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New lanostane-type triterpene acids from *wolfiporia extensa*

Gaimei She†, Nailiang Zhu†, Shuai Wang, Yang Liu, Yinying Ba, Changqing Sun and Renbing Shi*

Abstract

Backgroud: 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 regard as major secondary metabolites from dried sclerotia of *W. extensa*.

Results: Three new lanostane-type triterpene acids, 3-*epi*-benzoyloxyl-dehydrotumulosic acid (**1**), 3-*epi*-(3′-*O*-methyl malonyloxy)-dehydrotumulosic acid (**2**) and 3-*epi*-(3′-hydroxy-3′-methylglutaryloxyl)-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*-benzoyloxyl-dehydrotumulosic acid (**1**), 3-*epi*-(3′-*O*-methyl malonyloxy)-dehydrotumulosic acid (**2**) and 3-*epi*-(3′-hydroxy-3′-methylglutaryloxyl)-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_2O 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_3OH. The molecular formula was determined as $C_{38}H_{52}O_5$ from its positive HRESI-MS ($[M + H]^+$, *m/z* 589.3864) and ^{13}C-NMR spectrum. The UV spectrum showed absorption at 234 nm, indicating the presence of a $\Delta^{7,9(11)}$ diene moiety, which was further supported by an absorption band at 1641 cm^{-1} in the IR spectrum. Strong IR absorption at 3400 and 1710 cm^{-1} indicated the carboxyl group in **1** [13]. The ^{1}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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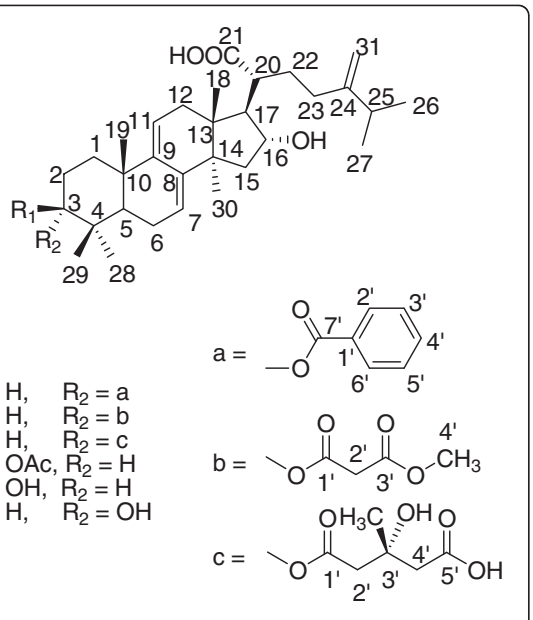

Figure 1 Structure of compounds 1–6.

1.06 and 1.48, each 3 H, s), two oxygen-bearing methylenes [δ 4.52 (1 H, t, $J = 6.8\,Hz$) and δ 5.09 (1 H, br s)], one terminal methylene group at δ 4.84 (1 H, s) and 4.97 (1 H, s), two olefinic methylenes at [δ 5.39 (1 H, d, $J = 5.6\,Hz$) and δ 5.64 (1 H, br s)], together with signals from typical benzoyl group [δ 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 [δ 178.6 (C-21)], two carbons from terminal methylene group [δ 107.0 (C-31) and 156.1 (C-24)], four olefinic carbons [δ 116.7 (C-11), 120.8 (C-7), 142.9 (C-8) and 146.0 (C-9)], two oxygenated methylenes [δ 79.0 (C-3) and 76.4 (C-16)], seven methyl carbons [δ 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 [δ 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 (δ 79.0) in **1**, from (δ 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 (δ 5.09) with the signal from C-7′ (δ 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-3β at (δ 5.09, 1 H, br s) with Me-29β at (δ 0.95, 3 H, s) revealed the benzoyl linked the α position of C-3 in compound **1**. On the basis of the above evidence, the structure of **1** was elucidated as 3α-benzoyl-16α-dihydroxyl-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_3OH. 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 [δ 41.9 (–CH_2-), 166.4 (–COO-) and 167.6 (–COO-)] and a methoxyl group [δ 52.2 (–OCH_3)]. HMBC experiment showed correlations between methoxyl proton (δ 3.63) with 3′-C (δ 166.4, from malonyl group) indicated the methyl malonate group [14]. The HMBC experiment of **2** revealed the correlation between H-3 (δ 4.86) and C-1′ (δ 167.6), indicated the 3-substitution. Thus, compound **2** was established as 3-α-methyl-malonyl-16α-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 [δ 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_3)] along with ^{13}C-NMR showed signals [δ 171.4 (C-1′), 46.3 (C-2′), 69.9 (C-3′), 46.4 (C-4′), 174.6 (C-5′), and 28.4 (–CH_3)]. 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_3) (OH)-CH_2-COOH)]$^-$. The HMBC correlations of H-3 (δ 4.94 br s) with C-1′ (δ 171.4) confirmed that the 3-hydroxy-3-methylglutaryloxyl 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-methylglutaric 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-α-(3′-hydroxy-3′-methylglutaryloxy)-16α-dihydroxy-lanost-7, 9(11), 24 (31)-trien-21-oic acid, named as 3-*epi*-(3′-hydroxy-3′-methylglutaryloxyl)-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 Scientifi,

Table 1 ^{1}H-NMR data of 1–3 (at 500 or 600 MHz, in C_5D_5N; δ in ppm, *J* in *Hz*)

position	1	2	3
1	1.73, m 1.82, td (9.6, 3.2)	1.65, m 1.75, 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) 1.85, d (12.0)	1.78, ddd (15.6, 6.4, 2.8)1.86, m
3	5.09, br s	4.86, br s	4.94, br s
5	1.88, dd (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 (15.6, 5.2) 2.66, d (16.8)	2.42, dd (18.0, 6.6) 2.66, d (18.0)	2.42, dd (17.2, 6.8) 2.66, d (16.4)
15	1.95, d (12.4) 2.47, dd (12.8, 9.2)	1.91, d (13.2) 2.45, t (3.9)	1.91, m 2.45, 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 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 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) 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) 3.08, d (14.4)
5′	7.35, t (7.6)	–	–
6′	8.18, d (7.2)	–	–
-CH_3	–	–	1.71, s

USA) with KBr pellets. ^{1}H- and ^{13}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}H-NMR experiments, and 125 and 150 MHz for ^{13}C-NMR experiment, respectively. Coupling constants are expressed in Hertz (*Hz*) and chemical shifts are given on a δ (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}C-NMR Data of 1–3 (at 125 or 150 MHz, in C_5D_5N; δ 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 precoated plates (Qingdao Haiyang Chemical Co., Qingdao, China). The TLC plate was monitored by spraying with 10% H_2SO_4 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_2O (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 $CHCl_3/CH_3OH$ (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/$CHCl_3$ (8:1 and 4:1, each 1 L), and ODS eluted with a step gradient of H_2O/MeOH (1:0 → 0:1), and PTLC (Cyclohexane/$CHCl_3$/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 ($CHCl_3$/EtOAc, 8:1) and ODS (CH_3OH/H_2O, 75:25 → 85:15), eluted with $CHCl_3/CH_3OH$ (50:1), to obtain **5** (20 mg) and **6** (10 mg) from Fr **2**. Fr **3** was subjected to CC on silica gel ($CHCl_3$/EtOAc, 4:1), and preparative TLC on silica gel ($CHCl_3$/EtOAc/HOAc, 1:1:0.1) to obtain **3** (20 mg).

3-epi-benzoyloxyl-dehydrotumulosic acid (1)

Colourless needles; ^{1}H-NMR (in pyridine-d_5): see Table 1. ^{13}C-NMR (in pyridine-d_5): see Table 2. IR (KBr) cm^{-1}: 3400, 2928, 1710, 1641, 1279, 1175, 895, 800. UV λMeOH max nm (log): 234 (4.32). HRESI-MS (*m/z*): 589.3864 $[M + H]^+$, calcd for $C_{38}H_{53}O_5$, 589.3893. ESI-MS (*m/z*) (rel. int.): 587.3 $[M - 1]^-$ (100), 417.0 (23), 338.9 (4), 208.8 (13).

3-epi-(3'-O-methyl malonyloxy)-dehydrotumulosic acid (2)

Colourless needles; ^{1}H-NMR (in pyridine-d_5): see Table 1. ^{13}C-NMR (in pyridine-d_5): see Table 2. IR (KBr) cm^{-1}: 3416, 2960, 1736, 1707, 1641, 1254, 1152, 891, 800. UV λMeOH max nm (log): 243 (4.16). HRESI-MS (*m/z*): 607.3605 $[M + Na]^+$, calcd for $C_{35}H_{52}O_7Na$, 607.3611.

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

Colourless needles; $[\alpha] = -7.6$ ($c = 0.1705$, pyridine); ^{1}H-NMR (in pyridine-d_5): see Table 1. ^{13}C-NMR (in pyridine-d_5): see Table 2. IR (KBr) cm^{-1}: 3389, 2962, 1707, 1642, 1205, 1176, 891, 802, 780, 770. UV λMeOH max nm (log): 244 (4.13); HRESI-MS (*m/z*): 651.3880 $[M + Na]^+$, calcd for $C_{37}H_{56}O_8Na$, 651.3873. ESI-MS (*m/z*) (rel. int.): 627.5 $[M - 1]^-$ (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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