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New triterpenoid saponins from the roots of *Gypsophila pacifica* Kom.

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ABSTRACT

Six new triterpenoid saponins (**1–6**) have been isolated from the roots of *Gypsophila pacifica* Kom. Their structures were established on the basis of extensive NMR (^{1}H, ^{13}C, TOCSY, HSQC, and HMBC) and ESIMS studies.

1. Introduction

The genus *Gypsophila*, a genus of the family Caryophyllaceae, comprises more than 150 species distributed throughout the world. Some of these species have long been used as pharmaceutical and ornamental plants.[1] *Gypsophila pacifica* Kom. is a small perennial herb widely distributed in the northeast regions of China. Its roots have been used as a substitute for the traditional Chinese medicine Yin-Chai-Hu (roots of *Stellaria dichotoma* var. Lanceolata Bge) to treat fever, consumptive disease, and infantile malnutrition syndrome.[2] A series of triterpenoid saponins have been reported from this plant during the 1960s.[3–5] Some triterpenoid saponins have been reported in our previous saponins investigation from this genus.[6] In our continuing search for triterpenoid saponins constituents, six new oleanane-type triterpenoid saponins were isolated from the roots of *G. pacifica*. The structural elucidation of these triterpenoid saponins was based on chemical methods and spectroscopic techniques.

2. Results and discussion

The 70% EtOH extract of the roots of *G. pacifica* was partitioned by water, EtOAc, and *n*-BuOH. The *n*-BuOH and H_2O soluble fractions were subjected to chromatographic purification over a silica gel column and repeated RP-C_{18} column, followed by prep-HPLC purification, respectively, to finally afford six new triterpenoid saponins (Chart 1).

Compound **1** was obtained as a white, amorphous powder. It was assigned the molecular formula $C_{74}H_{116}O_{39}$ from its negative-ion (HRESIMS *m/z* 1627.7022 $[M-H]^-$). On acid hydrolysis with 2 M HCl, **1** afforded the sugars and aglycone. The sugars were identified as D-glucuronic acid, D-galactose, D-xylose, D-fucose, L-rhamnose, and L-arabinose in the ratio of 1:1:2:1:1:2 based on GC–MS analysis of their chiral derivatives. And the aglycone was identified as gypsogenin by co-TLC comparison with standard sample, which was also confirmed on the basis of the ^{1}H and ^{13}C NMR (Table 1) referring to the reported data.[7] The downfield ^{13}C NMR chemical shift at δ_C 84.6 and the upfield ^{13}C NMR chemical shift at δ_C 176.3 suggested that **1** was a bidesmosidic saponin with glycosidic linkages at C-3 through an ether bond and at C-28 through an ester bond.

The anomeric proton signals at δ_H 6.45 (s), 6.01 (d, J = 8.2 Hz), 5.56 (d, J = 7.7 Hz), 5.34 (d, J = 7.7 Hz), 5.17 (d, J = 7.2 Hz), 5.04 (d, J = 7.0 Hz), 4.98 (d, J = 7.8 Hz), and 4.82 (d, J = 7.5 Hz) displayed the correlations with anomeric carbon signals at δ_C 101.1, 94.5, 104.0, 104.8, 105.0, 106.8, 106.7, and 103.8 in HSQC spectrum, respectively, which showed that **1** contained eight sugar units. The ^{1}H and ^{13}C NMR data (Table 2) of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the TOCSY, HSQC, and HMBC spectra obtained for this compound. The β-anomeric configurations for the D-glucuronic acid, D-fucose, D-galactose and D-xylose units, and the α-anomeric configurations for L-arabinose were determined by their large $^3J_{H1,H2}$ coupling constants at 7–8 Hz. And the α-anomeric configuration of L-rhamanose was judged by its C-5 (δ_C 67.8).[6] The sequence of the sugar residues was subsequently determined by HMBC experiments. The linkage of the sugar units at C-3 of the aglycone was established from the following HMBCs: H-1 of galactose (δ_H 5.56) with C-2 of glucuronic

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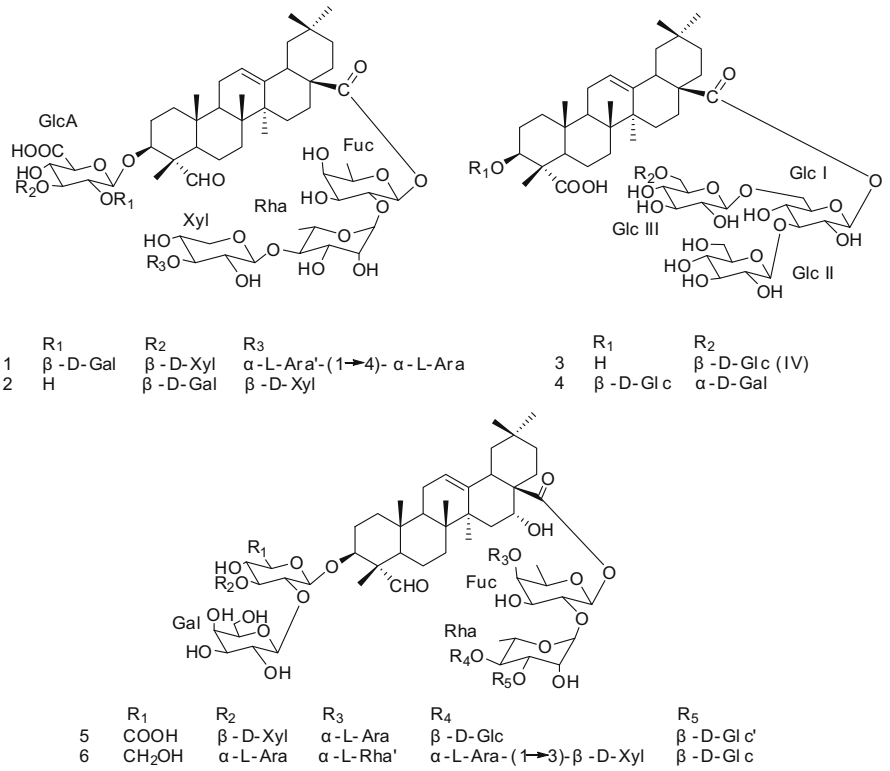

Chart 1. Structures of triterpenoid saponins **1**–**6**.

Table 1
^{13}C NMR (150 MHz, C_5D_5N) data for aglycone moieties of compounds **1–6**

Carbon	1	2	3	4	5	6
1	37.9	37.8	38.7	38.5	38.3	37.9
2	25.0	25.0	23.7	23.6	25.5	24.9
3	84.6	84.5	75.2	84.8	84.6	84.6
4	54.9	54.9	53.3	53.2	55.3	55.1
5	48.3	47.5	52.0	51.9	49.0	48.1
6	20.4	20.5	23.2	22.8	20.7	20.5
7	32.4	32.1	32.8	32.6	30.9	31.6
8	39.9	39.9	40.1	40.0	40.5	40.1
9	47.6	47.0	48.2	48.0	49.4	49.1
10	36.0	36.0	36.6	36.5	36.4	36.0
11	23.0	23.0	23.7	23.1	23.1	23.5
12	122.2	122.5	122.7	122.6	121.8	122.3
13	143.9	143.9	144.1	144.0	144.4	144.4
14	42.0	42.0	42.0	41.9	41.8	41.3
15	28.3	28.3	28.2	28.1	36.1	36.1
16	23.5	23.4	21.2	21.2	74.3	74.2
17	46.8	46.8	47.0	46.8	47.7	47.2
18	41.8	41.8	41.6	41.5	42.3	41.9
19	46.1	46.1	46.0	45.9	47.1	46.7
20	30.5	30.5	30.7	29.9	30.1	30.6
21	33.5	33.7	33.8	33.7	32.3	34.2
22	32.1	32.3	32.4	32.2	31.8	32.6
23	210.1	210.2	181.1	181.6	210.4	209.6
24	10.8	11.0	12.7	12.6	11.3	10.7
25	15.5	15.6	16.0	15.9	14.5	14.1
26	17.2	17.2	17.3	17.2	17.7	17.2
27	25.7	25.7	26.1	26.0	27.4	26.9
28	176.3	176.4	176.1	176.1	176.3	175.8
29	32.9	32.9	33.1	32.9	33.3	33.0
30	23.5	23.4	23.6	23.1	23.9	24.3

acid (δ_C 78.4), H-1 of xylose (δ_H 5.34) with C-3 of glucuronic acid (δ_C 86.0), and H-1 of glucuronic acid (δ_H 4.88) with C-3 of the aglycone (δ_C 84.6). Similarly, the sugar chain at C-28 was also established from the following HMBCs: H-1 of arabinose′ (δ_H 5.04) with C-4 of arabinose (δ_C 78.1), H-1 of arabinose (δ_H 5.17) with C-3 of xylose (δ_C 86.3), H-1 of xylose (δ_H 4.98) with C-4 of rhamnose (δ_C 85.4), H-1 of rhamnose (δ_H 6.45) with C-2 of fucose (δ_C 73.7), and H-1 of fucose (δ_H 6.01) with C-28 of the aglycone (δ_C 176.3) (Fig. 1). On the basis of the data obtained, the structure of **1** was established as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl gypsogenin 28-*O*-α-L-arabinopyranosyl-(1→4)-α-Larabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester.

Compound **2**, a white amorphous powder, exhibited in HRESIMS (*m*/*z* 1387.6192 $[M+Na]^+$), which was consistent with the molecular formula of $C_{64}H_{100}O_{31}$. Acid hydrolysis afforded gypsogenin and the monosaccharide components were identified as D-glucuronic acid, D-galactose, D-fucose, L-rhamnose, and D-xylose (1:1:1:1:2) by GC–MS. Similar to **1**, the aglycone of **2** was also determined to be gypsogenin with sugar linkages at positions C-3 and C-28 on the basis of the ^{1}H-, ^{13}C NMR (Table 1). The NMR data (Table 3) assignments of sugar moieties were accomplished by a combination of TOCSY, HSQC, and HMBC experiments. The exact linkage for the sugar units of **2** was established using the HMBC spectrum. From the long-range correlations H-1 (δ_H 5.43) of xylose′ and C-4 (δ_C 87.3) of xylose, H-1 (δ_H 4.97) of xylose and C-4 (δ_C 86.7) of rhamnose, H-1 (δ_H 6.38) of rhamnose and C-2 (δ_C 73.6) of fucose, and H-1 (δ_H 5.97) of fucose and C-28 (δ_C 176.4) of the aglycone, it was deduced that oligosaccharide at C-28 was consistent with the data in the literature[8] as well. The sequence of the sugar chain at C-3 of the aglycone was also established by a combination of H-1 (δ_H 5.13) of galactose and C-3 (δ_C 87.7) of glucuronic acid, and H-1 (δ_H 4.65) of glucuronic acid and C-3 (δ_C 84.6) of the aglycone in HMBC spectrum. From the above evidences, the structure of **2** was elucidated as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyl gypsogenin 28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester.

The HRESIMS of compound **3** showed a quasimolecular ion peak at *m*/*z* 1157.5367 $[M+Na]^+$, compatible with the molecular formula

Table 2
^{13}C and ^{1}H NMR data for sugar moieties of **1**, **5,** and **6** (C_5D_5N)

Position	**1**			**5**			**6**	
	δ_C	δ_H (multi, *J* in Hz)		δ_C	δ_H (multi, *J* in Hz)		δ_C	δ_H (multi, *J* in Hz)
3-O-Sugars			3-O-Sugars			3-O-Sugars		
GlcA			GlcA			Glc		
1	103.8	4.82 (d, 7.5)	1	103.7	4.93 (d, 7.5)	1	103.4	5.45 (d, 7.8)
2	78.4	4.33	2	78.2	4.37	2	78.1	4.31
3	86.0	4.21	3	86.2	4.28	3	85.9	4.14
4	71.5	4.45	4	71.2	4.42	4	71.3	4.02
5	77.1	4.64	5	77.2	4.54	5	75.6	3.97
6	171.7		6	172.4		6	62.5	4.46,4.30
Gal			Gal			Gal		
1	104.0	5.56 (d, 7.7)	1	104.2	5.56(d, 7.6)	1	103.9	5.58 (d, 7.6)
2	73.7	4.47	2	73.5	4.49	2	73.5	4.46
3	75.2	4.14	3	75.4	4.19	3	75.2	4.23
4	70.0	4.56	4	70.4	4.58	4	70.8	4.60
5	76.3	3.98	5	76.4	4.07	5	76.2	3.97
6	61.4	4.52,4.40	6	61.3	4.45	6	61.7	4.53,4.40
Xyl			Xyl			Ara		
1	104.8	5.34 (d, 7.7)	1	105.3	5.34 (d, 7.7)	1	105.1	5.21 (d, 7.2)
2	75.1	3.96	2	75.2	3.98	2	72.3	4.44
3	78.3	4.13	3	78.5	4.13	3	74.8	4.15
4	70.7	4.13	4	70.7	4.06	4	68.3	4.24
5	67.0	4.23,3.70	5	67.2	4.17,3.68	5	67.8	4.26,3.45
28-O-Sugars			28-O-Sugars			28-O-Sugars		
Fuc			Fuc			Fuc		
1	94.5	6.01 (d, 8.2)	1	95.3	6.03 (d, 8.2)	1	94.7	6.08 (d, 8.2)
2	73.7	4.67	2	73.8	4.62	2	73.7	4.69
3	76.4	4.17	3	76.6	4.16	3	75.9	4.17
4	72.9	3.96	4	82.1	3.97	4	82.3	3.96
5	72.3	3.89	5	72.4	3.93	5	71.4	3.91
6	16.6	1.48 (d, 6.0)	6	16.9	1.71 (d, 6.2)	6	15.6	1.71 (d, 6.3)
Rha			Rha			Rha		
1	101.1	6.45 (s)	1	102.0	6.07 (s)	1	101.3	6.32 (s)
2	71.5	4.82	2	71.2	4.72	2	71.7	4.82
3	72.1	4.64	3	82.7	4.68	3	83.0	4.52
4	85.4	4.30	4	78.1	4.34	4	78.4	4.36
5	67.8	4.42	5	68.7	4.45	5	68.7	4.47
6	18.2	1.65 (d,4.5)	6	18.1	1.76 (d, 6.1)	6	18.8	1.76 (d, 6.2)
Xyl			Glc			Xyl		
1	106.7	4.98 (d, 7.8)	1	105.1	5.49 (d, 7.7)	1	106.2	5.14 (d, 7.8)
2	75.3	3.97	2	75.3	4.06	2	75.4	3.92
3	86.3	4.03	3	78.6	4.15	3	86.4	3.95
4	68.6	4.08	4	71.4	4.66	4	69.1	4.05
5	67.0	4.23,3.44	5	78.2	3.94	5	66.8	4.14,3.70
			6	62.7	4.50,4.30			
Ara			Glc′			Glc		
1	105.0	5.17 (d, 7.2)	1	105.2	5.34 (d, 7.8)	1	104.7	5.32 (d, 7.8)
2	73.2	4.43	2	75.5	4.06	2	75.1	3.90
3	74.0	4.23	3	78.6	4.16	3	78.4	4.10
4	78.1	4.27	4	71.5	4.02	4	71.7	3.98
5	66.3	4.47,3.79	5	78.3	3.93	5	78.2	3.90
			6	62.5	4.51,4.24	6	62.5	4.46,4.30
Ara′			Ara			Ara		
1	106.8	5.04 (d, 7.0)	1	102.9	5.46 (d, 7.0)	1	106.6	5.02 (d, 7.8)
2	72.9	4.47	2	73.8	4.52	2	71.8	4.44
3	74.3	4.12	3	74.7	4.24	3	74.8	4.15
4	69.4	4.23	4	70.0	4.35	4	69.8	4.24
5	66.7	4.23,3.70	5	67.2	4.26,3.44	5	67.8	4.26,3.45
						Rha′		
						1	103.4	4.90 (s)
						2	73.9	4.88
						3	72.6	4.72
						4	73.4	4.57
						5	72.6	4.53
						6	16.9	1.69 (d, 6.3)

$C_{54}H_{86}O_{25}$. On acid hydrolysis, **3** afforded gypsogenic acid as the aglycone[8] (Table 1), and D-glucose as component sugars by GC–MS. The long-range correlations between δ_H 1.54 (H-24) and δ_C 181.1 indicated that δ_C 181.1 belonged to C-23 of the aglycone, so δ_C 176.1 belonged to C-28. The chemical shifts of δ_C 176.1 (C-28) revealed that the sugar chain was attached to C-28 of the aglycone. The sequences of the tetrasaccharide unit and sugar–aglycone linkage were determined from the HMBC spectrum of **3**, which afforded key ^{1}H–^{13}C long-range correlations between H-1 of Glc I (δ_H 6.18) and C-28 (δ_C 176.1) of the aglycone, H-1 (δ_H

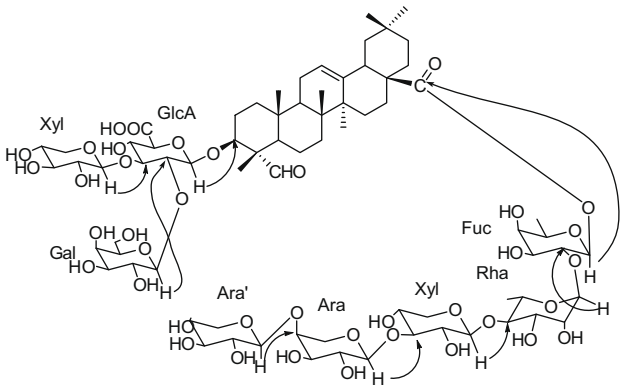

Figure 1. Key HMBCs of **1**.

5.24) of Glc II and C-3 (δ_C 88.4) of Glc I, H-1 (δ_H 4.98) of Glc III and C-6 (δ_C 69.0) of Glc I, H-1 (δ_H 5.07) of Glc IV and C-6 (δ_C 68.9) of Glc III, respectively. Accordingly, compound **3** was elucidated as gypsogenic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl ester.

Compound **4** was obtained as an amorphous powder. The molecular formula of **4** was determined to be $C_{60}H_{96}O_{30}$ from data of the HRESIMS (*m/z* 1319.5914 $[M+Na]^+$). ^1H and ^{13}C NMR spectra indicated that compound **4** had the same aglycone to **3**. The chemical shifts of δ_C 84.8 (C-3) and δ_C 176.1 (C-28) revealed that compound **4** was a bidesmosidic glycoside. Among the five sugar units in the molecule, four were identified as D-glucose, and one as D-galactose by GC–MS after acid hydrolysis of **4**. The β anomeric configurations for the glucose units were determined from their $^3J_{H1,H2}$ coupling constants (7–8 Hz), and the galactose unit was determined to be of a α-configuration based on the $^3J_{H1,H2}$ (3.5 Hz) values observed.[6] The HMBC spectrum of **4** showed correlations between H-1 (δ_H 5.42) of Gal-1 and C-6 (δ_C 68.2) of Glc III-6, between H-1 (δ_H 5.03) of Glc III-1 and C-6 (δ_C 68.5) of Glc I-6, and between H-1 (δ_H 5.24) of Glc II-1 and C-3 (δ_C 88.2) of Glc-6, which suggested the linkage of C-1 of Glc I to C-28 of the aglycone, C-1 of Glc II to C-3 of Glc I, C-1 of Glc III to C-6 of Glc I, and C-1 of Gal to C-6 of Glc III, respectively. The sequence of the sugar chain at C-3 of the aglycone was also established by a combination of δ_H 5.22 (H-1 of Glc) and δ_C 84.8

Table 3
^{13}C and ^1H NMR data for sugar moieties of **2**–**4** (C_5D_5N)

Position	**2**			**3**			**4**	
	δ_C	δ_H (multi, *J* in Hz)		δ_C	δ_H (multi, *J* in Hz)		δ_C	δ_H (multi, *J* in Hz)
3-O-Sugars			28-O-Sugars			3-O-Sugars		
GlcA			Glc I			Glc		
1	104.6	4.65 (d, 7.5)	1	95.0	6.18 (d, 7.8)	1	105.2	5.22 (d, 7.8)
2	79.4	4.26	2	72.6	4.09	2	75.1	4.01
3	87.7	4.17	3	88.4	4.19	3	78.3	4.19
4	73.8	4.51	4	68.8	4.28	4	71.9	4.14
5	79.2	4.43	5	78.2	3.88	5	78.2	3.91
6	172.3		6	69.0	4.57,4.32	6	62.7	4.51,4.41
						28-O-Sugars		
Gal			Glc II			Glc I		
1	106.0	5.13 (d, 7.5)	1	105.7	5.24 (d, 7.6)	1	94.9	6.19 (d, 8.0)
2	74.8	4.43	2	75.4	3.97	2	72.1	4.09
3	75.7	4.14	3	78.3	4.14	3	88.2	4.24
4	71.5	4.54	4	71.6	4.19	4	68.5	4.25
5	77.9	3.92	5	78.6	3.94	5	77.1	4.03
6	63.0	4.39	6	62.8	4.44,4.24	6	68.5	4.55,4.24
28-O-Sugars								
Fuc			Glc III			Glc II		
1	95.9	5.97 (d, 8.2)	1	105.3	4.98 (d, 7.8)	1	105.7	5.24 (d, 7.8)
2	73.6	4.64	2	75.2	3.96	2	75.5	4.02
3	77.5	4.13	3	78.4	4.18	3	78.3	4.18
4	74.4	3.94	4	71.5	4.00	4	71.9	4.13
5	73.6	3.87	5	77.6	3.84	5	78.2	3.98
6	18.1	1.47 (d, 6.2)	6	68.9	4.55,4.28	6	62.4	4.52,4.39
Rha			Glc IV			Glc III		
1	102.5	6.38 (s)	1	105.3	5.07 (d, 7.8)	1	105.0	5.03 (d, 7.8)
2	73.0	4.79	2	75.6	3.99	2	75.0	3.99
3	73.7	4.64	3	78.4	4.14	3	78.2	4.12
4	86.7	4.26	4	71.6	4.17	4	71.4	3.92
5	69.4	4.42	5	78.4	3.90	5	75.9	4.09
6	19.7	1.66 (d, 6.3)	6	62.7	4.44,4.24	6	68.2	4.46,4.34
Xyl						Gal		
1	108.8	4.97 (d, 7.6)				1	100.5	5.42 (d, 3.5)
2	76.5	3.99				2	70.5	4.69
3	87.3	4.03				3	71.4	4.58
4	71.0	4.13				4	70.9	4.62
5	68.4	4.17,3.46				5	72.6	4.583
						6	62.5	4.45,4.32
Xyl′								
1	105.1	5.43 (d, 7.2)						
2	76.2	3.96						
3	79.8	4.14						
4	70.4	4.04						
5	68.6	4.27,3.74						

(C-3) of the aglycone. On the basis of the above results, compound **4** is 3-*O*-β-D-glucopyranosyl gypsogenic acid 28-*O*-α-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl ester.

Compound 5 had a molecular composition $C_{76}H_{120}O_{42}$ as determined from HRESIMS analysis (quasimolecular ions at *m/z* 1727.7213 $[M+Na]^+$). Acid hydrolysis of **5** yielded quillaic acid, and D-glucuronic acid, D-galactose, D-xylose, D-fucose, L-rhamnose, L-arabinose, and D-glucose (1:1:1:1:1:1:2) as component sugars. Detailed NMR analysis established the aglycone to be quillaic acid[9] (Table 1). The ^1H and ^{13}C NMR data showed eight anomeric proton signals at δ_H 6.07 (s), 6.03 (d, *J* = 8.2 Hz), 5.56(d, *J* = 7.6 Hz), 5.49 (d, *J* = 7.7 Hz), 5.46 (d, *J* = 7.0 Hz), 5.34 (d, *J* = 7.7 Hz), 5.34 (d, *J* = 7.8 Hz), and 4.93 (d, *J* = 7.5 Hz) and the corresponding anomeric carbon signals at δ_C 95.3, 102.0, 104.2, 105.1, 102.9, 105.3, 105.2, and 103.7. The ^1H and ^{13}C NMR chemical shift assignments were accomplished by a combination of HSQC, TOCSY, and HMBC experiments. It was evident that **5** had the same trisaccharides linked to C-3 of the aglycone as in **1**, as the expected sequence correlations were observed. The linkage of the remaining five sugars at C-28 was determined from the following HMBCs. The long-range correlations observed in the HMBC spectrum between the ^1H NMR resonances at δ_H 5.46 (H-Ara-1) and the ^{13}C NMR resonances at δ_C 82.1 (C-Fuc-4), between δ_H 6.07 (H-Rha-1) and δ_C 73.8 (C-Fuc-2), between δ_H 5.33 (H-Glc-1) and δ_C 82.7 (C-Rha-3), between δ_H 5.49 (H-Glc′-1) and δ_C 78.1 (C-Rha-4), and between δ_H 6.03 (H-Fuc-1) and δ_C 176.3 (C-28), respectively, showed that the pentosaccharide residue *O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→4)]-β-D-fucopyranosyl was linked to the quillaic acid unit at C-28. On the basis of all the foregoing evidences, **5** was elucidated as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→4)]-β-D-fucopyranosyl ester.

Compound **6** as amorphous powder possessed the molecular formula $C_{81}H_{130}O_{44}$, as determined by HRESIMS in the positive-ion mode (HRESIMS *m/z* 925.3957 $[M+HCOOH-2H]^{2-}$). Acid hydrolysis of **6** afforded D-glucose, D-galactose, D-xylose, D-fucose, L-rhamnose, and L-arabinose in the ratio of 2:1:1:1:2:2 by GC–MS. The downfield chemical shifts of C-3 and upfield chemical shifts of C-28 of the aglycone and HMBCs revealed that **6** were bidesmosidic glycosides. The positions of the sugar residues were unambiguously defined by the HMBC experiment. Across peaks due to long-range correlations between C-3 (δ_C 84.6) of the aglycone and H-1 of Glc (δ_H 5.45), C-2 (δ_C 78.1) of Glc and H-1 of Gal (δ_H 5.58), and C-3 (δ_C 85.9) of Glc and H-1 of Ara (δ_H 5.21) indicated that the trisaccharide moiety of **6** attached to C-3 was established to be β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyl. Similarly, the hexasaccharide moiety of **6** attached to C-28 was established to be α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-fucopyranosyl. Therefore, the structure 3-*O*-β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyl quillaic acid 28-*O*-α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-fucopyranosyl ester was assigned to **6**.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO P-1020 polarimeter. IR (KBr-disks) spectra were recorded by Brucker Tensor 27 spectrometer. 1D and 2D NMR spectra were recorded at 300 K on Bruker, ACF-500 NMR instrument (^1H: 500 MHz, ^{13}C: 125 MHz), with TMS as internal standard. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESIMS) and a Micro Q-TOF MS (HRESIMS), respectively. TLC was performed on precoated silica gel G (Qingdao Haiyang Chemical Co. Ltd) and detection was achieved by 15% H_2SO_4–EtOH for saponins, and aniline–phthalate reagents for sugars. Sephadex LH-20 (Pharmacia) and RP-C_{18} (40–63 μm, Fuji) were used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-C_{18} column (200 × 20 mm i.d.) and 1100 Series Multiple Wavelength detector.

3.2. Plant material

The roots of *G. pacifica* were collected from Xifeng region, Liaoning Province, China, in October 2005. The botanical origin of material was identified by Professor Minjian Qin, Department of Medicinal Plants, China Pharmaceutical University, and the voucher specimens (No. 051020) were deposited at the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China.

3.3. Extraction and isolation

The roots of *G. pacifica* (8.9 kg) were ground into powders, and then extracted with 70% aqueous ethanol (v/v) three times (10 L, 2 h each) under reflux. After evaporation, the residue was suspended in water and partitioned by EtOAc, *n*-BuOH, and water. The *n*-BuOH-soluble portion (268 g) was fractionated by MCI gel, which was eluted with MeOH/H_2O (0:10, 3:7, 1:1, 7:3, and 10:0) to give five fractions (fractions 1–5); fractions 3 (MeOH/H_2O, 1:1) and 4 (MeOH/H_2O, 7:3) were further subjected to repeated RP-C_{18} column with MeOH/H_2O (4:6→9:1) and then the eluents of fraction 3.4 (MeOH/H_2O, 7:3) were further separated by HPLC (MeCN–0.05% TFA in H_2O, 32:68, UV detection at 210 nm), to yield pure **1** (5.6 mg, t_R = 14.5 min), and **2** (13 mg, t_R = 26.2 min), respectively. The eluents of fraction 4.4 (MeOH/H_2O, 7:3) were subjected to a silica gel column (200–300 mesh), which was eluted with $CHCl_3$/MeOH/H_2O (7:3:0.5) to give **3** (18 mg) and **4** (4.2 mg). The part of H_2O-soluble portion (50 g) was fractionated by MCI gel, which was eluted with MeOH/H_2O (0:10, 3:7, and 10:0) to give three fractions (fractions 1–3). Fraction 2 (3:7 MeOH/H_2O) was subjected to a silica gel column (100–200 mesh), which was eluted with $CHCl_3$/MeOH (1:1) and 100% MeOH to give fraction 2.1 and fraction 2.2. Fraction 2.2 (100% MeOH) was further separated by HPLC (MeCN–0.05% TFA in H_2O, 26: 74, UV detection at 210 nm) to yield pure **5** (3.7 mg, t_R = 18.0 min) and **6** (3.2 mg, t_R = 21.2 min), respectively.

3.3.1. Compound 1

White powder, $[\alpha]_D^{25}$ +6.5 (*c* 0.14; C_5H_5N); IR (KBr)ν_{max}: 3429, 2932, 1740, 1656, 1067 cm^{-1}. ^1H NMR (500 MHz, C_5D_5N) δ_H 0.74, 0.84, 0.86, 1.08, 1.23, 1.40 (3H each s, Me-25, 30, 29, 26, 27, 24), 3.12 (1H, br d, *J* = 10.8 Hz, 18-H), 4.00 (1H, m, 3-H), 5.38 (1H, br s, 12-H), 9.95 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^1H and ^{13}C NMR data of glycosidic part, see Table 2. ESIMS *m/z*: 1627 $[M-H]^-$, HRESIMS *m/z*: 1627.7022 $[M-H]^-$ (calcd for $C_{74}H_{116}O_{39}$, 1627.7020).

3.3.2. Compound 2

White powder, $[\alpha]_D^{25} \approx 0$ (*c* 0.20; C_5H_5N); IR (KBr)ν_{max}: 3403, 2945, 1739, 1676, 1062 cm^{-1}. ^1H NMR (500 MHz, C_5D_5N) δ_H 0.73, 0.84, 0.87, 1.01, 1.22, 1.38 (3H each, s, Me-25, 30, 29, 26, 27, 24), 3.09 (1H, m, 18-H), 3.96 (1H, m, 3-H), 5.39 (1H, br s, 12-H), 9.88 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^1H and ^{13}C NMR

data of glycosidic part, see Table 3. ESIMS *m/z*: 1363 $[M-H]^-$, HRESIMS *m/z*: 1387.6192 $[M+Na]^+$ (calcd for $C_{64}H_{100}O_{31}Na$, 1387.6141).

3.3.3. Compound 3

White powder, $[\alpha]_D^{25}$ +7.7 (*c* 0.20; C_5H_5N); IR(KBr)ν_{max}: 3410, 2931, 1738, 1649, 1064 cm^{-1}. ^1H NMR (500 MHz, C_5D_5N) δ_H 0.82, 0.83, 0.88, 1.04, 1.16, 1.54 (3H each, s, Me-30, 29, 25, 26, 27, 24), 3.16 (1H, d, *J* = 9.6 Hz, 18-H), 3.99 (1H, m, 3-H), 5.41 (1H, br s, 12-H); ^{13}C NMR data of the aglycone, see Table 1. ^1H and ^{13}C NMR data of glycosidic part, see Table 3. ESIMS *m/z*: 1133 $[M-H]^-$, HRESIMS *m/z*: 1157.5367 $[M+Na]^+$ (calcd for $C_{54}H_{86}O_{25}Na$, 1157.5350).

3.3.4. Compound 4

White powder, $[\alpha]_D^{25}$ +18.1 (*c* 0.14; C_5H_5N); IR(KBr)ν_{max}: 3427, 2923, 1748, 1660, 1076 cm^{-1}. ^1H NMR (500 MHz, C_5D_5N) δ_H 0.85, 0.87, 0.93, 1.04, 1.20, 1.34 (3H each, s, Me-30, 29, 25, 26, 27, 24), 3.17 (1H, m, 18-H), 4.02 (1H, m, 3-H), 5.41 (1H, m, 12-H); ^{13}C NMR data of the aglycone, see Table 1. ^1H and ^{13}C NMR data of glycosidic part, see Table 3. ESIMS *m/z*: 1295 $[M-H]^-$, HRESIMS *m/z*: 1319.5914 $[M+Na]^+$ (calcd for $C_{60}H_{96}O_{30}Na$, 1319.5879).

3.3.5. Compound 5

White powder, $[\alpha]_D^{25}$ +5.8 (*c* 0.14; C_5H_5N); IR(KBr)ν_{max}: 3431, 2941, 1724, 1653, 1065 cm^{-1}. ^1H NMR (500 MHz, C_5D_5N) δ_H 0.86, 0.93, 0.97, 1.08, 1.46, 1.75 (3H each, s, Me-25, 29, 30, 26, 24, 27), 3.32 (1H, m, 18-H), 4.00 (1H, m, 3-H), 5.25 (1H, br s, 16-H), 5.37 (1H, br s, 12-H), 9.90 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^1H and ^{13}C NMR data of glycosidic part, see Table 2. ESIMS *m/z*: 1703 $[M-H]^-$, HRESIMS *m/z*: 1727.7213 $[M+Na]^+$ (calcd for $C_{76}H_{120}O_{42}Na$, 1727.7146).

3.3.6. Compound 6

White powder, $[\alpha]_D^{25}$ +5.1 (*c* 0.24; C_5H_5N); IR(KBr)ν_{max}: 3428, 2937, 1729, 1662, 1069 cm^{-1}. ^1H NMR (500 MHz, C_5D_5N) δ_H 0.82, 0.95, 0.98, 1.06, 1.44, 1.74 (3H each, s, Me-25, 29, 30, 26, 24, 27), 3.34 (1H, t-like, 18-H), 4.04 (1H, m, 3-H), 5.23 (1H, br s, 16-H), 5.39 (1H, br s, 12-H), 9.73 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^1H and ^{13}C NMR data of glycosidic part, see Table 2. ESIMS *m/z*: 1805 $[M-H]^-$, HRESIMS *m/z*: 925.3957 $[M+HCOOH-2H]^{2-}$ (calcd for $C_{82}H_{130}O_{46}$, 925.3922).

3.4. Acid hydrolysis of 1–6 and determination of absolute configuration of monosaccharides[6]

Compound **1** (4 mg) was treated with 2 M HCl (4 ml) at 90 °C for 2 h. The reaction mixture was extracted with $CHCl_3$ (3 × 5 ml). The $CHCl_3$ extract was purified by chromatography on Sephadex LH-20 (2.0 × 100 cm). Comparing TLC with authentic samples, the aglycone of **1** was determined as gypsogenin. Acid hydrolysis of **2–6** was operated likewise, and the aglycone of **2** was determined to be gypsogenin, while that of **3–4** was gypsogenic acid and that of of **5–6** was quillaic acid.

Each remaining aqueous layer was concentrated to dryness to give a residue and was dissolved in pyridine (2 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution. The mixture was heated at 60 °C for 1 h, and trimethylchlorosilane (0.5 ml) was added, followed by heating at 60 °C for 30 min. Then, the solution was concentrated to dryness and taken up in water (1 ml × 3), followed by extraction with *n*-hexane (1 ml × 3). The supernatant was subjected to GC/MS analysis under the following conditions: Varian CP-3800 Gas Chromatograph equipped with a Saturn 2200 Mass detector (detection temperature 220 °C). Column: CP-sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 μm). Column temperature: 150–260 °C with the rate of 8 °C/min, and the carrier gas was He (0.8 ml/min), split ratio 1/10, injection temperature: 250 °C. Injection volume: 0.5 μl. The absolute configurations of the monosaccharides were confirmed to be L-arabinose, D-fucose, L-rhamnose, D-xylose, D-glucuronic acid, D-galactose, and D-glucose by comparison of the retention times of monosaccharide derivatives with those of standard samples: L-arabinose (12.67 min), D-fucose(12.85 min), L-rhamnose (12.67 min), D-xylose (11.89 min), D-galactose (14.32 min), and D-glucose (14.01 min), respectively.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.08.015.

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