Two new sesquiterpenes from cultures of the basidiomycete Agaricus arvensis

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

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

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

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms &
Two new sesquiterpenes from cultures of the basidiomycete *Agaricus arvensis*

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

<sup>a</sup>Key Laboratory of Medicinal Chemistry for Natural Resources, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, China; <sup>b</sup>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 anticancer 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<sub>15</sub>H<sub>24</sub>O<sub>4</sub> by HR-ESI-MS at m/z 291.1573 [M + Na]<sup>+</sup>, in combination with <sup>1</sup>H and <sup>13</sup>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<sup>-1</sup>, respectively. The <sup>1</sup>H NMR spectrum of 1 displayed signals for two tertiary methyl groups (δ<sub>H</sub> 0.73, s, H-13; 1.20, s, H-14), two oxymethylenes (δ<sub>H</sub> 3.61, dd, J = 10.9, 7.5 Hz, H-11a; 3.85, dd, J = 10.9, 2.5 Hz, H-11b; δ<sub>H</sub> 3.93, d, J = 12.3 Hz, H-12a; 4.24, d, J = 12.3 Hz, H-12b), and an olefinic proton (δ<sub>H</sub> 5.73, m, H-7). The

<sup>*</sup>Corresponding author. Email: jkliu@mail.kib.ac.in

© 2013 Taylor & Francis
13C NMR spectrum of 1, together with the DEPT, HMQC, and 1H NMR spectra, revealed 15 carbon resonances, including two methyl carbons (δC 13.9, C-13; 29.2, C-14), four methylene carbons (δC 40.2, C-1; 20.4, C-2; 38.9, C-3; 24.9, C-6), two methines (δC 51.4, C-5; 54.6, C-9), two quaternary carbons (δC 36.6, C-10; 43.9, C-4), two olefinic carbons (δC 125.5, C-7; 138.1, C-8), two oxygenated methylene carbons (δC 61.2, C-11; δC 66.9, C-12), and one keto carbon at δC 178.7 (C-15). In the 1H–1H 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. 1H and 13C NMR spectroscopic data of compounds 1 and 2 (δ in ppm, J in Hz).

<table>
<thead>
<tr>
<th>No.</th>
<th>δC</th>
<th>δH</th>
<th>δC</th>
<th>δH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.2</td>
<td>1.18 (m)</td>
<td>38.6</td>
<td>2.07 (m)</td>
</tr>
<tr>
<td>2</td>
<td>20.4</td>
<td>1.44 (m)</td>
<td>28.8</td>
<td>1.81 (m)</td>
</tr>
<tr>
<td>3</td>
<td>38.9</td>
<td>1.10 (m)</td>
<td>81.0</td>
<td>3.41 (m)</td>
</tr>
<tr>
<td>4</td>
<td>43.9</td>
<td>1.96 (m)</td>
<td>51.1</td>
<td>1.29 (m)</td>
</tr>
<tr>
<td>5</td>
<td>51.4</td>
<td>1.96 (m)</td>
<td>24.0</td>
<td>2.00 (m)</td>
</tr>
<tr>
<td>6</td>
<td>24.9</td>
<td>1.96 (m)</td>
<td>26.0</td>
<td>1.88 (m)</td>
</tr>
<tr>
<td>7</td>
<td>125.5</td>
<td>5.73 (m)</td>
<td>123.1</td>
<td>5.41 (m)</td>
</tr>
<tr>
<td>8</td>
<td>138.1</td>
<td>1.96 (m)</td>
<td>135.2</td>
<td>1.96 (m)</td>
</tr>
<tr>
<td>9</td>
<td>54.6</td>
<td>2.07 (overlapped)</td>
<td>58.0</td>
<td>1.80 (overlapped)</td>
</tr>
<tr>
<td>10</td>
<td>36.6</td>
<td>3.61 (dd, J = 10.9, 7.5)</td>
<td>36.3</td>
<td>3.61 (dd, J = 10.9, 7.5)</td>
</tr>
<tr>
<td>11</td>
<td>61.2</td>
<td>3.85 (dd, J = 10.9, 2.5)</td>
<td>60.7</td>
<td>3.78 (dd, J = 10.9, 6.7)</td>
</tr>
<tr>
<td>12</td>
<td>66.9</td>
<td>3.93 (d, J = 12.3)</td>
<td>22.3</td>
<td>1.76 (3H, s)</td>
</tr>
<tr>
<td>13</td>
<td>13.9</td>
<td>0.73 (3H, s)</td>
<td>16.0</td>
<td>0.78 (3H, s)</td>
</tr>
<tr>
<td>14</td>
<td>29.2</td>
<td>1.20 (3H, s)</td>
<td>23.2</td>
<td>1.17 (3H, s)</td>
</tr>
<tr>
<td>15</td>
<td>178.7</td>
<td>4.24 (d, J = 12.3)</td>
<td>64.4</td>
<td>4.18 (2H, m)</td>
</tr>
</tbody>
</table>

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-d6.
b At 600 and 150 MHz, in acetone-d6.
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 $\delta_C$ 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 $\alpha$-oriented, while C-11, C-13, and C-15 were $\beta$-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\textsubscript{15}H\textsubscript{26}O\textsubscript{3} as established by the HR-ESI-MS at $m/z$ 277.1772 [M + Na]$^+$. The $^1$H 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 3$\beta$-hydroxydrimenol [6], except that the methyl of C-15 in 2 was oxidized into a hydroxymethyl [H-15 4.18 (2H, m, H-15); $\delta_C$ 64.4 (t, C-15)], as indicated by the HMBC correlation from H-15 at $\delta_H$ 4.18 (2H, m) to C-4 at $\delta_C$ 42.8 (s). Detailed analysis of other 2D NMR data suggested that the other parts were similar to those of 3$\beta$-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\textsubscript{2}-15 to be $\beta$-oriented, while H-5, Me-15, and H-9 were $\alpha$-oriented (Figure 2). Thus, compound 2 was determined as 3$\alpha$,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\textsubscript{50} > 40 $\mu$M).

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% H2SO4 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%, KH2PO4 0.05%, and MgSO4 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 CHCl3–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–H2O, 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 (I)
Colorless oil; [α]D20 + 4.2 (c 0.30, MeOH). UV (MeOH) λmax 202, 231 nm; IR (KBr) νmax 3393, 2930, 2852, 1693, 1446, 1386, 1202, 987 cm−1; for 1H NMR (400 MHz, CDCl3) and 13C NMR (100 MHz, CDCl3) spectral data, see Table 1; positive HR-ESI-MS m/z: 291.1573 [M + Na]+ (calcd for C15H24O4Na, 291.1572).

3.4.2 3α,11,15-Trihydroxydrimene (2)
Colorless oil; [α]D20 − 12.0 (c 0.10, MeOH). UV (MeOH) λmax 204 nm; IR (KBr) νmax 3428, 2928, 2854, 1630, 1028, 696 cm−1; for 1H NMR (600 MHz, MeOD) and 13C NMR (150 MHz, CDCl3) spectral data, see Table 1; positive HR-ESI-MS m/z: 277.1772 [M + Na]+ (calcd for C15H26O3Na, 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% CO2 at
37°C. The cytotoxicity assay was carried out according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-lium 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