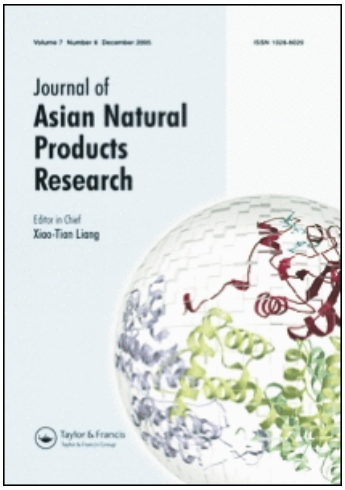

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Two new triterpenoid saponins from *Glochidion puberum*

Zhen Zhang[ab], Zhu-Lin Gao[b], Xin Fang[b], Yue-Hu Wang[b], Huai Xiao[a], Xiao-Jiang Hao[b], Guang-Ming Liu[a] and Hong-Ping He[b]*

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Two new triterpenoid saponins, puberosides A and B, were isolated from the aerial parts of *Glochidion puberum*. Their structures were elucidated as 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-22α-benzoyloxy-olean-12-ene-16α,28-diol (**1**) and 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-28-benzoyloxy-olean-12-ene-16α,22α-diol (**2**) by analysis of their spectroscopic data.

Keywords: *Glochidion puberum*; triterpenoid saponins; puberoside A; puberoside B

1. Introduction

Glochidion puberum (Linn.) Hutch., belonging to the family Euphorbiaceae, grows in tropical and subtropical forests. In China, this plant has been used for the treatment of feverish illness, malaria, dysentery, gastroenteritis, and so on [1]. Investigation of *Glochidion puberum* has been rarely carried out. So far, only 12 compounds, such as friedelin, lup-20 (29)-ene-1β-ol-3α-yl acetate, have been isolated [2].

The biological and therapeutic significance of this plant stimulated us to intensively study *Glochidion puberum* and two new triterpenoid saponins were isolated. This paper reports the isolation and the structural elucidation of the new compounds **1** and **2**.

2. Results and discussion

Compound **1** was obtained as an amorphous powder with a molecular formula of $C_{48}H_{72}O_{14}$ analyzed from its HRESIMS. The IR spectra showed absorption bands for hydroxyl group (3417 cm^{-1}), carbonyl group (1702 cm^{-1}), and aromatic ring (1603, 1585, and 1451 cm^{-1}) [3,4]. The ^{13}C NMR spectrum of **1** showed 48 signals, of which 30 were assigned to the triterpene aglycone moiety. The chemical shifts of two olefinic carbons at δ 124.2 and 143.5 and comparison of the remaining chemical shifts (Table 1) with those for pentacyclic triterpenes (22α-hydroxyerythrodiols) revealed the presence of the characteristic signals of an olean-12-ene triterpene [5]. The methylene resonance at δ 64.7 was diagnostic for the hydroxylated C-28, which corresponded to two proton signals at δ 3.67 and 3.99 in the HSQC spectrum. The absence of a methylene resonance at high field (*ca.* δ 15.0) [5,6] in the ^{13}C NMR spectrum and the appearance of the signal at δ 4.30 in the ^{1}H NMR spectrum of **1**, which correlated with C-28 in the HMBC spectrum, suggested the existence of 16-OH in **1** (Figure 2). The correlations between H-16 and H-28 in the ROESY spectrum revealed H-16 to be β-configuration, which indicated α-configuration of 16-OH. The lack of another

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Table 1. ^{1}H (400 Hz), ^{13}C (100 Hz), and HMBC NMR spectral data of compounds **1** and **2** (CH_3OH, ppm).

	1			***2***		
	C	H	HMBC (H → C)	C	H	HMBC (H → C)
1	38.3 (t)	1.81 (m) 0.97 (brs)		39.8 (t)	1.63 (m)	
2	27.0 (t)	1.88 (m) 1.74 (m)		27.1 (t)	1.83 (m) 1.69 (m)	
3	90.4 (d)	3.17 (dd, $J = 11.6, 4.0$)	23, 24, 1″	90.4 (d)	3.17 (dd, $J = 11.6, 4.4$)	
4	40.0 (s)			40.2 (s)		
5	56.9 (d)	0.80 (brs)		56.9 (d)	0.80 (brs)	
6	19.3 (t)	1.65 (m) 1.50 (m)		19.3 (t)	1.61 (m)	
7	33.9 (t)	1.45 (m) 1.67 (m)		33.6 (t)	1.40 (m)	
8	41.0 (s)			41.2 (s)		
9	48.0 (d)	1.64 (m)		48.1 (d)	1.58 (m)	
10	37.8 (s)			37.9 (s)		
11	24.7 (t)	1.99 (brs)		24.7 (t)		
12	124.2 (d)	5.39 (brs)		124.9 (d)	5.32 (brs)	
13	143.5 (s)			143.0 (s)		
14	44.2 (s)			44.2 (s)		
15	37.7 (t)	2.02 (m) 1.06 (m)		37.6 (t)	1.91 (m) 1.51 (m)	
16	69.4 (d)	4.30 (brs)	28	69.4 (d)	4.29 (brs)	
17	44.8 (s)			44.9 (s)		
18	43.4 (d)	2.40 (dd, $J = 13.6, 4$)		43.1 (d)	2.63 (brs)	
19	48.0 (t)	1.94 (m) 1.24 (m)		47.1 (t)	1.88 (m) 1.20 (m)	
20	31.0 (s)			31.0 (s)		
21	39.9 (t)	1.94 (m) 1.00 (m)		38.3 (t)	1.77 (2H, m)	
22	72.1 (d)	5.91 (brs)	16, 18, 20, 7′	72.1 (d)	5.90 (brs)	18
23	28.6 (q)	1.07 (3H, s)	3, 5	27.9 (q)	1.07 (3H, s)	3
24	17.0 (q)	0.86 (3H, s)	3, 5	17.0 (q)	0.86 (3H, s)	3
25	16.2 (q)	0.99 (3H, s)	9, 15	16.2 (q)	0.98 (3H, s)	
26	17.3 (q)	1.06 (3H, s)	7, 9	17.3 (q)	1.05 (3H, s)	

Table 1 – *continued*

	1			***2***		
	C	H	HMBC (H → C)	C	H	HMBC (H → C)
27	27.9 (q)	1.29 (3H, s)	8, 13, 15	27.9 (q)	1.30 (3H, s)	13
28	64.7 (t)	3.67 (brs)	22	64.7 (t)	4.69 (1H, d, $J = 11.2$)	18
		3.99 (d, $J = 11.2$)			4.33 (1H, d, $J = 11.2$)	22, 7′
29	34.3 (q)	0.93 (3H, s)	19, 21	34.3 (q)	0.92 (3H, s)	
30	27.6 (q)	1.03 (3H, s)	19, 21	27.5 (q)	1.01 (3H, s)	
1′	132.1 (s)			132.0 (s)		
2′, 6′	130.5 (d)	8.04 (d, $J = 7.3$)	4′, 7′	130.6 (d)	8.02 (d, $J = 7.3$)	4′, 7′
3′, 5′	129.7 (d)	7.49 (dd, $J = 7.3, 7.6$)		129.5 (d)	7.45 (dd, $J = 7.3, 7.4$)	
4′	134.1(d)	7.60 (t, $J = 7.3, 7.6$)		134.1 (d)	7.57 (t, $J = 7.3, 7.4$)	
7′	167.2 (s)			168.4 (s)		
1″	107.1 (d)	4.28 (d, $J = 7.6$)	3	107.1	4.27 (d, $J = 7.6$)	3
2″	72.1 (d)	3.74 (brs)		72.1 (d)	3.70 (m)	
3″	83.9 (d)	3.67 (brs)		83.8 (d)	3.63 (m)	
4″	69.5 (d)	4.05 (brs)		69.5 (d)	4.00 (brs)	
5″	66.7 (t)	3.89 (brs)		66.7 (t)	3.84 (m)	
		3.59 (brs)			3.54 (m)	
1‴	105.4 (d)	4.55 (d, $J = 7.2$)	3″	105.4 (d)	4.55 (d, $J = 7.2$)	3″
2‴	75.3 (d)	3.32 (brs)		75.3 (d)	3.27 (m)	
3‴	77.6 (d)	3.42 (brs)		77.6 (d)	3.28 (brs)	
4‴	71.1 (d)	3.37 (brs)		71.1 (d)	3.31 (m)	
5‴	77.9 (d)	3.33 (brs)		77.9 (d)	3.27 (m)	
6‴	62.3 (t)	3.81 (m)		62.3 (t)	3.81 (m)	
		3.71 (m)			3.67 (m)	

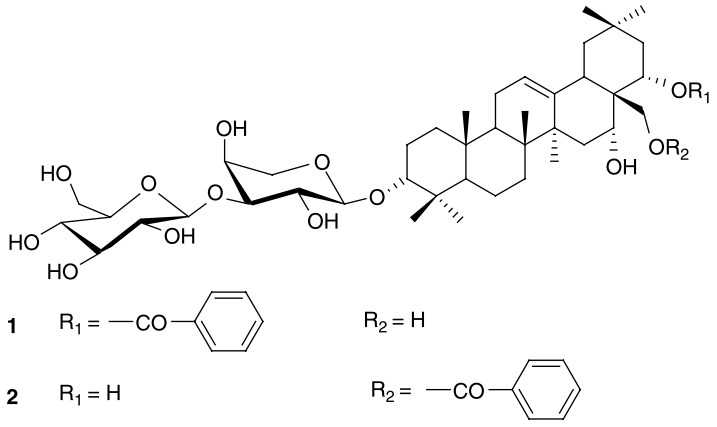

Figure 1. Structures of **1** and **2**.

methylene resonance at ca. δ 33.0 (C-22) [6] and the appearance of HMBC correlations between the proton at δ 5.91 (brs) and C-16, C-18, C-20 (Figure 2) indicated that a hydroxyl group should be allocated to C-22. The correlations between H-16 and H-22 in the ROESY spectrum revealed H-22 to be β-configuration, which indicated α-configuration of 22-OH. Moreover, the ^{1}H and ^{13}C NMR, DEPT, and HMBC spectra showed the existence of a benzoyl group at δ_H 8.04 (d, 2H, $J = 7.3$ Hz, H-2′, 6′), 7.49 (dd, 2H, $J = 7.3$ and 7.6 Hz, H-3′, 5′) and 7.60 (t, $J = 7.3$ and 7.6 Hz, H-4′), and at δ_c 132.1 (s), 130.5 (d), 129.7 (d), 134.1(d), 129.7 (d), and 130.5 (d) (Table 1 and Figure 1) [7], which was linked to C-22 by the HMBC correlation between C-7′ and H-22 (Figure 2).

The carbon at δ 90.4 in the ^{13}C NMR spectrum, showing a significant glycosidation shift, suggested the linkage of the sugar moiety to C-3 of the aglycone [8]. H-3 (δ 3.13) correlated with H-24 (δ 0.86) in the ROESY spectrum, indicating the α-configuration of C-3-OH. By means of HSQC, HMBC, and ^{1}H–^{1}H COSY spectra, compound **1** had two monosaccharides, arabinopyranose (δ_C 107.1, 72.1, 84.2, 69.5, and 66.7), and glucopyranose (δ_C 105.4, 75.3, 77.6, 71.1, 77.9, and 62.3) [9,10], respectively. The anomeric protons appearing at δ 4.28 (1H, d, $J = 7.6$ Hz) and δ 4.55 (1 H, d, $J = 7.2$ Hz), and their corresponding carbons resonating at δ_C 107.1 (C-1″) and 105.4 (C-1‴) from HSQC experiment, suggested the presence of α-L-arabinopyranosyl and β-D-glucopyranosyl groups [9,10]. The HMBC correlation between H-1″ [δ 4.28 (1H, d, $J = 7.6$ Hz)] and C-3 (δ_C 90.4) confirmed that the arabinopyranosyl unit was linked at C-3. The HMBC correlation between H-1‴ [δ 4.55 (1 H, d, $J = 7.2$ Hz)]

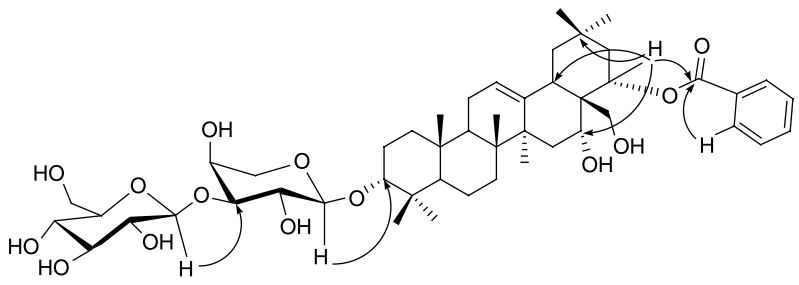

Figure 2. Key HMBC correlations of **1**.

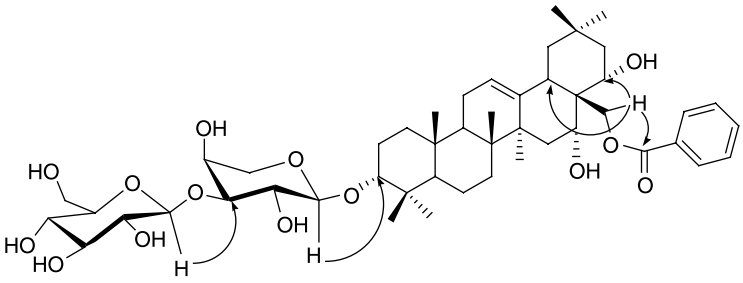

Figure 3. Key HMBC correlations of **2**.

and C-3″ (δ_C 83.9) revealed the 1 → 3 linkage of the two sugars. Thus, the structure of **1** was established as 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-22α-benzoyloxy-olean-12-ene-16α,28-diol.

Compound **2** was also obtained as an amorphous powder with a molecular formula of $C_{48}H_{72}O_{14}$ analyzed from its HRESIMS. The IR spectrum showed absorption band for hydroxyl group (3416 cm^{-1}), carbonyl group (1715 cm^{-1}), and aromatic ring (1603, 1587, and 1452 cm^{-1}) [2,3]. Comparison of the ^1H and ^{13}C spectral data of **2** with those of **1** indicated, similar to **1**, that compound **2** also included two monosaccharides and a benzoyl-substituted triterpenoid moiety. The difference between **1** and **2** was the benzoyl moiety that was allocated at C-28 (δ_C 64.7) in **2**, not C-22, and this can be confirmed by the HMBC correlations between H-28 and C-7′ (Figure 3). So, the structure of **2** was established as 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-28-benzoyloxy-olean-12-ene-16α,22α-diol.

3. Experimental

3.1 General experimental procedures

Optical rotations were carried out by using a Horiba SEAP-300 spectropolarimeter. UV spectra were measured on a Shimadzu 2401 PC. IR spectra were measured as KBr pellets by using a 577 spectrometer. FABMS and HRESIMS data were recorded by using a VG Auto Spec-3000 spectrometer and an API QSTAR PULSAR, respectively. 1D NMR spectra were recorded by using a Bruker AM-400 and 2D NMR spectra were recorded by using a Bruker DRX-500 instrument in which TMS was used as an internal standard for all measurements. Semipreparative HPLC was performed by using an Agilent 1100 liquid chromatograph equipped with a Zorbax SB-C_{18}, 9.4 mm × 25 cm column. Column chromatography was performed by using silica gel (200–300 mesh; Qingdao Marine Chemical Ltd. Co., Qinggao, China) and Sephadex LH-20 (Amershan Biosciences, Switzerland). Fractions were monitored by TLC (Qingdao Marine Chemical Ltd Co., Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. Solvents were distilled before use.

3.2 Plant material

The aerial parts of *Glochidion puberum* were collected in Xishuangbanna, Yunnan province of China, in March 2004. The plant was identified by Professor De-Ding Tao (Kunming Institute of Botany, Chinese Academy of Sciences), and a voucher specimen (Kun NO. 0615419) is deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried aerial parts of *G. puberum* (6 kg) were extracted with hot 95% EtOH. The EtOH extract was successively partitioned between H_2O and petroleum ether and between H_2O and EtOAc. The residue from the EtOAc layer (50 g) was subjected to silica

gel column chromatography and eluted with gradient petroleum ether–acetone to give four fractions (1–4). Fraction 1 was submitted to Sephadex LH-20 and eluted with MeOH to yield two fractions A (200 mg) and B (100 mg). Fraction A was separated by semipreparative HPLC (CH_3CN–H_2O, 60:40), yielding **1** (25 mg, t_R 12 min) and **2** (10 mg, t_R 8 min).

*3.3.1 3α-[(O-β-D-glucopyranosyl-(1 → 3)-O-α-L-arabinopyranosyl)oxy]-22α-benzoyloxy-olean-12-ene-16α, 28-diol (**1**)*

Amorphous powder; $[\alpha]_D^{23.6}$ + 19.67 (*c* 0.5, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 349 (2.20), 280 (3.56), 273 (3.64), 229 (4.78), 202 (4.80), and 194 (4.48) nm; IR (KBr) ν_{max} 3417, 1702, 1603, 1585, and 1451 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; FABMS *m/z* 871 $[M - H]^-$; HRESIMS *m/z* 871.4857 $[M - H]^-$ (calcd for $C_{48}H_{71}O_{14}$, 871.4843).

*3.3.2 3α-[(O-β-D-glucopyranosyl-(1 → 3)-O-α-L-arabinopyranosyl)oxy]-28-benzoyloxy-olean-12-ene-16α, 22α-diol (**2**)*

Amorphous powder; $[\alpha]_D^{23.6}$ − 19.67 (*c* 0.5, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 272 (3.70), 227 (4.73), and 202 (4.79) nm; IR (KBr) ν_{max} 3416, 1715, 1603, 1587, and 1452 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; FABMS *m/z* 871 $[M - H]^-$; HRESIMS *m/z* 871.4828 $[M - H]^-$ (calcd for $C_{48}H_{71}O_{14}$, 871.4843).

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