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Dammarane-type glycosides from *Gynostemma pubescens*

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Abstract

Triterpene saponins **1**–**8** were isolated from the aerial parts of *Gynostemma pubescens*. Among them, compounds **1**–**4**, **8** possess carboxylic groups at both C-21 and C-29, **5** contains a carboxylic group at C-21 and an aldehyde function at C-29, whereas **6** and **7** contain carboxylic groups at C-21 and hydroxymethylene groups at C-29. Their structural elucidation was accomplished by extensive spectroscopic methods including application of 1D (1H, ^{13}C, ^{13}C DEPT) and 2D NMR experiments (HMQC, HMBC, HSQC), HRESIMS analysis, as well as by chemical degradation.

Keywords: *Gynostemma pubescens*; Cucurbitaceae; Triterpenoid saponins

1. Introduction

Gynostemma pentaphyllum Makino, a Chinese herb called jiao-gu-lan, is used in traditional medicine to treat bronchitis and asthma. Phytochemical studies of this plant have identified more than 130 dammarane-type glycosides (Nagai et al., 1981; Takemoto et al., 1983a,b, 1984a,b,c,d,e; Simone et al., 1995; Hu et al., 1996, 1997; Yin and Hu, 2005; Yin et al., 2004a,b, 2006a,b), closely related to the ginseng saponin. Indeed, gypenosides III, IV, VIII, XII and malonyl gypenosides III and VIII are identical to ginsenosides Rb1, Rb3, Rd, F2, and malonyl ginsenosides RB1 and Rd. The pharmacological studies of the ethanolic extract of *G. pentaphyllum* and the isolated saponins have illustrated a variety of biological activities, including anti-inflammatory, anti-tumour, immunopotentiating, anti-ulcer, anti-oxidant and retardation of aging (Blumert and Liu, 1999; Li et al., 1993; Tanner et al., 1999). Products based on crude saponins from *G. pentaphyllum* have been used clinically in China and have been described as having insignificant toxicity (Jian, 1986; Attawish et al., 2004). The dammarane saponins, namely gypenosides, isolated from *G. pentaphyllum* are believed to be the active principles responsible for its biological activities and reported clinical efficacy of the *G. pentaphyllum* extract in the treatment of cardiovascular diseases and related disorders (Li et al., 1993; La Cour et al., 1995). The isolation of ginsenosides from *G. pentaphyllum* has earned the herb the name "Southern Ginseng" in China and has stimulated keen interest among phytochemists in searching for new, pharmacologically active gypenosides from this plant and other *Gynostemma* plants. The genus *Gynostemma* (Cucurbitaceae) comprises about 14 species in China. Previously, we have isolated and defined more than 40 new gypenosides from the aerial part of *G. pentaphyllum* (Hu et al., 1996, 1997; Yin and Hu, 2005; Yin et al., 2004a,b, 2006a,b) and *G. cardiospermum* (Yin et al., 2006a,b). Here, we report the isolation and structural characterization of eight new triterpene saponins **1**–**8** from the EtOH extract of *Gynostemma pubescens* (Gagnep) C.Y. Wu never reported before (Fig. 1).

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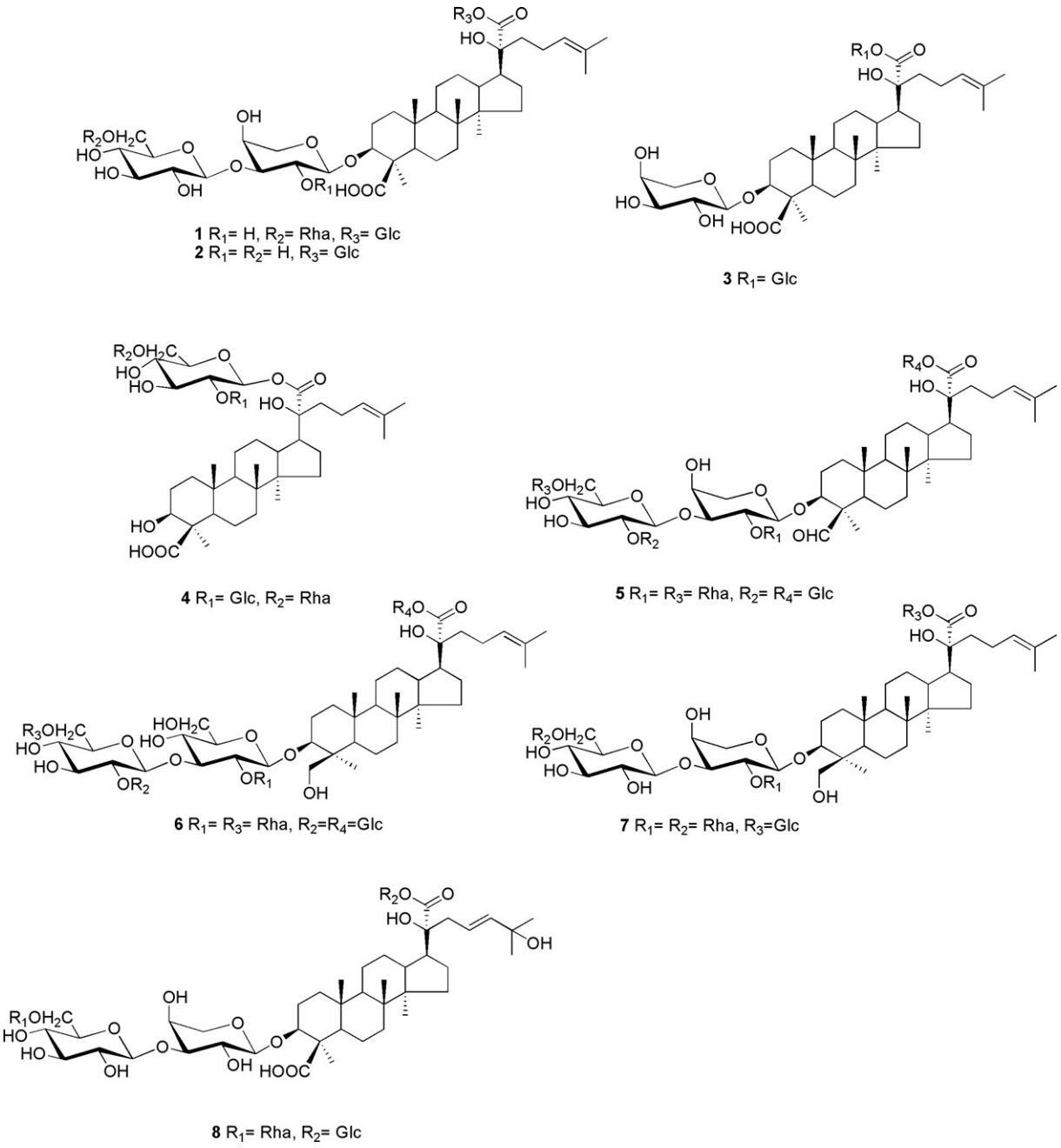

Fig. 1. Structures of **1**–**8**.

2. Results and discussion

Compound **1** was obtained as an amorphous powder. Its IR spectrum showed characteristic absorptions for hydroxyl (3400 cm^{-1}), carbonyl (1700 cm^{-1}), and glycosidic linkages (1000–1100 cm^{-1}). The molecular weight was determined from the HRESIMS at *m/z* 1130.2415 for the $[M+Na]^+$ ion (calcd. for $C_{53}H_{86}O_{24}Na$, 1130.2411 $[M+Na]^+$). The ^{13}C NMR spectra gave 53 signals, of which 23 were assigned to the sugar moiety and 30 to a triterpene moiety. The ^1H NMR spectrum of **1** showed seven signals assignable to aglycone methyl groups at δ 1.05–1.85, of which two were linked to sp^2 carbons (δ 1.65 and 1.70). The ^1H NMR spectrum of **1** also showed an olefinic proton at δ 5.45 (1H, *t*, $J = 6.2$ Hz). A 3β-hydroxyl substitution was evident from the chemical shift, and the *J* values of the proton ascribable to H-3α at δ 3.50 (1H, *dd*, $J = 11.9$ and 4.5 Hz). By comparison of its ^1H and ^{13}C NMR spectra with those of known dammarane-type saponins (Yin et al., 2004a,b), it was evident that **1** had two more carbonyl groups than the common triterpene saponins. One carbonyl carbon was assigned at C-21 due to HMBC correlations of H_2-22 (δ 1.10, 1.90, each *m*) with C-21 (δ 176.2), the other carbonyl carbon was assigned to C-29

due to its HMBC correlations of C-29 (δ 177.5) with H-3 (δ 3.50, 1H, *dd*, $J = 11.9$ and 4.5 Hz), H-28 (δ 1.85, 3H, *s*), and H-5 (δ 1.10, 1H, *m*) (Fig. 2).

Acid hydrolysis of compound **1** yielded compound **1a**, a H_2O adduct at C-24 and C-25 of the corresponding aglycone of **1**. All the ^1H and ^{13}C NMR spectroscopic data were assigned using HSQC, HMBC, ^1H-^1H COSY, and NOESY experiment (Tables 1 and 2). In the NOESY spectrum, CH_3-28 (δ 1.78, 3H, *s*) exhibited prominent cross-peaks with H-3 (δ 3.45, 1H, *dd*, $J = 12.5$ and 5.0 Hz) and H-5 (δ 1.10, 1H, *m*) (Fig. 3), also indicating that a carbonyl group was located at C-29. The absolute configuration at C-20 was established as *S* by comparing the NMR spectroscopic data with model compounds (Yin and Hu, 2005).

Based on the above evidence, the aglycone of **1** was elucidated to be 3β,20*S*-dihydroxydammar-24-en-21,29-dioic acid.

Hydrolysis of compound **1** yielded L-arabinose, L-rhamnose and D-glucose in a ratio of 1:1:2 by GC analysis of the leucine derivatives of the component monosaccharides compared with the leucine derivatives of the reference sugars. The NMR spectroscopic data indicated an α-configuration for the arabinosyl unit [δ 5.05 (1H, *d*, $J = 5.0$ Hz, H-1 of ara); δ 107.2 (C-1 of ara)], an α-configuration for the rhamnosyl unit [δ 5.50 (1H, *br s*, H-1 of rha); δ 102.8 (C-1 of rha)], a β-configuration for the glucosyl uint [δ 5.53 (1H, *d*, $J = 7.8$ Hz, H-1 of glc); δ 105.8 (C-1 of glc)], and a β-configuration for the other glucosyl unit [δ 6.30 (1H, *d*, $J = 8.1$ Hz, H-1 of glc′); δ 94.5 (C-1 of glc′)]. The linkage sites and sequences of the four saccharides and the aglycone were deduced from an HMBC experiment. Correlations were observed between H-1 (δ 5.05, 1H, *d*, $J = 5.0$ Hz) of the arabinose and C-3 (δ 89.1) of the aglycone, H-1 (δ 5.53, 1H, *d*, $J = 7.8$ Hz) of the glucose and C-3 (δ 81.0) of the arabinose, H-1 (δ 5.50, 1H, *br s*) of the rhamnose and C-6 (δ 68.1) of the glucose and H-1 (δ 6.30, 1H, *d*, $J = 8.1$ Hz) of the other glucose and C-21 (δ 176.2) of the aglycone (Fig. 2). All proton and carbon signals of the aglycone and each sugar moiety in compound **1** were assigned using HSQC, ^1H–^1H COSY, HMQC, HMBC NMR experiments (see Tables 1 and 2). Thus, **1** was elucidated as (20*S*)-3β,20-dihydroxydammar-24-en-21,29-dioic acid-3-*O*-{[α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

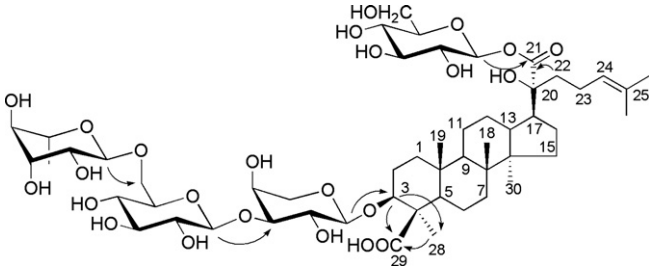

Fig. 2. Key HMBC relationships for compound **1**.

Compound **2** was purified as an amorphous powder. The HRESIMS sodiated molecular ion at *m/z* 984.0953 suggested a molecular formula of $C_{47}H_{76}O_{20}$ (calcd. for $C_{47}H_{76}O_{20}Na$, 984.0951 $[M+Na]^+$). The IR spectrum showed the same pattern as that of **1**. Comparison of the ^1H and ^{13}C NMR spectra of **2** with **1** indicated that they had the same aglycone. Analysis of the NMR and ESI-mass spectra established that compound **2** contained one pentose and two hexose units. Hydrolysis of **2** yielded D-glucose and L-arabinose. Presence of one L-arabinose and two D-glucose units per molecule of compound **2** was thus indicated. Chemical shifts, the multiplicity of the signals, the absolute values of the coupling constants and the magnitude in the ^1H NMR, and also the ^{13}C NMR spectroscopic data (Tables 1 and 2), indicated a β-configuration at the anomeric positions for the glucopyranosyl units and an α-configuration for the arabinosyl unit. The ^{13}C NMR spectroscopic data allowed assignment of the pyranose form to D-glucose and L-arabinose. Assignment of all proton and carbon NMR signals were also made using the NMR experiments described above. The linkage site and sequences of the saccharide moieties and that of the aglycone were also determined using an HMBC experiment. Based on the above results, the structure of compound **2** was identified as (20*S*)-3β,20-dihydroxydammar-24-en-21,29-dioic acid-3-*O*-{[β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

Compound **3** was obtained as an amorphous powder. The HRESIMS showed a sodiated molecular ion at *m/z* 821.9545, indicating a molecular formula of $C_{41}H_{66}O_{15}$ (calcd. for $C_{41}H_{66}O_{15}Na$, 821.9541 $[M+Na]^+$). Comparison of the ^1H and ^{13}C NMR spectra of **3** with **1** indicated that they had the same aglycone. The difference between **3** and **1** was that **3** had only one D-glucose and one L-arabinose. Chemical shifts, the multiplicity of the signals, the absolute values of the coupling constants and the magnitude in the ^1H NMR and ^{13}C NMR spectroscopic data (Tables 1 and 2), indicated a β-configuration at the anomeric positions for the glucopyranosyl unit and an α-configuration for the arabinosyl unit. From the HMBC spectrum, the H-1 (δ 6.50, 1H, *d*, $J = 7.9$ Hz) of the glucosyl unit was linked to C-21 (δ 177.0) of the aglycone and the H-1 (δ 5.00, 1H, *d*, $J = 5.7$ Hz) of the arabinosyl unit was linked to C-3 (δ 89.2) of the aglycone. Thus, compound **3** was determined as (20*S*)-3β,20-dihydroxydammar-24-en-21,29-dioic acid-3-*O*-[α-L-arabinopyranosyl]-21-*O*-β-D-glucopyranoside.

The HRESIMS of **4** exhibited a sodiated molecular ion at *m/z* 998.1218, establishing a molecular formula of $C_{48}H_{78}O_{20}$ (calcd. for $C_{48}H_{78}O_{20}Na$, 998.1220 $[M+Na]^+$). From the ^1H and ^{13}C NMR spectra, **4** had a similar aglycone to that of **1**. The signals for the oxymethine proton (H-3) at δ 3.40 and carbon (C-3) at δ 78.6 were shifted downfield compared to those of **1** (δ 3.50, δ 89.1), indicating that no saccharide was connected to C-3 in the aglycone. The difference between **4** and **1** was in the saccharide chain. Hydrolysis of **4** yielded L-glucose and

Table 1
^{1}H NMR (500 MHz) spectroscopic data of compounds **1a**, **1**–**8** in C_5D_5N[a]

Position	**1a**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	1.03 *m*, 1.75 *m*	1.10 *m*, 1.90 *m*	0.90 *m*, 1.73 *m*	0.85 *m*, 1.75 *m*	2.10 *m*, 2.45 *m*	0.95 *m*, 1.75 *m*	1.05 *m*, 1.70 *m*	0.90 *m*, 1.75 *m*	1.10 *m*, 1.90 *m*
2	1.50 *m*, 2.17 *m*	1.54 *m*, 2.23 *m*	1.55 *m*, 2.23 *m*	1.60 *m*, 2.27 *m*	1.55 *m*, 2.20 *m*	1.5 0 *m*, 2.23 *m*	1.55 *m*, 2.24 *m*	1.50 *m*, 2.20 *m*	1.52 *m*, 2.20 *m*
3	3.45 *dd* (12.5, 5.0)	3.50 *dd* (11.9, 4.5)	3.50 *dd* (12.3, 3.9)	3.50 *dd* (11.4, 4.8)	3.40 *dd* (11.1, 5.7)	3.55 *dd* (11.6, 4.7)	3.55 *br d* (11.3)	3.50 *br d* (10.0)	3.50 *br d* (11.5)
5	1.10 *m*	1.10 *m*	1.23 *m*	1.10 *m*	1.05 *m*	1.05 *m*	0.90 *m*	0.88 *m*	1.15 *m*
6	2.10 *m*, 2.35 *m*	2.05 *m*, 2.13 *m*	2.04 *m*, 2.10 *m*	2.03 *m*, 2.10 *m*	2.08 *m*, 2.30 *m*	1.80 *m*	1.80 *m*	1.75 *m*	1.45 *m*, 1.75 *m*
7	1.45 *m*, 1.65 *m*	1.25 *m*, 1.63 *m*	1.35 *m*, 1.60 *m*	1.38 *m*, 1.63 *m*	1.40 *m*, 1.58 *m*	1.25 *m*, 1.45 *m*	1.30 *m*, 1.55 *m*	1.30 *m*, 1.52 *m*	1.38 *m*, 1.63 *m*
9	1.43 *m*	1.42 *m*	1.38 *m*	1.40 *m*	1.42 *m*	1.42 *m*	1.43 *m*	1.42 *m*	1.42 *m*
11	1.25 *m*, 1.55 *m*	1.50 *m*, 1.80 *m*	1.38 *m*, 1.60 *m*	1.39 *m*, 1.63 *m*	1.41 *m*, 1.70 *m*	1.50 *m*, 1.72 *m*	1.45 *m*, 1.72 *m*	1.25 *m*, 1.70 *m*	1.42 *m*, 1.73 *m*
12	2.05 *m*, 2.20 *m*	2.00 *m*, 2.15 *m*	2.05 *m*, 2.10 *m*	2.05 *m*, 2.20 *m*	2.05 *m*, 2.10 *m*	2.00 *m*, 2.10 *m*	2.02 *m*, 2.05 *m*	2.00 *m*, 2.10 *m*	2.05 *m*, 2.13 *m*
13	2.30 *m*	2.30 *m*	2.30 *m*	2.30 *m*	2.30 *m*	2.25 *m*	2.27 *m*	2.30 *m*	2.35 *m*
15	1.23 *m*, 1.75 *m*	1.15 *m*, 1.75 *m*	1.20 *m*, 1.70 *m*	1.14 *m*, 1.75 *m*	1.20 *m*, 1.70 *m*	1.10 *m*, 1.75 *m*	1.15 *m*, 1.73 *m*	1.10 *m*, 1.75 *m*	1.20 *m*, 1.70 *m*
16	2.05 *m*, 2.50 *m*	2.24 *m*, 3.00 *m*	2.23 *m*, 2.90 *m*	2.25 *m*, 2.98 *m*	2.00 *m*, 2.45 *m*	2.20 *m*, 2.95 *m*	2.30 *m*, 2.85 *m*	1.75 *m*, 2.81 *m*	2.30 *m*, 2.95 *m*
17	2.57 *m*	2.65 *m*	2.65 *m*	2.55 *m*	2.56 *m*	2.60 *m*	2.70 *m*	2.60 *m*	2.65 *m*
18	1.07 *s*	1.07 *s*	1.10 *s*	1.05 *s*	1.10 *s*	0.90 *s*	1.05 *s*	1.02 *s*	1.05 *s*
19	1.10 *s*	1.15 *s*	1.13 *s*	1.10 *s*	1.20 *s*	0.68 *s*	1.02 *s*	0.80 *s*	1.13 *s*
22	2.20 *m*, 2.30 *m*	1.10 *m*, 1.90 *m*	1.73 *m*, 2.65 *m*	2.23 *m*, 2.30 *m*	1.22 *m*, 1.95 *m*	2.13 *m*, 2.45 *m*	2.10 *m*, 2.45 *m*	2.08 *m*, 2.45 *m*	2.90 *dd* (5.5, 12.1), 3.20 *dd* (8.5, 12.1)
23	1.97 *m*, 2.32 *m*	2.45 *m*, 2.70 *m*	2.41 *m*, 2.65 *m*	2.55 *m*, 2.75 *m*	2.45 *m*, 2.70 *m*	2.45 *m*, 2.70 *m*	1.68 *m*	1.75 *m*	6.21 *ddd* (15.5, 8.5, 5.5)
24	1.85 *m*	5.45 *t* (6.2)	5.40 *t* (6.0)	5.30 *t* (6.0)	5.50 t (6.3)	5.45 *t* (6.2)	5.55 *t* (6.0)	5.50 *t* (6.3)	6.11 *d* (15.5)
26	1.43 *s*	1.70 *s*	1.70 *s*	1.75 *s*	1.70 *s*	1.75 *s*	1.77 *s*	1.77 *s*	1.60 *s*
27	1.43 *s*	1.65 *s*	1.63 *s*	1.75 *s*	1.65 *s*	1.70 *s*	1.73 *s*	1.63 *s*	1.60 *s*
28	1.78 *s*	1.85 *s*	1.83 *s*	1.78 *s*	1.70 *s*	1.50 *s*	1.55 *s*	1.40 *s*	1.85 *s*
29						10.45 *s*	4.48 *m*, 4.65 *m*	4.46 *m*, 4.65 *m*	
30	1.05 *s*	1.05 *s*	1.08 *s*	1.10 *s*	1.03 *s*	1.40 *s*	0.95 *s*	0.80 *s*	1.05 *s*
		C-3-Ara	C-3-Ara	C-3-Ara		C-3-Ara	C-3-Glc	C-3-Ara	C-3-Ara
1		5.05 *d* (5.0)	5.05 *d* (5.7)	5.00 *d* (5.7)		4.90 *d* (5.0)	5.03 *d* (7.9)	5.00 *d* (5.7)	5.05 *d* (4.8)
2		4.35 *m*	4.40 *m*	4.45 *m*		4.68 *m*	4.33 *m*	4.55 *m*	4.40 *m*
3		4.35 *m*	4.42 *m*	4.10 *m*		4.35 *m*	4.30 *m*	4.28 *m*	4.10 *m*
4		4.60 *m*	4.55 *m*	4.55 *m*		4.60 *m*	4.15 *m*	4.40 *m*	4.53 *m*
5		4.10 *m*, 4.37 *m*	4.00 *m*, 4.45 *m*	4.08 *m*, 4.47 *m*		4.30 *m*, 4.58 *m*	4.27 *m*	4.02 *m*, 4.57 *m*	3.98 *m*, 4.45 *m*
6							4.26 *m*, 4.52 *m*		
Rha									
1						6.10 *br s*	6.50 *br s*	5.75 *br s*	
2						4.38 *m*	4.22 *m*	4.20 *m*	
3						4.77 *m*	4.75 *m*	4.67 *m*	
4						4.30 *m*	4.28 *m*	4.30 *m*	
5						4.35 *m*	4.78 *m*	4.05 *m*	
6						1.70 *d* (6.2)	1.80 *d* (5.8)	1.77 *d* (6.2)	

(*continued on next page*)

Table 1 (*continued*)

Position	**1a**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Glc									
1		5.53 *d* (7.8)	5.52 *d* (7.7)			5.15 *d* (7.5)	5.10 *d* (7.6)	5.55 d (7.3)	5.50 *d* (7.4)
2		4.13 *m*	4.13 *m*			4.00 *m*	4.12 *m*	4.10 *m*	4.05 *m*
3		4.25 *m*	4.25 *m*			4.35 *m*	4.45 *m*	4.30 *m*	4.18 *m*
4		4.07 *m*	4.35 *m*			4.15 *m*	4.14 *m*	4.53 *m*	4.06 *m*
5		4.03 *m*	4.20 *m*			4.25 *m*	4.27 *m*	4.16 *m*	4.30 *m*
6		4.23 *m*, 4.35 *m*	4.42 *m*, 4.70 *m*			4.22 *m*, 4.58 *m*	4.24 *m*, 4.55 *m*	4.18 *m*, 4.61 *m*	4.15 *m*, 4.58 *m*
Glc′									
1						5.51 *d* (7.5)	5.54 *d* (7.7)		
2						4.10 *m*	4.20 *m*		
3						4.05 *m*	4.05 *m*		
4						4.25 *m*	4.23 *m*		
5						4.05 *m*	4.05 *m*		
6						4.38 *m*, 4.58 *m*	4.36 *m*, 4.55 *m*		
Rha′									
1		5.50 *br s*				5.50 *br s*	5.52 *br s*	5.50 *br s*	5.55 *br s*
2		4.17 *m*				4.53 *m*	4.53 *m*	4.63 *m*	4.18 *m*
3		4.55 *m*				4.78 *m*	4.44 *m*	4.53 *m*	4.56 *m*
4		4.30 *m*				4.27 *m*	4.28 *m*	4.25 *m*	4.28 *m*
5		4.35 *m*				4.36 *m*	4.28 *m*	4.30 *m*	4.38 *m*
6		1.73 *d* (6.2)				1.70 *d* (6.3)	1.73 *d* (6.1)	1.73 *d* (7.0)	1.68 *d* (5.8)
C-21-Glc″									
1		6.30 *d* (8.1)	6.50 *d* (7.6)	6.50 *d* (7.9)	6.25 *d* (8.1)	6.15 *d* (7.6)	6.05 *d* (7.2)	6.20 *d* (7.8)	6.20 *d* (7.7)
2		4.25 *m*	4.35 *m*	4.20 *m*	4.30 *m*	4.10 *m*	4.23 *m*	4.60 *m*	4.05 *m*
3		4.35 *m*	4.42 *m*	4.44 *m*	4.25 *m*	4.15 *m*	4.22 *m*	4.16 *m*	4.28 *m*
4		4.07 *m*	4.15 *m*	4.50 *m*	4.55 *m*	4.38 *m*	4.80 *m*	4.26 *m*	4.18 *m*
5		4.35 *m*	4.10 *m*	4.10 *m*	4.06 *m*	4.38 *m*	4.14 *m*	4.16 *m*	4.15 *m*
6		4.45 *m*, 4.65 *m*	4.45 *m*, 4.50 *m*	4.48 *m*, 4.46 *m*	4.10 *m*, 4.50 *m*	4.45 *m*, 4.62 *m*	4.38 *m*, 4.63 *m*	4.41 *m*, 4.65 *m*	4.40 *m*, 4.60 *m*
					Glc				
					5.52 *d* (7.8)				
					4.04 *m*				
					4.25 *m*				
					4.05 *m*				
					4.06 *m*				
					4.45 *m*, 4.55 *m*				
					Rha				
					5.50 *br s*				
					4.53 *m*				
					4.48 *m*				
					4.27 *m*				
					4.30 *m*				
					1.65 *d* (7.0)				

[a] Measured at 500 MHz; referenced to δ 7.58 (C_5D_5N); *J* values (Hz) in parentheses.

Table 2
^{13}C NMR (125 MHz) spectroscopic data for compounds **1a**, **1**–**8** in C_5D_5N[a]

Position	**1a**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	40.3	40.1	40.2	40.3	40.2	38.7	40.2	39.2	40.0
2	26.4	26.2	26.5	26.6	26.3	26.1	26.4	26.2	26.1
3	78.4	89.1	89.1	89.2	78.6	85.9	90.2	91.5	90.0
4	49.5	50.1	50.3	50.3	49.7	49.8	50.6	50.5	50.2
5	57.3	57.7	57.9	57.9	57.6	58.2	57.8	57.1	57.5
6	20.9	20.8	21.0	21.0	21.0	19.6	18.8	18.8	20.6
7	36.1	36.2	36.4	36.4	36.3	35.6	36.7	36.3	36.0
8	40.7	40.9	41.0	41.0	41.1	40.6	41.2	41.0	41.1
9	50.7	50.9	51.2	51.2	50.9	50.4	51.6	51.2	51.1
10	38.0	38.0	38.1	38.1	38.3	37.2	37.5	37.2	38.3
11	22.3	22.5	22.6	22.4	22.7	22.5	22.5	22.5	22.3
12	24.6	24.3	24.5	24.7	24.5	24.1	24.5	24.4	24.1
13	41.4	41.6	41.8	41.8	41.7	41.3	41.7	41.6	41.4
14	50.7	50.6	50.7	50.9	50.7	49.9	50.7	50.6	50.6
15	31.2	31.0	31.2	31.5	31.2	30.9	31.3	31.1	30.8
16	29.4	27.7	27.8	27.7	29.6	27.4	27.1	26.4	27.5
17	49.0	49.1	49.3	49.4	49.1	49.1	49.3	49.1	48.5
18	15.8	16.2	16.4	16.1	16.3	16.1	16.4	16.3	16.0
19	14.6	14.8	15.0	14.9	15.0	17.2	17.0	16.8	14.6
20	78.4	79.4	79.6	79.4	79.5	79.3	79.6	79.4	80.1
21	180.3	176.2	176.5	177.0	176.4	176.0	176.4	176.2	175.9
22	40.7	40.1	40.3	40.7	40.4	40.1	40.2	40.2	42.8
23	19.7	23.3	23.5	23.6	23.4	23.3	19.0	19.4	121.4
24	45.3	125.6	125.7	125.7	125.8	125.5	125.7	125.7	143.9
25	69.8	131.5	132.0	132.0	131.7	131.5	131.8	131.6	70.6
26	29.9	26.0	26.3	26.3	26.2	25.9	26.2	26.1	30.6
27	30.3	18.1	18.3	18.3	18.3	18.0	18.3	18.2	30.6
28	24.8	24.8	24.9	24.9	25.0	21.7	23.0	23.4	24.6
29	180.9	177.5	178.1	178.1	181.1	206.7	63.5	63.4	178.0
30	16.7	16.8	17.0	17.0	16.9	38.7	40.2	39.2	40.0
		C-3-Ara	C-3-Ara	C-3-Ara		C-3-Ara	C-3-Glc	C-3-Ara	C-3-Ara
1		107.2	107.3	107.4		104.8	104.9	102.8	107.8
2		74.3	74.4	74.4		74.7	76.7	76.5	74.7
3		81.0	80.8	79.4		82.4	89.2	80.8	82.1
4		68.2	68.6	68.7		68.4	71.0	66.3	68.4
5		65.2	65.5	65.5		65.3	78.7	61.5	65.7
6							63.0		
Rha									
1						102.1	101.1	101.7	
2						72.4	72.5	72.4	
3						72.6	72.6	73.0	
4						74.2	74.2	74.2	
5						70.2	70.5	71.0	
6						18.8	19.0	18.9	
Glc									
1		105.8	105.6			104.9	104.3	105.7	106.5
2		75.6	75.8			75.1	75.4	75.7	76.2
3		78.7	78.7			78.6	80.7	78.7	82.1
4		71.0	71.0			72.3	71.9	72.8	72.7
5		78.7	78.7			78.4	78.7	78.2	79.0
6		68.1	63.5			68.0	68.2	68.2	68.5
Glc′									
1						106.0	105.6		
2						75.6	75.8		
3						78.7	78.8		
4						71.7	70.3		
5						78.7	78.8		
6						62.7	62.8		
Rha′									
1		102.8				102.7	102.9	102.8	103.2

(*continued on next page*)

Table 2 (*continued*)

Position	1a	1	2	3	4	5	6	7	8
2		72.4				72.7	72.5	72.6	72.6
3		72.9				72.9	73.0	72.8	73.3
4		74.2				74.1	74.4	74.2	74.5
5		70.0				70.0	70.2	70.0	70.3
6		102.8				18.9	18.8	18.7	18.8
C-21-Glc″									
1		94.5	94.7	97.0	94.6	94.4	94.7	94.5	94.8
2		71.0	71.0	71.6	78.8	70.9	70.3	70.4	71.2
3		78.2	78.7	78.9	78.7	78.2	78.3	78.2	78.6
4		72.2	72.4	72.4	72.4	72.4	72.5	72.6	72.4
5		78.7	79.5	79.4	78.3	78.6	78.9	78.7	79.0
6		63.5	62.6	62.9	68.2	63.4	63.0	63.5	63.7
					Glc				
					105.6				
					75.7				
					78.7				
					71.0				
					78.3				
					63.5				
					Rha				
					102.8				
					72.4				
					73.0				
					74.2				
					70.1				
					19.0				

[a] Measured at 125 MHz; referenced to δ 135.9 (C_5D_5N).

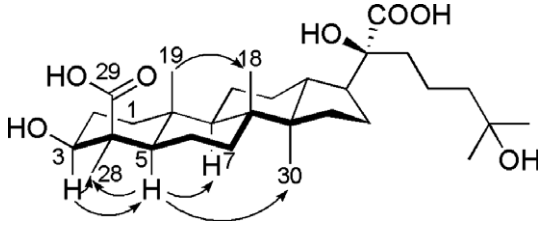

Fig. 3. Key NOESY relationships for compound **1a**.

L-rhamnose. By GC analysis of the acetate derivatives of the component monosaccharides, it was clear that **4** contained two units of D-glucose and one unit of L-rhamnose. Through an HMBC experiment, correlations were observed between H-1(δ 6.25, 1H, *d*, $J = 8.1$ Hz) of one glucose and C-21 (δ 176.4) of the aglycone as well as H-1 (δ 5.52, 1H, *d*, $J = 7.8$ Hz) of the other glucose and C-2 (δ 78.8) of the first glucose, and H-1 (δ 5.50, 1H, *br s*) of the rhamnose and C-6 (δ 68.2) of the first glucose. No cross-peaks were observed between saccharides and C-3 of the aglycone. Thus, compound **4** was elucidated as (20*S*)-3β,20-dihydroxydammar-24-en-21,29-dioic acid-21-*O*-[β-D-glucopyranoside(1 → 2)][α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranoside.

Compound **5**, an amorphous powder, displayed a sodiated molecular ion at *m/z* 1422.5213, corresponding to $[M\ (C_{65}H_{106}O_{32})+Na]^+$ in the HRESIMS spectrum. The ^{13}C NMR spectroscopic data were similar to that of compound **1** except for the presence of an aldehyde functionality (δ 206.7) instead of a carboxylic group (δ 177.5). This diagnostic signal exhibited a connectivity with the one proton singlet signal at δ 10.45 (*s*) in the HMQC spectrum, which in turn showed cross-peaks with C-3 (δ 85.9), C-4 (δ 49.8), C-5 (δ 58.2) and C-28 (δ 21.7) in the HMBC experiment. These observations clearly validated the presence of an aldehydic function at C-29. Hydrolysis of compound **1** yielded L-arabinose, L-rhamnose and D-glucose in a ratio of 1:2:3. Correlations from the HMBC experiment established the following linkages: H-1 (δ 4.90, 1H, *d*, $J = 5.0$ Hz) of the arabinose to C-3 (δ 85.9) of the aglycone; H-1 (δ 6.10, 1H, *br s*) of one rhamnose to C-2 (δ 74.7) of the arabinose; H-1 (δ 5.15, 1H, *d*, $J = 7.5$ Hz) of the first glucose to C-3 (δ 82.4) of the arabinose; H-1 (δ 5.51, 1H, *d*, $J = 7.5$ Hz) of the second glucose to C-2 (δ 78.6) of the first glucose; H-1 (δ 5.50, 1H, *br s*) of the other rhamnose to C-6 (δ 68.0) of the first glucose; and H-1 (δ 6.15, 1H, *d*, $J = 7.6$ Hz) of the third glucose to C-21 (δ 176.0) of the aglycone. Based on these facts, compound **5** was identified as (20*S*)-3β,20-dihydroxydammar-24-en-29-aldehyde-21-carboxylic acid-3-*O*-{[α-L- rhamnopyranosyl (1 → 2)]{[β-D-glucopyranosyl(1 → 2)][α-L- rhamnopyranosyl(1 → 6)]-β-D-glucopyranosyl(1 → 3)}-α-L-arabin-opyranosyl}-21-*O*-β-D-glucopyranoside.

The molecular formula of **6** was established as $C_{66}H_{110}O_{33}$ from the HRESIMS and ^{13}C spectroscopic data. Comparison of the ^1H and ^{13}C NMR spectra of **6** with **1** indicated that one carbonyl group of **1** was replaced

by a hydroxymethylene group. From the HMBC experiment, correlations were observed between the hydroxymethylene protons (δ 4.48, δ 4.65, 2H, each *m*) and C-3 (δ 90.2), C-4 (δ 50.6), C-5 (δ 57.8), and C-28 (δ 23.0). Thus, this hydroxymethylene group was assigned at C-29. The NMR and ESI-mass spectra indicated that compound **6** contained six hexose units. Hydrolysis of **6** yielded D-glucose and L-rhamnose in a ratio of 2:1. Therefore, compound **6** contained four D-glucose and two L-rhamnose. Through HMBC experiment results, the linkage sites and sequences of the saccharides and the aglycone were determined. Thus, compound **6** was elucidated as (20*S*)-3β,20,29-trihydroxydammar-24-en-21-carboxylic acid-3-*O*-{[α-L-rhamno-pyranosyl(1 → 2)]{[β-D-glucopyranosyl(1 → 2)]-[α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranosyl(1 → 3)}-β-D-glucopyranosyl}-21-*O*-β-D-glucopyranoside.

Comparison of the NMR spectroscopic data of **7** with **6** indicated that they had the same aglycone (Tables 1 and 2), with signals for the hydroxymethylene protons at (δ 4.46, δ 4.65) and the corresponding carbon C-29 at δ 63.4. The HRESIMS sodiated molecular ion appeared at *m*/*z* 1262.3967. Thus, compound **7** was determined to have the elemental composition $C_{59}H_{98}O_{27}$ (calcd. for $C_{59}H_{98}O_{27}Na$, 1262.3961 $[M+Na]^+$). Hydrolysis of **7** yielded L-arabinose, D-glucose and L-rhamnose in a ratio of 1:2:2. The linkage site and sequences of the saccharides and the aglycone were also determined using HMBC experiment results. Compound **7** was thus established as (20*S*)-3β,20,29-trihydroxydammar-24-en-21-carboxylic acid-3-*O*-{[α-L-rhamnopyranosyl(1 → 2)][α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyran-oside.

The HRESIMS sodiated molecular ion at *m*/*z* 1146.2364 of **8** established its molecular formula as $C_{53}H_{86}O_{25}$ (calcd. for $C_{53}H_{86}O_{25}Na$, 1146.2359 $[M+Na]^+$). The ^{13}C NMR spectroscopic data obtained for **8** was very similar to that of **1**, except for the side chain (C-22 to C-27). Moreover, it showed two methine olefinic carbon resonances instead of signals for one methine olefinic carbon and one quaternary olefinic carbon found in **1**. The two olefinic proton signals at δ 6.11 (1H, *d*, $J = 15.5$ Hz) and δ 6.21 (1H, *ddd*, $J = 15.5$, 8.5 and 5.5 Hz) indicated the presence of a *trans* double bond connected to a methylene group at δ 2.90 (1H, *dd*, $J = 5.5$ and 12.1 Hz) and δ 3.20 (1H, *dd*, $J = 8.5$ and 12.1 Hz) as suggested by spin-decoupling experiments. From the HMBC spectrum, one oxygen-bearing quaternary olefinic carbon signal at δ 70.6 was correlated with the hydrogen at δ 6.11 (1H, *d*, $J = 15.5$ Hz) and δ 1.60 (3H, *s*). According to literature (Yoshikawa et al., 1987), it could be judged that the olefinic functional group was at C-23 and C-24, and one hydroxyl functional group was at C-25. Hydrolysis of **8** yielded L-arabinose, D-glucose and L-rhamnose in a ratio of 1:2:1. The linkage site and sequences of the saccharides and the aglycone were also determined using an HMBC experiment. Based on the above results, compound **8** was identified as 20(*S*)-3β,20,25-trihydroxydammar-23-en-21,29-dioic acid-3-*O*-{[α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

3. Conclusions

As a result of this investigation, the structures of eight new compounds from *G. pubescens* were identified. Among them, **1**–**4**, **8** possess carboxylic groups at both C-21 and C-29, **5** contains a carboxylic group at C-21 and an aldehyde function at C-29, **6** and **7** contain a carboxylic group at C-21 and a hydroxymethylene group at C-29. This kind of aglycone is rare in the Cucurbitaceae family, which may be the reason why there is no correlation of these saponins with any biological activities. The significant structural differences between the saponins occurring in *G. pubescens* and other Gynostemma species can be used as a chemical basis to differentiate them and which may explain possible difference in pharmacological effects and medicinal usages.

4. Experimental

4.1. Material

All solvents used were of chemical grade (Shanghai Chemical Plant). TLC: precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant). Column chromatography (CC): silica gel (200–300 mesh), MCI Gel CHP20P (75–150 μm; Mitsubishi Kasei Chemical Industries), C18 reversed-phase silica gel (20–45 μm: Fuji Silysia Chemical Ltd.), or Sephadex LH-20 (Pharmacia); FC = flash chromatography. Optical rotations: Perkin–Elmer 341 polarimeter. NMR Spectra; Bruker AMX-500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C); conventional pulse sequences for NOESY, HMQC, and HMBC; 200 ms mixing time for NOESY; chemical shifts δ in ppm, *J* in Hz: (D5) pyridine solns. (δ (C) 150.3, 155.9, 123.9). HR-ESI-MS (positive mode). Bruker-Atex-III spectrometer. GC/EI-MS; Shimadzu GC–MS-QP5050A; db-lcolumn, 0.25 mm (i.d.) × 30 m; column temp 2000; carrier gas N_2, flow rate of 32.2 ml/min; EI-MS detector.

4.2. Plant material

The aerial parts of *G. pubescens* (Gagnep) C.Y. Wu were collected in Sichuan province, PR China in May, 2002. The plant was identified by Jin-Gui Shen and a voucher specimen (No. 2002004) deposited at the herbarium of Shanghai Research Center for Modernization of Traditional Chinese Medicine.

4.3. Extraction and isolation

The dried and powered aerial parts of *G. pubescens* (2.0 kg) were extracted successively with EtOH–H_2O (4:1, 3 × 5 l) at room temperature. Evaporation of the

EtOH–H_2O extract left a dark residue (80 g), which was subjected to silica gel (200–300 mesh) CC, eluted with $CHCl_3$–MeOH (100:10, 100:20, 100:30, 100:50, 100:100, and MeOH) to yield six fractions (A–F). Fraction B (5 g) was passed through a Sephadex LH-20 column and eluted with MeOH to give kaempferol (100 mg) and quercetin (80 mg). Fraction C (8 g) was subjected to MCI gel CHP 20P column chromatography, eluted with MeOH–H_2O (30:70, 40:60, 50:50, 60:40) to yield nine subfractions (C-1–9). Subfraction C-5 (1.5 g) was subjected to RP-18 flash CC, eluted with MeOH–H_2O (40:60) to afford compound **3** (72 mg). Fraction D (21 g) was subjected to MCI gel CHP 20P CC, eluted with MeOH–H_2O (30:70, 40:60, 50:50, 60:40, and 70:30) to yield five subfractions (D-1–5). Subfraction D-4 (4.6 g) was purified by RP-18 flash CC, eluted with MeOH–H_2O (50:50, 55:45, 60:40, 65:35, 70:30, and 75:25) to afford compound **4** (72 mg), **2** (91 mg) and rutin (34 mg). Fraction E (26 g) was subjected to MCI gel CHP 20P CC, eluted with MeOH–H_2O (40:60, 50:50, 55:45, 60:40, 65:35, and 70:30) to yield six subfractions (E-1–6). Subfraction E-2 (1.3 g) was purified by RP-18 flash CC, eluted with MeCN–H_2O (20:80, 22:78, 24:76, and 30:70) to afford compound **8** (160 mg) and **5** (80 mg). E-3 (1.2 g) was subjected to RP-18 flash CC. Elution with MeOH–H_2O (40:60, 50:50, 55:45, and 60:40) to afforded compound **6** (110 mg) and **7** (60 mg). E-4 (4.6 g) was purified by RP-18 flash CC, eluted with MeCN–H_2O (20:80, 25:75, 30:70 and 35:65) to afford compound **1** (300 mg).

*4.4. (20S)-3β,20-Dihydroxydammar-24-en-21,29-dioic acid-3-O-{[α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl -(1 → 3)]-α-L-arabinopyranosyl}-21-O-β-D-glucopyranoside (**1**)*

Amorphous powder, $[\alpha]_D^{23}$ +9.0 (*c* 0.30, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr} cm^{-1}: 3410, 2932, 1749, 1637, and 1069. HRESIMS: *m/z* 1130.2415 (calcd. for $[M+Na]^+$, 1130.2411). GC analysis of sugar components, t_R 7.62, 8.87, and 13.96 min.

*4.5. (20S)-3β,20-Dihydroxydammar-24-en-21,29-dioic acid-3-O-{[β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranosyl}-21-O-β-D-glucopyranoside (**2**)*

Amorphous powder, $[\alpha]_D^{23}$ +20.0 (*c* 0.19, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr} cm^{-1}: 3415, 2933, 1735, 1637, and 1072. HRESIMS: *m/z* 984.0953 (calcd. for $[M+Na]^+$, 984.0951). GC analysis of sugar components, t_R 7.64 and 13.95 min.

*4.6. (20S)-3β,20-Dihydroxydammar-24-en-21,29-dioic acid-3-O-[α-L-arabinopyranosyl]-21-O-β-D-glucopyranoside (**3**)*

Amorphous powder, $[\alpha]_D^{23}$ +26.0 (*c* 0.26, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr} cm^{-1}: 3419, 2937, 1716, 1635, and 1074. HRESIMS: *m/z* 821.9545 (calcd. for $[M+Na]^+$, 821.9541). GC analysis of sugar components, t_R 7.61 and 13.98 min.

*4.7. (20S)-3β,20-Dihydroxydammar-24-en-21,29-dioic acid-21-O-[β-D-glucopyranoside(1 → 2)][α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside (**4**)*

Amorphous powder, $[\alpha]_D^{23}$ +11.0 (*c* 0.16, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr} cm^{-1}: 3423, 2935, 1741, 1637, and 1074. HRESIMS: *m/z* 998.1218 (calcd. for $[M+Na]^+$, 998.1210). GC analysis of sugar components, t_R 8.88 and 13.99 min.

*4.8. (20S)-3β,20-Dihydroxydammar-24-en-29-aldehyde-21-carboxylic acid-3-O-{[α-L-rhamno-pyranosyl(1 → 2)]{[β-D-glucopyranosyl(1 → 2)][α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranosyl(1 → 3)}-α-L-arabinopyranosyl}-21-O-β-D-glucopyranoside (**5**)*

Amorphous powder, $[\alpha]_D^{23}$ −8.0 (*c* 0.28, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr} cm^{-1}: 3423, 2929, 1718, 1637, and 1072. HRESIMS: *m/z* 1422.5213 (calcd. for $[M+Na]^+$, 1422.5211). GC analysis of sugar components, t_R 7.60, 8.84, and 13.95 min.

*4.9. (20S)-3β,20,29-Trihydroxydammar-24-en-21-carboxylic acid-3-O-{[α-L-rhamnopyranosyl(1 → 2)]{[β-D-glucopyranosyl(1 → 2)][α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranosyl(1 → 3)}-β-D-glucopyranosyl}-21-O-β-D-glucopyranoside (**6**)*

Amorphous powder, $[\alpha]_D^{23}$ −3.0 (*c* 0.17, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr} cm^{-1}: 3415, 2933, 1743, 1637, and 1074. HRESIMS: *m/z* 1454.5635 (calcd. for $[M+Na]^+$, 1454.5631). GC analysis of sugar components, t_R 7.60, 8.89, and 13.97 min.

*4.10. (20S)-3β,20,29-Trihydroxydammar-24-en-21-carboxylic acid-3-O-{[α-L-rhamnopyranosyl(1 → 2)][α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranosyl}-21-O-β-D-glucopyran-oside (**7**)*

Amorphous powder, $[\alpha]_D^{23}$ −15.0 (*c* 0.15, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr} cm^{-1}: 3415, 2935, 1747, 1637, and 1074. HRESIMS: *m/z* 1262.3967 (calcd. for $[M+Na]^+$, 1262.3961). GC analysis of sugar components, t_R 7.61, 8.85, and 13.93 min.

*4.11. (20S)-3β,20,25-Trihydroxydammar-23-en-21,29-dioic acid-3-O-{[α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranosyl}-21-O-β-D-glucopyranoside (**8**)*

Amorphous powder, $[\alpha]_D^{23}$ −15.0 (*c* 0.15, MeOH). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. IR ν_{Max}^{KBr}

cm^{-1}: 3415, 2933, 1743, 1637, and 1070. HRESIMS: *m/z* 1146.2366 (calcd. for $[M+Na]^+$, 1146.2362). GC analysis of sugar components, t_R 7.61, 8.87, and 13.94 min.

4.12. Acid hydrolysis of compounds 1–8 (Ito et al., 2004)

Compounds **1**–**8** (4 mg each) in 10% HCl–dioxane (1:1, 1 ml) were individually heated at 80 °C for 4 h in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (1 ml × 3). After concentration, each H_2O layer (monosaccharide portion) was examined by TLC with $CHCl_3$–MeOH–H_2O (55:45:10) and compared with authentic samples.

4.13. Determination of sugar components

The monosaccharide subunits were obtained by hydrochloric acid hydrolysis as described above. The sugar residue was then dissolved in anhydrous pyridine (1 ml) under argon, following which L-leucine methyl ester hydrocloride (2 mg) was added, and the mixture was warmed at 60 °C for 1 h. $NaBH_4$ (2 mg) was next added and the mixture was stirred for 1 h at ambient temperature. For trimethylsilylation, trimethylchlorosilane (0.2 ml) (Shengyu Chemical Ltd., Shanghai, PR China) was added and incubated at 60 °C for 30 min. The leucine derivatives obtained were subjected to GC analysis (column temperature 200 °C; injection temperature 250 °C; carrier gas N_2 at flow rate of 32.2 ml/min) to identify the monosaccharides. Derivatives of D-glucose, L-arabinose, and L-rhamnose eluted at 13.95, 7.62, and 8.87 min, respectively.

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References

Attawish, A., Chivapat, S., Phadungpat, S., Bansiddhi, J., Techadamrongsin, Y., Mitrijit, O., 2004. Chronic toxicity of *Gynostemma pentaphyllum*. Fitoterapia 75, 539–551.

Blumert, M., Liu, J.L., 1999. Jiaogulan China's "immortality" herb. Badger. Torchlight Publishing Inc., USA.

Hu, L.H., Chen, Z.L., Xie, Y.Y., 1996. New triterpenoid saponins from *Gynostemma pentaphyllum*. J. Nat. Prod. 59, 1143–1145.

Hu, L.H., Chen, Z.L., Xie, Y.Y., 1997. Dammarane-type glycosides from *Gynostemma pentaphyllum*. Phytochemistry 44, 667–670.

Ito, A., Chai, H.B., Kardono, L.B.S., Setowati, F.M., Afriastini, J.J., Riswan, S., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M., Swanson, S.M., Kinghorn, A.D., . Saponins from the bark of *Nephelium maingayi*. J. Nat. Prod. 67, 201–205.

Jian Su, New Medical College, 1986. The Dictionary of Chinese Medicine (Zhong Yao Da Ci Dian). Shanghai Science and Technology Press, Shanghai, China.

La Cour, B., Molgaard, P., Yi, Z., 1995. Traditional Chinese medicine in treatment of hyperlipidaemia. J. Ethnopharmacol. 46, 125–129.

Li, L., Jiao, L., Lau, B.H., 1993. Protective effect of gypenosides against oxidative stress in phagocytes, vascular endothelial cells and liver microsomes. Cancer Biother. 8, 263–272.

Nagai, M., Nagumo, S., Izawa, K., Sakurai, N., Inoue, T., 1981. Two glycosides of a novel dammarane alcohol from *Gynostemma pentaphyllam*. Chem. Pharm. Bull. 29, 779–783.

Simone, F., Tommasi, N., Sonia, P., Cosimo, P., 1995. New dammarane-type glycosides from *Gynostemma pentaphyllum*. J. Nat. Prod. 58, 512–519.

Takemoto, T., Arihara, S., Nakajima, T., Okuhira, M., 1983a. Studies on the constituents of *Gynostemma pentaphyllum* MAKINO. I: Structures of gypenoside I–XIV. Yakugaku Zasshi 103, 173–185.

Takemoto, T., Arihara, S., Nakajima, T., Okuhira, M., 1983b. Studies on the constituents of *Gynostemma pentaphyllum* MAKINO. II: Structures of gypenoside XV–XXI. Yakugaku Zasshi 103, 1015–1023.

Takemoto, T., Arihara, S., Yoshikawa, K., Nakajima, T., Okuhira, M., 1984a. Studies on the constituents of Cucurbitaceae plants. VII: On the saponin constituents of *Gynostemma pentaphyllum* MAKINO (3). Yakugaku Zasshi 104, 325–331.

Takemoto, T., Arihara, S., Yoshikawa, K., Nakajima, T., Okuhira, M., 1984b. Studies on the constituents of Cucurbitaceae plants. VIII: On the saponin constituents of *Gynostemma pentaphyllum* MAKINO (4). Yakugaku Zasshi 104, 332–339.

Takemoto, T., Arihara, S., Yoshikawa, K., Nakajima, T., Okuhira, M., 1984c. Studies on the constituents of Cucurbitaceae plants. IX: On the saponin constituents of *Gynostemma pentaphyllum* MAKINO (5). Yakugaku Zasshi 104, 724–730.

Takemoto, T., Arihara, S., Yoshikawa, K., Nakajima, T., Okuhira, M., 1984d. Studies on the constituents of Cucurbitaceae plants X. On the saponin constituents of Gynostemma pentaphyllum MAKINO. (6). Yakugaku Zasshi 104, 939–945.

Takemoto, T., Arihara, S., Yoshikawa, K., Kawasaki, J., Nakajima, T., Okuhira, M., 1984e. Studies on the constituents of Cucurbitaceae plants. XI: On the saponin constituents of *Gynostemma pentaphyllum* MAKINO (7). Yakugaku Zasshi 104, 1043–1049.

Tanner, M.A., Bu, X., Steimle, J.A., Myers, P.R., 1999. The direct release of nitric oxide by gypenosides derived from the herb *Gynostemma pentaphylum*. Nitric Oxide 3, 359–365.

Yin, F., Hu, L.H., 2005. Six new triterpene saponins with a 21,23-lactone skeleton from *Gynostemma pentaphyllum*. Helv. Chim. Acta. 88, 1126–1134.

Yin, F., Hu, L.H., Pan, R.X., 2004a. Novel dammarane-type glycosides from *Gynostemma pentaphyllum*. Chem. Pharm. Bull. 52, 1440–1444.

Yin, F., Hu, L.H., Lou, F.C., Pan, R.X., 2004b. Dammarane-type glycosides from *Gynostemma pentaphyllum*. J. Nat. Prod. 67, 942–952.

Yin, F., Zhang, Y.N., Yang, Z.Y., Chen, Q.Q., Hu, L.H., 2006a. Triterpene saponins from *Gynostemma cardiospermum*. J. Nat. Prod. 69, 1394–1398.

Yin, F., Zhang, Y.N., Yang, Z.Y., Hu, L.H., 2006b. Nine new dammarane saponins from *Gynostemma pentaphyllum*. Chem. Biodivers. 3, 771–782.

Yoshikawa, K., Takemoto, T., Arihara, S., 1987. Studies on the constituents of Cucurbitaceae plants. XVI: On the saponin constituents of *Gynostemma pentaphyllum* MAKINO (11). Yakugaku Zasshi 107, 262–267.