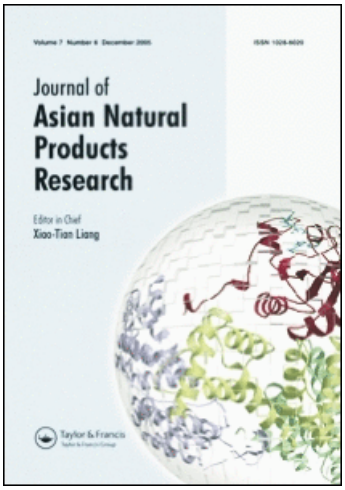

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One new triterpenoid from biotransformation product of glycyrrhizic acid[1]

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A new triterpenoid compound (**1**) and a known compound (**2**) were isolated from the product of biotransformation of glycyrrhizic acid by *Aspergillus niger*. On the basis of the 1D and 2D NMR (^{1}H–^{1}H COSY, HSQC, HMBC and NOESY) and MS spectrometry, their structures were established as 7β, 15α-dihydroxy-3,11-dioxo-oleana-12-en-30-oic acid (**1**) and 15α-hydroxy-3,11-dione-oleana-12-en-30-oic acid (**2**), respectively.

Keywords: triterpenoid; glycyrrhizic acid; *Aspergillus niger*; biotransformation

1. Introduction

Glycyrrhizic acid (GL), the main active component of licorice, *Glycyrrhiza glabra*, has been paid attention to for biological activities.[1–3] The derivatives of GL were prepared by biotransformation.[4–8] In this paper, we report the isolation and structural determination of a new triterpenoid (compound **1**) and a known triterpenoid (compound **2**)[8] from the biotransformed products of GL. The results of the extensive application of the 1D (^{1}H, ^{13}C NMR and DEPT) and 2D (^{1}H–^{1}H COSY, HSQC, HMBC and NOESY) NMR techniques were used to characterize the structures and establish the ^{1}H and ^{13}C resonance assignments of these two triterpenoids.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder. The HR-EI-MS spectrum of **1** showed the molecular ion at *m/z* 500.3126 $[M]^+$, indicating that the molecular formula is $C_{30}H_{44}O_6$, supported by the ^{1}H and ^{13}C NMR spectral data. The ^{1}H NMR spectrum of **1** in CD_3OD showed seven tertiary methyl proton signals at δ 0.78 (3H, s, CH_3-28), 0.98 (3H, s, CH_3-24), 1.03 (3H, s, CH_3-23), 1.08 (3H, s, CH_3-29), 1.09 (3H, s, CH_3-26), 1.15 (3H, s, CH_3-25), and 1.34 (3H, s, CH_3-27), and two methine proton signals at δ 4.02 (1H, dd, $J = 6.6$, 5.4 Hz, H-7) and 4.20 (1H, dd, $J = 6.6$, 5.4, H-15), indicative of the secondary alcoholic functionalities. The ^{13}C NMR spectrum showed 30 carbon signals. Comparing the ^{13}C NMR data of **1** with those of glycyrrhetic acid (GA), the new signals at δ 71.7, 67.7 and 219.4 suggested hydroxylation of two secondary carbons and a ketone group. The detailed analyses of the ^{1}H and ^{13}C NMR spectra with the aid of DEPT, ^{1}H–^{1}H COSY, HSQC and HMBC spectra of **1** were summarized in Table 1. The HMBC experiment showed long-range correlations between H-26 at δ 1.09 and C-14 at δ 51.4,

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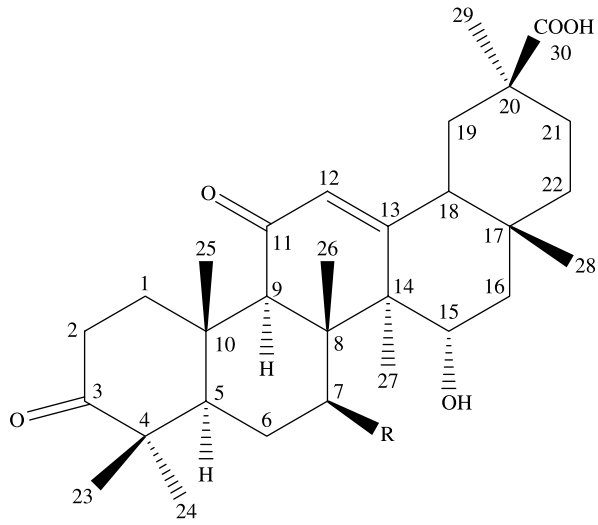
Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (δ in CD_3OD).

	1			**2**		
Position	δ_C	DEPT	δ_H (J in Hz)	δ_C	DEPT	δ_H (J in Hz)
1	40.3	CH_2	1.41 m, 2.74 m	40.9	CH_2	1.52 m, 2.83 m
2	35.0	CH_2	2.32 m, 2.48 m	35.1	CH_2	2.56 m
3	219.4	C	–	220.2	C	–
4	48.4	C	–	47.8	C	–
5	52.7	CH	1.48 m	55.9	CH	1.46 m
6	28.9	CH_2	1.61 m, 1.63 m	20.1	CH_2	1.56 m, 1.63 m
7	71.7	CH	4.02 dd 6.6, 5.4	36.1	CH_2	1.80 m, 1.97 m
8	52.5	C	–	47.8	C	–
9	62.3	CH	2.44 s	62.2	CH	2.59 s
10	38.0	C	–	38.1	C	–
11	200.4	C	–	201.7	C	–
12	129.7	CH	5.64 s	129.4	CH	5.66 s
13	172.5	C	–	173.3	C	–
14	51.4	C	–	50.5	C	–
15	67.7	CH	4.20 dd 6.6, 5.4	68.2	CH	4.24 dd 6.0, 5.4
16	36.1	CH_2	1.22 m, 2.02 m	36.0	CH_2	1.25 m, 2.09 m
17	33.1	C	–	33.4	C	–
18	50.9	CH	2.14 m	50.4	CH	2.20 m
19	42.3	CH_2	1.62 m, 1.80 m	42.1	CH_2	1.70 m, 1.87 m
20	44.9	C	–	44.8	C	–
21	32.0	CH_2	1.30 m, 1.88 m	32.0	CH_2	1.37 m, 1.40 m
22	38.7	CH_2	1.28 m, 1.36 m	38.8	CH_2	1.28 m, 1.36 m
23	27.0	CH_3	1.03 s	27.1	CH_3	1.09 s
24	21.7	CH_3	0.98 s	21.7	CH_3	1.05 s
25	16.2	CH_3	1.15 s	16.5	CH_3	1.25 s
26	13.1	CH_3	1.09 s	19.5	CH_3	1.22 s
27	18.5	CH_3	1.34 s	18.6	CH_3	1.42 s
28	29.7	CH_3	0.78 s	29.7	CH_3	0.87 s
29	28.7	CH_3	1.08 s	28.7	CH_3	1.18 s
30	180.2	C	–	180.2	C	–

C-8 at δ 52.5, C-9 at δ 62.3, and C-7 at δ 71.7; between H-27 at δ 1.34 and C-14 at δ 51.4, C-8 at δ 52.5, C-15 at δ 67.7, and C-13 at δ 172.5; between H-7 at δ 4.02 and C-6 at δ 28.9, C-26 at δ 13.1 and C-14 at δ 51.4; between H-15 at δ 4.20 and C-27 at δ 18.5, C-16 at δ 36.1, and C-8 at δ 52.5 and confirmed that the hydroxyl groups were attached to C-7 and C-15. The long-range correlations between C-3 at δ 219.4 and H-23 at δ 1.03, H-24 at δ 0.98, H-2 at δ 2.32 and 2.48, and H-1 at δ 2.74 in the HMBC spectrum indicated that the ketone group was attached to C-3. In the NOESY spectrum of **1**, the NOE correlations between H-7 at δ 4.02, H-6 at δ 1.63, H-5 at δ 1.48, H-9 at δ 2.44, and H-27 at δ 1.34 suggested α-configurations of H-7 and H-5; NOE correlations between H-15 at δ 4.20, H-16 at δ 1.22, H-26 at δ 1.09, and H-28 at δ 0.78 indicated β-configuration of H-15. On the basis of these data, compound **1** was identified as 7β, 15α-dihydroxy-3, 11-dioxo-oleana-12-en-30-oic acid (Figures 1 and 2).

Compound **2** was isolated as a white amorphous powder. The EI-MS spectrum of **2** showed the $[M]^+$ ion at m/z 484.2. Comparing the ^{13}C NMR data of **2** with those of **1**, the new signal at δ 36.1, suggesting that it lacked a hydroxyl group at C-7. The resonances of the protons and carbons detailed analysis of the ^{1}H and ^{13}C NMR spectra with the aid of DEPT,



1 R = – OH
2 R = – H

Figure 1. Structures of compounds **1** and **2**.

$^{1}H-^{1}H$ COSY, HSQC and HMBC of **2** were summarized in Table 1. From these data, compound **2** was identified as 15α-hydroxy-3,11-dioxo-oleana-12-en-30-oic acid. The structure of **2** was the same as 3-*oxo*-15α-hydroxyglycyrrhetinic acid,[8] but the NMR data were different (Figure 1 and Table 1).

3. Experimental

3.1 General experimental procedures

The NMR spectra were recorded on Varian ^{UNITY}INOVA 600 (599.8 MHz for ^{1}H NMR and 150.8 MHz for ^{13}C NMR) in CD_3OD, and the chemical shifts were calculated with

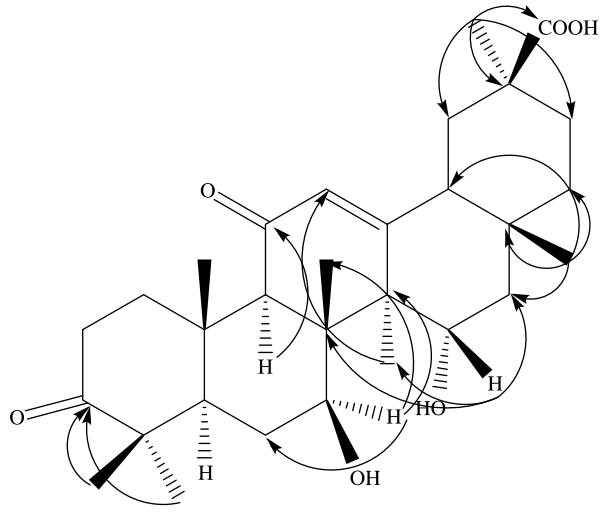


Figure 2. Key HMBC correlations of compound **1**.

tetramethylsilane as an internal standard. The HR-EI-MS, EI-MS and FAB-MS were recorded on Micromass ZabSpec.

HPLC was performed on Agilent 1100. Column: YMC-Pack ODS-A C_{18} (5 μm, 4.6 × 250 mm; YMC, Japan) and Lichrospher C_{18} (5 μm, 10 × 250 mm; Hanbon, China). Detector: DAD, Alltech ELSD 2000, temperature: 105°C, gas flow: 2.4 l/min. TLC was performed on precoated Kieselgel GF_{254} plate (0.2–0.25 mm, 100 × 200 mm; Qingdao Haiyang Chemical Co., Ltd, China) using $CHCl_3$–MeOH–H_2O, and detection was achieved by spraying 10% H_2SO_4–EtOH solution followed by heating. ODS-A (120 Å, 50 μm; YMC, Japan) columns were used for column chromatography.

GL (HPLC purity > 95%) and GA (HPLC purity > 98%) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products.

3.2 Extraction and isolation of compounds 1 and 2

The biotransformation product of *glycyrrhizic acid* by *Aspergillus niger* was extracted with MeOH. The extract was then concentrated under reduced pressure to give 2.4 g of residue that was dissolved in 50% CH_3OH, and the supernatant was chromatographed on ODS silica gel and eluted with a gradient mixture of CH_3OH–H_2O (1:1, 3:2, 7:3) to give fractions A (60.2 mg) and B (104 mg). Fraction A was chromatographed on preparation HPLC with CH_3OH–0.015% CF_3COOH (13:7) to yield compound **1** (6.0 mg), and fraction B was chromatographed with CH_3OH–0.015% CF_3COOH (17:8) to yield compound **2** (9.6 mg).

Compound **1**: White amorphous powder, HR-EI-MS: $m/z = 500.3126$ $[M]^+$ (calcd for $C_{30}H_{44}O_6$, 500.3138), FAB-MS: $m/z = 501.3$ $[M + 1]^+$. ^1H and ^{13}C NMR (CD_3OD) (Table 1).

Compound **2**: White amorphous powder, EI-MS: $m/z = 484.2$ $[M]^+$. ^1H and ^{13}C NMR (CD_3OD) (Table 1).

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Notes

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