Highly Oxygenated Lanostane Triterpenoids from the Fungus *Ganoderma applanatum*

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Two new highly oxygenated lanostane triterpenoids, ganoderic acid AP2 (1) and ganoderic acid AP3 (2), were isolated from the fruiting bodies of the fungus *Ganoderma applanatum* (Ganodermataceae), along with four known analogues, ganoderenic acids A, B, D and G (3—6). The structures of the new compounds were elucidated on the basis of extensive spectroscopic analysis.

Key words *Ganoderma applanatum*; ganoderic acid; lanostane triterpenoid

The fungus *Ganoderma lucidum* is a well-known Chinese crude drug that has been used clinically in China, Japan and Korea for a long time. More than 140 highly oxygenated lanostane-type triterpenoids have been isolated from the fruiting bodies, mycelia and spores of *G. lucidum*, some of them exhibiting a very broad spectrum of biological activities and pharmacological functions.[1)] Other *Ganoderma* spp. have also been used in traditional medicines for the treatment of cancer, hypertension, chronic bronchitis, diabetes, and arteriosclerosis and as a tonic or sedative. In the case of *G. applanatum* (=*Elfvingia applanata*), some highly oxygenated lanostane triterpenoids,[2—6)] such as ganoderenic acids, ganoderic acids, applanoxidic acids and elfvingic acids, were also isolated in addition to several meroterpenoids.[7,8)] In a continuation of the studies on the bioactive principles of higher fungi from China, we have conducted a further chemical study on *G. applanatum*. Two new highly oxygenated lanostane triterpenoids, ganoderic acid AP2 (**1**) and ganoderic acid AP3 (**2**), were isolated from the fruiting bodies along with four known analogues, ganoderenic acids A, B, D and G (**3**—**6**). We describe here the isolation and structure elucidation of the new triterpene acids.

Compound **1**, obtained as colorless, amorphous powder, has a molecule formula of $C_{34}H_{50}O_8$ based on the positive-ion HR-ESI-MS, showing a quasi-molecular ion peak at *m/z* 587.3573 $[M+H]^+$ (Calcd for $C_{34}H_{51}O_8$, 587.3583), and the ^{13}C-NMR (DEPT) spectrum. The IR spectrum showed the absorption bands of hydroxyl (3434 cm^{-1}), ester or/and carboxylic carbonyl (1742, 1726 cm^{-1}), α,β-unsaturated ketone carbonyl (1678 cm^{-1}) as well as double bonds (1643 cm^{-1}) groups. The ^{13}C-NMR spectrum (Table 1) exhibited 34 carbon signals, containing α,β-unsaturated carboxylic carbonyl and ketone carbonyl resonances at δ 172.7 (s), 191.6 (s), two double bonds at δ 127.2 (s), 140.1 (s), 144.2 (d), 161.5 (s), three oxygen-bearing methine carbons at δ 74.6 (d), 78.5 (d), 80.1 (d), as well as characteristic signals at δ 170.5 (s), 21.0 (q); 170.6 (s), 21.3 (q) due to two acetoxyls. The following signals in the ^{1}H-NMR spectrum (Table 1) were easily distinguishable: an olefinic triplet at δ 6.84 (br t, J=7.0 Hz), three oxygenated methine protons at δ 5.61 (s), 5.20 (dd, J=8.8, 6.6 Hz), 3.24 (dd, J=11.0, 5.1 Hz), two acetoxyl methyl singlets at δ 2.19 (s), 2.11 (s), a vinyl methyl broad singlet at δ 1.83 (br s), together with five tertiary methyls and two secondary ones at the upfield zone. The above-mentioned NMR data suggested that compound **1** was an acetylated lanostane-type triterpene acid, and this conclusion was also in accordance with the previous study in which the fungus was considered a rich source of lanostane derivatives.[2—6)]

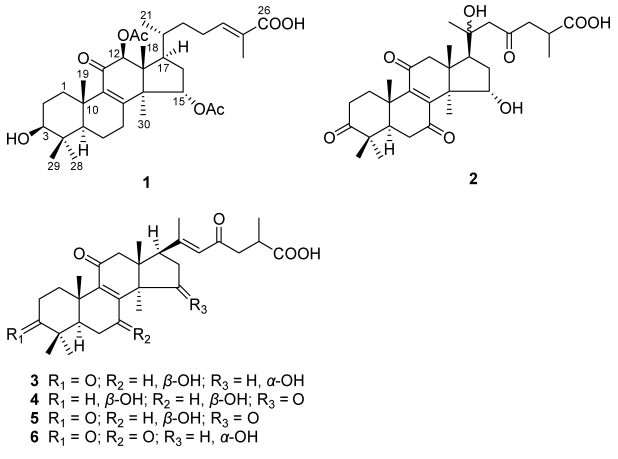

Fig. 1. Structures of Compounds **1**—**6** from *G. applanatum*

The signal at δ_H 3.24 (dd, J=11.0, 5.1 Hz) was typical for the H-3α in the overwhelming triterpenoids, and the observed HMBC correlations (Fig. 2) from this proton to two tertiary methyl cabons δ_C 15.7 (q, C-29), 28.2 (q, C-28) further indicated the presence of a hydroxyl at C-3. The olefinic triplet at δ_H 6.84 and the vinyl methyl broad singlet at δ_H 1.83 all correlated with the carboxylic carbon δ_C 172.7 in the HMBC spectrum, suggesting that α,β-unsaturated carboxyl group must be located at the bottom of the side chain. The important HMBC correlations: from δ_H 1.14 (s, Me-19) to δ_C 140.1 (s, C-9), from δ_H 1.41 (s, Me-30) to the downfield olefinic carbon δ_C 161.5 (s, C-8) were detected, consequently the other double bond and ketone carbonyl were doubtless emplaced at C-8 and C-11 (not at C-8 and C-7), respectively. The downfield oxygenated methine singlet proton at δ 5.61 was necessarily attached with an acetoxyl and assigned to H-12, considering the appearance of the HMBC correlations from the proton to δ_C 140.1 (s, C-9) and ketone carbon resonance δ_C 191.6 (s, C-11). Likewise, the remaining acetoxyl group was easily located at C-15 by the same means. The stereochemistry of **1** was deduced as 3β-hydroxy, 12β-acetoxy and 15α-acetoxy by the careful analysis of the ROESY spectrum (Fig. 3). The configuration of the double bond at C-

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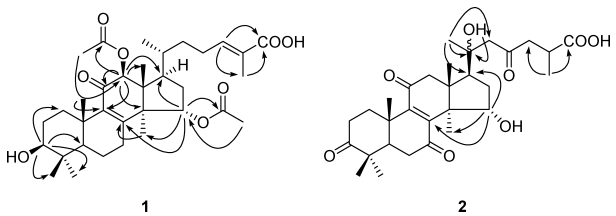
Table 1. NMR Spectral Data for Compounds **1** and **2** in $CDCl_3$

No.	Ganoderic acid AP2 (**1**)		Ganoderic acid AP3 (**2**)	
	δ_C	δ_H	δ_C	δ_H
1	33.8 (t)	1.02 (m, H_α) 2.80 (br d, 13.6, H_β)	35.0 (t)	1.81 (ddd, 14.2, 9.3, 6.6, H_α) 2.97 (m, H_β)
2	27.5 (t)	1.61 (m); 1.66 (m)	33.9 (t)	2.52 (m, H_β); 2.62 (m, H_α)
3	78.5 (d)	3.24 (dd, 11.0, 5.1)	215.4 (s)	
4	38.8 (s)		46.4 (s)	
5	51.3 (d)	0.88 (br d, 11.7)	49.0 (d)	2.27 (dd, 15.2, 2.4)
6	17.3 (t)	1.46 (m, H_β); 1.74 (m, H_α)	36.8 (t)	2.47 (m, H_α); 2.63 (m, H_β)
7	29.3 (t)	2.19 (m, H_β); 2.29 (m, H_α)	204.6 (s)	
8	161.5 (s)		150.4 (s)	
9	140.1 (s)		152.3 (s)	
10	37.5 (s)		39.1 (s)	
11	191.6 (s)		201.2 (s)	
12	80.1 (d)	5.61 (s)	52.1 (t)	2.69 (d, 17.0, H_β) 2.86 (d, 17.0, H_α)
13	51.5 (s)		47.8 (s)	
14	53.9 (s)		52.8 (s)	
15	74.6 (d)	5.20 (dd, 8.8, 6.6)	71.8 (d)	4.35 (dd, 10.0, 5.6)
16	33.6 (t)	1.74 (m); 2.26 (m)	30.2 (t)	1.67 (ddd, 14.2, 9.8, 5.6, H_α) 2.55 (m, H_β)
17	48.6 (d)	2.20 (m)	50.9 (d)	2.11 (dd, 9.8, 9.8)
18	12.3 (q)	0.92 (s)	18.9 (q)	1.05 (s)
19	19.0 (q)	1.14 (s)	17.5 (q)	1.26 (s)
20	34.1 (d)	1.49 (m)	73.3 (s)	4.81 (br s, OH)
21	19.7 (q)	0.94 (d, 6.6)	26.6 (q)	1.30 (s)
22	33.7 (t)	1.17 (m); 1.58 (m)	52.5 (t)	2.56 (d, 16.3); 2.64 (d,16.3)
23	26.3 (t)	2.11 (m); 2.24 (m)	211.2 (s)	
24	144.2 (d)	6.84 (br t, 7.0)	47.7 (t)	2.46 (m); 2.89 (m)
25	127.2 (s)		34.5 (d)	2.96 (m)
26	172.7 (s)		177.8 (s)	
27	12.0 (q)	1.83 (br s)	17.0 (q)	1.23 (d, 6.8)
28	28.2 (q)	1.03 (s)	27.2 (q)	1.15 (s)
29	15.7 (q)	0.82 (s)	20.2 (q)	1.12 (s)
30	19.8 (q)	1.41 (s)	20.6 (q)	1.18 (s)
$\underline{C}OCH_{3(12)}$	170.5 (s)			
$CO\underline{CH}_{3(12)}$	21.0 (q)	2.19 (s)		
$\underline{C}OCH_{3(15)}$	170.6 (s)			
$CO\underline{CH}_{3(15)}$	21.3 (q)	2.11 (s)		

The unambiguous assignments were made on the basis of HSQC, HMBC and ROESY experiments.



1 2

Fig. 2. Selected HMBC Correlations of Compounds **1** and **2**

24 was assigned to be *E*-orientation because of Me-27 characteristic upfield resonance at δ_C 12.0 (q). Therefore, the structure of **1** was elucidated as 12β,15α-diacetoxy-3β-hydroxy-11-oxolanost-8,24(*E*)-dien-26-oic acid, named ganoderic acid AP2.

Compound **2**, also obtained as colorless, amorphous powder, has a molecule formula of $C_{30}H_{42}O_8$ based on the negative-ion HR-ESI-MS, showing a quasi-molecular ion peak at *m*/*z* 529.2786 $[M-H]^-$ (Calcd for $C_{30}H_{41}O_8$, 529.2801), and the ^{13}C-NMR (DEPT) spectrum. The IR spectrum showed the absorption bands of hydroxyl (3444 cm^{-1}) and unsaturated carbonyl (1709, 1670 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectroscopic character (Table 1) suggested that compound **2** possessed a skeleton of lanostane triterpene acid, and the NMR signals were similar to those of ganoderenic acid G (**6**),[2] also isolated from this fungus. Nevertheless, there was a remarkable difference as follows: the signals at δ_C 155.6 (s, C-20), 124.5 (d, C-22), 197.4 (s, C-23) due to the unsaturated ketone group at the side chain of **6** were absent and replaced by a set of newly arised signals at δ_C 73.3 (s, C-20), 52.5 (t, C-22), 211.2 (s, C-23) in the ^{13}C-NMR spectra of **2**, indicating that this double bond was hydrogenated and substituted by hydroxyl at C-20, which was further supported by the fact that the HMBC correlations from the broad hydroxyl singlet δ_H 4.81 to δ_C 73.3 (s, C-20), from the methyl singlet δ_H 1.30 (s, Me-20) to δ_C 52.5 (t, C-22) were clearly detectable. The stereochemistry of 15-hydroxy was deduced as α-orientation by the observable ROESY correlation (Fig. 3) between H-15 and Me-18. Accordingly the structure of **2** was characterized as 15α,20ξ-dihydroxy-3,7,11,23-tetraoxolanost-8-en-26-oic acid, *i.e.*, ganoderic acid AP3. Complete assignments (Table 1) of the ^{1}H-NMR signals were unambiguously performed by careful analysis of HSQC, HMBC and ROESY experiments.

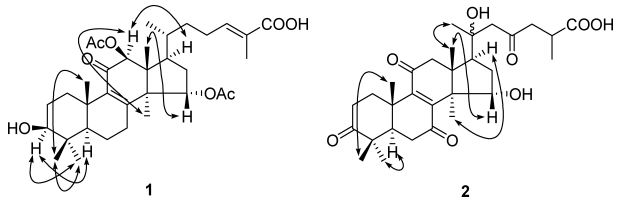


Fig. 3. Significant ROESY Correlations of Compounds **1** and **2**

Experimental

General Experimental Procedures Melting points were measured on a PHMK 79/2289 micro-melting point apparatus and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with a Bruker DRX-500 spectrometer in $CDCl_3$ at room temperature, and chemical shifts were referred to TMS as internal standard. EI-MS were taken on a Finnigan-MAT 90 instrument, and ESI-MS and HR-ESI-MS were recorded with an API QSTAR Pulsar i spectrometer.

Column chromatography was performed using silica gel (200—300 mesh, Qingdao Marine Chemical Inc., China) and Chromatorex C-18 (40—75 μm, Fuji Silysia Chemical Ltd., Japan). Fractions were monitored by Agilent 1100 reversed-phase HPLC (Zorbax SB-C-18 column, 5 μm, 4.6×150 mm, 30—100% CH_3CN in H_2O over 15 min, 1 ml/min).

Fungus Material The fresh fruiting bodies of *G. applanatum* were collected at the Gaoligong Mountains in Yunnan Province, China, in August 2006. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The fresh fruiting bodies (3.0 kg) were immersed in 12 l $CHCl_3$–MeOH (1/1, v/v) and left at room temperature for two weeks. Then the extraction was concentrated *in vacuo* to give a brown gum (70.0 g), which was fractionated by silica gel column using $CHCl_3$–MeOH gradient elution.

The fractions (5.0 g) eluted with 1—2% MeOH, mainly containing the triterpene acids, were further repeatedly separated by low pressure Chromatorex C-18 column chromatography. Purification of these fractions using different gradients afforded the new triterpenes, ganoderic acid AP2 (**1**; 38 mg; 80% MeOH in H_2O; t_R 10.3 min) and ganoderic acid AP3 (**2**; 25 mg; 50% MeOH; t_R 6.2 min), and four known analogues, ganoderenic acid A (**3**; 45 mg; 45% MeOH; t_R 5.9 min), ganoderenic acid B (**4**; 120 mg; 40% MeOH; t_R 5.5 min), ganoderenic acid D (**5**; 350 mg; 58% MeOH; t_R 7.1 min) and ganoderenic acid G (**6**; 65 mg; 60% MeOH; t_R 7.7 min).

Ganoderic Acid AP2 (**1**): Colorless, amorphous powder. mp 121—122 °C. $[\alpha]_D^{20}$ +84.3° (c=0.18, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm (log ε): 253 (3.90). IR (KBr) cm^{-1}: 3434, 2956, 2932, 2874, 1742, 1726, 1678, 1643, 1380, 1237, 1035. ^{1}H- and ^{13}C-NMR data: see Table 1. EI-MS *m/z*: 586 $[M]^+$ (9), 544 (4), 527 (9), 526 (10), 508 (4), 484 (11), 466 (17), 448 (13), 362 (13), 291 (43), 277 (100), 274 (37), 264 (35), 213 (217), 193 (80); HR-ESI-MS (pos.): 587.3573 $[M+H]^+$ (Calcd for $C_{34}H_{51}O_8$: 587.3583).

Ganoderic Acid AP3 (**2**): Colorless, amorphous powder. mp 118—119 °C. $[\alpha]_D^{15}$ +141.9° (c=0.35, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm (log ε): 263 (3.76). IR (KBr) cm^{-1}: 3444, 2956, 2922, 2852, 1709, 1670, 1464, 1389, 1233, 1177, 1054. ^{1}H- and ^{13}C-NMR data: see Table 1. EI-MS *m/z*: 400 (12), 382 (43), 367 (10), 364 (17), 340 (70), 339 (100), 321 (13), 311 (13), 300 (57), 301 (57); ESI-MS (neg.): 529 $[M-H]^-$, 399, 129; HR-ESI-MS (neg.): 529.2786 $[M-H]^-$ (Calcd for $C_{30}H_{41}O_8$: 529.2801).

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