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Three New Triterpenoids Containing Four-Membered Ring from the Fruiting Body of *Ganoderma sinense*

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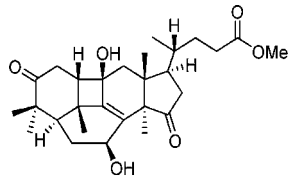
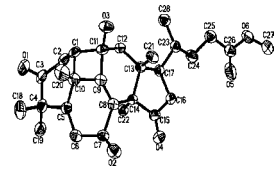
ABSTRACT



Methyl ganosinensate A



Methyl ganosinensate A (1), ganosinensic acid A (1a), and ganosinensic acid B (2), three new triterpenoids with an unusual four-membered ring skeleton produced by a bond across C-1 to C-11, were isolated from the fruiting body of *Ganoderma sinense*. Their structures were established on the basis of extensive spectroscopic methods, including 1D and 2D NMR techniques, and methyl ganosinensate A was confirmed by X-ray crystallographic analysis.

The genus *Ganoderma* has been used as folk medicine since ancient time. Among them, the well-known species of *Ganoderma lucidum* has been widely used as a health supplement, especially in China, Japan, and Korea. Therefore, most research has focused on *G. lucidum*, on its chemical and biologically active constituents. The species is known to be prolific producers of lanostane-type triterpenoids, and over 100 such triterpenoids have been isolated and characterized,[1] some of which are antiandrogen,[2] antitumor,[3] anti-HIV-1,[4] and anti-inflammatory[5] constituents.

In our previous research on this genus, we reported two novel 3,4-*seco*-trinorlanostane triterpenoids[6] and a new 18(13→12β) *abeo*-lanostadiene triterpenoid[7] from *G. fornicatum*.

To further discover structurally diverse and biologically significant compounds from the *G. sinense*, we examined its fruiting body, which led to the isolation of three new triterpenoids, methyl ganosinensate A (**1**), ganosinensic acid A (**1a**), and ganosinensic acid B (**2**), with an unusual four-membered ring produced by linkage of C-1 with C-11. These

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compounds provide us a new insight into the biosynthesis of triterpenoids with the lanostane skeleton. Here we describe the structure elucidation and propose a biosynthetic route.

The dried and powdered fruiting body of *G. sinense* (dry weight 50 kg) was extracted with MeOH by refluxing and concentrated in vacuo to give a crude extract (5 kg), which was then partitioned between H_2O and EtOAc. The EtOAc fraction (1.5 kg) was repeatedly chromatographed on silica gel, RP-18, and Sephadex LH-20 (MeOH) to yield methyl ganosinensate A (**1**, 12 mg), mixed **1a**, and **2** (31 mg). Further purification with HPLC led to the isolation of ganosinensic acid B (**2**, 13 mg) and ganosinensic acid A (**1a**, 8 mg) (for details, see the Supporting Information).

Methyl ganosinensate A (**1**)[8] was isolated as colorless cubes. Its molecular formula, $C_{28}H_{40}O_6$, was established on the basis of HRESIMS. The ^{1}H NMR showed the presence of six methyl signals at δ_H 1.68 (s), 1.37 (s), 1.31 (s), 1.12 (s), 1.02 (s), and 0.94 (d, $J = 6.4$ Hz) and a methoxyl signal at δ_H 3.64 (s) (Table 1). The ^{13}C and DEPT NMR spectra (Table 2) displayed 28 carbons, including 10 quaternary carbons (two sp^2 carbons at δ_C 136.4 and 154.7, one oxygenated sp^3 carbon at δ_C 83.5, and three carbonyls at δ_C 216.5, 215.0, and 174.1), five methines (one oxymethine at δ_C 68.3), six methylenes, and seven methyls. The data suggested that **1** was close to the structure of lucidenic acid A,[9] except for those of C-1 and C-11. Interestingly, the chemical shift of C-1 is no more than 40 ppm in a normal lanostane-type triterpenoid, while the chemical shift of C-1 in **1** is 57 ppm; the chemical shift of C-11 (83.5) is also quite different from the general data (δ_C 198−200). Furthermore, there was absence of one carbonyl and no adding of one unsaturated bond of **1**, but **1** and lucidenic acid A still retain the same degrees of unsaturation, and it could be deduced that **1** includes five rings instead of the usual four rings. This was further supported by HMBC correlations (Figure 1) observed from signals of H-1 (δ_H 2.77) to C-2 (δ_C 36.5), C-9 (δ_C 154.7), C-11 (δ_C 83.5), and C-19 (δ_C 18.8), as well as signals from H-12 (δ_H 2.13) to C-9, C-11, C-14 (δ_C 62.3), and C-18 (δ_C 17.7). These correlations allowed us to establish a four-membered ring E system, which could well explain the odd chemical shift of C-1 and C-11 and the degrees of unsaturation mentioned above. The five methyls were assigned by the key HMBC correlations observed from H-5 (δ_H 2.02) to C-28 (δ_C 27.1) and C-29 (δ_C 18.9), from H-12 to C-18, from H-1 to C-19, from H-30 (δ_H 1.31, s) to C-13, and from H-21 (δ_H 0.94, d, $J = 6.4$ Hz) to C-20 (δ_C 35.7) and C-17 (δ_C 46.8). Correlations from the signals H-28, H-29 to C-3 (δ_C 216.5); H-7 (δ_H 5.10) to C-8 (δ_C 136.4), C-9; H-30 to C-15 (δ_C 215.0), confirmed that the positions of two ketones and a hydroxyl groups were at C-3, C-15, and C-7, respectively. Comprehensive analysis of the ^{1}H−^{1}H COSY spectrum of **1** allowed the establishment of three structural fragments, as drawn with bold lines in Figure 1. The structure of **1** was further confirmed by X-ray crystallographic analysis.[10]

Table 1. ^{1}H NMR Assignments of **1**, **1a**, and **2**[a]

	δ_H (J in Hz) mult		
no.	**1**	**1a**	**2**
1	2.77 m	2.76 m	2.77 m
2α	3.01 t (12.3)	3.01 t (12.3)	2.99 t (12.3)
2β	2.50 dd (12.6, 7.5)	2.49 (overlapped)	2.41 (overlapped)
5	2.02 m	2.01 m	2.01 m
6α	2.33 m	2.34 m	2.35 m
6β	2.02 m	2.02 m	2.04 m
7	5.10 m	5.11 m	5.12 dd (8.5, 4.4)
12	2.13 m	2.15 m	2.16 m
16α	2.83 m	2.87 m	2.85 m
16β	2.15 m	2.24 m	2.16 m
17	2.10 m	2.14 m	2.26 m
18	1.37 s	1.38 s	1.39 s
19	1.68 s	1.68 s	1.67 s
20	1.58 m	1.70 m	2.37 m
21	0.94 d (6.4)	0.99 d (6.4)	1.11 (overlapped)
22	1.81 m, 1.38 m	2.62 m, 1.98 m	2.55 m, 2.44 m
23	2.43 m, 2.31 m	2.50 m, 1.51 m	
24			3.11 m, 2.62 m
25			3.3 m
27			1.35 d (7.5)
28	1.02 s	1.00 s	1.02 s
29	1.12 s	1.12 s	1.12 (overlapped)
30	1.31 s	1.30 s	1.30 s
OMe	3.64 s		

[a] Measured at 500 MHz in C_5D_5N.

The relative configuration of **1** was determined by the ROESY spectrum (Figure 1), in which the correlations of H-5/H-7 and H-1/H$_3$-19 indicated that 7-OH and H-1 was in β-orientation. From X-ray crystallographic analysis, H$_3$-19 and 11-OH have the same relative configuration, so 11-OH was in β-orientation. The conformation of **1** in solution as established by the ROESY spectrum was in good agreement with that in the solid state, as determined by X-ray study (Figure 2).

Ganosinensic acid A (**1a**),[11] obtained as colorless needles, was determined as $C_{27}H_{38}O_6$ on the basis of HRESIMS. Comparing signals of ^{13}C spectra with **1**, the only difference is the disappearance of a methoxyl group. So **1a** is supposed

(8) Methyl ganosinensate A (**1**): colorless cubes (petroleum ether.acetone 10:1); mp 215−217 °C; $[\alpha]^{25}_D = +171.3$ (c 0.1, CH_3OH); negative FAB m/z 471 $[M - H]^-$; HRESIMS m/z $[M + Na]^+$ 495.2728 (calcd for $C_{28}H_{40}O_6Na$, 495.2722); UV (CH_3OH) λ_{max} (log ε) 215 (3.55) nm; IR (KBr) ν_{max} 3400, 2930, 1717, 1695, 1368, 1257, 1156, 1043 cm^{-1}. NMR can be found in Table 1and 2.

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(10) Crystallographic data for **1**: $C_{28}H_{40}O_6$, $M = 472.60$, orthorhombic, space group $P2_12_12_1$, $a = 7.6814$ (1) Å, $b = 12.1284$ (1) Å, $c = 27.7022$ (3) Å, $V = 2580.82$ (5) Å^3, $Z = 4$, $d = 1.216$ g/cm^3, crystal dimensions $0.10 \times 0.10 \times 0.20$ mm was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω-2θ scans, $2\theta_{max} = 134.52°$), Mo Kα radiation. The total number of independent reflections measured was 3692, of which 3439 were observed ($||F||^2 \geq 2\sigma||F||^2$). Final indices: $R_1 = 0.0406$, $wR_2 = 0.1111$ ($w = 1/\sigma||F||^2$), $S = 1.035$. The crystal structure of **1** was solved by direct method SHELXS-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 755772). Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U.K.; fax (+44) 1223−336−033; or deposit@ccdc.cam.ac.uk)

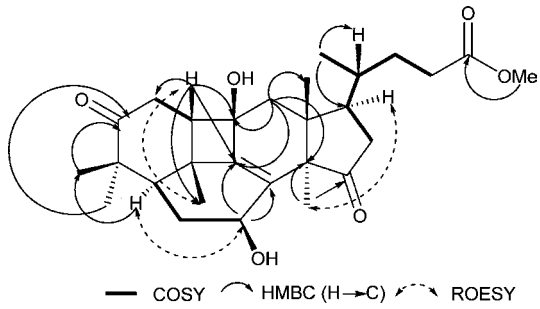


Figure 1. Key COSY, HMBC, and ROESY correlations of **1**.

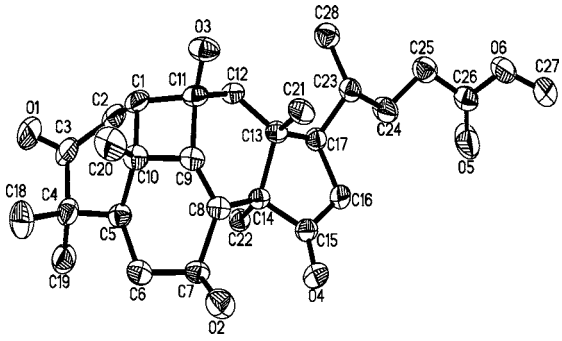


Figure 2. Single-crystal X-ray structure of **1**.

to possess a hydroxyl group at C-24, and this was confirmed by HMBC experiment.

Ganosinensic acid B (**2**)[12] was determined as $C_{30}H_{42}O_7$ on the basis of a combination of FABMS and HRESIMS. Inspection of spectral data of **2** revealed the presence of the same four-membered ring as in **1**, including the characteristic data of C-1 (δ_C 57.0, d) and C-11 (δ_C 83.5, s). This analysis suggested that **2** was also a triterpenoid with a four-membered ring. In the ^{13}C NMR spectrum, most of the signals in **2** were superimposable over those of **1**, except for the side chain moiety, where the former possesses an eight-carbon group and the latter possesses a five-carbon group. The ^{13}C NMR signals of the side chain of **2** were in good agreement with those of ganoderic acid C.[9] This was further supported by HMBC correlations observed from signals of H-27 (δ_H 1.11, d, $J = 8.2$ Hz) to C-24, C-25, and C-26, signals from H-25 (δ_H 2.37) to C-23, C-24, C-26, and C-27, as well as a proton spin system deduced from $^1H-^1H$ COSY correlations, H-24/H-25/H-27.

(11) Ganosinensic acid A (**1a**): colorless needles (petroleum ether/acetone 3:1); mp 206−208°; $[\alpha]^{25}_D = +167.2$ (*c* 0.1, CH_3OH); negative FAB *m/z* 457 $[M - H]^-$; HRESIMS *m/z* 481.2564 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_6Na$, 481.2566); UV (CH_3OH) λ_{max} (log ε) 214 (3.68) nm; IR (KBr) ν_{max} 3350, 2950, 1720, 1675, 1380, 1270, 1160, 1050 cm^{-1}. NMR can be found in Table 1and 2.

(12) Ganosinensic acid B (**2**): colorless powders; $[\alpha]^{25}_D = +140.8$ (*c* 0.1, CH_3OH); negative FAB *m/z* 513 $[M - H]^-$; HRESIMS *m/z* 537.2824 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_7Na$, 537.2828); UV (CH_3OH) λ_{max} (log ε) 216 (3.96) nm; IR (KBr) ν_{max} 3410, 2947, 1740, 1685, 1370, 1222, 1161, 1038 cm^{-1}. NMR can be found in Tables 1and 2

Table 2. ^{13}C NMR and DEPT Assignments of **1**, **1a**, and **2**[b]

	1	1a	2
1	57.0 d	57.0 d	57.0 d
2	36.5 t	36.5 t	36.5 t
3	216.5 s	216.5 s	216.4 s
4	47.0 s	47.0 s	47.1 s
5	55.4 d	55.4 d	55.5 d
6	29.4 t	29.4 t	29.4 t
7	68.3 d	68.4 d	68.3 d
8	136.4 s	136.3 s	136.3 s
9	154.7 s	154.7 s	154.8 s
10	48.6 s	48.6 s	48.6 s
11	83.5 s	83.6 s	83.5 s
12	37.4 t	37.4 t	37.4 t
13	45.6 s	45.6 s	45.7 s
14	62.3 s	62.4 s	62.4 s
15	215.0 s	215.3 s	214.8 s
16	41.0 t	41.1 t	41.0 t
17	46.8 d	46.9 d	46.6 d
18	17.7 q	17.7 q	17.7 q
19	18.8 q	18.8 q	18.8 q
20	35.7 d	35.9 d	32.6 d
21	18.6 q	18.7 q	20.3 q
22	31.4 t	31.9 t	49.8 t
23	31.1 t	31.8 t	208.9 t
24	174.1 s	176.1 s	46.9 t
25			35.7 t
26			178.4 s
27			17.7 q
28	27.1 q	27.1 q	27.1 q
29	18.9 q	18.9 q	18.9 q
30	20.6 q	20.7 q	20.6 q
OCH_3	51.4 q		

[b] Measured at 100 MHz in C_5D_5N.

The relative stereochemistry of **2** was constructed from the ROESY spectrum (Figure 3), together with 1D NMR data comparison with those of **1** (Tables 1 and 2). The configurations of H-7 and H-1 were deduced to be α-H and *β*-H, respectively.

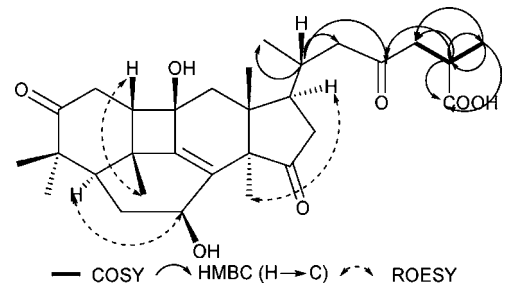
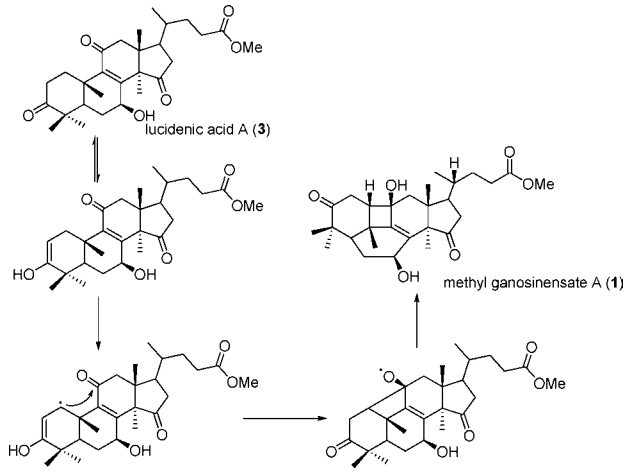


Figure 3. Key COSY, HMBC, and ROESY correlations of **2**.

According to the literature, methyl ganosinensate A (**1**), ganosinensic acid A (**1a**), and ganosinensic acid B (**2**) have a complex triterpenoid skeleton without precedent among known natural products. Especially, the four-membered ring

Scheme 1. Proposed Biogenetic Pathway for **1**



framework produced by a bond across C-1 to C-11 provides us a new insight into the biosynthesis of lanostane-type triterpenoid. The co-occurrence of the three triterpenoids and normal lanostane-type triterpenoids within the same plant raises the possibility that triterpenoids with a four-membered ring result from a further modification of an existing metabolite. Because the core skeleton of the three triterpenoids is still preserved in lucidenic acid A (**3**), and **3** is widely distributed in the genus *Ganoderma*, it is concluded that **3** should occur early in Scheme 1 as the biogenetic origin. Herein, we propose a new possible biosynthetic route of methyl ganosinensate A (**1**) in Scheme 1.

Considering some triterpenoids are known to have antitumor activity,[13,14] the antitumor activities of compounds **1**, **1a**, and **2** were evaluated with the HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines; however, neither of them showed significant inhibitory activity (IC_{50} > 40 μg/mL for the five cell lines).

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Supporting Information Available: Experimental procedures, 1D and 2D NMR spectra and crystallographic data of methyl ganosinensate A (**1**), ganosinensic acid A (**1a**), and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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