-F. Therefore, the structure of **1** was determined (Figure 1), and named stachybotrin G.

Compound **2** was also obtained as a white amorphous powder. Its structure was elucidated as stachybotrin by the comparison of the ^{1}H and ^{13}C NMR data (Table 1) of **2** with the NMR data reported by Kamalov *et al.*[8,9]

The cytotoxicity of compounds **1** and **2** were assayed *in vitro* against the human lung carcinoma A549 cell lines by the CCK8 colorimetric method as described in our previous papers.[10,11] As a result, compounds **1** and **2** exhibited cytotoxic activity with IC_{50} values of 17.4 and 9.5 μg ml^{-1}, respectively.

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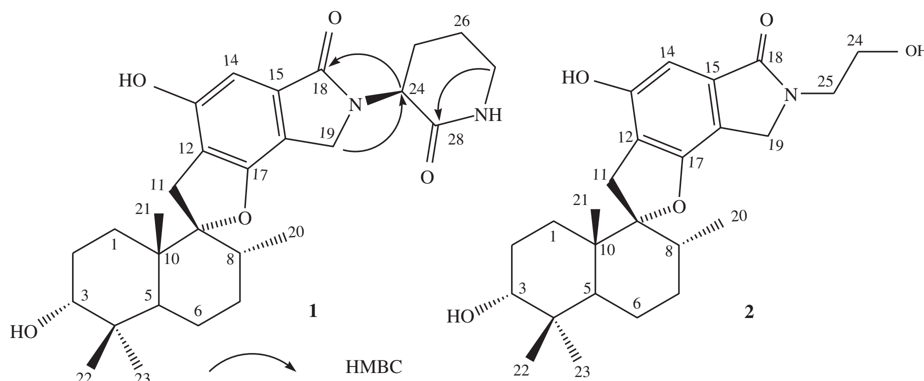

Figure 1 Structures of **1**, **2** and key HMBC correlations of **1**.

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