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Full Paper

Two Novel Lanostane Triterpenoids from *Ganoderma Sinense*

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Abstract: Two novel lanostane-type triterpenes, Ganolactone B and Ganoderiol A triacetate, were isolated from the fruiting bodies of *G. sinense*, together with six known compounds. The structures of the two new triterpenes were determined as 3*β*,7*β*-dihydroxy-11,15-dioxo-lanosta-8-en-24→20 lactone (**1**) and 3*β*,24,26-triacetoxy-5*α*-lanosta-7,9(11)-dien-25-ol (**2**), respectively, by chemical and spectroscopic means.

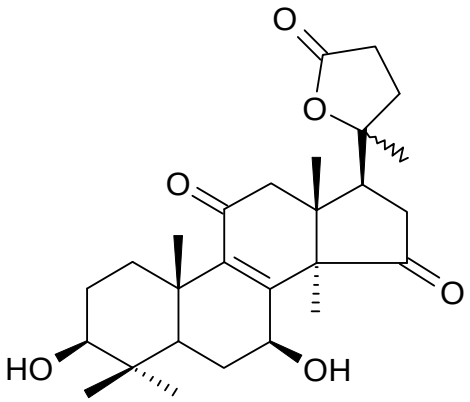
Keywords: Ganolactone B, Ganoderiol A triacetate, *Ganoderma Sinenes*.

Introduction

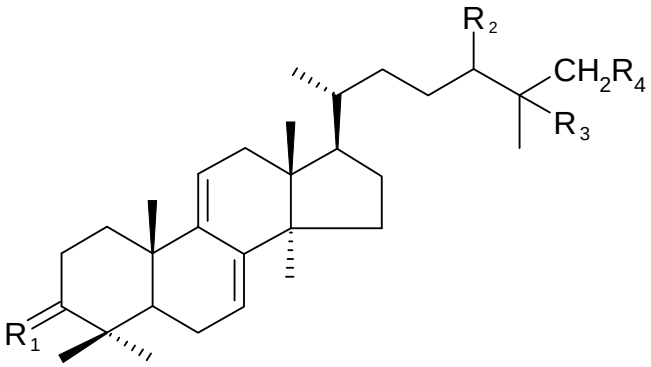
The fruiting body of the fungus *Ganoderma lucidum* Karst.(Polyporaceae) has attracted much attention as a well-known traditional Chinese medicine that has been used clinically in China, Japan, and Korea for a long time. Over the past recent two decades, more than 130 highly oxygenated lanostane-type triterpenoids have been isolated from the fruiting bodies, spores and culture mycelia of *Ganoderma* spp., including common fungal steroids derived from ergosterol. Some of them are known to possess varied bioactive properties. Other *Ganoderma* spp. have also been used in traditional Chinese, Japanese and Korean medicines, and their pharmacological activities as antitumor [1,2] and antihypertensive agents [3], their use in the treatment of chronic bronchitis and diabetes or their liver

protective [4], antioxidant [5] and anti-HIV activity [6] were studied. In this paper, we wish to report the structural elucidation by extensive spectroscopic analyses of two new components isolated together with known six compounds (Figure 1) from the fruiting bodies of *G. sinense*.

Figure 1.

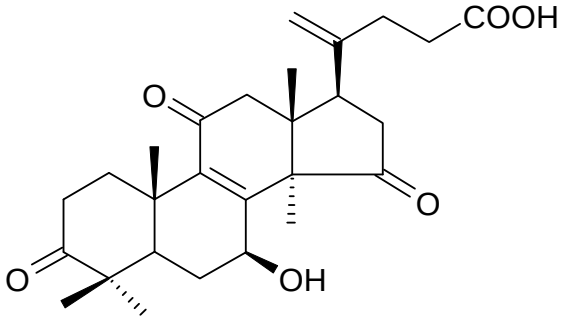


1

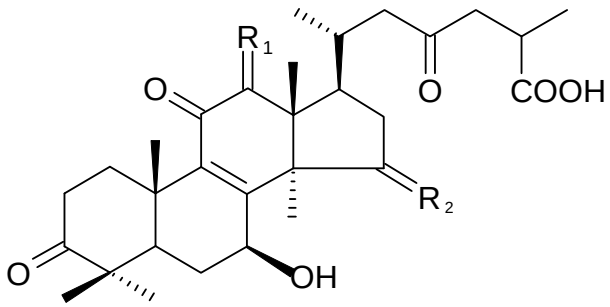


	R_1	R_2	R_3	R_4
2	OAc, H	OAc	OH	OAc
3	OH, H	OH	OH	OH
4	OH, H	△24(25)	OH	OH
5	O	OH	OH	OH

Figure 1. Cont.



6



	R_1	R_2
7	OH, H	O
8	H_2	OH, H

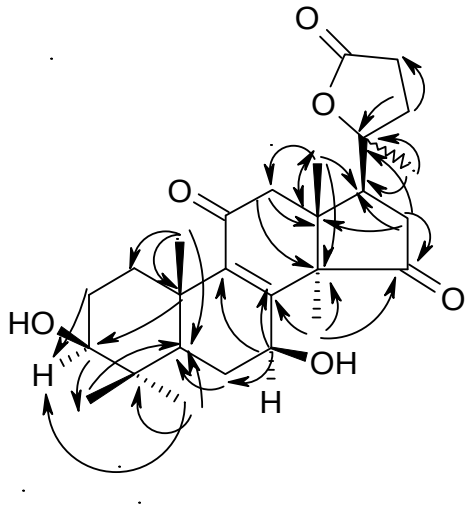
Results and Discussion

Dried chipped fruiting bodies of *G. sinense* were soaked overnight in acetone at room temperature to give an extract which was suspended in water and successively partitioned with petroleum ether, $CHCl_3$, EtOAc and n-BuOH, respectively. The $CHCl_3$ layer was chromatographed on silica gel to separate it into six fractions. Some of these were subjected to silica gel column chromatography to give two new triterpene compounds, which we have named Ganolactone B (3*β*,7*β*-dihydroxy-11,15-dioxo-lanosta-8-en-24→20 lactone, **1**), Ganoderiol A triacetate (3*β*,24,26-triacetoxy-5*α*-lanosta-7,9(11)-dien-25-ol, **2**), together with six known compounds: Ganoderiol A (**3**)[7], Ganodermatriol (**4**), Ganodermanontriol (**5**), 20(21)-dehydrolucidenic acid (**6**) [8], Ganoderic acid D (**7**) [9] and Ganoderic acid A (**8**) [10].

Ganolactone B (**1**): colorless needles, m.p. > 300°C, $[\alpha]_D^{19}$ 183°(c=0.3, MeOH). Compound **1** gave a quasi-molecular ion peak at *m/z* 459 $[M+H]^+$ in its positive FABMS spectrum, and was assigned a molecular formula of $C_{27}H_{38}O_6$, which was confirmed by HRESIMS ($C_{27}H_{38}O_6Na$, 481.2579; calcd. for $C_{27}H_{38}O_6Na$, 481.2566) and NMR spectral data (Table 1). The IR spectrum [3433 (br, OH), 1769, 1719, 1655 (C=O), 1207, 1143 and 1065 (C-O and COOC)] indicated the presence of hydroxyl and

carbonyl groups. The UV data [(MeOH) λ_{max}(logε) nm: 255 (0.17)] indicated the presence of an α,β-unsaturated carbonyl group. The ^{1}H-NMR spectrum of **1** (Table 1), analyzed together with the compound's HMQC spectrum, exhibited singlet signals (3H each) of six methyl groups at δ_H 1.07, 1.24, 1.36, 1.39, 1.40, 1.44 and two oxygenated methine protons at δ_H 3.46 (1H, dd, J=5.5, 11.0Hz, 3α-H) and 5.19 (1H, t, 8.4Hz, 7α-H). The ^{13}C-NMR spectrum displayed signals characteristic of six methyl groups, an oxygen-bearing quaternary carbon at δc 86.49 (s), two hydroxyl-bearing methine carbons at δc 77.59 (d) and 66.70 (d), an *α,β*-unsaturated C=O at δc 197.98 (s), 158.71 (s), 142.65 (s), a ketone carbon at δc 214.96 (s), and a carboxyl group at δc 176.69 (s). These data suggested a polyoxygenated lanostane-type triterpene with a structure very similar to Ganolactone A, including its chemical configuration at C-20 [11]. Ganolactone B (**1**) had a signal at δc 86.49 (s). This indicated that linking group was at C-20. The connectivities of **1** were established by interpretation of the HMBC spectrum (Figure 2). In the HMBC experiment, δc 86.49 (s, C-20) showed correlation with two secondary methyl group signals δ_H 2.81, 2.66 (each 1H, H-16), δ_H 2.68, 2.49 (each 1H, H-22) and one methyl signal δ_H 1.39 (3H, H-21). From the above observation and on the basis of all the above results the structure of Ganolactone B was established as 3β,7β-dihydroxy-11,15-dioxo-lanosta-8-en-24→20 lactone.

Figure 2. key HMBC(↷) correlations of Ganolactone B (**1**).



Compound **2** gave colorless needles, mp 232~233°C, $[\alpha]_D^{19}$ 80°(c=0.1, C_5D_5N) and a quasi-molecular ion peak at *m/z* 601 $[M+H]^+$ in its positive FABMS spectrum, and was thus assigned a molecular formula of $C_{36}H_{56}O_7$, which was confirmed by HRESIMS ($C_{36}H_{56}O_7Na$, 623.3923; calcd. for $C_{36}H_{56}O_7Na$, 623.3920) and NMR spectral data (Table 1). Its UV spectrum was similar to that of Ganoderiol A [7], indicating the presence a heteroannular diene system in this molecule. The IR spectrum [3492 (br, OH), 1735, 1719 (C=O), 1233, 1152 and 1031 (C-O and COOC)] indicated the presence of hydroxyl and carbonyl groups. The ^{1}H-NMR spectrum of **2** showed singlets for three acetyl methyl group at δ_H 2.05, 2.07 and 2.10 (3H each). The ^{13}C-NMR spectrum of **2** verified the presence of three carbons attached to oxygen at δc 68.45 (t), 76.64 (d) and 80.83 (d), three carbonyl carbons for acetyl moieties at δc 170.64 (s), 170.93 (s), and 171.11 (s), and three acetyl methyl carbons at δc 20.83 (q), 20.98 (q), and 21.29 (q). From these spectral data, the structure of this compound was

established as 3*β*,24,26-triacetoxy-5*α*-lanosta-7, 9(11)-dien-25-ol, for which we propose the name Ganoderiol A triacetate.

Table 1. ^{1}H-NMR and ^{13}C-NMR data of compounds **1** and **2**.

	1		**2**	
No.	δc	δ_H (J = Hz)	δc	δ_H (J = Hz)
	C_5D_5N		$CDCl_3$	
1	35.47 t	3.22 m (*α*)	35.41 t	-
		1.20 m (*β*)		-
2	28.59 t	1.89 m (*α*)	22.77 t	-
		1.89 m (*β*)		-
3	77.59 d	3.46 dd(10.96, 5.43) (*α*)	80.83 d	4.51 dd (12.68, 3.56) (*α*)
4	49.68 s	-	37.62 s	-
5	49.65 d	1.10 t(16.97, 12.99) (*α*)	49.26 d	-
6	28.14 t	2.37 m (*α*)	24.26 t	-
		1.90 m (*β*)		-
7	66.70 d	5.19 t(16.97, 12.99) (*α*)	119.97 d	5.45 m (*α*)
8	158.71 s	-	142.70 s	-
9	142.65 s	-	146.65 s	-
10	39.29 s	-	37.80 s	-
11	197.98 s	-	116.50 d	5.30 d (4.64)
12	50.94 t	3.09 d(16.88) (*α*)	37.24 t	-
		2.95 d(16.78) (*β*)		-
13	45.78 s	-	43.74 s	-
14	59.03 s	-	50.32 s	-
15	214.96 s	-	25.99 t	-
16	36.53 t	2.81 dd(18.30, 10.17) (*α*)	31.45 t	-
		2.66 dd(18.30, 10.32) (*β*)		-
17	49.65 d	2.65 dd(11.98, 7.26) (*α*)	50.77 d	-
18	18.64 q	1.40 s	16.92 q	0.54 s
19	19.40 q	1.36 s	22.82 q	0.99 s
20	86.49 s	-	36.45 d	-
21	25.79 q	1.39 s	18.56 q	0.94 d(5.28)
22	27.84 t	2.28 m (*α*)	32.62 t	-
		2.49 m (*β*)		-
23	34.20 t	2.08 m (*α*)	27.78 t	-
		1.85 m (*β*)		-
24	176.69 s	-	76.64 d	4.89 dd(9.80, 1.96)
25	-	-	73.27 s	-
26	-	-	68.45 t	-
27	-	-	20.19 q	1.19 s
28	25.39 q	1.44 s	28.08 q	0.86 s
29	16.47 q	1.24 s	15.66 q	0.86 s
30	28.75 q	1.07 s	25.53 q	0.94 s
OCOMe	-	-	170.64 s	-
	-	-	170.93 s	-
	-	-	171.11 s	-
OCOMe	-	-	20.83 q	2.05(3H, s)
	-	-	20.99 q	2.07(3H, s)
	-	-	21.29 q	2.10(3H, s)

Experimental

General

Melting points were measured on a XRB-1 micro hot-stage melting point apparatus and are uncorrected. Optical rotations were measured with a DIP-370 automatic polarimeter. UV spectra were measured with a Shimadzu double-beam instrument. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H-, ^{13}C-NMR and 2D-NMR spectra were recorded on a Bruker AM-400 MHz or a DRX-500 spectrometer with TMS as internal standard.

Plant Material

The fruiting bodies of *G. sinenes* were purchased from the Kunming natural medicine market, Yunnan Province, P. R. China in July 2005. The botanical identification was made by Prof. Yang Zhuliang (Kunming Institute of Botany, The Chinese Academy of Sciences).

Extraction and Isolation

Dried chipped fruiting bodies of *G. sinense* (8 kg) were soaked overnight in acetone at room temperature to afford an extract which was suspended in water and successively partitioned with petroleum ether (PE), $CHCl_3$, EtOAc, and n-BuOH, respectively. The $CHCl_3$ extracts were concentrated *in vacuo* to afford a residue (115 g), which was subjected to silica gel column chromatography, using a $CHCl_3$-Me_2CO gradient (from 100% $CHCl_3$ to $CHCl_3$-Me_2CO 1:1) as eluent to separate it into six fractions (Fr. A - F). Fr. D (19.5 g) was subjected repeatedly to silica gel chromatography and eluted stepwise with PE-Me_2CO (from 100% PE to PE-Me_2CO 1:1) to afford compounds **2** (17 mg), **3** (29 mg), **4** (22 mg) and **5** (13 mg), respectively. Fr. E (8.3 g) was rechromatographed on a silica gel column chromatography by elution with PE-Me2CO (5:1 v/v, 3:1, 1:1) to yield **1** (36 mg). Fr. F (4.7g) was rechromatographed on a silica gel column chromatography by elution with PE- Me_2CO (2:1 v/v, 1:2). Further purification of Fr. F-4 (1.7 g) was achieved by a reversed phase C-18 (from MeOH- H_2O 40% to 65%) to give compounds **6** (12 mg), **7** (11 mg) and **8** (9 mg), respectively.

Ganolactone B (**1**): colorless needles, m.p. > 300°C, $[\alpha]_D^{19}$ 183°(c=0.3, MeOH); positive FABMS *m/z* 459[M+H]$^+$, HRESIMS (calcd. for $C_{27}H_{38}O_6$: 458.2579); IR(KBr)cm^{-1}: 3433 (br, OH), 1769, 1719, 1655 (C=O), 1207, 1143 and 1065 (C-O and COOC); UV (MeOH) λ_{max} (logε) nm:255(0.17); ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

3β,24,26-Triacetoxy-5α-lanosta-7,9(11)-dien-25-ol (Ganoderiol A triacetate) (**2**): Colorless needles, mp 232~233°C, $[\alpha]_D^{19}$ 80°(c=0.1, C_5D_5N); positive FABMS *m/z* 601 [M+H]$^+$; HRESIMS (calcd. for $C_{36}H_{56}O_7$: 600.3920); IR (KBr) cm^{-1}: 3492 (br, OH), 1735, 1719 (C=O), 1233, 1152 and 1031 (C-O and COOC); UV (MeOH) λ_{max} (logε) nm: 243 (0.30); ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

Ganoderiol A (**3**): colorless needles, mp 232~234°C, $[\alpha]_D^{22}$ +20°(c=0.10, EtOH); HREIMS *m/z* 474.3740 (calcd for $C_{30}H_{50}O_4$: 474.3709); UV (EtOH) λ_{max} (logε) nm: 253 (8058), 244 (962), 253 (6518); IR (KBr) cm^{-1}: 3350, 2950, 2900, 2850, 1430, 1360, 1060; ^{1}H-NMR (C_5D_5N) δ_H: 5.55 (1H, m, H-7), 5.40 (1H, m, H-12), 3.83, 3.47 (1H each, d, *J*=10.6Hz, 26-H), 3.46 (1H, t, *J*=12.1Hz, C-24), 3.24 (1H, dd, *J*=10.5 and 5.0 Hz, H-3), 1.11 (3H, s, H-27), 1.01 (3H, s, H-19), 0.98 (3H, s, H-28), 0.92 (3H, d, *J*=6.2Hz H-21), 0.88 (3H, s, H-29), 0.88 (3H, s, H-30), 0.67 (3H, s, H-18); ^{13}C-NMR (C_5D_5N) δc: 36.40 (t, C-1), 28.86 (t, C-2), 78.12 (d, C-3), 39.37 (s, C-4), 49.83 (d, C-5), 23.56 (t, C-6), 120.99 (d, C-7), 143.03 (s, C-8), 146.61 (s, C-9), 37.85 (s, C-10), 116.58 (s, C-11), 38.14 (t, C-12), 44.13 (s,C-13), 50.68 (s, C-14), 28.17 (t, C-15), 31.91 (t, C-16), 51.50 (d, C-17), 16.64 (q, C-18), 23.11 (q, C-19), 37.15 (d, C-20), 19.06 (q, C-21), 34.40 (t, C-22), 28.94 (t, C-23), 77.23 (d, C-24), 74.79 (s, C-25), 69.28 (t, C-26), 20.15 (q,C-27), 28.94 (q, C-28), 16.07 (q, C-29), 25.89 (q, C- 30).

Ganodermatriol (**4**): white powder, mp 180~190°C; $[\alpha]_D^{22}$+9°(c=0.04, EtOH); HREIMS *m/z* 456.3612 (calcd for $C_{30}H_{48}O_3$: 456.3605); UV (EtOH) λ_{max} (logε) nm: 237 (4740), 245 (5400), 253 (3650); ^{1}H-NMR (C_5D_5N) δ_H: 5.56 (1H, *J*=7.5 , H-24), 5.48 (1H, m, H-7), 5.32 (1H brd, *J*=6.2Hz, H-11), 4.33 (2H, s, H-27), 4.21 (2H, s, H-26), 3.24 (3H, dd, *J*=10.6 and 4.8 Hz H-3), 1.01 (3H, s, H-19), 0.98 (3H, s, H-28), 0.91 (3H, d , *J*=6.2Hz, H-21), 0.88 (3H, s, H-29), 0.88 (3H, s, H-30), 0.67 (3H, s, H-18); ^{13}C-NMR (C_5D_5N) δc: 36.42 (t, C-1), 28.74 (t, C-2), 78.12 (d, C-3), 39.37 (s, C-4), 49.84 (d, C-5), 23.57 (t, C-6), 121.06 (d,C-7), 142.98 (s, C-8), 146.64 (s, C-9), 37.86 (s, C-10), 116.54 (s, C-11), 38.11 (t, C-12), 44.13 (s,C-13), 50.69 (s, C-14), 28.16 (t, C-15), 31.90 (t,C-16), 51.24 (d, C-17), 16.65 (q, C-18), 23.10 (q, C-19), 36.42 (d, C-20), 18.62 (q, C-21), 36.91 (t, C-22), 24.66 (t, C-23), 127.74 (d, C-24), 140.75 (s, C-25), 65.51 (t, C-26), 58.59 (q,C-27), 28.86 (q, C-28), 16.02 (q, C-29), 25.87 (q, C- 30).

Ganodermanontriol (**5**): colorless prisms, mp 168~170°C; $[\alpha]_D^{24}$+41°(c=0.20, MeOH); HREIMS *m/z* 472.3540 (calcd. for $C_{30}H_{48}O_4$: 472.3550); ^{1}H-NMR (C_5D_5N) δ_H: 5.51 (1H, d, *J*=6.2Hz, H-7), 5.39 (1H, d, *J*=5.9Hz, H-11), 3.83, 3.48 (1H each, d, *J*=11.3Hz, 26-H), 3.46 (1H, t, *J*=11.0Hz, C-24), 1.20 (3H, s, H-19), 1.13 (3H, s, H-28), 1.11 (3H, s, H-27), 1.09 (3H, s, H-29), 0.92 (3H, d, *J*=6.2Hz, H-21), 0.88 (3H, s, H-30), 0.60 (3H, s, H-18); ^{13}C-NMR (C_5D_5N) δc: 36.86 (t, C-1), 34.39 (t, C-2), 215.18 (s, C-3), 47.52 (s, C-4), 51.11 (d, C-5), 23.92 (t, C-6), 120.43 (d,C-7), 143.14 (s, C-8), 146.97 (s, C-9), 37.13 (s, C-10), 117.69 (s, C-11), 37.50 (t, C-12), 44.08 (s,C-13), 50.60 (s, C-14), 31.39 (t, C-15), 28.14 (t, C-16), 51.11 (d, C-17), 16.06 (q, C-18), 22.11 (q, C-19), 36.55 (d, C-20), 19.06 (q, C-21), 34.39 (t, C-22), 28.94 (t, C-23), 77.24 (d, C-24), 74.79 (s, C-25), 69.28 (t, C-26), 20.14 (q,C-27), 25.66 (q, C-28), 22.39 (q, C-29), 25.66 (q, C- 30).

20(21)-Dehydrolucidenic acid A (**6**): colorless needles, mp 135-137°C; $[\alpha]_D^{25}$+69.9°(c 0.20, $CHCl_3$); HREIMS *m/z*456.2512 (calcd. for $C_{27}H_{36}O_6$ [M], 456.2511); UV (MeOH) λ_{max} (logε) nm: 253 (3.78); IR (KBr) cm^{-1}: 3445, 1735, 1702, 1659, 897; ^{1}H-NMR ($CHCl_3$) δ_H: 5.06 (1H, s, H-21), 4.91 (1H, s, H-21), 1.39 (3H, s, H-30), 1.25 (3H, s, H-19), 1.13 (3H, s, H-28), 1.10 (3H, s, H-29), 0.89 (3H, s, H-19); ^{13}C-NMR ($CHCl_3$) δc: 35.64 (t, C-1), 34.27 (t, C-2), 216.57 (s, C-3), 46.78 (s, C-4),49.07 (d, C-5), 27.66 (t, C-6), 66.34 (d,C-7), 157.75 (s, C-8), 141.15 (s, C-9), 38.31 (s, C-10), 197.49 (s, C-11), 49.07 (t, C-12), 45.26 (s,C-13), 58.82 (s, C-14), 217.67 (s, C-15), 38.64 (t,C-16), 46.24 (d, C-17), 18.82 (q, C-18), 18.14 (q, C-19), 143.91 (s, C-20), 112.35 (t, C-21), 31.28 (t, C-22), 32.26 (t, C-23), 176.88 (s, C-24), 27.03(q, C-28), 20.78(q, C-29), 24.55(q, 30).

Ganoderic acid D (**7**): colorless needles, mp 201~203°C: $[\alpha]_D^{22}$ +185°(c=0.10, EtOH); HREIMS *m/z* 530.2875 (calcd. for $C_{30}H_{42}O_8$: 530.2881); UV (EtOH) λ_{max} (logε) nm: 253 (8800); IR (KBr) cm^{-1}: 3410, 3400~2500 (br), 1730, 1720, 1660; ^{1}H-NMR (C_5D_5N) δ_H: 5.16 (1H, dd, *J*=8.4 and 8.4Hz, H-7), 4.69 (1H, s, H-12), 1.47 (3H, s, H-28), 1.43 (3H, s, H-19), 1.41 (3H, d, *J*=6.7Hz, C-21), 1.36 (3H, d, *J*=7.3Hz, C-27), 1.22 (3H, s, H-18), 1.15 (3H, s, H-30), 1.07 (3H, s, H-29); ^{13}C-NMR (C_5D_5N) δc: 35.47 (t, C-1), 34.59 (t, C-2), 216.64 (s, C-3), 46.93 (s, C-4), 49.34 (d, C-5), 28.94 (t, C-6), 65.72 (d, C-7), 159.83 (s, C-8), 140.90 (s, C-9), 38.23 (s, C-10), 201.07 (s, C-11), 79.26 (d, C-12), 51.78 (s, C-13), 59.91 (s, C-14), 215.26 (s, C-15), 39.24 (t,C-16), 46.93 (d, C-17), 13.03 (q, C-18), 18.37 (q, C-19), 29.09 (d, C-20), 21.89 (q, C-21), 49.02 (t, C-22), 209.04 (s, C-23), 46.93 (t, C-24), 46.93 (d, C-25), 178.26 (s, C-26), 17.66 (q,C-27), 24.01 (q, C-28), 21.14 (q, C-29), 26.60 (q, C- 30).

Ganoderic acid A (**8**): colorless needles, mp 233~236°C; $[\alpha]_D^{22}$ +150°(c=0.13, $CHCl_3$); HREIMS *m/z* 516.3070 (calcd. for $C_{30}H_{42}O_8$: 516.3087); UV (EtOH) λ_{max} (logε) nm: 254 (3.70); IR (KBr) cm^{-1}: 3400, 2700~2300 (br), 1700, 1655, 1270, 1000; ^{1}H-NMR (C_5D_5N) δ_H: 5.23 (1H, dd, *J*=9.2 and 7.8Hz, H-15), 4.94 (1H, dd, *J*=9.7 and 7.6Hz, H-7), 1.51 (3H, s, H-28), 1.41 (3H, s, H-19), 1.33 (3H, d, *J*=7.3Hz, C-27), 1.15 (3H, s, H-30), 1.11 (3H, s, H-29), 1.07 (3H, s, H-18), 0.95 (3H, d, *J*=6.2Hz, C-21); ^{13}C-NMR (C_5D_5N) δc: 36.07 (t, C-1), 34.63 (t, C-2), 216.11 (s, C-3), 47.10 (s, C-4), 49.04 (d, C-5), 29.69 (t, C-6), 68.76 (d,C-7), 161.51 (s, C-8), 139.98 (s, C-9), 38.35 (s, C-10), 199.70 (s, C-11), 52.44 (t, C-12), 46.78 (s, C-13), 54.76 (s, C-14), 72.22 (d, C-15), 36.96 (t, C-16), 48.68 (d, C-17), 17.54 (q, C-18), 19.58 (q, C-19), 33.10 (d, C-20), 19.77 (q, C-21), 49.97 (t, C-22), 208.95 (s, C-23), 47.15 (t, C-24), 35.60 (d, C-25), 176.23 (s, C-26), 17.65 (q,C-27), 20.29 (q, C-28), 20.87 (q, C-29), 27.26 (q, C- 30).

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