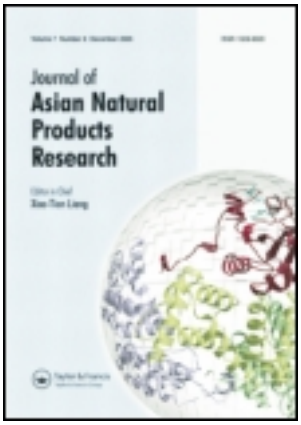

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Two new triterpene saponins from *Gynostemma pentaphyllum*

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Two new dammarane-type triterpene saponins, gypenbiosides A (**1**) and B (**2**), were isolated from the aerial parts of *Gynostemma pentaphyllum* (Thunb.) Makino. Their structural elucidations were accomplished mainly on the basis of the interrelation of spectroscopic methods, such as IR, HR-TOF-MS, and NMR. The cytotoxic activity was evaluated against one human cancer cell line HL-60 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Keywords: *Gynostemma pentaphyllum*; dammarane type; gypenbioside A; gypenbioside B; cytotoxic activity

1. Introduction

'Jiao-Gu-Lan,' the dried aerial parts of *Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae), is a herbal medicine with anticancer activity [1], widely distributed in China, Korea, and Japan, and has been used as a famous folk medicine in China. The biological active constituents are dammarane-type glycosides, called gypenosides, which are structurally correlated to the ginseng saponins [2–6]. In our series of studies on this anticancer natural medicine, we have found some active compounds [7–8]. As a continuation of our work for discovering more effective components of *G. pentaphyllum*, two new dammarane saponins, gypenbiosides A (**1**) and B (**2**), were isolated from the extract of the aerial parts of this plant (Figure 1). In this paper, we report the structural elucidation of the two new dammarane-type saponins as well as their antitumor activities.

2. Results and discussion

Compound **1**, obtained as white amorphous powder, showed a pseudo-molecular ion peak at *m/z* 789.4410 $[M + Na]^+$ in the HR-TOF-MS, pointing to the molecular formula $C_{41}H_{66}O_{13}$. The IR spectrum (KBr) showed peaks at 3427 cm^{-1} (OH) and 1635 cm^{-1} (C=C). On acid hydrolysis, it yielded glucose and xylose which were identified by thin layer chromatography (TLC) comparison. The ^1H NMR spectrum (Table 1) showed two anomeric protons at δ 4.92 (1H, d, $J = 7.9$ Hz, H-1′), 5.28 (1H, d, $J = 7.5$ Hz, H-1″)], an olefinic proton signal at δ 5.58 (1H, d, $J = 8.0$ Hz), and seven methyl proton signals at δ 0.74 (3H, s), 0.89 (3H, s), 0.92 (3H, s), 0.98 (3H, s), 1.30 (3H, s), 1.60 (3H, s), and 1.68 (3H, s). Analysis of the ^1H and ^{13}C NMR spectra (Table 1) established that **1** was a triterpene saponin with a 21,23-lactone skeleton. In the previously published literature [9], the carbon signals in the ^{13}C NMR spectral data closely resembled

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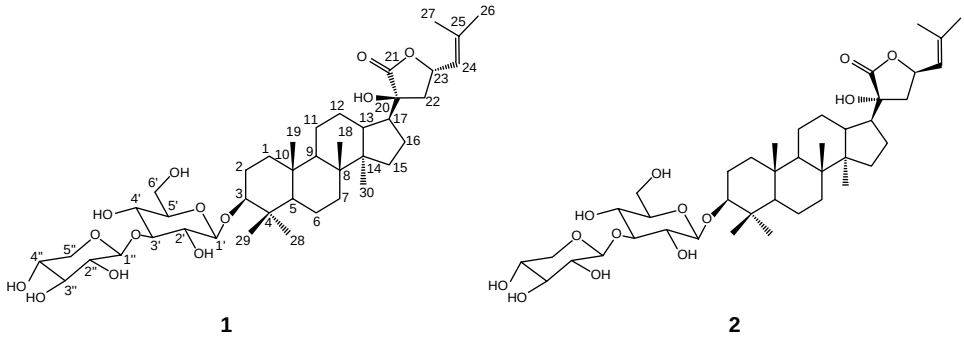

Figure 1. Structure of compounds **1** and **2**.

to those of (20*S*,23*S*)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside, but losing six signals due to L-rhamnose of the side chain. And the absolute configuration at C-20 of **1** was deduced to be *S* on the basis of the chemical shifts C-20 at δ 79.0 and C-17 at δ 45.8 [4]. ^{1}H–^{1}H NOESY interactions (Figure 2) and NMR analysis showed that the chemical environment of H_β-22 and H_α-22 is similar to each other except for the impact of OH-C-20, which shifted the signal of H_β-22 toward upfield. NOESY cross peaks were observed between H-23 and H_β-22, H-24 and H_α-22, Me-26 and H-24, Me-27 and H-23, suggesting that H-23 should be located on the β-side and the 2-methylpropenyl group on the α-side, which was also supported by the chemical shifts of C-23 at δ 74.1 and C-24 at δ 125.4 [9]. Thus, the absolute configuration at C-23 of **1** was deduced to be *S*. So, the aglycon part of **1** was determined as (20*S*,23*S*)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone. By GC analysis of the trimethylsilyl (TMS) ether derivatives of the component monosaccharides and comparing with the standard monosaccharides under the same condition, together with the analysis of the coupling constants in ^{1}H NMR spectrum, it was clear that **1** contained one unit of β-D-glucose and one unit of β-D-xylose. And the linkage sites and sequences of the two saccharides and the aglycon were confirmed by the 2D NMR experiments. In the HMBC spectrum (Figure 3), the cross peaks between H-1′ of the glucose at δ 4.92 and C-3 at δ 89.1 of the aglycon, H-1″ of the xylose at δ 5.28, and C-3′ at δ 87.8 of the glucose were observed. Thus, the structure of **1** was deduced as (20*S*,23*S*)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone 3-*O*-[β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside and named gypenbioside A.

Compound **2**, a white amorphous powder, showed a peak at *m*/*z* 789.4386 $[M + Na]^+$ in the HR-TOF-MS, pointing to the molecular formula $C_{41}H_{66}O_{13}$. The IR spectrum (KBr) showed peaks at 3426 cm^{-1} (OH) and 1637 cm^{-1} (C=C). On acid hydrolysis, it yielded glucose and xylose which were identified by TLC comparison. The ^{1}H NMR spectrum (Table 2) showed two anomeric protons at δ 4.90 (1H, d, $J = 7.8$ Hz, H-1′), 5.26 (1H, d, $J = 7.8$ Hz, H-1″)], an olefinic proton signal at δ 5.41 (1H, d, $J = 7.2$ Hz), and seven methyl proton signals at δ 0.81 (3H, s), 0.90 (3H, s), 0.99 (3H, s), 1.01 (3H, s), 1.31 (3H, s), 1.63 (3H, s), and 1.68 (3H, s). Analysis of ^{1}H and ^{13}C NMR spectra established that **2** was a triterpene saponin with a 21,23-lactone skeleton. Comparison of ^{1}H and ^{13}C NMR spectra (Table 2) of **2** with those of **1** indicated that they had the same aglycon and sugar

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **1** (in C_5D_5N, *J* in Hz).

Position	δ_C	δ_H (*J* in Hz)	HMBC correlations
1	39.7	1.44 (1H, m), 0.73 (1H, m)	
2	26.7	2.19 (1H, m), 1.80 (1H, m)	
3	89.1	3.35 (1H, dd, 12.0, 4.6)	C-1′, C-4, C-28, C-29
4	39.7		
5	56.3	0.72 (1H, m)	C-10
6	18.4	1.51 (1H, m), 1.37 (1H, m)	
7	35.7	1.48 (1H, m), 1.22 (1H, m)	
8	40.8		
9	51.1	1.24 (1H, m)	
10	37.0		
11	21.8	1.48 (1H, m), 1.25 (1H, m)	
12	25.8	2.01 (1H, m), 1.67 (1H, m)	
13	43.2	2.08 (1H, m)	
14	50.7		
15	31.7	1.60-1.68 (1H, m), 1.06-1.13 (1H, m)	
16	27.3	2.41 (1H, m), 1.34 (1H, m)	
17	45.8	2.53 (1H, m)	
18	15.6	0.92 (3H, s)	C-7, C-8, C-9, C-14
19	16.5	0.74 (3H, s)	C-1, C-5, C-9, C-10
20	79.0		
21	179.5		
22	40.7	2.70 (1H, dd, 13.1, 6.0), 2.27 (1H, dd, 13.1, 5.9)	C-17, C-20, C-21, C-23, C-24
23	74.1	5.49 (1H, m)	C-24, C-25
24	125.4	5.58 (1H, d, 8.0)	C-22, C-23, C-26, C-27
25	138.5		
26	25.6	1.60 (3H, s)	C-24, C-25, C-27
27	18.1	1.68 (3H, s)	C-24, C-25, C-26
28	28.0	1.30 (3H, s)	C-4, C-29
29	17.0	0.98 (3H, s)	C-4, C-28
30	16.5	0.89 (3H, s)	C-13, C-14, C-15
3-*O*-glc-1′	106.6	4.92 (1H, d, 7.8)	C-3, C-5′
2′	75.4	4.02 (1H, t, 7.8)	C-1″
3′	87.8	4.21 (1H, m)	C-1″, C-2″, C-4′
4′	69.6	4.11 (1H, m)	
5′	78.2	3.95 (m)	
6′	62.7	4.55 (1H, d, 10.8), 4.32 (1H, m)	
xyl(1 → 3)-1″	106.4	5.28 (1H, d, 7.5)	C-3′
2″	74.7	4.06 (1H, m)	C-1″
3″	78.3	4.17 (1H, m)	
4″	71.0	4.18 (1H, m)	
5″	67.5	4.29 (1H, m), 3.69 (1H, t, 10.3)	

moieties, and the only difference between them was in the absolute configurations of C-20 and C-23. Moreover, comparing with **1**, the downfield shifts at C-20 ($\Delta\delta + 2.2$), C-23 ($\Delta\delta + 1.1$), and C-25 ($\Delta\delta + 0.9$) and the upfield shift at C-21 ($\Delta\delta - 1.1$) and C-22 ($\Delta\delta - 1.6$) could also testify the difference. The carbon signals of the aglycon part in the ^{13}C NMR spectrum (Table 2) closely resembled to those of (20*R*,23*R*)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside

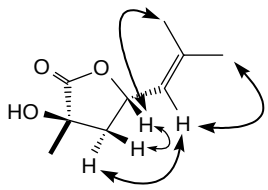

Figure 2. Key NOE correlations of compound **1**.

[9]. And the absolute configuration at C-20 of **2** was deduced to be *R* on the basis of the chemical shifts C-20 at δ 81.2, and C-17 at δ 45.4 [4]. Therefore, the structure of **2** was elucidated as (20*R*,23*R*)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone 3-*O*-[β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside and named gypenbioside B.

Compounds **1** and **2** were tested for *in vitro* cytotoxicity against a human promyelocytic leukemia cell line (HL-60) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [10]. Both of them showed cytotoxicity against HL-60 cells and their IC_{50} values ($\mu g\,ml^{-1}$) were 47.12 and 45.50, respectively.

3. Experimental

3.1 General experimental procedures

IR spectra were recorded on Bruker IFS-55 spectrophotometer (Karlsruhe, Germany). Optical rotations were measured on Perkin-Elmer polarimeter (Beijing, China). ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV-600 and ARX-300 spectrometer (Zurich, Switzerland). HR-TOF-MS were measured on BIC micro TOF-Q mass spectrometer (New York City, NY, USA). The GC was carried out on Agilent technologies 6890N apparatus, with OV-17 (30 m × 0.32 mm) column (Santa Clara, CA, USA). Preparative HPLC was carried out on Beijing CXTH3000 system, P3000 pump, UV3000 spectrophotometric detector at 203 nm, and Daisogel C_{18} reversed-phase column (10 μm, 30 × 250 mm) (Beijing Chuangxintongheng Company, Beijing, China). Column chromatographies were carried out with silica gel (SiO_2: 200–300 mesh, Qingdao Marine Chemical Group, Co., Qingdao, China) and macroporous resin D101 (Cangzhou Company, Hebei, China).

3.2 Plant material

The aerial parts of *G. pentaphyllum* were collected from Shaanxi province of China by Xi'an Tianyi Co. Ltd. A voucher specimen of the plant (No. 2007016) at our laboratory was identified by Prof. Qishi Sun of Shenyang Pharmaceutical University.

3.3 Extraction and isolation

Dried aerial parts of *G. pentaphyllum* (8.0 kg) were extracted with 75% EtOH (× 3), and the water soluble extract of the

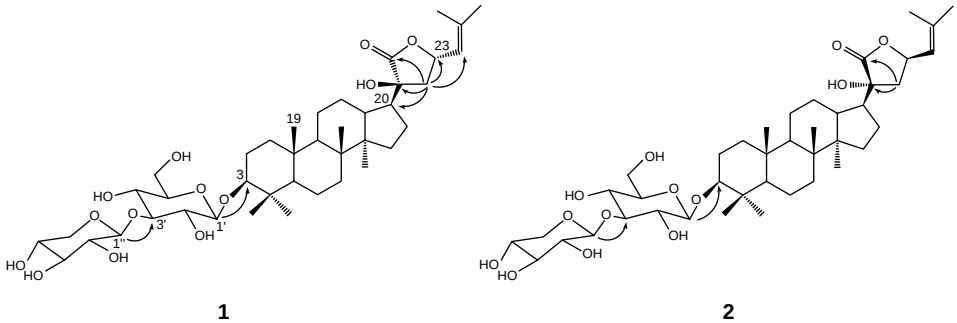

Figure 3. Key HMBC correlations of compounds **1** and **2**.

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **2** in C_5D_5N (*J* in Hz).

Position	δ_C	δ_H (*J* in Hz)	HMBC correlations
1	39.8	1.61 (1H, m), 0.98 (1H, m)	
2	26.8	2.23 (1H, m), 1.86 (1H, m)	
3	89.1	3.38 (1H, m)	C-1′, C-28, C-29
4	39.8		
5	56.4	0.74 (1H, m)	C-4
6	18.4	1.50 (1H, m), 1.39 (1H, m)	
7	35.7	1.49 (1H, m), 1.23 (1H, m)	
8	40.7		
9	51.1	1.33 (1H, m)	
10	37.1		
11	21.8	1.58 (1H, m), 1.30 (1H, m)	
12	26.3	2.01 (1H, m), 1.67 (1H, m)	
13	45.0	1.85 (1H, m)	
14	50.2		
15	31.7	1.60 (1H, m), 1.11 (1H, m)	
16	28.0	2.53 (1H, d, 11.5), 1.50 (1H, m)	
17	45.4	2.70 (1H, m)	
18	15.7	0.90 (3H, s)	C-8, C-13, C-14
19	16.5	0.81 (3H, s)	C-1, C-9, C-10
20	81.2		
21	178.4		
22	39.1	2.60 (1H, m), 2.12 (1H, m)	C-20, C-21
23	75.2	5.70 (1H, m)	
24	124.0	5.41 (1H, d, 7.2)	
25	139.4		
26	25.6	1.68 (3H, s)	C-24, C-25, C-27
27	18.2	1.63 (3H, s)	C-24, C-25, C-26
28	28.0	1.31 (3H, s)	C-3, C-4, C-5, C-29
29	16.8	1.01 (3H, s)	C-3, C-4, C-5, C-28
30	16.3	0.99 (3H, s)	C-8, C-14
3-*O*-glc-1′	106.5	4.90 (1H, d, 7.8)	C-3
2′	75.4	4.03 (1H, t, 7.8)	C-1′
3′	87.8	4.22 (1H, m)	C-1″, C-2′
4′	69.9	4.12 (1H, m)	
5′	78.2	3.96 (1H, m)	
6′	62.7	4.54 (1H, dd, 11.9, 2.4), 4.32 (1H, m)	
xyl(1 → 3)-1″	106.4	5.26 (1H, d, 7.8)	C-3′
2″	74.7	4.08 (1H, m)	C-1″
3″	78.3	4.17 (1H, m)	
4″	71.0	4.19 (1H, m)	
5″	67.5	4.29 (1H, m), 3.70 (1H, t, 9.6)	

plant was separated by a macroporous resin column to get the 70% EtOH eluates which upon drying afforded the total saponins (80 g). The total saponins were chromatographed repeatedly over silica gel with $CHCl_3$/MeOH/H_2O (7:2:1–7:3:1–7:4:1) to provide five fractions A–E. Fraction B (0.5 g) was separated into five fractions, frs C_a—C_e, by HPLC (80% MeOH). Fraction C_c (0.07 g) was then subjected to preparative RP-HPLC (85% MeOH), with the spectrophotometric detector at 203 nm, C_{18} reversed-phase *column* (10 μm, 30 × 250 mm; flow rate 14.0 ml min^{-1}), to yield **1** (15 mg, t_R 15 min) and **2** (18 mg, t_R 20 min).

*3.3.1 Gypenbioside A ((20S,23S)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone 3-O-[β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside; **1**)*

White amorphous powder. Libermann–Burchard and Molish reactions were positive. $[\alpha]_D^{28}$ −17.5 ($c = 0.54$, MeOH). IR (KBr) ν_{max}: 3427, 2943, 1756, 1635, 1388, 1043, and 618 cm^{-1}. ^1H and ^{13}C NMR spectral data (Table 1). HR-TOF-MS: *m/z* 789.4410 $[M + Na]^+$ (calcd for $C_{41}H_{66}O_{13}Na$, 789.4396).

*3.3.2 Gypenbioside B ((20R,23R)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone 3-O-[β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside; **2**)*

White amorphous powder. Libermann–Burchard and Molish reactions were positive. $[\alpha]_D^{28}$ +31.5 ($c = 0.52$, MeOH). IR (KBr) ν_{max}: 3427, 2943, 1756, 1636, 1451, 1386, 1080, 1042, 981, and 618 cm^{-1}. ^1H and ^{13}C NMR spectral data (Table 2). HR-TOF-MS: *m/z* 789.4386 $[M + Na]^+$ (calcd for $C_{41}H_{66}O_{13}Na$, 789.4396).

3.4 Acid hydrolysis of 1 and 2

Each compound (2 mg) was heated in 2.5 ml of 1 M HCl/MeOH (4:1) at 90°C for 6 h in a water bath. After cooling, the reaction mixture was diluted to 10 ml with water and then extracted with $CHCl_3$ (10 ml × 3). After concentration, the aqueous layer was examined by TLC ($CHCl_3$/-MeOH/H_2O 55:45:10) and compared with the authentic samples.

3.5 Determination of sugar components

The monosaccharide subunits were obtained by HCl hydrolysis as described above. The aqueous layer was concentrated to dryness to give a residue which was dissolved in pyridine (1 ml), and then hexamethyl disilazane (0.4 ml) and trimethylchlorosilane (0.2 ml) were added to the solution to obtain the TMS ethers. The mixture was refluxed at 20°C for 15 min, the solvent was dried, then dissolved in ethyl acetate (1 ml), and extracted with H_2O (1 ml). The aqueous layer was examined by GC (H_2 flame ionization detector, column temperature: 100–280°C, programmed increase: 10°C min^{-1}, carrier gas: N_2 (1.5 ml min^{-1}), injector and detector temperature: 280°C, injection volume: 1 μl, split ratio: 10:1). The derivatives of D-xylose and D-glucose were detected at t_R (min): 8.84 and 26.59 min, respectively. The standard monosaccharides were subjected to the same operation and GC analysis under the same condition.

3.6 Antitumor bioassay

Antitumor activity was evaluated by MTT assay as reported in the literature [10], with Rg_3 as the positive control.

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