

RESEARCH ARTICLE

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# New lanostane-type triterpene acids from *wolfiporia extensa*

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## Abstract

**Background:** Dried sclerotia of *Wolfiporia extensa* (Polyporaceae) is used to invigorate the spleen and to tranquilize the mind in Chinese herbal medicine. Lanostane-type triterpene acids were regarded as major secondary metabolites from dried sclerotia of *W. extensa*.

**Results:** Three new lanostane-type triterpene acids, 3-*epi*-benzoyloxy-dehydrotumulosic acid (**1**), 3-*epi*-(3'-*O*-methyl malonyloxy)-dehydrotumulosic acid (**2**) and 3-*epi*-(3'-hydroxy-3'-methylglutaryloxy)-dehydrotumulosic acid (**3**), were isolated from the sclerotia of *W. extensa*, together with 3 known lanostane derivatives (**4–6**). Their structures were elucidated on the basis of spectroscopic analysis, including 1D and 2D-NMR techniques.

**Conclusion:** Six lanostane derivatives including three new triterpene acids and three known compounds were reported from the sclerotia of *W. extensa* in this paper.

## Background

Dried sclerotia of *Wolfiporia extensa* (Polyporaceae), well known as 'Fu-Ling' in China, is used to invigorate the spleen and to tranquilize the mind in Chinese herb medicine [1]. In combination with some other herbs, it shows activities as diuretic, sedative and analgesic [2]. Lanostane-type triterpenes were reported as major secondary metabolites, which are characterized with hydroxyl groups at C-16 position, and with a C-21 carboxylic acid group. A number of lanostane-type triterpene acids have been reported from dried sclerotia of *W. extensa*, in which some lanostane derivatives showed activities in the anti-tumor, anti-inflammatory and anti-oxidant activities [3-9]. As part of our continuing research on chemical constituents from Traditional Chinese Medicine (TCM) [10-12], three new lanostane-type triterpene acids, 3-*epi*-benzoyloxy-dehydrotumulosic acid (**1**), 3-*epi*-(3'-*O*-methyl malonyloxy)-dehydrotumulosic acid (**2**) and 3-*epi*-(3'-hydroxy-3'-methylglutaryloxy)-dehydrotumulosic acid (**3**) were isolated from the dried sclerotia of *W. extensa*, together with three known lanostane derivatives (**4–6**) (Figure 1). Here we report the structure elucidation of the new compounds as follows.

## Results and discussion

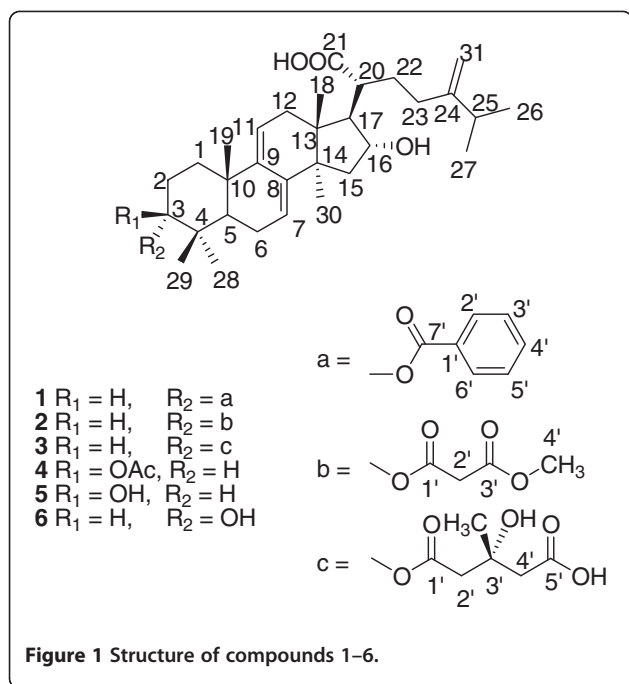
The dried sclerotia of *W. extensa* were extracted with 95% ethanol as described in Experimental part. The ethanolic extract was concentrated under reduced pressure to small volume and the solution was fractionated with a HPD-826 macroporous adsorptive resin column eluting with H<sub>2</sub>O and 90% EtOH. The 90% EtOH fraction was concentrated and repeatedly fractionated on reverse-phase ODS, and on silica gel column to obtain six lanostane-type triterpene acids (**1–6**). Of them, **4–6** were identified as known compounds, dehydropachymic acid (**4**) [7], dehydrotumulosic acid (**5**) [13] and 3-*epi*-dehydrotumulosic acid (**6**) [13] (Figure 1) by spectroscopic methods and comparison with reported data. Compounds **1–3** were identified as new compounds based on a detailed analysis of NMR as described below (Tables 1 and 2).

Compound **1** was obtained as a colourless crystal in CH<sub>3</sub>OH. The molecular formula was determined as C<sub>38</sub>H<sub>52</sub>O<sub>5</sub> from its positive HRESI-MS ([M + H]<sup>+</sup>, *m/z* 589.3864) and <sup>13</sup>C-NMR spectrum. The UV spectrum showed absorption at 234 nm, indicating the presence of a Δ<sup>7,9(11)</sup> diene moiety, which was further supported by an absorption band at 1641 cm<sup>-1</sup> in the IR spectrum. Strong IR absorption at 3400 and 1710 cm<sup>-1</sup> indicated the carbonyl group in **1** [13]. The <sup>1</sup>H-NMR spectrum of **1** showed signals from two secondary methyls (δ 0.97 and 0.99, each 3 H, d, *J* = 6.8 Hz), five tertiary methyls (δ 0.92, 0.95, 1.04,

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**Figure 1** Structure of compounds 1–6.

1.06 and 1.48, each 3 H, s), two oxygen-bearing methylenes [ $\delta$  4.52 (1 H, t,  $J=6.8$  Hz) and  $\delta$  5.09 (1 H, br s)], one terminal methylene group at  $\delta$  4.84 (1 H, s) and 4.97 (1 H, s), two olefinic methylenes at [ $\delta$  5.39 (1 H, d,  $J=5.6$  Hz) and  $\delta$  5.64 (1 H, br s)], together with signals from typical benzoyl group [ $\delta$  8.18 (2 H, d,  $J=7.2$  Hz), 7.35 (2 H, d,  $J=7.6$  Hz), 7.46 (1 H, t,  $J=7.4$  Hz)] (Table 1).  $^{13}$ C-NMR and DEPT spectra of **1** showed signals from 38 carbons, including one carboxyl carbon [ $\delta$  178.6 (C-21)], two carbons from terminal methylene group [ $\delta$  107.0 (C-31) and 156.1 (C-24)], four olefinic carbons [ $\delta$  116.7 (C-11), 120.8 (C-7), 142.9 (C-8) and 146.0 (C-9)], two oxygenated methylenes [ $\delta$  79.0 (C-3) and 76.4 (C-16)], seven methyl carbons [ $\delta$  17.6 (C-18), 21.9 (C-27), 22.0 (C-26), 22.4 (C-29), 22.7 (C-19), 26.6 (C-30) and 28.1 (C-28)], signals from benzoyl group [ $\delta$  165.9 (C-7'), 133.2 (C-4'), 131.4 (C-1'), 129.8 (C-2', 6'), and 128.9 (C-3', 5')], and signals from other fifteen carbons (see Table 2). The aforementioned NMR features were similar to those of 3-*epi*-dehydrotumulosic acid (**6**), except for the existence of an additional set of signals arising from the benzoyl group in **1** [13].

The downfield shift at C-3 ( $\delta$  79.0) in **1**, from ( $\delta$  75.1) in **6**, suggested that the additional benzoyl group was linked to C-3 position of dehydrotumulosic acid moiety. It was further confirmed by the HMBC experiment which showed correlation between H-3 ( $\delta$  5.09) with the signal from C-7' ( $\delta$  165.9) of the benzoyl groups.

The relative configuration was established by  $^1$ H-NMR and the NOESY experiment, in which the H-3 appeared as a broad singlet, the NOESY correlations of H-3 $\beta$  at ( $\delta$  5.09, 1 H, br s) with Me-29 $\beta$  at ( $\delta$  0.95, 3 H, s) revealed the

benzoyl linked the  $\alpha$  position of C-3 in compound **1**. On the basis of the above evidence, the structure of **1** was elucidated as 3 $\alpha$ -benzoyl-16 $\alpha$ -dihydroxy-lanost-7, 9(11), 24(31)-trien-21-oic acid, named as 3-*epi*-benzoyloxyl-dehydrotumulosic acid.

Compound **2** was obtained as a colourless needle in CH<sub>3</sub>OH. Careful comparison of  $^{13}$ C-NMR spectra of **1** and **2** indicate that both have a similar lanostane skeleton with different substitution group (details in Table 2). Unlike compound **1** with a benzoyl group, compound **2** showed signals from a malonyl group [ $\delta$  41.9 (–CH<sub>2</sub>–), 166.4 (–COO–) and 167.6 (–COO–)] and a methoxyl group [ $\delta$  52.2 (–OCH<sub>3</sub>)]. HMBC experiment showed correlations between methoxyl proton ( $\delta$  3.63) with 3'-C ( $\delta$  166.4, from malonyl group) indicated the methyl malonate group [14]. The HMBC experiment of **2** revealed the correlation between H-3 ( $\delta$  4.86) and C-1' ( $\delta$  167.6), indicated the 3-substitution. Thus, compound **2** was established as 3- $\alpha$ -methyl-malonyl-16 $\alpha$ -dihydroxy-lanost-7, 9(11), 24(31)-trien-21-oic acid, named as 3-*epi*-(3'-O-methyl malonyloxy)-dehydrotumulosic acid.

The  $^{13}$ C-NMR spectra of **3** showed signals from a lanostane skeleton similar to those of **1** and **2** (Table 2), except with different substitution groups. Except signals from lanostane skeleton in compound **3**,  $^1$ H-NMR showed signals at [ $\delta$  3.12 (1 H, d,  $J=15.2$  Hz, H-2'), 3.16 (1 H, d,  $J=15.2$  Hz, H-2'), 3.02 (1 H, d,  $J=14.4$  Hz, H-4'), 3.08 (1 H, d,  $J=14.4$  Hz, H-4') and 1.71 (3 H, s, –CH<sub>3</sub>)] along with  $^{13}$ C-NMR showed signals [ $\delta$  171.4 (C-1'), 46.3 (C-2'), 69.9 (C-3'), 46.4 (C-4'), 174.6 (C-5'), and 28.4 (–CH<sub>3</sub>)]. Those signals were assigned to 3-hydroxy-3-methylglutaryl group based on HMQC and HMBC spectra data. It was further confirmed from ESI-MS experiment, which showed fragment ions at  $m/z$  525.4 [M-H-102 (CH (CH<sub>3</sub>) (OH)-CH<sub>2</sub>-COOH)]. The HMBC correlations of H-3 ( $\delta$  4.94 br s) with C-1' ( $\delta$  171.4) confirmed that the 3-hydroxy-3-methylglutaryl group was at C-3 in **3** (Figure 1). The compound **3** is levorotatory. The *R*-configurations of C(3') in **3** was deduced by comparing of the compound **3** specific rotation features with those of (+)-3-*epi*-dehydrotumulosic acid, and (3' *S*)-(+)-3-hydroxy-3-methylglutamic acid, which are dextrorotatory [8,13]. These evidences indicated *R*-configuration of C(3') in compound **3**. As stated above, the structure of **3** was indicated as 3- $\alpha$ -(3'-hydroxy-3'-methylglutaryl)-16 $\alpha$ -dihydroxy-lanost-7, 9(11), 24(31)-trien-21-oic acid, named as 3-*epi*-(3'-hydroxy-3'-methylglutaryl)-dehydrotumulosic acid.

## Experimental

### General experimental procedures

Optical rotations were measured on a P-1020 Polarimeter (JASCO, Tokyo, Japan). UV spectra were obtained on a UV 210A Shimadzu spectrometer. IR spectra were recorded on an FT-IR spectrometer (Nicolet iS10, Thermo Scientific,

**Table 1  $^1\text{H-NMR}$  data of 1–3 (at 500 or 600 MHz, in  $\text{C}_5\text{D}_5\text{N}$ ;  $\delta$  in ppm,  $J$  in Hz)**

| position         | 1   | 2                                      | 3                                      |
|------------------|---|--|--|
| 1                | 1.73, m 1.82, td<br>(9.6, 3.2)            | 1.65, m 1.75,<br>dd (13.8, 3.0)        | 1.69, m 1.88, m                        |
| 2                | 1.91, m 1.97, m                           | 1.79, dt (6.6, 3.0)<br>1.85, d (12.0)  | 1.78, ddd (15.6, 6.4,<br>2.8) 1.86, m  |
| 3                | 5.09, br s                                | 4.86, br s                             | 4.94, br s                             |
| 5                | 1.88, dd<br>(10.0, 4.0)                   | 1.68, t (5.1)                          | 1.76, dd (9.2, 6.4)                    |
| 6                | 2.08, m 2.09, m                           | 2.00, m 2.01, m                        | 2.02, m 2.03, m                        |
| 7                | 5.64, br s                                | 5.57, br s                             | 5.57, br s                             |
| 11               | 5.39, d (5.6)                             | 5.38, d (6.0)                          | 5.39, d (6.0)                          |
| 12               | 2.42, dd<br>(15.6, 5.2)<br>2.66, d (16.8) | 2.42, dd (18.0, 6.6)<br>2.66, d (18.0) | 2.42, dd (17.2, 6.8)<br>2.66, d (16.4) |
| 15               | 1.95, d (12.4)<br>2.47, dd (12.8, 9.2)    | 1.91, d (13.2)<br>2.45, t (3.9)        | 1.91, m 2.45,<br>dd (12.4, 8.8)        |
| 16               | 4.52, t (6.8)                             | 4.51, t (7.2)                          | 4.52, t (6.8)                          |
| 17               | 2.86, dd (11.2, 5.6)                      | 2.85, dd (11.4, 6.0)                   | 2.84, dd (11.2, 5.6)                   |
| 18               | 1.06, s                                   | 1.05, s                                | 1.04, s                                |
| 19               | 1.04, s                                   | 0.99, s                                | 1.00, s                                |
| 20               | 2.95, td (10.8, 2.4)                      | 2.94, dd (10.8, 3.0)                   | 2.92, td (10.8, 2.0)                   |
| 22               | 2.46, m 2.68, m                           | 2.51, m 2.63, m                        | 2.42, m 2.61, m                        |
| 23               | 2.37, m 2.55,<br>br d (11.6)              | 2.38, m 2.54, m                        | 2.38, m 2.54, m                        |
| 25               | 2.29, m                                   | 2.29, m                                | 2.27, m                                |
| 26               | 0.97, d (6.8)                             | 0.97, d (6.6)                          | 0.97, d (6.8)                          |
| 27               | 0.99, d (6.8)                             | 0.98, d (6.6)                          | 0.99, d (6.8)                          |
| 28               | 0.92, s                                   | 0.87, s                                | 0.90, s                                |
| 29               | 0.95, s                                   | 0.90, s                                | 0.96, s                                |
| 30               | 1.48, s                                   | 1.42, s                                | 1.41, s                                |
| 31               | 4.84, br s 4.97,<br>br s                  | 4.83, br s 4.97, br s                  | 4.83, br s 4.96, br s                  |
| 2'               | 8.18, d (7.2)                             | 3.60, s                                | 3.12, d (15.2)<br>3.16, d (15.2)       |
| 3'               | 7.35, t (7.6)                             | –                                      | –                                      |
| 4'               | 7.46, t (7.4)                             | 3.63, s                                | 3.02, d (14.4)<br>3.08, d (14.4)       |
| 5'               | 7.35, t (7.6)                             | –                                      | –                                      |
| 6'               | 8.18, d (7.2)                             | –                                      | –                                      |
| -CH <sub>3</sub> | –   | –                                      | 1.71, s                                |

USA) with KBr pellets.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectrum was recorded in pyridine- $d_5$  with Bruker AM-400, DRX-500 and VARIAN INOVA-600 spectrometers operating at 400, 500 and 600 MHz for  $^1\text{H}$ -NMR experiments, and 125 and 150 MHz for  $^{13}\text{C}$ -NMR experiment, respectively. Coupling constants are expressed in Hertz (Hz) and chemical shifts are given on a  $\delta$  (ppm) scale with tetramethylsilane as internal standard. Negative ion ESI-MS and HRESI-MS

were recorded on an AutoSpec 3000 spectrometer (VG, Manchester, UK). Column chromatography separations were performed using HPD-826 (Cangzhou Bon Adsorber Technology Co., Cangzhou, China), Chromatorex ODS

**Table 2  $^{13}\text{C-NMR}$  Data of 1–3 (at 125 or 150 MHz, in  $\text{C}_5\text{D}_5\text{N}$ ;  $\delta$  in ppm)**

| Position | 1     | 2     | 3     |
|----------|-------|-------|-------|
| 1        | 31.2  | 30.8  | 31.1  |
| 2        | 23.5  | 23.2  | 23.4  |
| 3        | 79.0  | 79.6  | 78.2  |
| 4        | 37.7  | 36.8  | 36.7  |
| 5        | 45.3  | 44.7  | 44.8  |
| 6        | 23.2  | 23.1  | 23.1  |
| 7        | 120.8 | 120.8 | 120.7 |
| 8        | 142.9 | 142.7 | 142.8 |
| 9        | 146.0 | 146.0 | 146.0 |
| 10       | 37.2  | 37.6  | 37.6  |
| 11       | 116.7 | 116.6 | 116.5 |
| 12       | 36.2  | 36.2  | 36.2  |
| 13       | 45.1  | 45.1  | 45.1  |
| 14       | 49.5  | 49.5  | 49.5  |
| 15       | 44.4  | 44.4  | 44.4  |
| 16       | 76.4  | 76.4  | 76.4  |
| 17       | 57.6  | 57.6  | 57.6  |
| 18       | 17.6  | 17.6  | 17.6  |
| 19       | 22.7  | 22.6  | 22.7  |
| 20       | 48.5  | 48.5  | 48.5  |
| 21       | 178.6 | 178.7 | 178.6 |
| 22       | 31.4  | 31.4  | 31.4  |
| 23       | 33.2  | 33.2  | 33.2  |
| 24       | 156.1 | 156.0 | 156.1 |
| 25       | 34.1  | 34.1  | 34.1  |
| 26       | 22.0  | 22.0  | 22.0  |
| 27       | 21.9  | 21.8  | 21.8  |
| 28       | 28.1  | 27.9  | 28.1  |
| 29       | 22.4  | 22.3  | 22.5  |
| 30       | 26.6  | 26.6  | 26.6  |
| 31       | 107.0 | 107.0 | 107.2 |
| 1'       | 131.4 | 167.6 | 171.4 |
| 2'       | 129.8 | 41.9  | 46.3  |
| 3'       | 128.9 | 166.4 | 69.9  |
| 4'       | 133.2 | 52.2  | 46.4  |
| 5'       | 128.9 | –     | 174.6 |
| 6'       | 129.8 | –     | –     |
| 7'       | 165.9 | –     | –     |
| 3'-Me    | –     | –     | 28.4  |

(Fuji Silysia Chemical Co., Greenville, USA) and Silica gel (Qingdao Haiyang Chemical Co., Qingdao, China) as adsorbants. TLC was carried on silica gel G pre-coated plates (Qingdao Haiyang Chemical Co., Qingdao, China). The TLC plate was monitored by spraying with 10% H<sub>2</sub>SO<sub>4</sub> solution in ethanol followed by heating.

#### Fungal material

The dried sclerotia of *W. extensa* were collected from Hebei Guang Ming Prepared Medicinal Herbs Co., Ltd, China and identified by Prof. Yu-Ting Cheng (Beijing University of Chinese Medicines). An authentic sample was kept in School of Chinese Pharmacy, Beijing University of Chinese Medicines.

#### Extraction and isolation

The dried sclerotia of *W. extensa* (17.5 kg) were powdered and extracted with exhaustively 95% EtOH under reflux. The EtOH extract was concentrated to the small volume (3 L), and applied on a HPD-826 macroporous adsorptive resin (15 Kg, 18 cm × 150 cm), eluting with H<sub>2</sub>O (60 L) and 90% EtOH (80 L). The 90% EtOH fraction was concentrated under reduced pressure, and the residue (60 g) was subjected to column chromatography (CC) on silica gel eluted with CHCl<sub>3</sub>/CH<sub>3</sub>OH (4:1 to 1:1, 5 L) to obtain eight fractions (Fr 1–Fr 8). Fr 1, was further fractionated on silica gel eluted with cyclohexane/CHCl<sub>3</sub> (8:1 and 4:1, each 1 L), and ODS eluted with a step gradient of H<sub>2</sub>O/MeOH (1:0 → 0:1), and PTLC (Cyclohexane/CHCl<sub>3</sub>/HOAc, 3:1:0.1) to give **1** (20 mg), **2** (10 mg) and **4** (10 mg). Fraction 2 was fractionated repeatedly on Silica gel (CHCl<sub>3</sub>/EtOAc, 8:1) and ODS (CH<sub>3</sub>OH/H<sub>2</sub>O, 75:25 → 85:15), eluted with CHCl<sub>3</sub>/CH<sub>3</sub>OH (50:1), to obtain **5** (20 mg) and **6** (10 mg) from Fr 2. Fr 3 was subjected to CC on silica gel (CHCl<sub>3</sub>/EtOAc, 4:1), and preparative TLC on silica gel (CHCl<sub>3</sub>/EtOAc/HOAc, 1:1:0.1) to obtain **3** (20 mg).

#### 3-epi-benzoyloxyl-dehydrotumulosic acid (1)

Colourless needles; <sup>1</sup>H-NMR (in pyridine-*d*<sub>5</sub>): see Table 1. <sup>13</sup>C-NMR (in pyridine-*d*<sub>5</sub>): see Table 2. IR (KBr) cm<sup>-1</sup>: 3400, 2928, 1710, 1641, 1279, 1175, 895, 800. UV λMeOH max nm (log): 234 (4.32). HRESI-MS (*m/z*): 589.3864 [M + H]<sup>+</sup>, calcd for C<sub>38</sub>H<sub>53</sub>O<sub>5</sub>, 589.3893. ESI-MS (*m/z*) (rel. int.): 587.3 [M - 1]<sup>-</sup> (100), 417.0 (23), 338.9 (4), 208.8 (13).

#### 3-epi-(3'-O-methyl malonyloxyl)-dehydrotumulosic acid (2)

Colourless needles; <sup>1</sup>H-NMR (in pyridine-*d*<sub>5</sub>): see Table 1. <sup>13</sup>C-NMR (in pyridine-*d*<sub>5</sub>): see Table 2. IR (KBr) cm<sup>-1</sup>: 3416, 2960, 1736, 1707, 1641, 1254, 1152, 891, 800. UV λMeOH max nm (log): 243 (4.16). HRESI-MS (*m/z*): 607.3605 [M + Na]<sup>+</sup>, calcd for C<sub>35</sub>H<sub>52</sub>O<sub>7</sub>Na, 607.3611.

#### 3-epi-(3'-hydroxy-3'-methylglutaryloxyl)-dehydrotumulosic acid (3)

Colourless needles; [α] = -7.6 (*c* = 0.1705, pyridine); <sup>1</sup>H-NMR (in pyridine-*d*<sub>5</sub>): see Table 1. <sup>13</sup>C-NMR (in pyridine-*d*<sub>5</sub>): see Table 2. IR (KBr) cm<sup>-1</sup>: 3389, 2962, 1707, 1642, 1205, 1176, 891, 802, 780, 770. UV λMeOH max nm (log): 244 (4.13); HRESI-MS (*m/z*): 651.3880 [M + Na]<sup>+</sup>, calcd for C<sub>37</sub>H<sub>56</sub>O<sub>8</sub>Na, 651.3873. ESI-MS (*m/z*) (rel. int.): 627.5 [M - 1]<sup>-</sup> (100), 525.4 (5).

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

GS carried out the chemical analysis-structure elucidation and drafted the Manuscript; NZ carried out the chemical studies; SW employed in the several chemical assays of extraction and isolation; YL worked at the part of experimental design; YB engaged in the part of chemical analysis-structure elucidation; CS carried out the part of chemical assays of extraction and isolation; SR conceived of the study and its design and coordination of the scientific teams. All authors have read and approved the final manuscript.

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