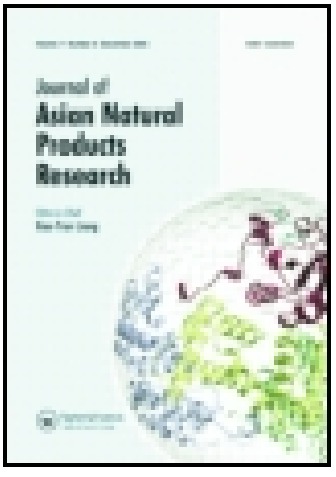

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Two new sesquiterpenoids from cultures of the basidiomycete *Tremella foliacea*

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Two new sesquiterpenoids, trefoliol B (**1**) and trefoliol C (**2**), together with known echinocidin A (**3**), were isolated from cultures of the basidiomycetes *Tremella foliacea.* The new structures were elucidated on the basis of extensive spectroscopic methods. At the same time, trefoliol B (**1**) and echinocidin A (**3**) were tested for their cytotoxicities against five human cancer cell lines and for their inhibitory activities against isozymes of 11β-hydroxysteroid dehydrogenases (11β-HSD). No compound showed significant activity ($IC_{50} > 40\ \mu M$). Compound **1** showed moderate inhibitory activities against 11β-HSD1 (human $IC_{50} = 13.1\ \mu M$; mouse $IC_{50} = 91.8\ \mu M$).

Keywords: *Tremella foliacea*; trefoliols B and C; cytotoxicities; 11β-HSD

1. Introduction

The basidiomycetes *Tremella foliacea* is an edible fungus with gelatinous fruiting bodies [1]. Our previous studies on the secondary metabolites of *T. foliacea* resulted in the isolation of trefolane A, an unprecedented skeleton sesquiterpenoid with a 5/6/4 tricyclic ring system [2]. A continuous investigation on the chemical constituents of this fungus led to the isolation of one new cadinane sesquiterpenoid, trefoliol B (**1**) and one new cucumane sesquiterpenoid, trefoliol C (**2**), together with a known protoilludane sesquiterpenoid, echinocidin A (**3**) [3] (Figure 1). Their structures were established by extensive spectroscopic methods. In previous bioactive studies, many metabolites from higher fungi were found to show cytotoxicities or inhibitory activities against isozymes of 11β-hydroxysteroid dehydrogenases (11β-HSD). For instance, terreumols A, C, and D, three meromonoterpenoids from fruiting bodies of *Tricholoma terreum*, showed comparable cytotoxicities with those of cisplatin [4]. Craterellin A, a merosesquiterpenoid from cultures of *Craterellus odoratus*, showed significant inhibitory activity against human 11β-hydroxysteroid dehydrogenases 11β-HSD2 with IC_{50} value of 1.5 μg/ml [5]. While catathelasmols C, D, and E, three pentanol derivatives from cultures of *Catathelasma imperial*, showed inhibitory activities against 11β-HSD1 and 1β-HSD2 [6]. Therefore, we also evaluated cytotoxicities of compounds **1** and **3** against five human cancer cell lines and their inhibitory activities against 11β-HSD.

2. Results and discussion

Compound **1**, a colorless oil, gave a molecular formula of $C_{15}H_{26}O_2$ by HR-ESI-MS at *m/z* 261.1834 $[M + Na]^+$, with three degrees of unsaturation. The IR

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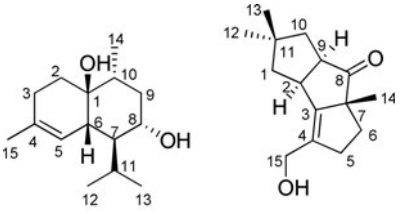

1 2

Figure 1. Sesquiterpenoids from cultures of *T. foliacea*.

absorption bands at 3361 and 1682 cm^{-1} were characteristic for hydroxy and double-bond functionalities. The ^{1}H NMR data (Table 1) showed the presence of a tertiary methyl (δ_H 1.71), three secondary methyls (δ_H 0.98, 1.00, and 1.06), an oxymethine (δ_H 3.59), and a trisubstituted olefinic proton (δ_H 5.44). The ^{13}C NMR and DEPT spectra (Table 1) displayed 15 carbon resonances comprising one oxygenated quaternary carbon, one trisubstituted double bond, one oxygenated methine, as well as four methyls, three methylenes, and four methines. The abovementioned data exhibited similarities with those of *epi*-cubenol [7]. Compound **1** was readily identified as a hydroxyl substituted derivative of *epi*-cubenol at C-8, as supported by the HMBC correlations from H-8 (δ_H 3.59) to C-7 (δ_C 55.2, d), C-9 (δ_C 40.9, t), and C-11 (δ_C 26.5, d) and the $^{1}H-^{1}H$ COSY correlations of H-7/H-8/H_2-9 (Figure 2). The ROESY experiment suggested that the relative configuration of **1** was the same to that of *epi*-cubenol, while the ROESY correlation of H-8/H-10 indicated that the 8-OH in **1** to be α-oriented. Therefore, compound **1** was established to be trefoliol B.

Compound **2** was obtained as a colorless oil with the molecular formula of $C_{15}H_{22}O_2$ based on the HREIMS at *m/z* 234.1623 $[M]^+$, corresponding to five degrees of unsaturation. The ^{1}H NMR data (Table 1) exhibited signals corresponding to three tertiary methyls (δ_H 0.99, 1.14, 1.38), and an

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** ($CDCl_3$, δ in ppm and *J* in Hz).

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1		72.1 s	1.57–1.59 m 1.81–1.83 m	47.2 t
2	1.61–1.63 m 1.72–1.74 m	21.8 t	3.51 dd (9.4, 9.8)	39.9 d
3	2.06–2.08 m	26.4 t		147.2 s
4		134.1 s		135.9 s
5	5.44 d (4.7)	122.5 d	2.43–2.45 m 2.73–2.75 m	33.8 t
6	1.74–1.76 m	46.2 d	1.80–1.83 m 1.93–1.95 m	36.4 t
7	1.19–1.21 m	55.2 d		62.3 s
8	3.59 ddd (4.2, 6.4, 10.6)	71.0 d		223.2 s
9	1.24–1.26 m 1.81–1.83 m	40.9 t	3.15 dd (9.6, 9.6)	57.9 d
10	1.75–1.77 m	39.0 d	1.59–1.61 m 1.96–1.98 m	45.2 t
11	2.17–2.19 m	26.5 d		43.6 s
12	1.00 d (7.2)	19.2 q	1.14 s	28.6 q
13	1.06 d (7.2)	21.0 q	0.99 s	26.8 q
14	0.98 d (8.0)	14.9 q	1.38 s	24.3 q
15	1.71 s	23.5 q	4.23 d (13.0) 4.32 d (13.0)	59.9 t

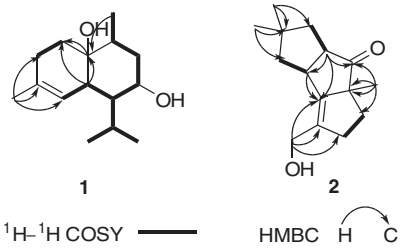

Figure 2. Selected 2D NMR correlations of **1** and **2**.

oxymethylene (δ_H 4.23, 4.32). The ^{13}C and DEPT NMR spectra (Table 1) displayed 15 carbons including a ketone carbonyl group, two sp^2 quaternary carbons, two sp^3 quaternary carbons, two methines, five methylenes (one oxygenated), and three methyls. The data suggested that **2** was a cucumane-type sesquiterpenoid similar to cucumin G [8], except for a hydroxyl group at C-15 in **2** instead of the hydroxyl group at C-5 in cucumin G, which was supported by the HMBC correlations from H-15 to C-3 (δ_C 147.2, s), C-4 (δ_C 135.9, s), and C-5 (δ_C 33.8, t) (Figure 2). The relative configuration of **2** was determined by ROESY correlations of H-12 with H-2 and H-9, 14-Me with H-5β and H-6β, which indicated that 14-Me was β-oriented, while H-2 and H-9 were α-oriented. Accordingly, compound **2** was established to be trefoliol C.

Compounds **1** and **3** were evaluated for their cytotoxicities against five human cancer cell lines using the MTT method as reported previously [9]. Unfortunately, no compound showed significant activity (IC_{50} values $> 40\,\mu M$). In addition, the inhibitory effects of **1** and **3** on human and mouse 11β-HSD1 were also investigated. As a result, compound **1** showed moderate inhibitory activities against 11β-HSD1 (human $IC_{50} = 13.1\,\mu M$; mouse $IC_{50} = 91.8\,\mu M$).

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). IR spectra were taken on a Bruker Tensor 27 FT–IR spectrometer (Bruker, Karlsruher, Germany) with KBr pellets. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker AV-400, DRX-500, and Bruker Avance III 600 MHz instruments (Bruker) with tetramethylsilane (TMS) as an internal standard at room temperature. HR-EI-MS and HR-ESI-MS were measured on a Waters AutoSpec Primier P776 instrument (Waters, Milford, MA, USA) and a Bruker HCT/Esquire (Bruker) instrument, respectively. Silica gel (200–300 mesh, Qingdao Marine Chemical Ltd, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for open column chromatography (CC). Fractions were monitored by TLC. Spots were visualized by heating silica gel plates immersed in vanillin-H_2SO_4 in ethanol.

3.2 Fungal material and cultivation conditions

T. foliacea was provided and fermented by Dr Zheng-Hui Li, Kunming Institute of Botany. A voucher specimen (No. 45869) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The culture medium consisted of glucose (5%), peptone from porcine meat (0.15%), yeast powder (0.5%), KH_2PO_4 (0.05%), and $MgSO_4$ (0.05%). The fungus was grown in seeding tank (inoculation volume 10%, 250 rpm, 24 °C, aeration 1.0 vvm, 6 days). Fermentation was carried out in a fermenter (60-L working volume) for 20 days.

3.3 Extraction and isolation

The culture broth (25 L) of *T. foliacea* was filtered, and the filtrate was extracted three times with EtOAc, while the mycelium was extracted three times with $CHCl_3$-MeOH (1:1). The EtOAc layer, together with the mycelium extraction,

was concentrated under reduced pressure to give a crude extract (40 g). The extract was subjected to CC over silica gel (200–300 mesh) eluted with a gradient of petroleum ether–acetone (1:0 → 0:1) to obtain 13 fractions (1–13). Fraction 6 (1.3 g) was separated by silica gel eluted with petroleum ether–acetone (8:1 → 6:1, v/v), then purified by reversed-phase RP-18 (MeOH-H_2O, 6:4–7:3) and Sephadex LH-20 (Me_2CO) CC to afford **1** (4.0 mg), **2** (2.5 mg), and **3** (14.0 mg).

3.3.1 *Trefoliol B (**1**)*

A colorless oil; $[\alpha]_D^{25}$ + 58.5 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3361, 2958, 2928, 1682, 1451, 1025 cm^{-1}; ^1H (400 MHz); and ^{13}C NMR (100 MHz) spectral data ($CDCl_3$) see Table 1; positive ion HR-ESI-MS: *m/z* 261.1834 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1830).

3.3.2 *Trefoliol C (**2**)*

A colorless oil; $[\alpha]_D^{25}$ −4.0 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3420, 2935, 1725, 1641, 1453, 1045 cm^{-1}; ^1H (400 MHz); and ^{13}C NMR (150 MHz) spectral data ($CDCl_3$) see Table 1; HREIMS: *m/z* 234.1623 $[M]^+$ (calcd for $C_{15}H_{22}O_2$, 234.1620).

3.4 *Cytotoxicity assay*

Five human cancer cell lines: breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells, were used in the cytotoxic assay. Cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) or in Dulbecco's Modified Eagle Medium (DMEM) (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates [9]. Briefly, 100 μl of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before the addition of drug with initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, and 8 μM in triplicates for 48 h, with cisplatin (Sigma, St Louis, MO, USA) as positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method [10].

3.5 *Inhibition on 11β-HSD1 activity assays*

The inhibition activity of compounds on human or mouse 11β-HSD1 enzymatic activities was determined in the scintillation proximity assay (SPA) using microsomes containing 11β-HSD1 as described in previous studies [11]. Briefly, the full lengths cDNAs of human or murine 11β-HSD1 were isolated from the cDNA libraries provided by the NIH Mammalian Gene Collection and cloned into a pcDNA3 expression vector. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected after cultivation in the presence of 700 μg/ml of G418. The microsomal fraction overexpressing 11β-HSD1 was prepared from the HEK-293 cells stably transfected with 11β-HSD1 and used as the enzyme source for SPA. Microsomes containing human or mouse 11β-HSD1 were incubated with nicotinamide adenine dinucleotide phosphate (NADPH) and [3H] cortisone, and then the product [^3H] cortisol was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads. All experiments were done in duplicate with glycyrrhetinic acid as a positive control. IC_{50} (± SD, $n = 2$) values were calculated using Prism

Version 4 (GraphPad Software, San Diego, CA, USA). IC_{50} values of glycyrrhetinic acid (positive control) are 5.41 and 8.42 nM for mouse 11β-HSD1 and human11β-HSD1, respectively.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] B.J. Robets and M. Spooner, *Kew Bull.* **59**, 95 (2004).
[2] J.H. Ding, T. Feng, Z.H. Li, X.Y. Yang, H. Guo, X. Yin, G.Q. Wang, and K. Liu, *Org. Lett.* **14**, 4976 (2012).
[3] Y. Shiono, T. Seto, and M. Kamata, *Z. Naturforsch.* **59**, 925 (2004).
[4] X. Yin, T. Feng, Z.H. Li, Z.J. Dong, Y. Li, and K. Liu, *J. Nat. Prod.* **76**, 1365 (2013).
[5] L. Zhang, Y. Shen, F. Wang, Y. Leng, and K. Liu, *Phytochemistry* **71**, 100 (2010).
[6] L. Zhang, Y. Shen, H.J. Zhu, F. Wang, H.J. Zhu, F. Wang, Y. Leng, and K. Liu, *J. Antibiot.* **62**, 239 (2009).
[7] Y. Ohta and Y. Hirose, *Tetrahedron Lett.* **22**, 2073 (1967).
[8] V. Hellwig, J. Dasenbrock, S. Schumann, W. Steglich, K. Leonhardt, and T. Anke, *Eur. J. Org. Chem.* **1**, 73 (1998).
[9] T. Mosmann, *J. Immunol. Methods* **65**, 55 (1983).
[10] L.J. Reed and H. Muench, *Am. J. Hygiene* **27**, 493 (1938).
[11] H. Yang, W. Dou, J. Lou, Y. Leng, and J. Shen, *Bioorg. Med. Chem. Lett.* **18**, 1340 (2008).