Four New Sesquiterpenoids from Cultures of the Fungus

Phellinidium sulphurascens

Zhen-Zhu Zhao · He-Ping Chen · Tao Feng · Zheng-Hui Li · Ze-Jun Dong · Ji-Kai Liu

Abstract Four new sesquiterpenoids, namely 12-hydroxy-3-oxodrimenol (1), 11-hydroxyacetoxydrim-7-en-3β-ol (2), 2,6-dimethyl-7,10-epoxy-10-hydroxymethylodeca-2,11-dien-6-ol (3), and 7,10-epoxy-2,6,10-trimethylodeca-2,11-diene-4,6-diol (4), along with fourteen known compounds, were isolated from the cultures of Phellinidium sulphurascens. The structures of compounds 1–4 were established on the basis of extensive spectroscopic analysis. All of them were evaluated for their cytotoxic activities.

Keywords Phellinidium sulphurascens · Drimane · 7,10-Epoxy-2,6,10-trimethylodeca-2,11-diene

1 Introduction

The wood-decaying fungus Phellinidium sulphurascens is a rare species belonging to the genus Phellinidium which is close-related to the genus Phellinus taxonomically. So far, only seven species of this genus were discovered in China, including P. sulphurascens [1]. In addition, the genus Phellinus has plenty of bioactive secondary metabolites involved in different types, such as sesquiterpenoids, steroids, pigments and polysaccharides [2–5]. Most of these metabolites showed significant vascular-relaxing [2], antiviral [3], estrogenic and anti-estrogenic [4], and antitumor activities [5]. However, the fungus P. sulphurascens has not been chemically investigated until now. As our continuous search for bioactive natural products from higher fungi, it is of importance to carry out the chemical investigation on cultures of P. sulphurascens, which resulted in the isolation of four new sesquiterpenoids (Fig. 1), namely 12-hydroxy-3-oxodrimenol (1), 11-hydroxyacetoxydrim-7-en-3β-ol (2), 2,6-dimethyl-7,10-epoxy-10-hydroxymethylodeca-2,11-dien-6-ol (3), and 7,10-epoxy-2,6,10-trimethylodeca-2,11-diene-4,6-diol (4), together with fourteen known compounds, from the EtOAc extract of the cultures of this fungus. All compounds were evaluated for their cytotoxicities against five human cancer cell lines.

2 Results and Discussion

Compound 1, obtained as amorphous powder, possessed a molecular formula C15H24O3, as deduced from HREIMS at m/z 252.1723 [M]+ (calcd for C15H24O3, 252.1725). The IR spectrum showed absorption bands at 3440 cm⁻¹ for hydroxy and 1701 cm⁻¹ for carbonyl functional groups. The 1H NMR spectrum of compound 1 showed three methyl singlets (δH 1.02, 1.04 and 1.08), two O-bearing methylene signals (δH 3.70, ddd, J = 11.1, 5.0, 2.5 Hz; δH...
3.90, ddd, J = 11.1, 6.9, 5.0 Hz and δ<sub>H</sub> 3.96, dd, J = 12.2, 6.9 Hz; δ<sub>H</sub> 4.27, dd, J = 12.2, 5.3 Hz), and one olefinic proton (δ<sub>H</sub> 5.78, d, J = 5.1 Hz). The <sup>13</sup>C NMR spectrum of 1 showed fifteen carbon resonances, which were ascribed to a trisubstituted double bond, three methyl, five methylenes (two oxygenated), two sp<sup>3</sup> methines, two sp<sup>3</sup> quaternary carbons and one carboxyl (δ<sub>C</sub> 215.1) (Table 1). The chemical shift values of 1D NMR of 1 were quite similar to those of the known compound 3β,12-dihydroxydrimenol (1a) [6], which suggested compound 1 possessed a drimane sesquiterpenoid skeleton. The notable difference between 1a and 1 was that the hydroxy group at C-3 (δ<sub>C</sub> 79.7) in 1a was oxidized into a carbonyl group (δ<sub>C</sub> 215.1) in 1, which caused the downfield shifts of C-4 from δ<sub>C</sub> 39.8 in 1a to 47.9 in 1, and C-2 from δ 28.1 in 1a to 38.7 in 1. The above assignment was further supported by the HMBC correlations from δ<sub>H</sub> 1.08 (Me-14, s) and 1.02 (Me-15, s) to δ<sub>C</sub> 215.1 (s, C-3) (Fig. 2), as well as IR absorption band at 1701 cm<sup>-1</sup> and mass data analysis. In the ROESY spectrum (Fig. 2), cross peaks between Me-15/H-5, H-5/H-9, Me-13/H-11 were observed, which suggested that both Me-13 and H-11 should be β oriented. Therefore, compound 1 was established as 12-hydroxy-3-oxodrimenol (1).

Compound 2 was isolated as white powder. The molecular formula was established to be C<sub>17</sub>H<sub>28</sub>O<sub>4</sub> on the basis of HREIMS at m/z 296.1974 [M]<sup>+</sup> (calcd for 296.1988, C<sub>17</sub>H<sub>28</sub>O<sub>4</sub>). The IR spectrum showed absorptions at 3442 cm<sup>-1</sup> for hydroxy, 1725 cm<sup>-1</sup> for carbonyl, and 1631 cm<sup>-1</sup> for double bond functional groups. The <sup>1</sup>H NMR (Table 1) spectrum showed resonances for four methyl singlets (δ<sub>H</sub> 0.81, 0.87, 0.98, 1.65), two O-bearing methylene signals (δ<sub>H</sub> 4.25, dd, J = 11.5,6.3 Hz; δ<sub>H</sub> 4.39, dd, J = 11.5, 3.3 Hz and δ<sub>H</sub> 4.13, dd, J = 12.7, 5.4 Hz; δ<sub>H</sub> 4.14, dd, J = 12.7, 5.4 Hz), one O-bearing methane signal (δ<sub>H</sub> 3.26, dd, J = 11.3, 3.2 Hz), and one olefinic proton (δ<sub>H</sub> 5.52 m). The <sup>13</sup>C NMR spectrum, along with the DEPT and HSQC spectra, classified the functionalities as a trisubstituted double bond, four methyl, five sp<sup>3</sup> methylenes (two oxygenated), three sp<sup>3</sup> methines (one oxygenated), two sp<sup>3</sup> quaternary carbons, and one carbonyl (δ<sub>C</sub> 173.5) (Table 1). These NMR spectroscopic data of 2 resembled those of a coexisting known compound acetoxydrim-7-en-3β-ol (2a) [7], except for the chemical shift of C-17 was 60.9 (CH<sub>2</sub>) in 2 instead of 21.3 ppm (CH<sub>3</sub>) in 2a. Therefore, compound 2 was established as a hydroxy derivative of 2a, which was also supported by the HMBC correlations from δ<sub>H</sub> 4.13 (1H, d, J = 5.1 Hz) and 4.14 (1H, d, J = 5.1 Hz) to δ<sub>C</sub> 173.5 (C-16, s) (Fig. 2), as well as mass data analysis. In the ROESY spectrum (Fig. 2), the cross peak of H-5/Me-15 suggested that C-14 was β oriented, while the cross peak of Me-15/H-3, as well as the constant coupling of H-3 (δ<sub>H</sub> 3.26, dd, J = 11.3, 3.2 Hz), indicated 3-OH to be β oriented. Besides, the cross peaks of Me-14/ H-2, H-2/Me-13, Me-13/H-11 proved that H-9 was α oriented and Me-13 was β oriented. Therefore, compound 2 was elucidated as 11-hydroxyacetoxydrim-7-en-3β-ol.

Compound 3, a colorless oil, had an [M]<sup>+</sup> peak at m/z 254.1873 in the HREIMS (calcd for 254.1882), corresponding to a molecular formula C<sub>15</sub>H<sub>26</sub>O<sub>3</sub>. 1D NMR spectra in combination with HSQC spectrum demonstrated 15 carbons, which were classified into three methyl, six methylenes (one olefinic carbon, one oxygenated carbon), three methines (two olefinic carbons) and three quaternary carbons (one olefinic carbon) (Table 2). All these data suggested that compound 3 was a sesquiterpenoid possessing the same skeleton with 2,6,10-trimethyl-7,10-

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Fig. 1 Structures of compounds 1–4, 1a, and 2a
epoxy-2,11-dodecadien-6-ol [8]. The only difference between them was that one proton of Me-15 (δC 24.2, q) in 2,6,10-trimethyl-7,10-epoxy-2,11-dodecadien-6-ol was replaced by a hydroxyl (δC 67.4, t) in 3. This change was supported by the HMBC correlations of H-15 (δH 3.47, d, J = 11.4, 4.4 Hz, δC 38.8, t) and H-15/C-10 (δC 85.8, s) and H-15/C-11 (δC 141.1, d) (Fig. 2). In the ROESY spectrum (Fig. 2), correlations of H-15/H-9b (δH 1.97) and H-9b/H-7 indicated that H-15 and H-7 were in the same side. Therefore, compound 3 was identified as 2,6-dimethyl-7,10-epoxy-10-hydroxymethyldodeca-2,11-diene-6-ol.

Compound 4 was isolated as a colorless oil. Its molecular formula was determined as C15H26O3 based on the HRESIMS at m/z 277.1772 (calcd for 277.1780, [M + Na]+). Its NMR data were similar to those of 2,6,10-trimethyl-7,10-epoxy-2,11-dodecadien-6-ol [8]. Compared with compound 3, one proton at C-4 of compound 4 was substituted by a hydroxy, while Me-15 of 4 was not oxygenated. This change was supported by 1H-1H COSY correlation of H-4/H-5 and HMBC correlation of H-4/C-2 (Fig. 2). The ROESY data suggested the other parts of 4 were same to those of 3 (Fig. 2). Therefore, the structure of compound 4 was elucidated as 7,10-epoxy-2,6,10-trimethyldodeca-2,11-diene-4,6-diol.

Fourteen known compounds were identified as 3,11-dihydrodrimane [9], 3β,11,12-trihydroxy-drimene (1a), 3β-hydroxydrimenol, 11,12-dihydroxydrimene [6], acetoxydrim-7-en-3β-ol (2a) [7], 3-κeto-drimenol [10], drimane-2,11-diol [11], 11-hydroxydrim-8-en-7-one [12], (−)-drimenol [13], 15-hydroxy-Γ-murolol [14], 2β-hydroxy-δ-cadinol [15], 2β-hydroxy-α-cadinol [16].

### Table 1

| No. | 1 | 1a<sup>a</sup> | 2 | 2a<sup>b</sup> |
|-----|---|---|---|---|
| 1   | δH | δC | δH | δC |
| 1   | 2.76, ddd (14.5, 14.5, 5.3) | 35.1, t | 38.8 | 1.62, overlapped |
| 2   | 2.14, ddd (14.5, 3.7, 3.7) | 38.7, t | 28.1 | 1.99, overlapped |
| 3   | 1.63, dd (11.8, 4.6) | 51.8, d | 50.7 | 1.22, dd (11.8, 4.8) |
| 4   | 2.14, overlapped | 24.4, t | 24.3 | 1.96, m |
| 5   | 1.59, ddd (14.5, 13.3, 3.7) | 215.1, s | 79.5 | 3.26, dd (11.3, 3.2) |
| 6   | 2.14, overlapped | 47.9, s | 39.8 | 3.88, s |
| 7   | 5.78, d (5.1) | 212.0, d | 126.4 | 5.52, m |
| 8   | 139.3, s | 138.4 | 131.8, s |
| 9   | 2.14, overlapped | 54.7, d | 55.8 | 2.05, m |
| 10  | 36.2, s | 36.6 | 35.9, s |
| 11  | 3.70, ddd (11.1, 5.0, 2.5) | 60.9, t | 61.2 | 4.25, dd (11.5, 6.3) |
| 12  | 3.96, dd (12.2, 6.9) | 66.7, t | 67.0 | 1.65, s |
| 13  | 1.04, s | 14.4, q | 15.0 | 0.81, s |
| 14  | 1.08, s | 22.5, q | 15.9 | 0.87, s |
| 15  | 1.02, s | 25.7, q | 28.7 | 0.98, s |
| 16  | 173.5, s | 171.2 |
| 17  | 4.13, dd (12.7, 5.4) | 60.9, t | 21.3 |
| 11-OH | 4.34, dd (5.0, 5.0) | 4.14, dd (12.7, 5.4) |
| 12-OH | 4.20, dd (6.9, 5.3) |
| 17-OH | 2.36, dd (5.4, 5.4) |

<sup>a</sup> Literature data
<sup>b</sup> Experimental data
<sup>c</sup> Spectra were measured in acetone-d₆
<sup>d</sup> Spectra were measured in CDCl₃
cadinol [17], and 2-(5′-ethenyltetrahydro-5′-methylfuran-2′-yl)-6-methylhept-5-en-ol [18] by comparison of spectroscopic data with those reported in the literature.

Unfortunately, none of them showed significant inhibitory activities against the five human cancer cell lines (> 40 μmol).

3 Experimental

3.1 General Experimental and Procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter. IR and UV spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets and a Shimadzu UV-2401PC instrument, respectively. 1D and 2D NMR spectra were obtained on a Bruker Avance III 600 MHz spectrometer. ESIMS and HREIMS were measured on a Waters Xevo TQ-S spectrometer and a Waters Autospec Premier P776 spectrometer, respectively. HRESIMS was measured on an Agilent G6230 TOF MS spectrometer. Silica gel 200–300 mesh (Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Medium pressure liquid chromatography (MPLC) was performed on a Büchi Sepacore System equipping pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortecnih AG, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan). Preparative high performance liquid chromatography (Prep-HPLC) was performed on an Agilent 1260 liquid chromatography system equipped with a Zorbax SB-C18 column (5 μm, 9.4 × 150 mm).

3.2 Fungus Material

The fungus *Phellinidium sulphurascens* was collected from Changbai Mountain, Jilin Province, China in 2009, and were identified by Prof. Yu-Cheng Dai (Institute of Microbiology, Beijing Forestry University). The culture medium to ferment this fungus consist of glucose (5%),

Fig. 2 Key 2D NMR correlations of 1–4
peptone from porcine meat (0.15 %), yeast powder (0.5 %), KH₂PO₄ (0.05 %) and MgSO₄ (0.05 %). Five hundred 500-mL Erlenmeyer flasks each containing 350 mL of above-mentioned culture medium were inoculated with *P. sulphurascens* strains, respectively. Then they were incubated on rotary shakers at 24 °C and 150 rpm for 25 days in dark environment.

### 3.3 Extraction and Isolation

The culture broth (20 L) of *P. sulphurascens* was filtered, and the filtrate was extracted four times with ethyl acetate (EtOAc). Meanwhile, the mycelium was extracted by CHCl₃/MeOH (1:1) for three times. The EtOAc layer together with the decolorized extract was concentrated under reduced pressure to afford a crude extract (6.0 g). Then this residue was extracted four times with ethyl acetate (EtOAc) connected by Sephadex LH-20 column chromatography (acetone) to afford six fractions (C2a–C2c). Fraction C2c was separated by Sephadex LH-20 column chromatography (MeOH) to afford three subfractions (A–G). Fraction C (0.8 g) was separated by Sephadex LH-20 column chromatography (acetone-MeOH/H₂O from 20:80 to 100:0) to give seven main fractions (A–G). Fraction C (0.8 g) was separated by Sephadex LH-20 column chromatography (acetone-MeOH/H₂O from 20:80 to 100:0) to give seven main fractions (A–G). Fraction C (0.8 g) was separated by Sephadex LH-20 column chromatography (acetone-MeOH/H₂O from 20:80 to 100:0) to give seven main fractions (A–G).

#### 3.3.1 12-Hydroxy-3-oxodrimenol (1)

Amorphous powder, [z]¹⁸D = -56.9 (c = 0.03, MeOH). IR (KBr) νmax cm⁻¹: 3440, 2971, 2933, 1701, 1630, 157, 1426, 1384, 1033. For ¹H NMR (600 MHz, acetone-d₆) and ¹³C NMR (150 MHz, acetone-d₆) spectroscopic data, see Table 1. HREIMS m/z: 252.1723 [M]+ (calcd for C₁₅H₂₆O₃, 254.1873).

#### 3.3.2 11-Hydroxyacetoxydrim-7-en-3-β-ol (2)

Amorphous powder, [z]¹⁸D = +6.2 (c = 0.03, MeOH). IR (KBr) νmax cm⁻¹: 3442, 2958, 2923, 2854, 1725, 1631, 1448, 1428, 1383, 1104, 1034. ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) spectroscopic data, see Table 1. HREIMS m/z: 296.1974 [M]+ (calcd for C₁₇H₂₈O₄, 296.1988).

#### 3.3.3 2,6-Dimethyl-7,10-epoxy-10-hydroxymethylidodeca-2,11-dien-6-ol (3)

Colorless oil, [z]¹⁸D = +5.9 (c = 0.03, MeOH). IR (KBr) νmax cm⁻¹: 3445, 2957, 2922, 2852, 1634, 1384, 1099, 1036. ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) spectroscopic data, see Table 2. HREIMS m/z: 254.1873 [M]+ (calcd for C₁₃H₂₆O₃, 254.1882).

#### 3.3.4 7,10-Epoxy-2,6,10-trimethylidodeca-2,11-diene-4,6-diol (4)

Colorless oil, [z]¹⁸D = +0.4 (c = 0.01, MeOH). IR (KBr) νmax cm⁻¹: 3432, 2957, 2923, 2853, 1630, 1463, 1384, 1111, 1038. ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) spectroscopic data, see Table 2.

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**Table 2** ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data of 3 and 4 in CDCl₃ (δ in ppm, J in Hz)

| No.  | C      | H      | C      | H      |
|------|--------|--------|--------|--------|
| 3    | 1.78, q| 1.62, s| 18.4, q| 1.68, s|
| 2    | 131.9, s| 136.6, s| 125.9, d| 5.11, br. d (8.4)|
| 4    | 22.3, t| 2.10, m| 73.7, d| 4.77, ddd (10.6, 8.4, 6.4)|
| 5    | 37.5, t| 1.51, ddd (13.7, 11.6, 5.2)| 47.8, t| 2.06, dd (13.1, 6.4)|
| 6    | 72.9, s| 73.1, s| 87.9, d| 3.59, dd (10.3, 3.2)|
| 7    | 86.9, d| 3.81, dd (9.0, 6.0)| 1.59, overlapped 3.3, m|
| 8    | 25.9, t| 1.81, overlapped 26.5, t| 1.81, ddd (14.9, 11.1, 5.6)|
| 9    | 32.6, t| 1.97, overlapped 39.1, t| 1.62, overlapped 1.62, overlapped|
| 10   | 85.8, s| 80.3, s| 145.2, d| 5.90, dd (17.5, 10.9)|
| 11   | 141.1, d| 5.83, dd (17.5, 10.9)| 111.8, t| 5.22, dd (17.5, 1.1)|
| 12   | 115.0, 0.4 (13.7, 11.6, 5.4)| 5.12, dd (10.9, 1.1)| 5.05, dd (10.9, 1.1)|
| 13   | 25.9, q| 1.68, s| 26.0, q| 1.72, s|
| 14   | 24.4, q| 1.25, s| 28.2, q| 1.29, s|
| 15   | 67.4, t| 3.47, dd (11.4, 4.4)| 3.42, dd (11.4, 6.2)|
| 15-16| 3.43, dd (6.2, 4.4)| 3.43, dd (6.2, 4.4)|

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HRESIMS m/z: 277.1772 [M+Na]^+ (calcd for C_{15}H_{26}NaO_{3}, 277.1780).

3.4 Cytotoxicity

The cytotoxicity assay was performed according to the MTT method in 96-well microplates. Five human cancer cell lines: human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7 and human colon cancer SW480 cells were used in the cytotoxicity assay. All the cells were cultured in RPMI-1640 or DMEM (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO\(_2\) at 37°C. 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 drug addition, while suspended cells were seeded just before drug addition with an initial density of 1 × 10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μmol in triplicates for 48 h, with cisplatin (sigma, USA) as a positive control (IC\(_{50}\): SW480, 12.0 μmol; SMMC-7721, 10.2 μmol; HL-60, 3.1 μmol; MCF-7, 17.5 μmol; A-549, 9.1 μmol). After compound treatment, cell viability was detected and cell growth curve was graphed.

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Conflict of interest The authors declare no conflicts of interest.

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