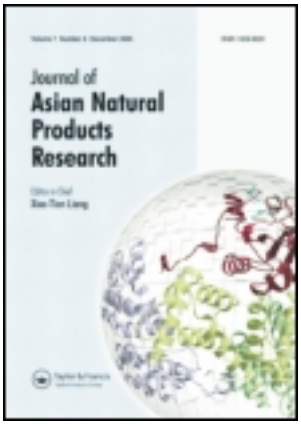

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Two new sesquiterpenes from cultures of the basidiomycete Agaricus arvensis

Jiang-Yuan Zhao [a b], Jian-Hai Ding [b], Zheng-Hui Li [b], Ze-Jun Dong [b], Tao Feng [b], Hong-Bin Zhang [a] & Ji-Kai Liu [b]

[a] Key Laboratory of Medicinal Chemistry for Natural Resources, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming, 650091, China

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China

Published online: 19 Feb 2013.

To cite this article: Jiang-Yuan Zhao, Jian-Hai Ding, Zheng-Hui Li, Ze-Jun Dong, Tao Feng, Hong-Bin Zhang & Ji-Kai Liu (2013) Two new sesquiterpenes from cultures of the basidiomycete Agaricus arvensis, Journal of Asian Natural Products Research, 15:3, 305-309, DOI: 10.1080/10286020.2013.764287

To link to this article: http://dx.doi.org/10.1080/10286020.2013.764287

Journal of Asian Natural Products Research, 2013
Vol. 15, No. 3, 305–309, http://dx.doi.org/10.1080/10286020.2013.764287

Two new sesquiterpenes from cultures of the basidiomycete *Agaricus arvensis*

Jiang-Yuan Zhao[ab], Jian-Hai Ding[b], Zheng-Hui Li[b], Ze-Jun Dong[b], Tao Feng[b], Hong-Bin Zhang[a] and Ji-Kai Liu[b]*

[a]*Key Laboratory of Medicinal Chemistry for Natural Resources, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, China;* [b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

(*Received 26 October 2012; final version received 4 January 2013*)

Two new drimane sesquiterpenoids, 11,12-dihydroxy-15-drimeneoic acid (**1**) and 3α,11,15-trihydroxydrimene (**2**), were isolated from cultures of the basidiomycete *Agaricus arvensis*, together with one known compound 3β,11,12-trihydroxydrimene (**3**). Their structures were established by means of spectroscopic analysis.

Keywords: drimane sesquiterpenoid; *Agaricus arvensis*; basidiomycete

1. Introduction

Agaricus arvensis, commonly known as the horse mushroom, is one of the largest *Agaricus* species [1]. The horse mushroom is regarded as one of the most delicious edible fungi, although the fruiting bodies of this and other yellow-staining *Agaricus* species often have a build-up of heavy metals, such as cadmium and copper [2]. Antioxidant activity has been investigated for this and some other *Agaricus* species [3], and in China it is claimed to have anti-cancer properties and has been used to cure lower back pain and pain in tendons and veins [4]. But little work has been done on the chemical constituents of *A. arvensis.* During our search for naturally occurring bioactive secondary metabolite from higher fungi in China, we investigated the cultures of *A. arvensis*, which led to the isolation of two new drimane-type sesquiterpenoids 11,12-dihydroxydrimene-15-oic acid (**1**) and 3α,11,15-trihydroxydrimene (**2**), together with one known compound 3β,11,12-trihydroxydrimene (**3**) (Figure 1). Their structures have been elucidated on the basis of spectroscopic analysis, especially 2D NMR experiments. Compounds **1** and **2** showed no significant activity against five human cancer cell lines.

2. Results and discussion

Compound **1** was obtained as a colorless oil. Its molecular formula was determined to be $C_{15}H_{24}O_4$ by HR-ESI-MS at *m/z* 291.1573 $[M + Na]^+$, in combination with ^1H and ^{13}C NMR spectral data (Table 1). The IR spectrum revealed the existence of hydroxyl and carbonyl groups due to absorption bands at 3393 and 1693 cm^{-1}, respectively. The ^1H NMR spectrum of **1** displayed signals for two tertiary methyl groups (δ_H 0.73, s, H-13; 1.20, s, H-14), two oxymethylenes (δ_H 3.61, dd, $J = 10.9$, 7.5 Hz, H-11a; 3.85, dd, $J = 10.9$, 2.5 Hz, H-11b; δ_H 3.93, d, $J = 12.3$ Hz, H-12a; 4.24, d, $J = 12.3$ Hz, H-12b), and an olefinic proton (δ_H 5.73, m, H-7). The

*Corresponding author. Email: jkliu@mail.kib.ac.in

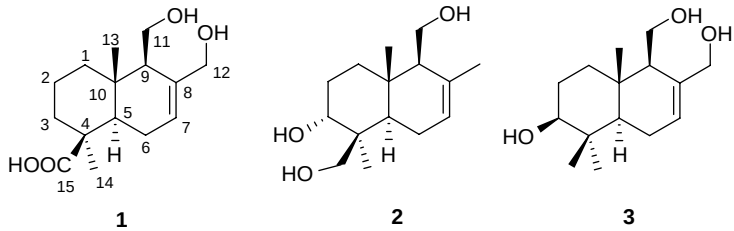

Figure 1. Structures of compounds **1**–**3**.

^{13}C NMR spectrum of **1**, together with the DEPT, HMQC, and ^{1}H NMR spectra, revealed 15 carbon resonances, including two methyl carbons (δ_C 13.9, C-13; 29.2, C-14), four methylene carbons (δ_C 40.2, C-1; 20.4, C-2; 38.9, C-3; 24.9, C-6), two methines (δ_C 51.4, C-5; 54.6, C-9), two quaternary carbons (δ_C 36.6, C-10; 43.9, C-4), two olefinic carbons (δ_C 125.5, C-7; 138.1, C-8), two oxygenated methylene carbons (δ_C 61.2, C-11; δ_C 66.9, C-12), and one keto carbon at δ_C 178.7 (C-15). In the ^{1}H–^{1}H COSY spectrum, three fragments were established by the correlations of H-1/H-2/H-3, H-5/H-6/H-7, and H-9/H-11. In the HMBC spectrum, the key correlations from H-2 to C-4 and H-3 to C-15 suggested the linkage of C-15 to C-4, the correlations of H-11 with C-8 and H-12 with C-7 revealed the connections of C-11 to C-9 and C-12 to C-8, respectively (Figure 2). The data mentioned above

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **2** (δ in ppm, J in Hz).

	1[a]		**2**[b]	
No.	δ_C	δ_H	δ_C	δ_H
1	40.2 (t)	1.18 (m) 2.04 (m)	38.6 (t)	2.07 (m) 1.29 (m)
2	20.4 (t)	1.44 (m) 1.96 (m)	28.8 (t)	1.81 (m) 1.74 (m)
3	38.9 (t)	1.10 (m) 2.13 (m)	81.0 (d)	3.41 (m)
4	43.9 (s)		42.8 (s)	
5	51.4 (d)	1.46 (m)	51.6 (d)	1.29 (m)
6	24.9 (t)	2.25 (m) 2.60 (m)	24.0 (t)	2.00 (m) 1.88 (m)
7	125.5 (d)	5.73 (m)	123.1 d	5.41 (m)
8	138.1 (s)		135.2 (s)	
9	54.6 (d)	2.07 (overlapped)	58.0 (d)	1.80 (overlapped)
10	36.6 (s)		36.3 (s)	
11	61.2 (t)	3.61 (dd, J = 10.9, 7.5) 3.85 (dd, J = 10.9, 2.5)	60.7 (t)	3.78 (dd, J = 10.9, 6.7) 3.58 (dd, J = 10.9, 5.6)
12	66.9 (t)	3.93 (d, J = 12.3) 4.24 (d, J = 12.3)	22.3 (q)	1.76 (3H, s)
13	13.9 (q)	0.73 (3H, s)	16.0 (q)	0.78 (3H, s)
14	29.2 (q)	1.20 (3H, s)	23.2 (q)	1.17 (3H, s)
15	178.7 (s)		64.4 (t)	4.18 (2H, m)
3-OH				4.65 (d, J = 4.8)
11-OH				3.38 (m)
15-OH				3.85 (dd, J = 8.4, 1.8)

[a] At 400 and 100 MHz, in acetone-d_6.
[b] At 600 and 150 MHz, in acetone-d_6.

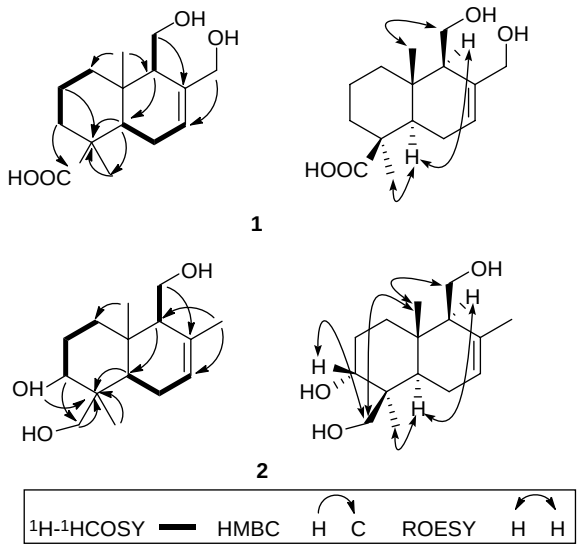

Figure 2. Key 2D NMR correlations of compounds **1** and **2**.

suggested that compound **1** possessed a drimane sesquiterpenoid skeleton related to that of 11,12-dihydroxydrimene [5], except that the methyl of C-15 was oxidized into a carboxyl group at δ_C 178.7 (s, C-15), which was supported by the key HMBC correlation from H-3 to C-15 (Figure 2). The relative configuration was inferred from ROESY interactions between H-5/H-14, H-5/H-9, and H-13/H-11, which suggested that H-5 and C-14 were α-oriented, while C-11, C-13, and C-15 were β-oriented (Figure 2). Hence, compound **1** was determined as 11,12-dihydroxy-15-drimeneoic acid.

Compound **2**, a colorless oil, possessed a molecular formula $C_{15}H_{26}O_3$ as established by the HR-ESI-MS at *m/z* 277.1772 $[M + Na]^+$. The ^1H and ^{13}C NMR spectral data (Table 1) were very similar to those of **1**, implying that they shared the same drimane skeleton. Analysis of the NMR spectral data indicated that compound **2** was closely related to 3β-hydroxydrimenol [6], except that the methyl of C-15 in **2** was oxidized into a hydroxymethyl [δ_H 4.18 (2H, m, H-15); δ_C 64.4 (t, C-15)], as indicated by the HMBC correlation from H-15 at δ_H 4.18 (2H, m) to C-4 at δ_C 42.8 (s). Detailed analysis of other 2D NMR data suggested that the other parts were similar to those of 3β-hydroxydrimenol (Figure 2). In the ROESY spectrum, the correlations of H-5/H-14, H-5/H-9, H-3/H-15, H-11/H-13, and H-13/H-15 indicated H-3, Me-13, and CH_2-15 to be β-oriented, while H-5, Me-15, and H-9 were α-oriented (Figure 2). Thus, compound **2** was determined as 3α,11,15-trihydroxydrimene.

Compounds **1** and **2** were evaluated for their cytotoxicities against five human cancer cell lines: MCF-7 breast, SMMC-7721 hepatocellular carcinoma, HL-60 myeloid leukemia, SW480 colon cancer, and A-549 lung cancer. Unfortunately, no significant activity was detected ($IC_{50} > 40$ μM).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy using KBr pellets. NMR

spectra were run on Avance III 600, Bruker DRX-500, and Bruker AM-400 spectrometers with tetramethylsilane as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HR-ESI-MS were obtained on an API-Qstar-Pulsar-1 spectrometer. Column chromatography (CC) was carried out on silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd, Qingdao, China), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), and RP-18 (20–45 μm, Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan). Fractions were monitored by thin-layer chromatography (GF 254, Qingdao Haiyang Chemical Co. Ltd), and spots were visualized by 10% H_2SO_4 in ethanol.

3.2 Fungus material

The fungi *A. arvensis* were collected from Deqin County, Yunnan province, China, in August 2005, and identified by Prof. Zhu-Liang Yang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). Voucher specimens have been deposited at the Herbarium of the Kunming Institute of Botany, CAS. The mycelial cultures were derived from tissue plugs.

3.3 Cultivation

The culture medium consisted of glucose 5%, peptone 0.15%, yeast 0.5%, KH_2PO_4 0.05%, and $MgSO_4$ 0.05% in 1 liter of deionized water (pH 6.5 before autoclaving). The fungus was grown in Erlenmeyer flasks (500 with 300 ml of medium). Fermentation was carried out in a rotary shaker at 24°C and 150 rpm for 40 days.

3.4 Extraction and isolation

The culture broth (25 liters) of *A. arvensis* was filtered, and the filtrate was extracted three times with ethyl acetate (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 (16 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 nine fractions (1–9). Fraction 5 (2.1 g) was separated by RP-18 (MeOH–H_2O, 3:7 → 9:1) to give five subfractions (A–E). Subfraction C (120 mg) was purified by Sephadex LH-20 (acetone) CC to afford **1** (5.5 mg) and **2** (1.2 mg).

3.4.1 11,12-Dihydroxydrimene-15-oic acid (**1**)

Colorless oil; $[\alpha]_D^{12.9}+4.2$ (*c* 0.30, MeOH). UV (MeOH) λ_{max}: 202, 231 nm; IR (KBr) ν_{max}: 3393, 2930, 2852, 1693, 1446, 1386, 1202, 1032, 987 cm^{-1}; for ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 1; positive HR-ESI-MS *m/z*: 291.1573 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_4Na$, 291.1572).

3.4.2 3α,11,15-Trihydroxydrimene (**2**)

Colorless oil; $[\alpha]_D^{12.7}$ −12.0 (*c* 0.10, MeOH). UV (MeOH) λ_{max}: 204 nm; IR (KBr) ν_{max}: 3428, 2928, 2854, 1630, 1028, 696 cm^{-1}; for ^1H NMR (600 MHz, MeOD) and ^{13}C NMR (150 MHz, MeOD) spectral data, see Table 1; positive HR-ESI-MS *m/z*: 277.1772 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1779).

3.5 Cytotoxicity assay

Five human cancer cell lines, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW480, and lung cancer A-549 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1460 or DMEM medium (Hyclone, Logan, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at

37°C. The cytotoxicity assay was carried out according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates [7]. Briefly, 100 μl adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.064, 0.32, 1.6, 8, and 40 μmol in triplicates for 48 h, with cisplatin (Sigma-Aldrich, St. Louis, Mo, USA) and taxol (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) as positive controls. 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 [8].

Acknowledgments

This work was financially supported by National Basic Research Program of China (973 Program, 2009CB522300) and the National Natural Science Foundation of China (U1132607).

References

[1] O. Miller, *Mushrooms of North America* (E.P. Dutton, New York, 1984), Vol. 12.
[2] R. Courtecuisse and B. Duhem, *Mushrooms and Toadstools of Britain and Europe*. *Collins Field Guides*, Harper Collins, London, 29 (1995).
[3] L. Barros, M.J. Ferreira, B. Queirós, I.C.F.R. Ferreira, P. Baptista, *Food Chem.* **13**, 413 (2007).
[4] J. Ying, *Icones of Medicinal Fungi from China*, (Science Press, Beijing, 1987).
[5] G.D. Brown and J. Essent, *Oil Res.* **7**, 705 (1995).
[6] D. Xu, Y. Shen, Z.Y. Zhou, R. Liu, Y. Leng, J.K. Liu, *Chem. Pharm. Bull.* **57**, 433 (2009).
[7] T.J. Mosmann, *Immunol. Methods* **65**, 55 (1983).
[8] L.J. Reed and H. Muench, *Am. J. Hyg.* **27**, 493 (1983).