NOTE

Two new pentanorlanostane metabolites from a soil fungus *Curvularia borreriae* strain HS-FG-237

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Fungi are ubiquitous microorganisms in the soil inhabitants and have been considered as a promising research area for secondary metabolites in recent years. Indeed, 38% bioactive compounds that have been isolated from microbes so far are produced by fungi.¹ As illustrated by a number of important products of the pharmaceutical industry such as penicillins, cephalosporins, mevastatin and lovastatin,² fungi are also an important source of useful secondary metabolites. During the course of study of fungi imperfecti as potential sources of new bioactive secondary metabolites, several new compounds have been obtained.^{3,4} Further investigation of an isolate of *Curvularia borreriae* strain HS-FG-237 collected from samples of loess taken from vegetable plots in Xiangtan of Hunan Province, China, led to the isolation of two new pentanorlanostane metabolites (1–2, Figures 1 and 2), named as curvalarols A (1) and B (2). Here, the report deals with the details of the isolation, structure elucidation and the cytotoxicity of the two new compounds.

The soil fungus *C. borreriae* strain HS-FG-237 was isolated from samples of loess taken from vegetable plots collected from Xiangtan of Hunan Province, China. It was provided and identified as *C. borreriae* by the professor Tianyu Zhang at the Shandong Agricultural University, China. The strain HS-FG-237 has been deposited in the Pharmaceutical Research Culture Collection, Zhejiang Hisun Group Co., Ltd, with accession No.: HS-FG-237.

The working strain was grown and preserved on Potato Dextrose Agar (PDA) slant and incubated under static conditions at 24 °C for 6–7 days. The stock culture was transferred into 1-liter Erlenmeyer flasks containing 250 ml of the seed medium consisting of potato starch (Huling, Huzhou Zhanwang Pharmaceutical Co., Ltd, Zhejiang, China) 3%, glucose (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) 4%, peptone (Sincere, Shanghai Sincere Biotech Co. Ltd, Shanghai, China) 0.4%, MgSO₄·7H₂O (Sinopharm Chemical Reagent Co., Ltd) 0.1%, KH₂PO₄ (Sinopharm Chemical Reagent

Co., Ltd) 0.2% (natural pH) and incubated at 24 °C for 48 h, shaken at 250 r.p.m. Then, 1 liter of the culture was transferred into a 50-liter fermentor (containing 30-liter fermentation broth), which was composed of peptone 0.5%, potato starch 0.5%, yeast extract (Oxoid Ltd, Basingstoke, Hampshire, UK) 0.2%, NaCl (Sinopharm Chemical Reagent Co., Ltd) 0.4%, KH₂PO₄ 0.1%, MgSO₄·7H₂O 0.05%, CaCO₃ (Sinopharm Chemical Reagent Co., Ltd) 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 liters of air per hour.

The mycelial cake and supernatant were separated from the fermentation broth (30 liters) by centrifuge. The resulting cake was extracted with MeOH (4 liters) and the supernatant was subjected to a Diaion HP-20 resin column and eluted with 95% EtOH (7 liters). The mixture of MeOH soluble and EtOH eluents was evaporated under reduced pressure to obtain a crude extract (21.4 g) at 50 °C. The crude extract was subjected to a silica gel (Qingdao Haiyang Chemical Group, Qingdao, Shandong, China; 100–200 mesh) column eluted with CHCl₃–MeOH mixture (100:0–50:50, v/v) to give five fractions (Fr.1 to Fr.5) based on the TLC (HSGF 254, 200 × 100, Yantai Chemical Industry Institute, Shandong, China) profiles under UV or by heating after spraying with sulfuric acid–ethanol, 5:95 (v/v). The Fr.2 was chromatographed on a silica gel column and eluted with a stepwise gradient of CHCl₃–MeOH (99:1, 97:3, 95:5, 93:7, 90:10, v/v) successively to yield two subfractions (Fr.2-1 to Fr.2-2) based on the TLC profiles. The Fr.2-2 was subjected to a Sephadex LH-20 gel column (GE Healthcare, Glenside, UK) eluted with EtOH and detected by TLC to give one fraction (Fr.2-2-1). After concentration, the evaporated Fr.2-2-1 was dissolved with MeOH and a white undissolved substance was presented in Fr.2-2-1. So, the Fr.2-2-1 was filtered to afford a white precipitate and methanol-soluble fraction. The white precipitate dissolved in CHCl₃/MeOH (1:1, v/v) was further purified by semi-preparative HPLC (Agilent 1100, Zorbax

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SB-C18, 5 μ m, 250 $\times$ 9.4 mm i.d.; 1.5 ml min⁻¹; 220 nm; Agilent, Palo Alto, CA, USA) eluting with MeOH–H₂O (85:15, v/v) to obtain compound **1** (t_R 20.2 min, 8.5 mg). The methanol-soluble fraction was subsequently isolated by semi-preparative HPLC using MeOH–H₂O (93:7, v/v) to obtain compound **2** (t_R 14.5 min, 7.0 mg). The ESIMS and HRESIMS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Milford, MA, USA). ¹H and ¹³C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ¹H and 100 MHz for ¹³C) spectrometer (Rheinstetten, Germany).

Compound **1**, a white amorphous powder with m.p. 194–195 °C and $[\alpha]_D^{25}$ 43° (*c* 0.05, EtOH), had a molecular formula of C₂₆H₄₂O₄ as deduced from the HRESIMS (m/z 441.2962 [M + Na]⁺, calcd 441.2975 for [C₂₆H₄₂NaO₄]⁺). The UV spectrum of **1** showed absorption maxima at 209 nm (log ϵ 3.58). The IR spectrum showed absorption bands due to the hydroxyl group (3380 cm⁻¹) and carbonyl group (1719 cm⁻¹). The ¹H NMR (400 MHz, CDCl₃) spectrum of **1** indicated five singlet methyl group (δ 0.67 (s), 0.81 (s), 0.92 (s), 0.99 (s), and 1.00 (s)), an aliphatic doublet methyl (δ 1.29 (d, *J* = 7.0 Hz)), two oxygenated methine protons (δ 3.24 (dd, *J* = 4.5, 11.6 Hz), 4.11 (t, *J* = 7.8 Hz)) and a methoxy group (δ 3.65 (s)). Its ¹³C and DEPT NMR spectra revealed 26 carbon resonances including an ester carbonyl at δ 178.2 (s); two oxygenated methine carbons at δ 72.2 (s), 78.8 (s); two *sp*² quaternary carbons at δ 134.1 (s), 135.5 (s); four aliphatic quaternary carbons at δ 36.9 (s), 38.8 (s), 48.8 (s) and 52.3 (s); three aliphatic methines at δ 41.3 (d), 48.7 (d) and 50.4 (d); and one methoxy carbon at δ 51.2 (q), in addition to six methyls and seven methylenes. Except the methoxy group, the above ¹H and ¹³C NMR data (Table 1) indicated that **1** should be a tetracyclic pentanorlanostane or sesterterpenoid analog. Comparison of the ¹H and ¹³C NMR data of **1** with those of 23,24,25,26,27-pentanorlanost-8-ene-3 β ,22-diol (**3**),^{5,6} suggested that **1** was structurally related to **3**. The differences between **1** and **3** could be explained by the replacement of the hydroxymethyl and C-12 methylene in **3** by a methoxycarbonyl group and an oxymethine, respectively, in **1**. This result was further confirmed by the HMBC correlations (Figure 3) from δ_H 3.65 (OCH₃) and 1.29 (H₃-21) to δ_C 178.2 (C-22) and from δ_H 0.67 (H₃-18) to δ_C 72.2 (C-12). As a consequence, the gross structure of **1** was established (Figure 1).

The relative configuration of **1** was assigned by analogy with **3** and the NOESY correlation (Figure 3) between H-3 and H-5, H₃-28, between H₃-30 and H-17, H-12, and between H₃-18 and H-20.

Figure 1 The structures of compounds **1** and **3**.

Compound **2** was obtained as colorless oil with $[\alpha]_D^{25}$ 21° (*c* 0.08, EtOH). Its molecular formula was established to be C₂₅H₃₈O₂ by the HRESIMS data (m/z 371.2950 [M + H]⁺, calcd 371.2945). The UV spectrum of **2** showed absorption maxima at 226 nm (log ϵ 3.63), 215 nm (log ϵ 3.62). The ¹H NMR spectrum of **2** display five singlet methyl group at δ 0.76 (s), 0.89 (s), 1.08 (s), 1.16 (s) and 1.23 (s), a *sp*³ doublet methyl at

Table 1 ¹H and ¹³C NMR data for compounds **1** and **2** (in CDCl₃)

Position	δ_H (J in Hz)		δ_C	
	1	2	1	2
1	1.24 (m) 1.72 (m)	7.26 (d, 10.4)	35.7 (t)	156.5 (d)
2	1.60 (m) 1.68 (m)	5.90 (d, 10.4)	27.8 (t)	126.0 (d)
3	3.24 (dd, 4.5, 11.6)		78.8 (s)	205.0 (s)
4			38.8 (s)	44.3 (s)
5	1.02 (dd, 2.1, 12.9)	1.91 (m)	50.4 (d)	47.6 (d)
6	1.49 (m) 1.68 (m)	1.75 (m)	18.1 (t)	18.3 (t)
7	2.02 (m)	2.18 (m)	26.1 (t)	25.5 (t)
8			134.1 (s)	136.4 (s)
9			135.5 (s)	131.0 (s)
10			36.9 (s)	40.3 (s)
11	2.58 (m) 1.72 (m)	2.24 (m)	33.9 (t)	22.4 (t)
12	4.11 (t, 7.8)	1.81 (m)	72.2 (s)	30.7 (t)
13			48.8 (s)	44.7 (s)
14			52.3 (s)	49.6 (s)
15	1.20 (m) 1.68 (m)	1.26 (m) 1.67 (m)	31.0 (t)	30.8 (t)
16	1.68 (m) 1.90 (m)	1.42 (m) 1.94 (m)	25.4 (t)	27.6 (t)
17	2.15 (m)	1.61 (m)	48.7 (d)	46.8 (d)
18	0.67 (s)	0.76 (s)	9.4 (q)	16.0 (q)
19	0.99 (s)	1.24 (s)	19.1 (q)	23.1 (q)
20	2.70 (m)	1.59 (m)	41.3 (d)	39.4 (d)
21	1.29 (d, 7.0)	1.05 (d, 6.2)	18.8 (q)	16.8 (q)
22		3.36 (dd, 6.0, 10.7) 3.65 (dd, 2.6, 10.7)	178.2 (s)	68.1 (t)
28	0.81 (s)	1.16 (s)	15.4 (q)	26.2 (q)
29	1.00 (s)	1.08 (s)	28.0 (q)	21.4 (q)
30	0.92 (s)	0.89 (s)	24.1 (q)	23.9 (q)
OCH ₃	3.65 (s)		51.2 (q)

Figure 2 The structure of compound **2**.

Figure 3 The key HMBC and NOESY correlations of **1** and **2**.

δ 1.05 (d) and two olefinic protons at δ 5.90 (1H, d, $J = 10.4$ Hz) and 7.26 (1H, d, $J = 10.4$ Hz). Its ^{13}C NMR spectra showed 25 carbon signals composing six methyl carbons, seven aliphatic methylene carbons, one of which was bearing an oxygen function (δ 68.1), three aliphatic methine carbons, four sp^3 quaternary carbons, two sp^2 methines, two sp^2 quaternary carbons and a conjugated carbonyl (δ 205.0). An α,β -unsaturated ketone was present by analysis of the HMBC correlations (Figure 3) from δ_{H} 7.26 (H-1) to δ_{C} 205.0 (C-3) and the NMR data. Except the α,β -unsaturated ketone moiety, the ^1H and ^{13}C NMR data (Table 1) of **2** was similar to those of **3** and suggested that **2** was also a derivative of **3**. By detailed analysis of the NMR data of **2** and **3**, it was revealed that the secondary alcohol and two methylenes in **3** were substituted by the α,β -unsaturated ketone. The HMBC correlations from H_3 -19 to δ_{C} 156.5 (C-1) and from H_3 -28 and H_3 -29 to δ_{C} 205.0 (C-3) indicated the placement of the α,β -unsaturated ketone in **2**. Thus, the planar structure of **2** was established as shown in Figure 2.

The relative stereochemistry of **2** was assigned as those of **1** and **3** by analysis of the NMR data and the NOESY correlations (Figure 3) from H_{28} to H-5, from H_3 -30 to H-17 and from H-18 to H-20.

The cytotoxicities of compounds **1** and **2** were assayed *in vitro* against the A549, K562 and MDA-MB-231 cell lines by the CCK8 colorimetric method as described in our previous papers.^{7,8} Compound **1** exhibited cytotoxic activity with IC_{50} values of 21.9, 23.5 and $17.7 \mu\text{g ml}^{-1}$, respectively. The values of compound **2** were 15.3, 11.1 and $13.3 \mu\text{g ml}^{-1}$.

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