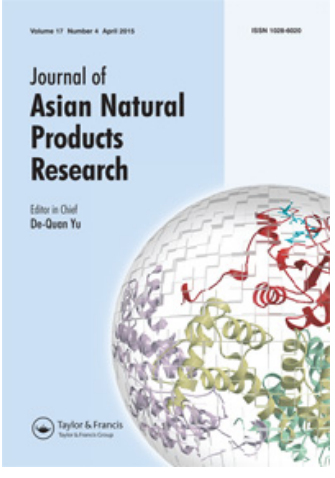

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Two new compounds from Ganoderma lucidum

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Two new compounds from *Ganoderma lucidum*

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Two pairs of new enantiomers, lucidulactones A and B (**1** and **2**), and two known compounds were isolated from *Ganoderma lucidum*. Their structures were determined by means of spectroscopic methods. The chiral HPLC was used to separate the (−)- and (+)-antipodes of the new compounds.

Keywords: *Ganoderma lucidum*; lactones; enantiomers

1. Introduction

Ganoderma lucidum (Lingzhi), belonging to the genus *Ganoderma* which includes about 80 species, is a well-known traditional medicine used for centuries in the Orient for the treatment of various diseases including cancer, asthma, and sleep disorders [1]. In recent years, hundreds of compounds have been identified from this genus, and many of them are triterpenoids and polysaccharides [2–4]. Recently, we have identified a meroterpenoid from the fruiting bodies of *G. lucidum* which exhibited significant inhibitory effect on the phosphorylation of Smad3 [5]. Encouraged by this discovery, we undertook an in-depth study on this species which resulted in the isolation of four compounds including two new lactones (Figure 1). Herein, we describe their isolation and structure characterization.

2. Results and discussion

Compound **1** had the molecular formula $C_{12}H_{14}O_6$ derived from its HR-EI-MS at *m/z* 254.0782 $[M]^+$, ^{13}C NMR and DEPT spectra, indicating six degrees of unsaturation. The ^{13}C NMR and DEPT spectra (Table 1) showed 12 carbons attributed to two methoxyl, one oxygenated methylene, four methine including two olefinic and one oxygenated, four olefinic quaternary carbons, and one carbonyl carbon. In addition to a benzene ring and carbonyl, there should be one ring in the structure. The COSY spectrum showed cross peaks of H-3/H-4/H-5. The architecture of **1** was assembled by HMBC correlations, which were H-2′, H-6′/C-4, OCH_3/C-3′, 5′, H-5/C-2, C-3, C-4, C-1′, H-3/C-2, C-4, C-5, and C-1′ (Figure 2). C-2 and C-5 are linked via an ether bond evidenced from HMBC correlations of H-5/C-2 (δ_C 176.7). The relative configuration was determined by ROESY correlations of H-3/H-2′, H-6′ (Figure 3), and the large coupling constant of H-3 ($J = 11.1$ Hz), characteristic of a trans relationship of H-3 with H-4. The structure of **1** was therefore assigned as shown, named lucidulactone A. The opti-

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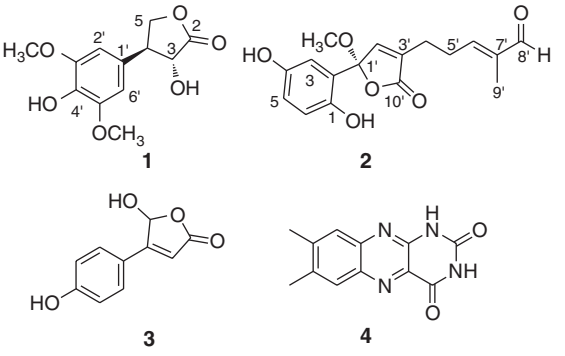

Figure 1. The structures of compounds **1**–**4**.

cal rotation of **1** indicated a racemic nature, which was subsequently separated by HPLC on a chiral phase to afford two enantiomers, (+)-**1** and (−)-**1**.

The molecular formula of compound **2** was established as $C_{17}H_{18}O_6$ from its HR-EI-MS at *m/z* 318.1109 $[M]^+$, ^{13}C NMR, and DEPT spectra, indicative of nine degrees of unsaturation. The ^1H and ^{13}C NMR spectral data of **2** (Table 1) were similar to those of fornicin A [4]. However, compared to fornicin A, in addition to the presence of a methoxyl group in **2**, an oxygenated methine and a methyl in fornicin A were, respectively, replaced by an oxygenated quaternary carbon (δ_C 107.7) and an aldehyde (δ_C 195.2) in **2**. HMBC (Figure 2) correlation of OCH_3/C-1′ suggested that the methoxyl group was located at C-1′. The ^1H–^1H COSY correlations of H-4′/H-5′/H-6′ and the HMBC correlations of H-8′/C-6′, C-7′, C-9′ indicated the presence of a terminal aldehyde. The ROESY correlation of H-6′/H-8′ (Figure 3) suggested that the geometry of double bond is trans. The optical

Table 1. ^1H and ^{13}C NMR spectral data for compounds **1** and **2** (600 MHz for ^1H and 150 MHz for ^{13}C, **1** in DMSO-d_6 and **2** in acetone-d_6).

	1			**2**	
No.	δ_H, *J* (Hz)	δ_C	No.	δ_H, *J* (Hz)	δ_C
2		176.7 s	1		151.1 s
3	4.57 dd (11.1, 7.0)	72.4 d	2		123.4 s
4	3.37–3.40 m	49.3 d	3	6.95 d (2.8)	114.3 d
5a	4.45–4.47 m	69.0 t	4		148.7 s
5b	4.14 dd (11.1, 8.8)		5	6.73 dd (8.6, 2.8)	118.0 d
1′		127.2 s	6	6.76 d (8.6)	118.3 d
2′, 6′	6.69 s	105.3 d	1′		107.7 s
3′, 5′		148.0 s	2′	7.48 s	147.4 d
4′		134.8 s	3′		135.2 s
3-OH	6.07 d (7.0)		4′	2.53–2.56 m	24.4 t
4′-OH	8.32 s		5′	2.70–2.73 m	27.2 t
3′,5′-OCH_3	3.74 s	56.0 q	6′	6.61 t (7.2)	153.0 d
			7′		140.8 s
			8′	9.38 s	195.2 d
			9′	1.67 s	9.2 q
			10′		171.4 s
			OCH_3	3.26 s	51.8 q

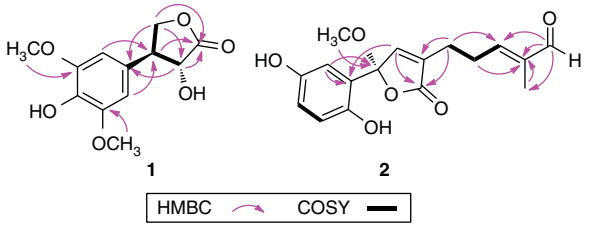

Figure 2. The COSY and HMBC correlations of **1** and **2**.

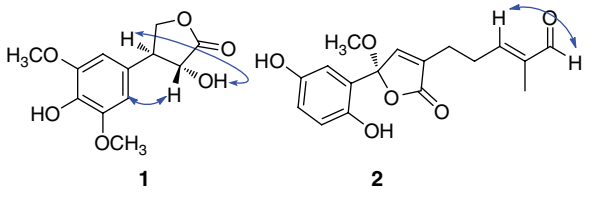

Figure 3. Key ROESY correlations of **1** and **2**.

rotation of **2** indicated a racemic nature, which was subsequently separated by HPLC on a chiral phase to afford two enantiomers, (+)-**2** and (−)-**2**, named lucidulactone B.

The known compounds were identified as hydroxybutenolide (**3**) [6] and lumichrome (**4**) [7], respectively, by comparison of their spectroscopic data with literature data.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter (Horiba, Kyoto, Japan). UV spectra were recorded on a Shimadzu UV-2401PC spectrometer (Shimadzu, Kyoto, Japan). NMR spectra were recorded on a Bruker AV-600 spectrometer (Bruker, Karlsruhe, Germany), with TMS as an internal standard. EI-MS and HR-EI-MS were collected by AutoSpec Premier P776 spectrometer (Waters, Milford, MA, USA). ESI-MS were collected by API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA). Semi-preparative HPLC was carried out using an Agilent 1200 liquid chromatograph (Agilent, Santa Clara, CA, USA), the column used was a 250 mm × 9.4 mm, i.d., 5 μm, Zorbax SB-C_{18} and a 250 mm × 4.6 mm, i.d., 5 μm, Daicel Chiralpak IC. Column chromatography was performed on silica gel (200 − 300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China), on C-18 silica gel (40–60 μm; Daiso Co., Osaka, Japan), MCI gel CHP 20P (75 − 150 μm, Mitsubishi Chemical Industries, Tokyo, Japan), and on Sephadex LH-20 (Amersham Pharmacia, Uppsala, Biosciences, Sweden).

3.2 Plant material

G. lucidum was purchased from the Culture Base of Bei-Zhi-Tang Co., Ltd in Jilin Province, China, in July 2012. A voucher specimen (CHYX-0579) has been deposited at the State Key Laboratory of Photochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried and powdered *G. lucidum* (80 kg) was extracted with 95% EtOH under reflux (2 × 3601 × 2 h) to give a crude extract, which was suspended in water followed by extracting with EtOAc

to afford a EtOAc-soluble fraction (1.1 kg). The EtOAc extract was divided into seven parts Fr. 1–Fr. 7 by a silica gel column eluted with a gradient of $CHCl_3$/MeOH (100:1 to 1:1). Fr. 3 (51 g) was fractionated by an MCI gel CHP 20P column eluting with gradient aqueous MeOH (20:80 to 100:0) to provide 11 portions Fr. 3.1–Fr. 3.11. Fr. 3.2 (8.4 g) was separated by Sephadex LH-20 (MeOH) followed by an RP-18 column (MeOH/H_2O, 40:60 to 50:50), and semi-preparative HPLC (MeOH/H_2O, 25:75) to give compounds **1** (Rt = 16.5 min, 5.5 mg) and **3** (Rt = 19.4 min, 5 mg). Fr.3.3 (31 mg) was purified by semi-preparative HPLC (MeOH/H_2O, 38:62) to give compounds **2** (Rt = 14.2 min, 2 mg) and **4** (Rt = 20.8 min, 10 mg). Compound **1** is a racemic mixture which was further purified by semi-preparative HPLC on a chiral phase (*n*-hexane/ethanol, 65:35, flow rate: 1 ml/min) to yield compounds (+)-**1** (Rt = 10.5 min, 1.3 mg) and (−)-**1** (Rt = 13.6 min, 1.5 mg). In the same manner as that of **1**, compound **2** was further purified via HPLC on a chiral phase (*n*-hexane/ethanol, 75:25, flow rate: 1 ml/min) to yield (+)-**2** (Rt = 13.8 min, 0.5 mg) and (−)-**2** (Rt = 15.6 min, 0.4 mg).

3.3.1 *Lucidulactone A (**1**)*

Yellowish gum; $\{[\alpha]_D^{22} + 67.2$ ($c = 0.14$, MeOH), (+)-lucidulactone A$\}$; $\{[\alpha]_D^{23} - 82.0$ ($c = 0.15$, MeOH), (−)-lucidulactone A$\}$. UV (MeOH) λmax (log ε) 272 (3.15), 206 (4.59) nm. For ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see Table 1. ESI-MS: *m/z* 253 $[M\text{-}H]^-$. HR-EI-MS: *m/z* 254.0782 $[M]^+$ (calcd for $C_{12}H_{14}O_6$, 254.0790).

3.3.2 *Lucidulactone B (**2**)*

Yellowish gum; $\{[\alpha]_D^{22} + 17.7$ ($c = 0.04$, MeOH), (+)-lucidulactone B$\}$; $\{[\alpha]_D^{23} - 19.1$ ($c = 0.05$, MeOH), (−)-lucilacton B$\}$. UV (MeOH) λmax (log ε) 310 (3.43), 226 (4.31), 201 (4.19) nm. For ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see Table 1. EI-MS: *m/z* 318 $[M]^+$. HR-EI-MS: *m/z* 318.1109 $[M]^+$ (calcd for $C_{17}H_{18}O_6$, 318.1103).

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Note

1. These authors contributed equally to this paper.

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