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Triterpenoids of *Ganoderma theaecolum* and their hepatoprotective activities

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Five new lanostane triterpenoids, ganoderic acid XL_1 (**1**), ganoderic acid XL_2 (**2**), 20-hydroxyganoderic acid AM_1 (**3**), ganoderenic acid AM_1 (**4**) and ganoderesin C (**5**), together with five known triterpenoids (**6**–**10**) were isolated from the fruiting bodies of *Ganoderma theaecolum*. Chemical structures were elucidated on the basis of spectroscopic evidence, including 1D, 2D NMR, mass spectrometric data and circular dichroism spectra. Compounds **1**, **4**, **5**, **8**, **9** and **10** (10 μM) exhibited hepatoprotective activities against DL-galactosamine-induced cell damage in HL-7702 cells.

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1. Introduction

Ganoderma theaecolum, which belongs to the family Ganoderma, is used as *Ganoderma lucidum*. *Ganoderma* sp. has been reported to have antimicrobial, anticytotoxic, antioxidant, anti-staphylococcal [1] and immunomodulatory activities in folks of Asian countries. Lanostane triterpenoids, the main bioactive compounds of the genus *Ganoderma* were reported to possess anti-HIV [2], antitumor, anti-oxidation, anti-inflammatory [3] and antiaging activities [4]. On account of their potential medicinal value, much attention had been paid to the search for significant pharmacological constituents from this genus. A systematic research on chemical constituents and their biological activities of *G. theaecolum* has been carried out. Five new lanostane triterpenoids (**1**–**5**) and five known triterpenoids (**6**–**10**) were isolated from the fruiting bodies of *G. theaecolum* (Fig. 1). Compound **4** possessed an unprecedented $\triangle^{17,20}$ double bond. Details of the structure elucidation and their hepatoprotective activities are reported herein.

2. Experimental details

2.1. General experimental procedures

Optical rotations were measured on a Jasco P-2000 polarimeter. UV spectra were collected with a Jasco V-650 spectrophotometer. ECD spectra were recorded on a JASCO J-815 spectrometer. IR spectra were recorded on a Nicolet 5700 spectrometer by an FT-IR microscope transmission method. NMR measurements were performed on VNS-500, and Bruker AV400 spectrometers. HRESIMS were obtained using an Agilent 1100 series LC/MSD ion trap mass spectrometer. TLC was carried out with GF254 plates (Qingdao Marine Chemical Factory). Column chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Factory) and LiChroprep RP-18 gel. HPLC was performed on a YMC-Pack ODS-A column (250 × 20 mm, 5 μm).

2.2. Plant material

The fruiting bodies of *Ganoderma theaecolum* were collected in Wuzhishan City, Hainan Province, P. R. China, in July 2012. A voucher specimen (no. S-2421) has been deposited at the herbarium of the Institute of Materia Medica, Chinese Academy

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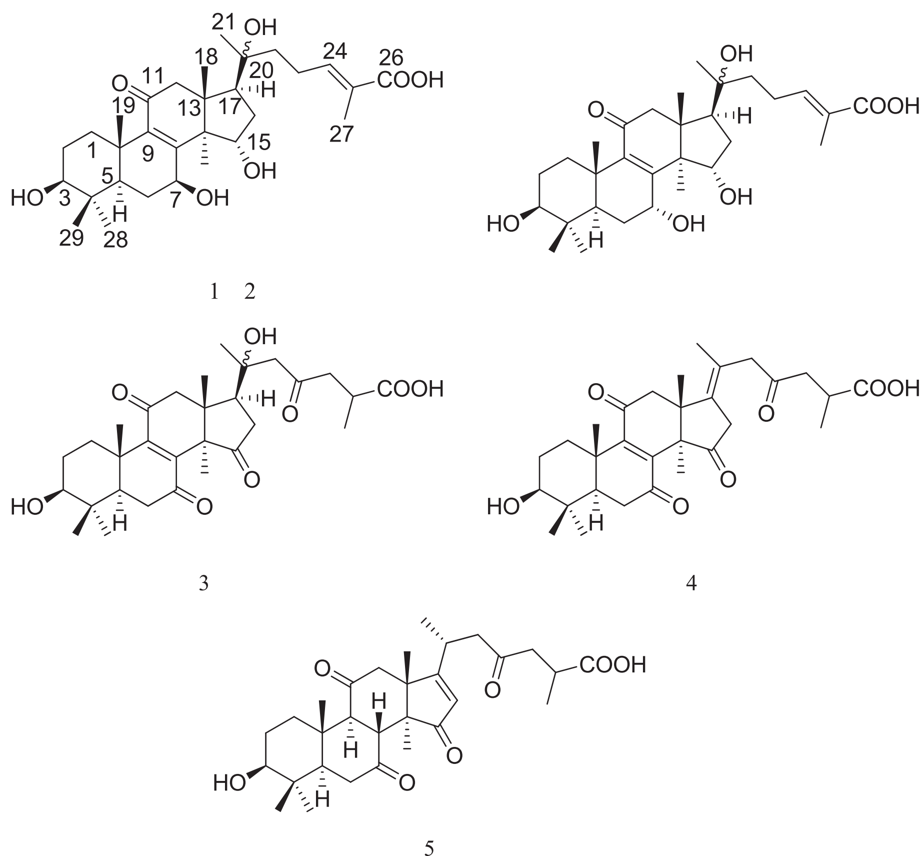

Fig. 1. Structures of new compounds **1**–**5**.

of Medical Sciences and Peking Union Medical College, and was identified by Professor Zhang Xiao-Qing in Institute of Microbiology, Chinese Academy of Sciences.

2.3. Extraction and isolation

Air-dried and powered fruiting bodies of *G. theaecolum* (20 kg) were extracted with 95% EtOH (3 × 140 L, at reflux, 2 h, 1 h, 1 h), then removal of solvent under reduced pressure. The ethanolia extract was subjected to a macroporous resin column chromatography (i.d. 12 × 150 cm, 7 L) that was eluted with 30% (21 L), 50% (21 L), 70% (21 L), and 95% EtOH (21 L), respectively. The 50% EtOH eluate (354 g) was then subjected to silica gel CC (4 kg, 12 × 150 cm) with mixtures of $CHCl_3$–MeOH (100 → 0:1 v/v) as eluent to yield 13 crude fractions. Fraction 3 (50 L, 40 g, 50:1) was chromatographed on a silica gel column (Petroleum Ether–Acetone, 9:1; 8:2; 7:3; 6:4; 1:1; 0:1) to give four subfractions 3_1–3_4. Subfraction 3_1 was further separated by silica gel CC (2.1 × 56 cm), eluted with Petroleum Ether–Acetone 8:2 (1 L) to yield **6** (90 mg) and **9** (12 mg). Subfraction 3_2 was crystallized after two silica gel columns CC [a, 2.4 × 66 cm, Petroleum Ether–Acetone, 8:2 (1.5 L); 7:3 (2 L); 6:4 (2 L); 1:1 (2 L); b, 2.2 × 72 cm, Petroleum Ether–Acetone 8:2 (6 L)] to afford **7** (37 mg). Subfraction 3_3 was repeatedly separated by silica gel CC [a, 2.6 × 87 cm, Petroleum Ether–Acetone, 9:1 → 1:1 (2 L, each); b, 1.3 × 65 cm, CH_2Cl_2–MeOH 60:1 (1 L); 50:1 (0.5 L); 40:1 (0.5 L); 30:1 (1 L); 20:1 (1 L); 10:1 (1 L)] and RP-18 column CC (1.9 × 60 cm, 200 g, 55:45 → 100:0 MeOH–H_2O) to afford **4** (65 mg), **8** (30 mg) and **12** (10 mg). Subfraction 2_4 was crystallized in MeOH to afford **10** (20 mg). Compounds **5** (19 mg) and **11** (10 mg) were purified by silica gel CC (300 g, 2.2 × 72 cm) from fraction 3, eluted with Petroleum Ether–Acetone (2:1,7.5 L). Fraction 4 was subjected to silica gel column chromatography (Petroleum Ether–Acetone, 6:4,15 L), then fraction 4_3 was separated by silica gel CC (4.6 × 70 cm), eluted with Petroleum Ether–Acetone (2:1, 2 L), and further purified by preparative HPLC using 78:22 MeOH–H_2O (6 mL/min) to give compound **1** (20 mg, 45.7 min). Finally, fraction 5 was chromatographed on two silica gel columns [a, 2.5 × 88 cm, CH_2Cl_2–MeOH, 20:1 → 1:1 (6 L, each); b, 1.6 × 65 cm, Petroleum Ether–Acetone, 7:3 (1 L); 6:4 (1 L); 1:1 (2 L)], and then purified by preparative HPLC using 30:70 MeCN–H_2O (6 mL/min) to yield compound **3** (5 mg, 42.7 min); compound **2** (5 mg, 275 min) was purified by preparative HPLC using 20:80 MeCN–H_2O (6 mL/min).

Ganoderic acid XL_1 (**1**): white, amorphous powder; $[\alpha]_D^{20}$ + 68.8 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε): 217 (4.02) nm, 254 (3.80) nm; MeOH); ECD (MeOH) 245 (Δε +5.3), 346 (Δε −1.0); ν_{max}: 3358, 2973, 1686, 1649, 1382, 1048 cm^{-1}; ^1H and ^{13}C NMR data (pyridine-d_5), see Tables 1 and 2; negative ESIMS *m/z* 517 $[M - H]^-$; negative HRESIMS *m/z* 517.3174 $[M - H]^-$ (calcd. for $C_{30}H_{45}O_5$, *m/z* 517.3171).

Ganoderic acid XL_2 (**2**): white, amorphous powder; $[\alpha]_D^{20}$ + 93.1 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε): 216 (4.02) nm, 254 (3.79) nm; ECD (MeOH) 214 (Δε +1.9), 258 (Δε +33.2), 353 (Δε −5.5); IR (KBr) ν_{max}:3418, 2954,

Table 1
^{1}H NMR data for compounds **1–5**[a].

No.	1	2	3	4	5
1	3.21 m	3.49[b]	3.06 m	2.81 m	3.22 m
	1.21 m	1.46 m	1.30 m	1.18 m	1.20 m
2	2.00 m	1.99 m	1.90 m	1.72 m	1.96 m
3	3.52dd (7.0,4.5)	3.54dd (7.0,5.0)	3.42dd (5.0,6.0)	3.24 m	3.39dd (7.5,4.5)
5	1.24d (13.0)	1.84[b]	1.75 m	1.54dd (12.4,2.0)	1.35dd (11.5,2.5)
6	2.41 m	2.14[b]	2.75 m	2.66 m	2.50 m
		1.80[b]	2.69 m	2.58 m	
7	5.03dd (7.5,2.5)	4.88 s	–	–	–
8	–	–	–	–	3.45d (12.5)
9	–	–	–	–	2.66d (12.5)
12	3.15d (16.0)	3.15d (17.5)	3.23 m	3.05 m	2.95 m
	2.95 m	2.85d (17.5)	3.06 m	2.85 m	2.53 m
15	–	–	–	–	–
	5.45 t (8.5)	5.06dd (6.8,3.0)	–	–	–
16	2.45 m	2.46 m	3.09 m	3.20d (21.6)	5.90s
	2.17[b]	2.42 m	2.99 m	2.76d (21.6)	–
17	2.44 m	2.49 t (10.0)	3.00 m	–	–
18	1.60s	1.43 s	1.40s	0.95 s	1.16 s
19	1.54 s	1.32 s	1.36 s	1.30s	1.42 s
20	–	–	–	–	2.96 m
21	1.44 s	1.42 s	1.70s	1.62 s	1.00d (6.5)
22	1.85 m	1.75[b]	2.94 m	3.27d (6.0)	2.90 m
	1.72 m		2.86 m		2.73 m
23	2.96[b]	3.02 m	–	–	–
	2.17 m	2.17 m	–	–	–
24	7.23 t (7.0)	7.23 t (7.0)	3.14 m	2.93 m	3.11 m
	–	–	2.64dd (13.0,4.5)	2.46dd (13.2,4)	2.53 m
25	–	–	3.02 m	3.00 m	3.26 m
27	2.05 s	2.05 s	1.31d (7.0)	1.25d (7.2)	1.31d (7.0)
28	1.29 s	1.35 s	1.12 s	1.02 s	1.08 s
29	1.10s	1.12 s	1.03 s	0.88 s	1.01 s
30	1.66 s	1.73 s	1.78 s	1.66 s	1.86 s

[a] ^{1}H NMR data were measured at 400 MHz in $CDCl_3$ for **4**; at 500 MHz in C_5D_5N for **1**, **2**, **3** and **5**.
[b] Overlapped signals.

1690, 1647, 1457, 1377, 1166 cm^{-1}; ^{1}H and ^{13}C NMR data (pyridine-d_5), see Tables 1 and 2; negative ESIMS m/z 517 [M − H]$^-$; negative HRESIMS m/z 517.3177 [M − H]$^-$ (calcd. for $C_{30}H_{45}O_7$, m/z 517.3171).

20-hydroxy-ganoderic acid AM$_1$ (**3**): yellow, amorphous powder; $[\alpha]_D^{20}$ + 47.2 (c 0.68, MeOH); UV (MeOH) λ_{max} (log ε): 204 (3.83) nm, 261 (3.79) nm; ECD (MeOH) 222 (Δε +2.8), 273 (Δε +9.3), 304 (Δε −6.5); IR (KBr) ν_{max}: 3267, 1752, 1697, 1380, 1242, 1127 cm^{-1}; ^{1}H and ^{13}C NMR data (pyridine-d_5), see Tables 1 and 2; negative ESIMS m/z 529 [M − H]$^-$; negative HRESIMS m/z 529.2799 [M − H]$^-$ (calcd. for $C_{30}H_{41}O_8$, m/z 529.2807).

Ganoderenic acid AM$_1$ (**4**): yellow, amorphous powder; $[\alpha]_D^{20}$ + 20.2 (c 0.45, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.90) nm, 254 (3.77) nm; ECD (MeOH) 224 (Δε +4.3), 249 (Δε −4.1), 276 (Δε +3.9), 304 (Δε −3.0); IR (KBr) ν_{max}: 3378, 2942, 1762, 1715, 1693, 1670, 1412, 1197,

1166, 1019 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Tables 1 and 2; positive ESIMS m/z 513 [M + H]$^+$; positive HRESIMS m/z 513.2847 [M + H]$^+$ (calcd. for $C_{30}H_{41}O_7$, m/z 513.2852).

Ganoderesin C (**5**): white, amorphous powder; $[\alpha]_D^{20}$ − 47.4 (c 0.70, MeOH); UV (MeOH) λ_{max} (log ε): 236 (3.84) nm; ECD (MeOH) 226 (Δε +7.0), 253 (Δε −3.8), 312 (Δε −0.7); IR (KBr) ν_{max}: 3401, 2972, 2942, 2876, 1708, 1596, 1381, 1011 cm^{-1}; ^{1}H and ^{13}C NMR data (pyridine-d_5), see Tables 1 and 2; negative ESIMS m/z 513 [M − H]$^-$; negative HRESIMS m/z 513.2864 [M − H]$^-$ (calcd. for $C_{30}H_{41}O_7$, m/z 513.2858).

2.4. Protective effect on damage induced by DL-galactosamine in HL-7702 cells

Hepatoprotective effects against DL-galactosamine-induced HL-7702 cells damage of compounds **1–10** were determined by the MTT colorimetric assay [5]. Each cell suspension of 2×10^4 cells in 200 μL of RPMI 1640 containing fetal calf serum (10%), penicillin (100 U/mL), and streptomycin (100 μg/mL) was placed in a 96-well microplate and precultured for 24 h at 37 °C under a 5% CO_2 atmosphere. Fresh medium (100 μL) containing bicyclol and test samples were added, and the cells were cultured for 1 h. Then, the cultured cells were exposed to 25 mM DL-galactosamine for 24 h. Then, 100 μL of 0.5 mg/mL MTT was added to each well after the withdrawal of the culture medium and incubated for an additional 4 h. The resulting formazan was dissolved in 150 μL of DMSO after aspiration of the culture medium. The optical density (OD) of the formazan solution was measured on a microplate reader at 492 nm.

3. Results and discussion

Ganoderic acid XL$_1$ (**1**) was isolated as an amorphous powder. Its molecular formula, $C_{30}H_{46}O_7$, was established by negative-ion HRESIMS. The ^{1}H NMR data and ^{13}C NMR data (Tables 1 and 2) revealed resonances for seven methyl singlets (δ_H 1.10, 1.29, 1.44, 1.54, 1.60, 1.66, 2.05, each 3H, s; δ_C 16.7, 28.8, 26.1, 19.8, 19.5, 20.7, 12.7), three oxymethines [δ_H 3.52

Table 2
^{13}C NMR data for compounds **1–5**[a].

Position	1	2	3	4	5
1	35.5	35.4	34.0	33.5	36.7
2	29.0	29.1	28.2	27.4	28.5
3	77.6	78.1	76.6	77.5	77.6
4	39.3	39.6	39.5	39.2	40.7
5	49.9	46.8	51.4	51.6	55.3
6	28.8	27.8	36.8	36.5	40.4
7	69.5	68.2	199.6	200.0	206.0
8	160.4	161.5	146.9	145.9	48.6
9	141.7	141.2	151.4	150.9	62.3
10	39.1	39.8	40.8	40.4	39.0
11	200.3	200.3	200.4	199.6	206.8
12	53.5	53.8	50.2	47.9	46.1
13	48.2	48.7	45.3	48.6	52.9
14	55.1	54.7	57.9	56.4	55.9
15	72.5	72.1	208.2	206.6	206.2
16	24.1	24.3	35.4	40.0	123.5
17	51.4	52.3	47.2	134.8	182.8
18	19.5	19.6	18.1	22.4	28.9
19	19.8	17.9	17.8	18.1	14.3
20	73.7	73.7	72.8	122.7	28.7
21	26.1	25.9	27.3	22.0	19.6
22	43.3	43.4	55.5	47.4	47.9
23	31.7	32.9	209.2	206.0	207.4
24	142.4	142.6	48.3	45.6	46.9
25	128.9	129.1	35.6	34.6	35.7
26	170.5	170.8	178.5	179.9	178.4
27	12.7	12.9	17.4	16.9	17.8
28	28.8	29.0	28.1	27.9	28.4
29	16.7	17.1	16.0	15.6	16.1
30	20.7	22.3	22.0	23.9	22.7

[a] ^{13}C NMR data were measured at 400 MHz in $CDCl_3$ for **4**; at 500 MHz in C_5D_5N for **1**, **2**, **3** and **5**.

(1H, dd, J = 7.0, 4.5 Hz), δ_C 77.6; δ_H 5.03 (1H, dd, J = 7.5, 2.5 Hz), δ_C 69.5 and δ_H 5.45 (1H, t, J = 8.5 Hz), δ_C 72.5], an oxyquaternary carbon (δ_c 73.7), one α, β-unsaturated ketone group (δ_C 200.3, 160.4, 141.7), a carboxylic acid group (δ_C 170.5) and two substituted olefinic carbons [δ_C 142.4 (C-24) and 128.9 (C-25)]. The above data displayed signals characteristic of a lanostane triterpene. The HMBC correlations of δ_H 1.29 (s, H-28) with δ_C 77.6 (C-3); δ_H 5.03 (dd, J = 7.5, 2.5 Hz, H-7) with δ_C 160.4 (C-8) and 141.7 (C-9); δ_H 2.45 (m, H-16) with δ_C 72.5 (C-15) and the methyl singlet δ_H 1.44 (s, H-21) with δ_C 73.7 (C-20) and 51.4 (C-17) indicated that the hydroxyl groups were located at C-3, C-7, C-15 and C-20. The carbonyl group at C-11 was proven by correlations from δ_H 3.15 (d, J = 16.5 Hz, H_1-12) and δ_H 2.95 (overlapped, H_2-12) to C-11 by HMBC. In addition, it was confirmed that H-3, H-5, H-7, H-28, H-30 were in α-orientation and H-15, H-18, H-19, H-29 were in β-orientation by NOESY correlations between H-3/H-5, H-3/H-28, H-5/H-7, H-7/H-30 H-15/H-18 and H-19/H-29 (Fig. 2). The absolute configurations were assigned as 13R, 14R from positive and negative Cotton effects at 245 nm ($\Delta\varepsilon = +5.3$) for a $\pi \rightarrow \pi^*$ transition and 346 ($\Delta\varepsilon = -1.0$) nm for a $n \rightarrow \pi^*$ transition, respectively, in the ECD spectrum on the basis of the octant rule for the α, β-unsaturated ketone group [6] (Fig. 3). Thus, the structure of compound **1** was identified as (3S,5S,7S,10S,13R,14R,15S,17R,24E)-3,7,15,20-tetrahydroxy-11-oxo-5-lanost-8,24-dien-26 oic acid.

The molecular formula $C_{30}H_{46}O_7$ for ganoderic acid XL_2 (**2**) was also determined by HRESIMS. A comparison of the ^{1}H and ^{13}C NMR data (Table 1) of **2** were highly resembled to those of **1**, and the HMBC correlations from H-28 to C-3; from H-7 to C-8 and C-9; from H-16 to C-15 and from H-21 to C-20 and C-17 were also quite similar to those for **1**. The α-orientation of the hydroxyl group at C-7 was deduced by the ^{1}H and ^{13}C NMR shifts of H-5, H-6 and C-5, C-6 (shifted by $\Delta\delta_H$ +0.60, −0.27 and $\Delta\delta_C$ −3.1, −1.0 ppm, respectively) between **2** and **1**, and δ_H 5.03 (dd, J = 7.5, 2.5 Hz) in **1** was replaced by δ_H 4.88 (s, H-7) in **2**, and it was further determined by the correlations between H-7 and H-18 in the NOESY spectrum. The relative configurations of H-3 and H-15 were assigned as α-orientation and β-orientation by NOESY correlations between H-3/H-28, H-5/H-28 and H-15/H-18. Similar ECD data indicated that **2** possessed the same absolute configurations as **1**. Consequently, the structure of **2** was (3S,5S,7R,10S,13R,14R,15S,17R,24E)-3,7,15,20-tetrahydroxy-11-oxo-5-lanost-8,24-dien-26-oic acid.

20-hydroxy-ganoderic acid AM_1 (**3**) was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{30}H_{42}O_8$ by HRESIMS. The ^{1}H NMR spectrum (Table 1) showed the presence of six methyl singlets at δ_H 1.03 (s), 1.12 (s), 1.36 (s), 1.40 (s), 1.70 (s) and 1.78 (s), one doublet methyl at δ_H 1.31 (d, J = 7.0 Hz), one oxymethine hydrogen at δ_H 3.42 (dd, J = 6.0, 5.0 Hz, H-3). The ^{13}C NMR spectra (Table 2) of **3** revealed thirty carbon resonances owed to seven methyls (δ_c 16.0, 28.0, 17.8, 18.1, 27.3, 22.0, 17.4), one oxymethine carbon (δ_c 76.6), a oxyquaternary carbon (δ_c 72.8), four carbonyl carbons (δ_c 199.6, 200.4, 208.2, 209.2), one carboxylic acid carbon (δ_c 178.5) and two substituted olefinic carbons [δ_C 146.9 (C-8) and 151.4 (C-9)]. The above data were highly resembled to those of ganoderic acid AM_1 [7]. The difference between them was a newly signal at δ_c 72.8, which indicated the appearance of an additional hydroxyl group. The position of it at C-20 was proved by correlations between δ 1.70 (s, H-21) and δ 72.8 (C-20) (Fig. 2) in the HMBC spectrum and the HMBC correlation of δ_H 1.12 (s, H-28) with δ_C 76.6 (C-3) showed that the oxymethine hydroxyl group was located at C-3. The relative stereochemistry of **3** was established by the NOESY spectral analysis. The correlations between H-3/H-5, H-3/H-28 and H-5/H-28 suggested 3-OH was in β-orientation. Hence, the structure of **3** was assigned as (3S,5S,10S,13R,14R,17R)-3,20-dihydroxy-7,11,15,23-tetraoxo-5-lanost-8-en-26-oic acid.

Ganoderenic acid AM_1 (**4**) was isolated as a yellow powder. Its HRESIMS spectrum exhibited $[M + H]^+$ peak at m/z 513.2847, which indicated a molecular formula of $C_{30}H_{40}O_7$. The ^{1}H NMR and ^{13}C NMR data for **4** were quite similar to those of **3**, except for a double bond signal (δ_C 134.8 and 122.7) replaced the oxyquaternary carbon signal (δ_C 72.8). The HMBC correlations from δ_H 1.62 (s, H-21), δ_H 3.27 (d, J = 6.0 Hz, H-22), δ_H 2.76 (d, J = 21.6 Hz, H-16) and δ_H 3.20 (d, J = 21.6 Hz, H-16) to δ_C 122.7 (C-20), and from δ_H 0.95 (s, H-18), δ_H 1.62 (s, H-21), δ_H 2.76 (d, J = 21.6 Hz, H-16), δ_H 3.20 (d, J = 21.6 Hz, H-16) and δ_H 3.27 (d, J = 6.0 Hz, H-22) to δ_C 134.8 (C-17) suggested that the double bond was located at C-17 and C-20 (Fig. 2). The relative configuration of H-3 was assigned as α-orientation by NOESY correlations between H-3/H-5, H-3/H-28, and H-5/H-28. Accordingly, the structure of **4** was identified as (3S,5S,10S,13R,14R)-3-hydroxy-7,11,15,23-tetraoxo-5-lanost-8,17 (20) -dien-26-oic acid.

Ganoderesin C (**5**) was established the molecular formula $C_{30}H_{42}O_7$ by HRESIMS. The NMR data of **5** were similar with those of **4**. However, detailed comparison of NMR data of **5** with those of **4** showed the absence of a double bond signal at δ_C

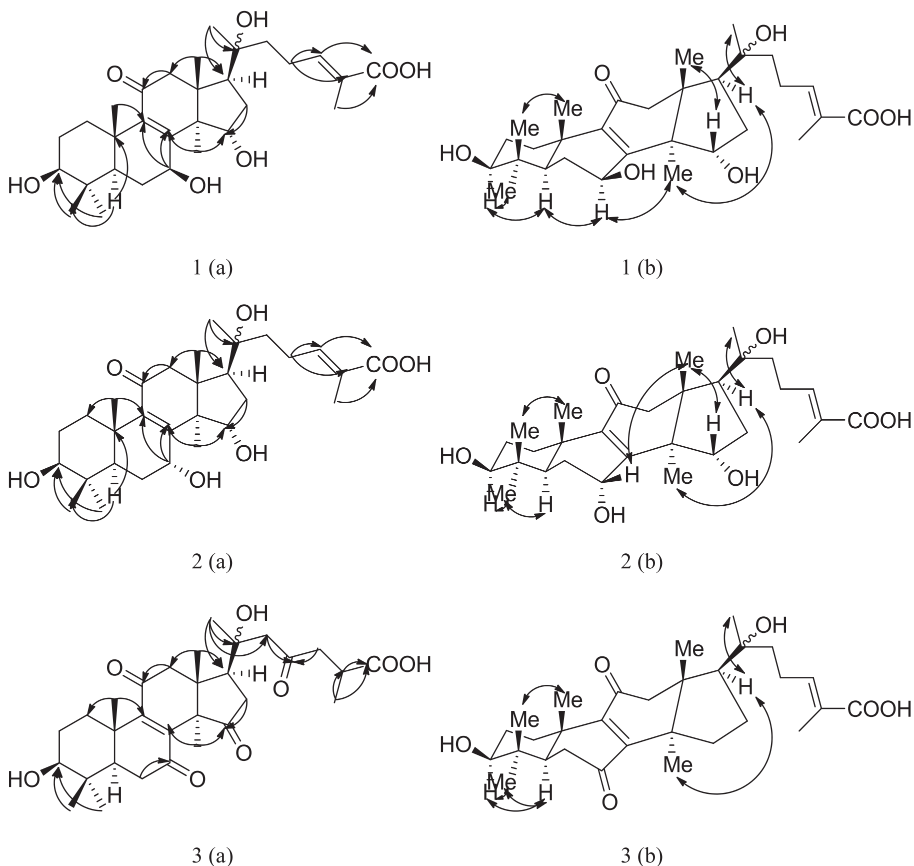

Fig. 2. Selected HMBC (⌒) (a) and NOESY (⌒) (b) correlations of **1**–**3**.

150.9 and 145.9. Meanwhile, the position of a double bond at C-16 and C-17 rather than at C-17 and C-20 was confirmed by correlations from δ_H 5.90 (s, H-16), 1.16 (s, H-18) and 1.00 (d, $J = 6.5$ Hz, H-21) to δ_C 182.8 (C-17) in the HMBC spectrum. The relative configurations of H-8 as β-oriented, H-3 and H-9 as α-oriented were determined by the NOESY correlations between

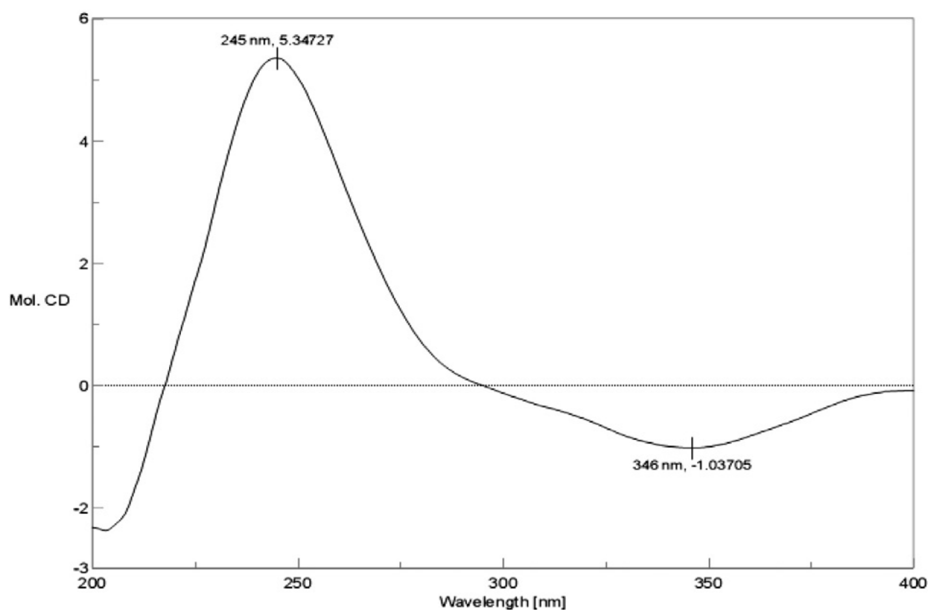

Fig. 3. The CD spectra of compound **1** in MeOH.

Table 3
Hepatoprotective effects of compounds **1**, **4**, **5**, **8**, **9** and **10** on the survival rate of HL-7702 cells injured by DL-GalN (10 μM).

Compound	OD value	Survival rate (%)
Normal	0.947 ± 0.065	100
Model	0.417 ± 0.033	43
Bicyclol[a]	0.526 ± 0.048	55*
1	0.765 ± 0.069	80**
4	0.525 ± 0.043	55*
5	0.679 ± 0.053	75*
8	0.500 ± 0.039	58*
9	0.575 ± 0.049	65*
10	0.502 ± 0.036	76*

* $P < 0.05$ vs. model.
** $P < 0.01$ vs. model.
[a] Positive control substance.

H-3/H-5, H-3/H-28, H-5/H-28, H-5/H-9, H-19/H-29, H-8/H-19 and H-8/H-18 (Fig. 2). In the ECD spectrum of 5, a negative Cotton effect at 253 nm for a $\pi \rightarrow \pi^*$ transition and a negative Cotton effect at 312 nm for a $n \rightarrow \pi^*$ transition suggested the 13R, 14R on the basis of the octant rule for the α, β-unsaturated cyclopentanone group [6]. Therefore, the structure of **5** was characterized as (3S,5S,8R,9S,10S,13R,14R)-3-hydroxy-7,11,15,23-tetraoxo-5-lanost-16-en-26-oic acid.

The known compounds were evaluated by comparing their MS and NMR spectrum data with literature data, and were identified as lucidone F (**6**) [8], lucidone A (**7**), lucidone B (**8**) [9], ganoderenic acid B (**9**) [10] and ganoderic acid C_2 (**10**) [11].

To assess the biological activities of 10 triterpenoids, a human hepatic cell (HL-7702) injury model induced by DL-galactosamine (GalN) was adopted. Bicyclol, a hepatoprotective drug in clinic was used as a positive control. As shown in Table 3, compound **1, 4, 5, 8, 9** and **10** at a concentration of 10 μM showed hepatoprotective activities.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.08.004.

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