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Letter

Minor Compounds from Fungus *Ganoderma cochlear*

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ABSTRACT

Objective To study the chemical constituents of the fungus *Ganoderma cochlear*. **Methods** The compounds were isolated by using MCI gel CHP 20P, Sephadex LH-20, RP-18 column chromatography, and preparative TLC. The structures were identified by means of spectroscopic methods. **Results** Two phenolic normeroterpenoid and meroterpenoid, cochlearols C and D (**1** and **2**), together with six benzene derivatives, 3-methoxy-4-hydroxy-phenylethanol (**3**), 4-hydroxyacetophenone (**4**), *p*-hydroxycinnamic methyl ester (**5**), 2-methoxy-4-hydroxybenzaldehyde (**6**), 4-hydroxy-3-methoxy benzoic acid (**7**), and 2-hydroxy-5-ethoxybenzoic acid (**8**), were isolated from the fruiting bodies of *Ganoderma cochlear*. **Conclusion** Compounds **1** and **2** are new phenolic normeroterpenoid and meroterpenoid, respectively.

Key words

Ganoderma cochlear; Ganodermataceae; phenolic meroterpenoid

1. Introduction

Fungal species of genus *Ganoderma* are mainly distributed over subtropical and tropical regions. So far more than 80 species have been found and studied (Dou et al, 2014). Many species of *Ganoderma* are known as Lingzhi in China and renowned for their wide use for the treatment of a variety of disorders such as cancer and sleep disorder (Yang and Feng, 2013). Chemical investigations on Lingzhi mushrooms have been extensively conducted in the last decades, which revealed the presence of triterpenoids, polysaccharides, alkaloids, lectins, and peptides. Among those, triterpenoids and polysaccharides are considered to be major components in this genus (Russel and Paterson, 2006; Aryantha and Adinda, 2002). We had identified a novel meroterpenoid with a rotary door shape from *G. lucidum* (Yan et al, 2013), which aroused the interest in the related scientific community. In the course of our focus on searching bioactive meroterpenoids from species of *Ganoderma*, the title species was investigated, two pairs of novel meroterpenoids with antifibrotic activity were isolated and characterized (Dou et al, 2014). Further in-depth study on this species led to the isolation of two new phenolic meroterpenoids, cochlearols C and D (compounds **1** and **2**) together with six small molecules. Their isolation and structure identification are described below.

2. Materials and methods

2.1 General experimental procedures

Column chromatography was performed on silica gel (200−300 mesh; Qingdao Marine Chemical Inc., China), C-18

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silica gel (40–60 μm; Daiso Co., Japan), MCI gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries, Japan), and Sephadex LH-20 (Amersham Pharmacia, Sweden). UV spectra were recorded on a Shimadzu UV−2401PC Spectrometer. IR spectra were recorded on a Bruker Tensor−27 Instrument using KBr pellets. Semi-preparative HPLC was carried out using an Agilent 1200 liquid chromatography, the column used was a 250 mm × 9.4 mm, i.d., 5 μm. NMR spectra were recorded on a Bruker AV−600 spectrometer with TMS as an internal standard. ESI-MS and HR-EI-MS were collected by AutoSpec Premier P776 spectrometer.

2.2 Fungal materials

Ganoderma cochlear was purchased from Tongkang Pharmaceutical Co., Ltd. in Guangdong Province, China, in September 2013. The material was identified by Prof. Zhu-liang Yang at Kunming Institute of Botany, Chinese Academy of Sciences, and a voucher specimen (CHYX−0570) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

2.3 Extraction and isolation

The powders of fruiting bodies of *G. cochlear* (100 kg) were extracted with 70% EtOH at room temperature and concentrated under reduced pressure (6 × 300 L × 48 h) to give a crude extract (10 kg), which was suspended in water followed by extracting with EtOAc to afford an EtOAc soluble extract. This extract (2 kg) was separated by a silica gel column eluted with gradient $CHCl_3$-MeOH, (100:1–1:1) to provide seven portions (Frs. 1–7). Fr. 4 (140 g) was fractionated into seven parts (Frs. 4.1–4.7) by Sephadex LH-20 (MeOH- H_2O, 80:20), of which Fr. 4.7 (25 g) was separated by a RP-18 column (MeOH-H_2O, 10:90–60:40) followed by semi- preparative HPLC (MeOH-H_2O, 80:20) to give compound **1** (11 mg). Fr 4.1 (10.8g) was subjected to a RP-18 column (MeOH-H_2O, 10:90–70:30) followed by purification using semi-preparative HPLC (MeOH-H_2O, 40:60) to give compounds **3** (3.6 mg), **4** (8.3 mg), **5** (2.6 mg), and **6** (2.9 mg). Fr 4.5 (25 g) was subjected to a RP-18 column (MeOH-H_2O, 40:60–90:10) followed by semi-preparative HPLC (MeOH- H_2O, 30:70) purification to give compounds **7** (5.2 mg) and **8** (3.2 mg). Fr. 5 (340 g) was fractionated into five portions (Frs. 5.1–5.5) by a MCI gel CHP 20P column eluted with gradient aqueous MeOH (10%–50%). Among them, Fr. 5.4 (22 g) was further divided into five portions (Frs. 5.4.1−5.4.5) by Sephadex LH-20 (MeOH-H_2O, 80:20). Purification of Fr. 5.4.5 (2.3 g) by an RP-18 column (MeOH-H_2O, 10:90–60:40) and semi-preparative HPLC (MeOH-H_2O, 73:27) gave compound **2** (12 mg).

3. Results and discussion

Compound **1**: Yellow amorphous solid; UV (MeOH) λ_{max} (logε) 372 (3.84), 328 (3.90), 244 (4.32), 202 (4.31) nm. IR (KBr) ν_{max} 3426, 2925, 1592, 1484, 1447, 1374, 1228, 1185, 1070 cm^{-1}; ESI-MS: *m/z* 327 $[M–H]^-$. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data were shown in Table 1. Compound **1** had a molecular formula of $C_{20}H_{24}O_4$ derived from its HR-ESI-MS at *m/z* 327.1602 $[M–H]^-$, indicating nine degrees of unsaturation. The ^{13}C-NMR and DEPT spectra (Table 1) of compound **1** showed 20 carbons attributed to three methyls, four methylenes, five olefinicmethyines, seven olefinic quaternary carbons, and one carbonyl carbon. The structure of compound **1** was constructed mainly by 2D NMR experiments. The ^{1}H-^{1}H COSY spectrum (Figure 1) showed the existence of fragments H-5/H-6, H-4′/H-5′/H-6′, and H-8′/H-9′/H-10′. The HMBC correlations (Figure 2) of H-12′/C-10′, C-11′, C-13′, and H-13′/C-10′, C-11′, C-12′ revealed the position of CH_3-12′ and CH_3-13′, of H-5′, H-6′, H_3-14′/C-7′ and H_3-14′/C-6′ positioned CH_3-14′ at C-7′. HMBC correlations of H-6′/C-8′ and H-8′/C-14′, in consideration of the above mentioned ^{1}H-^{1}H COSY correlations, suggested the linkage of two isoprenyl groups as shown in Figure 2. The ^{1}H-NMR showed a typical ABX system [δ_H 7.16 (dd, J = 8.9, 2.7 Hz, H-5), δ_H 7.45 (d, J = 8.9 Hz, H-6), δ_H 7.31 (d, J = 2.7 Hz, H-3)], indicating an 1,2,4-trisubstituted aryl group. The HMBC correlations of H-5/C-1 (δ_C 153.5), C-4 (δ_C 150.2) indicated that C-1 and C-4 could be oxygenated. The HMBC correlation of H-3/C-1′ suggested that the carbonyl group was connected to the aryl group. The chemical shifts of C-2′ (δ_C 137.8) and C-3′ (δ_C 153.4) indicated a $\Delta^{2'}$ double bond with C-2′ and C-3′ being

Table 1 ^{1}H-NMR and ^{13}C-NMR data of compound 1 in DMSO-d_6 (600 MHz and 150 MHz, J in Hz)

Position	Compound **1**	
	δ_C	δ_H
1	153.5 s	
2	121.7 s	
3	107.8 d	7.31 d (2.7)
4	150.2 s	
5	123.3 d	7.16dd (8.9, 2.7)
6	119.5 d	7.45 d (8.9)
1′	172.2 s	
2′	137.8 s	
3′	153.4 s	
4′	29.3 t	2.76 t (7.0)
5′	25.1 t	2.37 m
6′	122.1 d	5.15 t (6.8)
7′	137.1 s	
8′	39.6 t	1.89 m
9′	26.6 t	1.93 m
10′	124.1 d	4.98 t (6.5)
11′	131.5 s	
12′	17.6 q	1.49 brs
13′	25.1 q	1.57 brs
14′	15.9 q	1.52 brs
15′		
4-OH		9.87 brs
2′-OH		8.63 brs