Triterpenoid Saponins from Roots of *Gypsophila pacifica*

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Abstract: Objective To study the chemical constituents from the roots of *Gypsophila pacifica*. **Methods** The chemical constituents were isolated by various column chromatographic methods and their structures were identified by spectral data together with physicochemical analysis. **Results** Five compounds were isolated and identified as 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucuronopyranosyl gypsogenin 28-*O*-β-*D*-xylopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside (**1**), 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-β-*D*-xylopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside (**2**), 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucuronopyranosyl gypsogenin 28-*O*-β-*D*-glucopyranosyl-(1→3)-[β-*D*-xylopyranosyl-(1→4)]-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside (**3**), 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*-xylopyranosyl-(1→4)]-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside (**4**), 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-α-*L*-arabinopyranosyl-(1→4)-α-*L*-arabinopyranosyl-(1→3)-β-*D*-xylopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside (**5**). **Conclusion** The five compounds are isolated from this plant for the first time.

Key words: Caryophyllaceae; *Gypsophila pacifica*; triterpenoid saponins
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Introduction

Gypsophila pacifica Kom. is a small perennial herb in family Caryophyllaceae, widely distributed in the Northeast regions of China (Lu, 1994). Its roots have been used as a substitute for the traditional Chinese medicine, roots of *Stellaria dichotoma* L. var. *lanceolata* Bge. (*Yinchaihu*) to treat fever, consumptive disease, and infantile malnutrition syndrome (The Chinese Medicine Dictionary, 1977). Its roots have also been used as a herbal remedy for diabetes in folk medicines of China. A survey on the literature showed that triterpenoid saponins were the major constituents in the genus *Gypsophila* L. (Khorlin, Ovodov, and Kochetkov, 1962; Kochetkov, Khorlin, and Ovodov, 1963; Kochetkov and Khorlin, 1966; Sun *et al*, 2005). In order to clarify its chemical components thoroughly, we mainly studied its *n*-BuOH fraction and obtained a series of compounds. Five known triterpenoid saponins were identified by further analyses, they are compound **1** (7 mg), compound **2** (10 mg), compound **3** (8 mg), compound **4** (6 mg), and compound **5** (20 mg) isolated from this plant for the first time.

Materials and methods

Materials

The roots of *G. pacifica* were collected from Xifeng region, Liaoning Province, China, in October, 2005. The identity was confirmed by Prof. QIN Min-jian in Research Department of Traditional Chinese Medicinal Resources, China Pharmaceutical University, and the voucher specimen (No. 051020) was deposited at the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China.

NMR spectra were recorded at ACF-500 NMR instrument (^{1}H-NMR: 500 MHz, ^{13}C-NMR: 125 MHz), with TMS as internal standard. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS). TLC was performed on precoated silica gel H (Qingdao Haiyang Chemical Co., Ltd.) and detection was achieved by 15% H_2SO_4-EtOH for saponins, aniline-phthalate reagents for sugars. Sephadex LH-20 (Pharmacia) and RP-C_{18} (40–63 μm,

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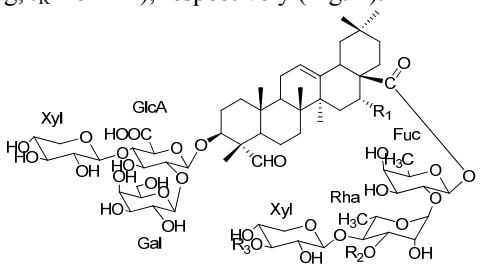
FuJi) were used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-C_{18} column (200 mm × 20 mm, 15 μm) and 1100 Series Multiple Wavelength detector.

Extraction and isolation

The roots of *G. pacifica* (8.9 kg) were ground into powder, and then extracted with 70% aqueous ethanol 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%, 30%, 50%, 70%, and 100%) to give five fractions (fractions 1–5); fractions 3 and 4 were further subjected to repeated RP-C_{18} column with MeOH-H_2O (40%→90%). Fraction 3 was further separated by HPLC (MeCN-0.05% TFA in H_2O, 32∶68, UV detection at 210 nm), and yielded pure compounds **1** (7 mg, t_R = 54 min), **2** (10 mg, t_R = 48 min), **3** (8 mg, t_R =28 min), **4** (6 mg, t_R =20 min), and **5** (20 mg, t_R = 9 min), respectively (Fig. 1).



	R_1	R_2	R_3
1	H	H	H
2	OH	H	H
3	H	Glc	H
4	OH	Glc	H
5	OH	H	Ara'-(1→4)-Ara

Fig. 1 Structures of triterpenoid saponins 1–5

Results

Compound **1**: white amorphous powder; ESI-MS *m/z* 1363 $[M-H]^-$. ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.79 (3H, s, Me), 0.88 (3H, s, Me), 0.92 (3H, s, Me), 1.04 (3H, s, Me), 1.23 (3H, s, Me), 1.47 (3H, s, Me), 1.45 (d, *J* = 6.0 Hz, Fuc-H-6), 1.70 (d, *J* = 4.5 Hz, Rha-H-6), 3.28 (1H, br d, *J* =11.6 Hz, 18-H), 4.90 (d, *J* = 7.5 Hz, GlcA-H-1), 5.34 (1H, br s, 12-H), 5.40 (d, *J* = 7.5 Hz, Xyl-H-1), 5.46 (d, *J* = 7.7 Hz, Xyl′-H-1), 5.54 (d, *J* = 7.8 Hz, Gal-H-1), 6.01 (d, *J* = 8.0 Hz, Fuc-H-1), 6.31 (s, Rha-H-1), 9.97(1H, s, 23-H). ^{13}C-NMR (C_5D_5N, 125 MHz) see Tables 1 and 2. Compound **1** was identified as 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucuronopyranosyl-gypsogenin 28-*O*-β-*D*-xylopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside by comparison of the spectral data with the literature (Ghezala, Tomofumi, and Marie-Aleth, 2001).

Table 1 ^{13}C-NMR (C_5D_5N, 125 MHz) data for aglycone moieties of compounds 1–5

Carbon	1	2	3	4	5
1	38.5	38.5	39.0	38.0	37.9
2	25.8	25.8	26.4	25.3	25.0
3	85.1	84.3	85.5	84.4	84.0
4	55.6	55.6	56.0	55.2	55.0
5	49.2	49.2	49.7	49.1	49.0
6	21.2	21.2	21.5	20.5	20.4
7	32.8	32.8	33.4	32.8	32.6
8	40.6	40.6	41.1	40.4	40.0
9	48.3	48.3	48.8	48.6	48.2
10	36.7	36.7	37.1	36.1	36.0
11	24.2	24.2	24.4	22.0	22.5
12	122.9	122.5	123.4	122.3	121.9
13	144.6	144.1	145.0	144.8	144.4
14	42.8	42.8	43.1	42.0	41.8
15	29.0	35.7	29.0	35.8	36.0
16	24.1	74.4	24.7	75.0	74.7
17	47.6	47.6	47.3	47.5	47.2
18	42.5	41.5	42.8	41.0	41.3
19	46.8	46.8	47.9	46.9	46.7
20	31.2	31.2	31.7	30.3	30.6
21	34.4	35.4	34.8	35.7	35.8
22	33.0	32.0	33.4	31.6	31.8
23	210.9	209.9	211.1	209.5	209.7
24	11.7	11.7	12.1	11.0	10.7
25	16.2	16.2	16.6	15.8	15.6
26	17.9	17.9	18.2	17.5	17.2
27	26.4	26.4	26.9	26.7	26.9
28	177.1	176.2	177.3	176.1	175.9
29	33.6	33.6	34.0	33.2	33.0
30	24.2	24.2	24.7	24.4	24.2

Compound **2**: white amorphous powder; ESI-MS *m/z* 1379 $[M-H]^-$. ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.81 (3H, s, Me), 0.89 (3H, s, Me), 0.93 (3H, s, Me), 1.05 (3H, s, Me), 1.22 (3H, s, Me), 1.44 (3H, s, Me), 1.43 (d, *J* = 6.2 Hz, Fuc-H-6), 1.71 (d, *J* = 4.8 Hz, Rha-H-6), 3.24 (1H, br d, *J* =12.6 Hz, 18-H), 4.89 (d, *J* = 7.7 Hz, GlcA-H-1), 5.25 (1H, br s, 12-H), 5.42 (d, *J* = 7.7 Hz, Xyl-H-1), 5.47 (d, *J* = 7.6 Hz, Xyl′-H-1), 5.53 (d, *J* = 7.4 Hz, Gal-H-1), 5.54 (1H, br s, 16-H), 6.02 (d, *J* = 7.8 Hz, Fuc-H-1), 6.34 (s, Rha-H-1), 9.91(1H, s, 23-H). ^{13}C-NMR (C_5D_5N, 125 MHz) see Tables 1 and 2. Compound 2 was characterized as 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-β-*D*-xylopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside by comparison of the spectral data with the literature (Bukharov *et al*, 1973).

Compound **3:** white amorphous powder; ESI-MS *m/z*: 1525 $[M-H]^-$. ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.81 (3H, s, Me), 0.87 (3H, s, Me), 0.91 (3H, s, Me), 1.07 (3H, s, Me), 1.22 (3H, s, Me), 1.49 (3H, s, Me), 1.45 (d, *J* = 6.3 Hz, Fuc-H-6), 1.65 (d, *J* = 6.1 Hz, Rha-H-6), 3.25 (1H, br d, *J* =11.6 Hz, 18-H), 4.86(d, *J* = 7.5 Hz, GlcA-H-1), 4.95 (d, *J* = 7.6 Hz, Glc-H-1), 5.19 (d, *J* = 7.1 Hz, Xyl′-H-1), 5.32 (1H, br s, 12-H), 5.34 (d, *J* = 7.7 Hz, Xyl-H-1), 5.55 (d, *J* = 7.7 Hz, Gal-H-1), 6.01 (d, *J* = 8.2 Hz, Fuc-H-1), 6.44 (s, Rha-H-1),

Table 2 ^{13}C-NMR (C_5D_5N, 125 MHz) data for sugar moieties of compounds 1–5

Carbon			1	2	3	4	5
3-*O*-	GlcA	1	104.4	104.1	105.1	104.9	103.6
		2	79.0	78.3	79.5	78.9	78.6
		3	86.3	85.8	87.1	86.7	86.5
		4	72.1	71.7	71.8	72.0	71.2
		5	77.1	76.7	78.3	78.5	77.2
		6	171.3	171.8	171.4	171.2	171.7
	Gal	1	104.7	104.9	104.1	104.3	104.0
		2	74.2	73.8	73.6	73.8	73.9
		3	75.8	75.3	75.5	75.6	75.3
		4	70.7	70.0	70.1	70.3	70.0
		5	76.8	76.0	76.3	76.7	76.4
		6	62.2	61.3	61.5	61.7	61.4
	Xyl	1	105.4	105.5	105.0	104.9	104.8
		2	75.8	75.1	75.6	75.4	75.2
		3	79.2	78.4	78.6	78.8	78.4
		4	71.3	70.7	71.2	71.0	70.7
		5	67.9	67.1	67.7	67.5	67.2
28-*O*-	Fuc	1	95.2	94.6	95.0	94.8	94.6
		2	74.4	73.8	75.1	74.7	73.5
		3	77.1	76.7	75.8	76.8	76.4
		4	73.7	73.1	73.0	73.2	73.0
		5	72.9	72.4	72.1	72.5	72.3
		6	16.4	15.6	17.0	17.1	16.8
	Rha	1	101.8	101.1	101.4	101.5	101.0
		2	72.1	71.7	71.2	71.9	71.7
		3	74.1	73.5	82.2	82.5	74.4
		4	86.3	86.5	78.9	78.6	83.2
		5	68.6	68.0	69.1	69.3	68.0
		6	18.9	17.2	18.8	18.9	18.3
	Xyl	1	107.2	106.9	105.3	105.4	105.9
		2	75.8	75.1	75.6	75.5	75.1
		3	79.0	78.4	79.8	79.3	85.8
		4	71.3	70.7	70.6	70.2	69.5
		5	67.9	67.0	67.3	67.1	67.1
	Glc	1			105.5	105.6	
		2			75.8	75.6	
		3			78.5	78.8	
		4			71.9	71.4	
		5			78.8	78.3	
		6			63.0	62.9	
	Ara	1					105.2
		2					72.8
		3					74.1
		4					78.3
		5					66.3
	Ara’	1					106.9
		2					73.0
		3					73.1
		4					68.7
		5					66.7

9.92(1H, s, 23-H). ^{13}C-NMR (C_5D_5N, 125 MHz) see Tables 1 and 2. Compound **3** was identified as 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucuronopyranosyl gypsogenin 28-*O*-β-*D*-glucopyranosyl-(1→3)-[β-*D*-xylopyranosyl-(1→4)]-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside by comparison of the spectral data with the literature (Mohamed, Tomofumi, and Marie-Aleth, 2007).

Compound **4:** white amorphous powder; ESI-MS *m/z*: 1541 $[M-H]^-$. ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.79 (3H, s, Me), 0.88 (3H, s, Me), 0.92 (3H, s, Me), 1.06 (3H, s, Me), 1.24 (3H, s, Me), 1.46 (3H, s, Me), 1.46 (d, J = 6.3 Hz, Fuc-H-6), 1.69 (d, J = 6.2 Hz, Rha-H-6), 3.25 (1H, br d, J =11.6 Hz, 18-H), 4.89 (d, J = 7.5 Hz, GlcA-H-1), 4.99 (d, J = 7.6 Hz, Glc-H-1), 5.21 (d, J = 7.1 Hz, Xyl′-H-1), 5.32 (1H, br s, 12-H), 5.33 (d, J = 7.7 Hz, Xyl-H-1), 5.54 (1H, br s, 16-H), 5.56 (d, J = 7.7 Hz, Gal-H-1), 6.02 (d, J = 8.0 Hz, Fuc-H-1), 6.34 (s, Rha-H-1), 9.96(1H, s, 23-H). ^{13}C-NMR (C_5D_5N, 125 MHz) see Tables 1 and 2. Compound **4** was characterized 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*-xylopyranosyl-(1→4)]-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside by comparison of the spectral data with the literature (Frechet *et al*, 1991).

Compound **5:** white amorphous powder; ESI-MS *m/z*: 1643 $[M-H]^-$. ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.80 (3H, s, Me), 0.94 (3H, s, Me), 0.98 (3H, s, Me), 1.04 (3H, s, Me), 1.41 (3H, s, Me), 1.74 (3H, s, Me), 1.46 (d, J = 6.5 Hz, Fuc-H-6), 1.68 (d, J = 5.9 Hz, Rha-H-6), 3.25 (1H, br d, J =11.6 Hz, 18-H), 4.90 (d, J = 7.4 Hz, GlcA-H-1), 5.02 (d, J = 7.4 Hz, Xyl′-H-1), 5.11 (d, J = 7.0 Hz, Ara′-H-1), 5.14 (d, J = 7.0 Hz, Ara-H-1), 5.31 (1H, br s, 12-H), 5.32 (d, J = 7.7 Hz, Xyl-H-1), 5.54 (1H, br s, 16-H), 5.54 (d, J = 7.5 Hz, Gal-H-1), 5.97 (d, J = 8.2 Hz, Fuc-H-1), 6.40 (s, Rha-H-1), 9.85(1H, s, 23-H). ^{13}C-NMR (C_5D_5N, 125 MHz) see Tables 1 and 2. Compound **5** was identified as 3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucuronopyranosyl quillaic acid 28-*O*-α-*L*-arabinopyranosyl-(1→4)-α-*L*-arabinopyranosyl-(1→3)-β-*D*-xylopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-fucopyranoside by comparison of the spectral data with the literature (Frechet *et al*, 1991).

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