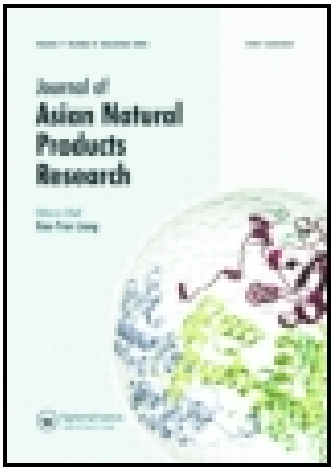

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Chain terpenoids isolated from cultures of basidiomycete Phellinus sp.

Jiang-Bo He[ab], Xiao-Man Lv[c], Zheng-Hui Li[a], Shen Zhang[a], Dong-Bao Hu[a], Rong-Hua Yin[a], Zheng-Zhu Zhao[a], Tao Feng[a] & Ji-Kai Liu[a]
[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[b] School of Medicine, Kunming University, Kunming 650214, China
[c] College of Elementary Medicine, Yunnan University of Traditional Chinese Medicine, Kunming 650500, China
Published online: 29 Jan 2015.

Journal of Asian Natural Products Research, 2015
http://dx.doi.org/10.1080/10286020.2014.1003184

Chain terpenoids isolated from cultures of basidiomycete *Phellinus* sp.

Jiang-Bo He[ab], Xiao-Man Lv[c], Zheng-Hui Li[a], Shen Zhang[a], Dong-Bao Hu[a], Rong-Hua Yin[a], Zheng-Zhu Zhao[a], Tao Feng[a]* and Ji-Kai Liu[a]*

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;* [b]*School of Medicine, Kunming University, Kunming 650214, China;* [c]*College of Elementary Medicine, Yunnan University of Traditional Chinese Medicine, Kunming 650500, China*

(*Received 7 December 2014; final version received 25 December 2014*)

Two new sesquiterpenoids (phellinuins H and I), together with five known compounds, were isolated from cultures of mushroom *Phellinus* sp. Their structures were elucidated based on comparison of nuclear magnetic resonance and MS data and those reported in the literature. All of these compounds were tested for cytotoxicity against five cancer cell lines (HL-60, SMMC-721, A-549, MCF-7, and SW-480).

Keywords: mushroom; *Phellinus* sp; sesquiterpenoids; phellinuins H and I; cytotoxicity

1. Introduction

Mushrooms of the genus *Phellinus* are used for traditional medicines, while the produced natural products played important roles in the biological activity [1,2]. For instance, the well-known compound phellinsin A was a novel chitin synthases inhibitor produced by *Phellinus* sp. [3], and the crude extract of *Phellinus* sp. showed immuno-enhancing activity [4]. Sonoko Atsumi isolated 1,6-*epi*-cyclophellitol from a culture filtrate of *Phellinus* sp., which showed significant α-glucosidase inhibition [5]. As part of our ongoing investigation to discover structurally novel and bioactive natural compounds from mushrooms, two new chain sesquiterpenoids and five known compounds (Figure 1) were isolated from cultures of *Phellinus* sp. All compounds were tested for their cytotoxic activities against human tumor cell lines HL-60, SMMC-7721, A549, MCF-7, and SW480 *in vitro*. Herein, we report the isolation and structural elucidation of these compounds.

2. Results and discussion

Compound **1** was isolated as a colorless oil. Its molecular formula $C_{15}H_{28}O_4$ was derived from HR-EI-MS at *m/z* 272.1998 $[M]^+$ (calcd for $C_{15}H_{28}O_4$, 272.1988), indicating two degrees of unsaturation. The IR spectrum showed a wide absorption band at 3425 cm^{-1} corresponding to hydroxyl groups. ^{13}C NMR and DEPT spectra showed 15 carbon resonances attributed to 4 methyl groups, 5 methylenes, 3 methines (one of them belonging to a double bond), and 3 quaternary carbons (including 2 oxygenated carbons and 1 olefinic carbon). The $^1H-^1H$ COSY spectrum showed spin systems of H-1 (δ_H 3.86)/H-2 (δ_H 3.66), H-4 (δ_H 1.66-1.70)/H-5 (δ_H 1.80–1.84)/H-6 (δ_H 3.43), and H-8 (δ_H 1.59)/H-9 (δ_H 1.99–2.04)/H-10 (δ_H 5.13) as shown in Figure 2. Furthermore, the planar structure of **1** was established by detailed analysis of HMBC data (Figure 2), which gave a backbone of a highly oxygenated chain-like sesquiterpenoid. However, a key

*Corresponding authors. Emails: fengtao@mail.kib.ac.cn; jkliu@mail.kib.ac.cn

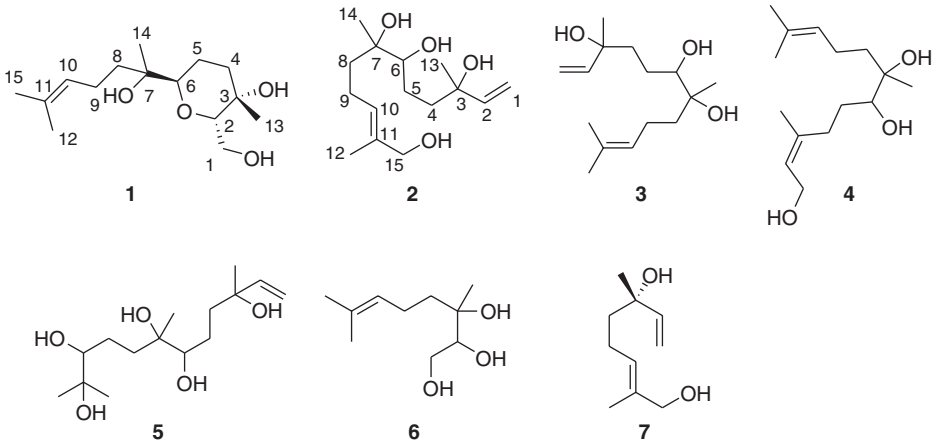

Figure 1. The structures of compounds **1**–**7**.

HMBC correlation from H-2 at δ_H 3.66 (1H, dd, $J = 9.0$ and 4.2 Hz) to C-6 at δ_C 76.4 (d) suggested that an ether bond was established between C-2 and C-6 (Figure 2). The ROESY correlations of Me-13/H-2 and H-1/H-6 suggested that Me-13 and H-2 were in the same side, while H-1 and H-6 were in the other side (Figure 2). Accordingly, the structure of **1** was assigned as shown, named phellinuin H.

Compound **2** was obtained as a colorless oil, and showed a *quasi*-molecular-ion peak at *m/z* 272.1975 $[M]^+$ in the HR-EI-MS, corresponding to the molecular formula $C_{15}H_{28}O_4$, with two degrees of unsaturation. IR spectrum revealed the existence of hydroxyl groups and double bond due to the absorption bands at 3431 and 1634 cm^{-1}. The ^1H NMR spectrum showed signals for three methyls at δ_H 1.66 (3H, s), 1.27 (3H, s), and 1.14 (3H, s), together with signals for olefinic protons at δ_H 4.96 and 5.20. The ^{13}C NMR and DEPT spectrum indicated the presence of three methyls, six methylenes (one olefinic carbon), three methines, and three quaternary carbons (including two oxygenated carbons and one olefinic carbon). $^1H-^1H$ COSY correlations showed three structure fragments as shown in Figure 2. All these data suggested that compound **2** might be a chain-like sesquiterpenoid with two double bonds and four oxygenated carbons, while the structure was finally determined by analyses of HMBC correlations. In the HMBC spectrum, correlations from H-12 at δ_H 1.66 (s) to C-10 (δ_C 127.3), C-11 (δ_C 136.0), C-15 (δ_C 69.2), from H-14 at δ_H 1.14 (s) to C-6 (δ_C 85.9), C-7 (δ_C 74.4), C-8 (δ_C 39.9), and

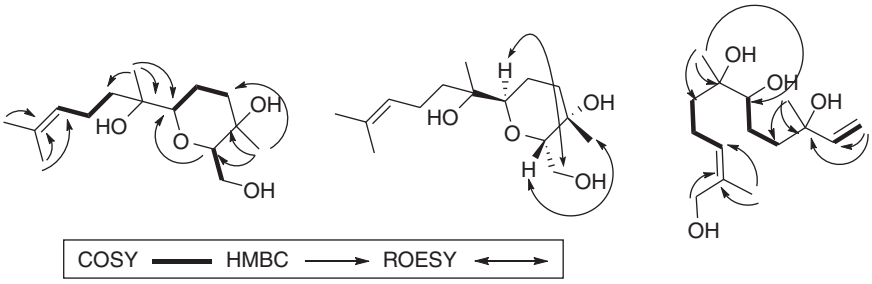

Figure 2. Key 2D NMR correlations of **1** and **2**.

from H-13 at δ_H 1.27 (s) and C-3 (δ_C 84.3) suggested that three methyls were connected to C-3, C-7, and C-11. The other HMBC correlations established the structure of **2** as shown in Figure 1. The ROESY correlation of H-10/H-15 suggested that the double bond between C-10 and C-11 to be *E* form. However, the stereoconfigurations of other chair centers could not be determined currently. Therefore, the structure of compound **2** was characterized and named phellinuin I.

The known compounds were identified as 3,7,11-trimethyldodeca-1,10-diene-3,6,7-triol (**3**) [6], 3,7,11-trimethyldodeca-2,10-diene-1,6,7-triol (**4**) [7], 2,3,6,7,10,11-pentaol (**5**) [8], 3,7-dimethyl-6-octene-1,2,3-triol (**6**) [9], 3,7-dimethylocta-1,6-dien-3,8-diol (**7**) [10], respectively, by comparison of their spectroscopic data with those reported in the literature. All compounds were evaluated for their cytotoxicities against five human cancer cell lines using the MTT method, and cisplatin was used as the positive control. However, no compound showed significant activity (IC_{50} values >40 μmol).

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. NMR spectra were recorded using Avance III 600 spectrometer (Bruker). HR-EI-MS were measured on a Waters Autospec Premier P776 mass spectrometer (Waters, Milford, MA, USA). Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), RP-18 (40–60 μm, Daiso Co., Osaka, Japan), and Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden). Fractions were monitored by TLC and spots were visualized by heating silica gel plates immersed in vanillin–H_2SO_4 in EtOH.

3.2 Plant material

Fruiting bodies of *Phellinus* sp. were collected at Jingdong, Yunnan Province, China in 2003 and identified by Prof. Zhu-Liang Yang (Kunming Institute of Botany). A voucher specimen (No. CGBWSHF00118) was deposited at the herbarium of Kunming Institute of Botany. Culture medium: glucose (5%), pork peptone (0.15%), yeast (0.5%), KH_2PO_4 (0.05%), Mg_2SO_4 (0.05%); the initial pH was adjusted to 6.0, and the fermentation was first carried out on an Erlenmeyer flask for 6 days till the mycelium biomass reached the maximum. Later it was transferred to a fermentation tank (20 L) at 24°C and 250 rpm for 20 days, and ventilation was set to 1.0 vvm (vvm: air volume/culture volume/min).

3.3 Extraction and isolation

The culture broth (20 L) was concentrated under vacuum, and extracted three times with EtOAc. The organic layer was evaporated *in vacuum* to give a crude extract (1.8 g), which was separated by Sephadex LH-20 CC (MeOH) to afford fractions A–D. Fraction B (1.5 g) was separated by reversed-phase C18 column (MeOH–H_2O, 20–100%) to give sub-fractions B1 and B5. The sub-fraction B1 (20 mg) was further purified by silica gel CC ($CHCl_3$–MeOH, 10:1) to yield **5** (2.3 mg). Fraction B2 (52.0 mg), separated by Sephadex LH-20 (MeOH), provided B2-1 (38.0 mg), which was further isolated and purified by silica gel ($CHCl_3$–MeOH, 40:1) to obtain **1** (11.0 mg). Fraction B3 (87.0 mg) was purified by Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$–MeOH, 40:1) to get **2** (2.8 mg) and **3** (8.6 mg). The sub-fraction B4 (32.0 mg) was purified by reversed-phase C18 (MeOH–H_2O, 30%) to obtain **4** (5.0 mg). Fraction B5 (112.0 mg), eluted with Sephadex LH-20 (MeOH) CC, was further separated on silica gel ($CHCl_3$–MeOH, 50:1) to give **6** (12.0 mg) and **7** (10.0 mg)

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of **1** and **2** in methanol-d_4 (δ in ppm, J in Hz).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1	3.61 dd (11.4, 4.2) 3.86 dd (11.4, 9.0)	60.2	4.96 dd (10.8, 1.8) 5.20 dd (17.4, 1.8)	111.9
2	3.66 dd (9.0, 4.2)	83.7	5.99 dd (17.4, 10.8)	145.9
3		69.4		84.3
4	1.66–1.70 m	34.2	1.79–1.84 m	39.0
5	1.46–1.50 m 1.80–1.84 m	21.7	1.87–1.90 m	27.3
6	3.43 dd (12.0, 2.4)	76.4	3.88 t (6.6)	85.9
7		75.1		74.4
8	1.45 td (13.7, 4.8) 1.59 td (13.7, 4.8)	38.8	1.50–1.52 m	39.9
9	1.99–2.04 m 2.09–2.15 m	23.3	2.13–2.17 m	22.8
10	5.13 t (8.4)	126.2	5.41 td (7.2,1.2)	127.3
11		132.2		136.0
12	1.68 s	26.1	1.66 s	13.8
13	1.09 s	26.3	1.27 s	26.0
14	1.18 s	23.2	1.14 s	22.6
15	1.63 s	17.9	3.91 s	69.2

3.3.1 *Phellinuin H (**1**)*

Colorless oil; $[\alpha]_D^{16.0} + 18.6$ ($c = 0.001$ MeOH); IR (KBr) ν_{max} 3425, 2965, 2927, 2857, 1632, 1455 cm^{-1}; ^{1}H NMR data (see Table 1); ^{13}C NMR data (see Table 1); HR-EI-MS: m/z 272.1998 (calcd for $C_{15}H_{28}O_4$, 272.1988).

3.3.2 *Phellinuin I (**2**)*

Colorless oil; $[\alpha]_D^{20.6} + 2.8$ ($c = 0.002$ MeOH); IR (KBr) ν_{max} 3431, 2971, 2929, 1634, 1412, 1032 cm^{-1}; ^{1}H NMR data (see Table 1); ^{13}C NMR data (see Table 1); HR-EI-MS: m/z 272.1975 (calcd for $C_{15}H_{28}O_4$, 272.1988).

3.4 *Cytotoxic bioassay*

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7, and SW-480. All cells were cultured in Roswell Park Memorial Institute-1640 or Dulbecco's Modified Eagle Medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA) at 37°C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-5 (3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Sigma, St. Louis, MO, USA). Briefly, 100 μl of adherent cells was seeded into each well of a 96-well cell culture plate and were allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/ml in 100 μl medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin (Sigma) as the positive control. After the incubation, MTS (100 μg) was added to each well, and the incubation continued for 4 h at 37°C. The cells were lysed with 100 μl of 20% sodium dodecyl sulfate–50% N,N-dimethylformamide after removal of 100 μl medium. The optical density of the lysate was measured at 490 nm in a 96-well

microtiter plate reader (Bio-Rad 680, Bio-Rad, Hercules, CA, USA). The IC_{50} value of each compound was calculated by the Reed and Muench's method.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] N. Langlois, J. Razafimbelo, R.Z. Andriamialisoa, J. Pusset, and G. Chauvière, *Heterocycles* **22**, 2453 (1984).
[2] I.K. Lee, G.S. Seo, N.B. Jeon, H.W. Kang, and B.S. Yun, *J. Antibiot.* **62**, 631 (2009).
[3] E.I. Hwang, B.S. Yun, Y.K. Kim, B.M. Kwon, H.G. Kim, H.B. Lee, W.J. Jeong, and S.U. Kim, *J. Antibiot.* **53**, 903 (2000).
[4] Q.B. Wang, Y. Yang, S. Zhou, Y.F. Liu, J.S. Zhang, Q.J. Tang, N. Feng, and D. Wu, *Nat. Prod. Res. Dev.* **22**, 873 (2010).
[5] S. Atsumi, C. Nosaka, Y. Ochi, H. Iinuma, and K. Umezawa, *Cancer Res.* **53**, 4896 (1993).
[6] D.S. Holmes, D.M. Ashworth, and J.A. Robinson, *Helv. Chim. Acta* **73**, 260 (1990).
[7] M. Kodama, S. Yoshio, T. Tabata, Y. Deguchi, Y. Sekiya, and Y. Fukuyama, *Tetrahedron Lett.* **38**, 4627 (1997).
[8] G. Vidari, A. Giori, A. Dapiaggi, and G. Lanfranchi, *Tetrahedron Lett.* **34**, 6925 (1993).
[9] A. Bongini, G. Cardillo, M. Orena, G. Porzi, and S. Sandri, *J. Org. Chem.* **47**, 4626 (1982).
[10] H. Morikawa, R. Kasai, H. Otsuka, E. Hirata, T. Shinzato, M. Aramoto, and Y. Takeda, *Chem. Pharm. Bull.* **52**, 1086 (2004).