Two New Monodesmosidic Triterpene Saponins from *Gypsophila oldhamiana*

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Two new monodesmosidic triterpene saponins were isolated from the roots of *Gypsophila oldhamiana* (Caryophyllaceae). Their structures were elucidated on the basis of spectral data to be quillaic acid, α-L-arabinopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester (1), and vaccaric acid, β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester (2). Compound 1 showed a significant enhancement of granulocyte phagocytosis *in vitro*.

Key words *Gypsophila oldhamiana*; Caryophyllaceae; triterpenoid saponin; quillaic acid; vaccaric acid; immunologic activity

Gypsophila oldhamiana Miq. (Caryophyllaceae) is widely distributed in Shanxi and Shandong province in China. The roots of this plant have been used as an alternative remedy to the most common traditional Chinese medicine Yin-Chai-Hu (roots of *Stellria dichotoma* var. Lanceolata Bge) and utilized to treat heat due to yin deficiency, hectic and tidal fever, and infantile malnutrition syndrome in China.[1)] Previous chemical investigation on the roots of this species led to the isolation of saponins, sterols, and fatty acid.[2—5)] As a part of our search for bioactive saponins from traditional Chinese medicine, we present in this report the isolation and structural elucidation of two new saponins (**1**, **2**) from the roots of *G. oldhamiana*. In addition, their immunologic properties were investigated.

Results and Discussion

The 70% EtOH extract of the roots of *G. oldhamiana* was partitioned with water and $CHCl_3$. The water layer was chromatographed successively over marcoporous resin D101, silica gel, and ODS-C_{18} to yield compounds **1** and **2**, which were identified as quillaic acid, α-L-arabinopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester (**1**), and vaccaric acid, β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester (**2**) (Fig. 1).

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{57}H_{90}O_{25}$ determined from its pseudomolecular ion peak at *m/z* 1175.5833 $[M+H]^+$ (Calcd 1175.5849) in the HR-FAB-MS. The IR spectrum showed absorption at 3600—3300 cm^{-1} (–OH) and 1724 cm^{-1} (ester carbonyl). The alkaline hydrolysis of **1** yielded quillaic acid identified by TLC in comparison with an authentic sample[6)] and an oligosaccharide. Acid hydrolysis of **1** afforded quillaic acid and monosaccharides, D-fucose, L-rhamnose, D-xylose, and L-arabinose identified by gas chromatography in comparison with standard monosaccharides.[6)] The proton and carbon signals (Tables 1, 2) were assigned by a combination analysis of 1D and 2D NMR (HSQC and HMBC). The aglycone moiety was further identified by comparison with literature data.[2)] The chemical shifts of C-3 (δ 71.7) and C-28 (δ 176.3) indicated that **1** was a monodesmosidic glycoside in which the sugar moiety was connected at C-28.[7)] In addition, five sugar anomeric carbons were detected at δ 94.9, 101.1, 106.1, 105.3, and 106.9 in the ^{13}C-NMR spectrum, attached to protons at δ 5.90 (d, J=8.1 Hz), 6.27 (br s) 5.05 (d, J=6.4 Hz), 5.06 (d, J=7.6 Hz), and 4.91 (d, J=7.3 Hz), respectively, in the HSQC experiment. After analyzing the ^{1}H- and ^{13}C-NMR data and the correlations between ^{1}H and ^{13}C of the individual sugars observed in HMBC spectrum, the sugar components were identified as one β-D-fucopyranosyl, one α-L-rhamnopyranosyl, one β-D-xylopyranosyl, and two α-L-arabinopyranosyl units, and the signals for the two methyl groups (δ 1.42, d, J=6.4 Hz; 1.54, d, J=6.3 Hz) could be assigned to Me- in Fuc and Rha, respectively. The β-anomeric configurations of the fucose and xylose units and the α-anomeric configurations of two arabinose ($^4C_1$ configuration) units were determined from their $^3J_{H1,H2}$ coupling constants (7—8 Hz) and ^{13}C-NMR data.[8)] The α-anomeric configuration of rhamnose was judged by its C_5 (δ 68.3).[9)] The sequence of the oligosaccharide chains in **1** was determined from the HMBC spectrum. The cross-peaks in the HMBC spectrum between the ^{1}H-NMR signals at $\delta_{H(Fuc\text{-}1)}$ 5.90 and the ^{13}C-NMR signals at $\delta_{C\text{-}28}$ 176.3, $\delta_{H(Rha\text{-}1)}$ 6.27, and

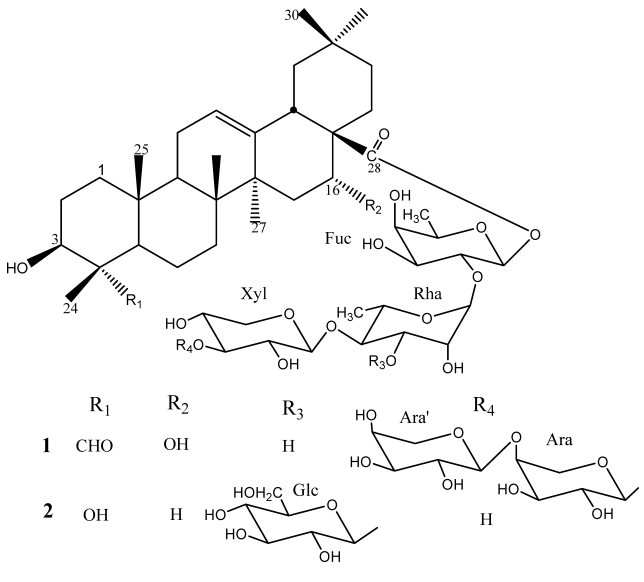

Fig. 1. The Structures of **1** and **2**

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Table 1. ^{1}H- and ^{13}C-NMR Data of Aglycon for **1** and **2** (in C_5D_5N)[a]

Position	1		2	
	^{13}C	^{1}H (*J*/Hz)	^{13}C	^{1}H (*J*/Hz)
1	38.7	1.00, 1.53	38.9	1.03, 1.51
2	27.0	1.86, nd[b]	28.6	1.71, 1.96
3	71.7	4.02	79.5	4.05
4	56.4	—	75.4	—
5	47.9	1.35	56.4	1.39
6	21.2	nd, nd	18.0	1.55, nd
7	33.0	1.53, nd	32.8	1.56, 1.60
8	40.4	—	40.2	—
9	47.2	1.80	48.2	1.66
10	36.2	—	37.9	—
11	23.9	1.80, 1.96	24.0	1.88, 1.90
12	122.3	5.54 (br s)	122.7	5.39 (br s)
13	144.5	—	144.0	—
14	42.2	—	42.3	—
15	36.2	1.20, 2.15	28.2	1.29, 2.03
16	74.2	5.10	23.6	1.80, 2.08
17	49.3	—	47.1	—
18	41.7	3.34 (m)	42.1	3.09 (br d, 13.6)
19	47.5	2.68 (t, 13.6)	46.4	1.75 (t, 13.6)
20	31.9	—	30.8	—
21	36.0	1.24, 2.12	34.0	1.18, 1.20
22	30.8	nd	32.4	1.71, 2.00
23	207.9	9.53 (s)	—	—
24	9.7	1.26 (3H, s)	17.8	1.40 (3H, s)
25	15.9	0.87 (3H, s)	15.4	0.92 (3H, s)
26	17.5	1.02 (3H, s)	17.4	1.14 (3H, s)
27	27.2	1.72 (3H, s)	25.9	1.11 (3H, s)
28	176.3	—	176.4	—
29	33.2	0.88 (3H, s)	33.2	0.82 (3H, s)
30	24.6	0.94 (3H, s)	23.8	0.86 (3H, s)

a) The assignments were based on the HSQC and HMBC experiments (125 MHz for ^{13}C- and 500 MHz for ^{1}H-NMR). *b*) nd: not determined.

Table 2. NMR Data for Sugar Moieties of **1** and **2** (in C_5D_5N)[a,b]

	1			2	
	^{13}C	^{1}H (*J*/Hz)		^{13}C	^{1}H (*J*/Hz)
Fuc			Fuc		
1	94.9	5.90 (d, 8.1)	1	95.1	5.95 (d, 8.1)
2	<u>73.8</u>	4.54 (dd, 9.0, 8.1)	2	<u>75.3</u>	4.40 (dd, 8.5, 8.1)
3	76.4	4.11 (dd, 9.0, 3.2)	3	75.3	4.04 (m)
4	73.1	3.94 (d, 3.2)	4	72.9	3.90 (m)
5	72.5	3.86 (br d, 6.4)	5	72.3	3.80 (br d, 6.6)
6	16.9	1.42 (3H, d, 6.4)	6	16.9	1.43 (3H, d, 6.6)
Rha			Rha		
1	101.1	6.27 (br s)	1	102.0	5.85 (br s)
2	71.8	4.70 (dd, 3.0, 1.5)	2	71.0	5.18 (br s)
3	72.5	4.58 (dd, 9.4, 3.0)	3	<u>82.4</u>	4.88 (br d, 8.5)
4	<u>83.5</u>	4.28 (t, 9.4)	4	<u>78.8</u>	4.48 (t, 8.5)
5	68.3	4.36 (dd, 9.4, 6.3)	5	69.1	4.46 (dd, 8.5, 5.5)
6	18.5	1.54 (3H, d, 6.3)	6	18.9	1.67 (3H, d, 5.5)
Xyl			Xyl		
1	106.1	5.05 (d, 7.4)	1	105.5	5.43 (d, 7.9)
2	75.2	3.89 (dd, 9.0, 7.4)	2	75.5	3.98 (dd, 10.7, 7.9)
3	<u>86.8</u>	3.94 (m)	3	79.5	4.05 (t, 10.7)
4	68.9	3.94 (m)	4	71.3	4.14 (m)
5	66.8	3.35 (dd, 14.5, 5.5)	5	67.2	3.34 (dd, 12.2, 5.3)
		4.10 (d, 5.5)			4.14 (d, 5.3)
Ara			Glc		
1	105.3	5.06 (d, 7.6)	1	105.2	5.40 (d, 8.0)
2	73.2	4.34 (dd, 9.0, 7.6)	2	75.9	3.89 (t, 8.0)
3	74.2	4.14 (dd, 9.0, 3.5)	3	78.6	4.11 (t, 8.0)
4	<u>78.8</u>	4.24 (m)	4	72.1	4.05 (m)
5	66.5	3.72 (dd, 11.5, 5.1)	5	78.3	3.92 (m)
		4.41 (d, 5.1)	6	63.0	4.16 (m)
Ara′					4.48 (m)
1	106.9	4.91 (d, 7.3)			
2	72.9	4.39 (dd, 9.8, 7.3)			
3	74.5	4.04 (dd, 9.8, 3.2)			
4	69.6	4.18 (m)			
5	67.2	3.63 (dd, 11.1, 5.0)			
		4.16 (d, 5.0)			

a) The assignments were based upon ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC spectra. *b*) ^{13}C chemical shifts of substituted residues are underlined.

$\delta_{C\,(Fuc\text{-}2)}$ 73.8; $\delta_{H\,(Xyl\text{-}1)}$ 5.05 and $\delta_{C\,(Rha\text{-}4)}$ 83.5; $\delta_{H\,(Ara\text{-}1)}$ 5.06 and $\delta_{C\,(Xyl\text{-}3)}$ 86.8; and $\delta_{H\,(Ara'\text{-}1)}$ 4.91 and $\delta_{C\,(Ara\text{-}4)}$ 78.8 showed that the pentasaccharide moiety α-L-arabinopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl was linked to the quillaic acid unit at C-28. These sugar linkages were confirmed by glycosidation shifts (^{13}C chemical shifts of substituted residues are underlined in Table 2). On the basis of the above results, the structure of **1** was determined to be quillaic acid, α-L-arabinopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester.

Compound **2**, white amorphous powder, had the molecular formula $C_{52}H_{84}O_{21}$ determined from its pseudomolecular ion peak at *m/z* 1045.5564 $[M+H]^+$ (Calcd 1045.5583) in the HR-FAB-MS. The IR spectrum showed absorption at 3600—3300 cm^{-1} (–OH) and 1720 cm^{-1} (ester carbonyl). Acid hydrolysis of **2** afforded D-fucose, L-rhamnose, D-xylose, and D-glucose identified by gas chromatography analysis with standard monosaccharides. The assignments of the NMR data (Table 1) for the aglycone moiety were readily recognized on the basis of 2D NMR experiments (HSQC, HMBC, and NOESY), in accordance with those of oleanolic acid,[10] except for the absence of 23-Me and a salient downshift (+36.4) at C-4 (δ 75.4), which indicated that the aglycone is an oleanolic acid derivative with replacement of 23-Me by a hydroxyl function. The HMBC spectrum (Fig. 2) of **2** confirmed the position of the hydroxyl function, showing significative cross-peaks due to $^{2}J_{C-H}$ and $^{3}J_{C-H}$ correlations between H-24 (δ 1.40, 3H, s) and C-3 (δ 79.5), and C-4 (δ 75.4) and C-5 (δ 56.4), but without cross-peaks between H-24 and other carbons (C-23 was absent). The α-configuration of the hydroxyl group was evident from the NOESY spectrum, which showed significant correlation through space interaction between H-25 and H-24 (Fig. 2). Base upon the above information, the aglycone moiety of **2** was established to be 3β, 4α-dihydroxy-23-norolean-12-en-28-oic acid, a nortriterpenoid sapogenin called vaccaric acid, which has been only isolated as an aglycone of saponins from *Vaccaria segetalis* (Neck) Garcke (Caryophyllaceae).[11] In addition to the signals of the aglycone, the ^{13}C-NMR spectra of **2** displayed four anomeric carbons at δ 95.1, 102.0, 105.5, and 105.2, attached protons at δ 5.95 (d, *J*=8.1 Hz), 5.85 (br s), 5.43 (d, *J*=7.9 Hz), and 5.40 (d, *J*=8.0 Hz), respectively in the HSQC experiment. The tetrasaccharide moiety linked to the vaccaric acid unit at C-28 was determined to be β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl in comparison with the literature data for similarly linked sugar moieties,[12] which was confirmed by HMQC, HMBC, and NOESY experiments (Fig. 2). Therefore the structure of **2** was determined to be vaccaric acid, β-D-glucopyranosyl-(1→3)-[β-D-

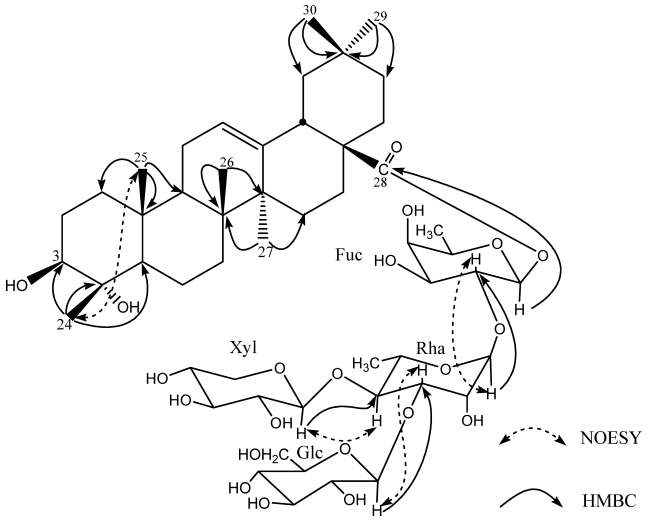

Fig. 2. Selected HMBC and NOESY Correlations of Compound **2**

xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester.

Since some saponins from plants in the Caryophyllaceae have been reported to exert immunomodulant activities,[13,14] compounds **1** and **2** were tested in an *in vitro* lymphocyte proliferation test system according to a reported procedure,[15,16] and in the *in vitro* T cell activation assay according to the reported method.[17] Only **1** showed an immunomodulatory effect dependent on concentration. In the concentration range of 10—100 μg/ml, compound **1** showed a significant enhancement of granulocyte phagocytosis (40—75%). In the concentration range of 100 ng/ml—1 pg/ml, the same substance exerted an immunosuppressive effect (65—22%) in the T cell activation assay.

Experimental

Optical rotations were measured with a JASCO P-1020 polarimeter (cell length: 1.0 dm). IR spectra (KBr discs) were recorded with a Nicolet Impact-410 spectrometer. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap Mass spectrometer (ESI-MS), and a JEOL-HX-110 spectrometer (HR-FAB-MS) using *m*-nitrobenzyl alcohol as a matrix. 1D and 2D NMR spectra were recorded in C_5D_5N at 300 K on Bruker ACF-500 NMR (^{1}H: 500 MHz, ^{13}C: 125 MHz) spectrometers, with tetramethylsilane (TMS) as an internal standard, in which coupling constants were given in Hz. Gas chromatography was done on a GC-14B Gas Chromatograph (Shimadzu), with an SE30 capillary column (12 m, 0.22 mm i.d.), hydrogen flame ionization detector (FID, 270 °C), column temperature of 170—250 °C with the rate of 5 °C/min, and the carrier gas was N_2 (30 ml/min). TLC was performed on precoated silica gel 60 F_{254} (Qingdao Haiyang Chemical Co. Ltd.) plates, and detection was achieved by 20% H_2SO_4–EtOH for saponins. Silica gel H (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (20—100 μm, Pharmacia), macroporous resin D101 (pore size B 13—14 nm, 26—60 mesh), and ODS-C_{18} (40—63 μm, Fuji) were used for column chromatography.

Plant Material The roots of *G. oldhamiana* (Miq.) were collected in the suburbs of Jinan, Shandong province, China, in September 2000, and identified by Prof. Yun-Yao Li of the Department of Pharmacognosy, Shandong University. A voucher specimen (no. 000902) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation The air-dried roots of *G. oldhamiana* (4 kg) were powdered and refluxed three times with 70% EtOH. After concentration *in vacuo*, the residue was suspended in H_2O and partitioned between $CHCl_3$ and water. The water layer was further chromatographed over a macroporous resin D101 column eluted initially with water, and then with 45% and 95% EtOH to give fractions 1 (66.0 g) and 2 (17.5 g). Fraction 1 was subjected to silica column chromatography (ϕ10×60 cm, 100—200 mesh, 900 g), which was eluted with $CHCl_3$–MeOH–H_2O (100 : 30 : 1→100 : 100 : 1) to give four subfractions. The first subfraction (30 mg) was further submitted to ODS-C_{18} column chromatography (ϕ1.5×30 cm, 40 g) eluted with MeOH–H_2O (8 : 2, v/v) to afford **1** (20 mg, 10—45 ml eluate) and **2** (5.1 mg, 60—75 ml eluate).

Compound **1**: White amorphous powder; $C_{57}H_{90}O_{25}$, $[\alpha]_D^{25}$ +8.1° (c=0.10, CH_3OH); IR (KBr): ν_{max} 3600—3300 (OH), 2934 (CH), 1724 (C=O ester), 1450, 1384, 1300, 1060 cm^{-1}; ESI-MS: *m/z* 1173 $[M-H]^-$, HR-FAB-MS *m/z* 1175.5833 $[M+H]^+$ (Calcd 1175.5849); ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz), see Tables 1 and 2.

Compound **2**: White amorphous powder; $C_{52}H_{84}O_{21}$, $[\alpha]_D^{25}$ +5.9° (c=0.10, CH_3OH); IR (KBr): ν_{max} 3600—3300 (OH), 2931 (CH), 1720 (C=O ester), 1451, 1385, 1310, 1074 cm^{-1}; ESI-MS: *m/z* 1044 $[M-H]^-$, HR-FAB-MS *m/z* 1045.5564 $[M+H]^+$ (Calcd 1045.5583); ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz), see Tables 1 and 2.

Alkaline Hydrolysis of 1 Compound **1** (10 mg) in 1 N KOH 2 ml was heated at 80 °C for 2 h. After cooling, the reaction mixture was neutralized with 1 N HCl and then extracted with CH_2Cl_2 (3×10 ml). The organic layers were washed with H_2O and then combined to yield quillaic acid (3 mg) identified by co-TLC with authentic sample in $CHCl_3$/MeOH (20 : 1, R_f: 0.25). The water layer was condensed to yield an oligosaccharide.

Acid Hydrolysis of 1 and 2 A solution of saponin **1** (2 mg) in 2 N HCl (2 ml) was refluxed in a water bath for 3 h. After this period, the reaction mixture was diluted with H_2O (5 ml) and extracted with CH_2Cl_2 (3×5 ml). The extraction was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. Quillaic acid was identified by co-TLC with authentic sample. The acid aqueous solution was neutralized with 0.5 M KOH and concentrated *in vacuo*. The residue was dissolved in pyridine (0.2 ml) and treated with 1-(trimethylsilyl) imidazole at room temperature for 2 h. After partitioning between hexane (1 ml) and H_2O (1 ml), the hexane extract was analyzed with gas chromatography. Derivatives of D-fucose, L-rhamnose, D-xylose, and L-arabinose were detected by comparison of the retention time with that of the authentic samples prepared in the same manner. Using the same method, the monosaccharides from **2** (2 mg) were identified as D-fucose, L-rhamnose, D-xylose, and D-glucose.

Bioassay The granulocyte phagocytosis assay was performed according to the published method.[15,16] The T cell activation assay was performed as reported.[17]

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