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TRITERPENOID SAPONINS FROM *GYPSOPHILA OLDHAMIANA*

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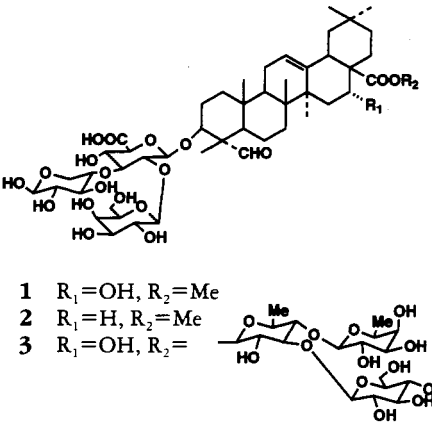
ABSTRACT.—Three new triterpenoid saponins were isolated from the roots of *Gypsophila oldhamiana*. Their structures were elucidated, using a combination of homonuclear and heteronuclear 2D nmr and fabms, as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid methyl ester [**1**], 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl gypsogenin methyl ester [**2**], and 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-{*O*-β-D-fucopyranosyl(1→4)-β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl} ester [**3**].

The roots of *Gypsophila oldhamiana* Miq. (Caryophyllaceae) have been used as an alternative remedy to the well-known Chinese traditional medicinal herb, *Stellaria dichotoma* L. var. *lanceolata*, for the treatment of fever, consumptive disease, and infantile malnutrition in the People's Republic of China, and a triterpenoid saponin from *Gypsophila pacifica* has been shown to reduce the concentration of serum cholesterol in atherosclerotic rabbits (1). As part of our studies on the saponins of Chinese traditional medicinal herbs, we have isolated three new triterpenoid saponins [**1–3**] from *Gypsophila oldhamiana*. This paper reports their isolation and structural elucidation.



1 R_1=OH, R_2=Me
2 R_1=H, R_2=Me
3 R_1=OH, R_2=

Fabms permitted assignments of the molecular formulas of **1** as $C_{48}H_{74}O_{20}$ and of **2** as $C_{48}H_{74}O_{19}$. The ^{13}C-nmr spectra of **1** and **2** showed that the aglycone moieties were based on olean-12-ene with a methoxy carbonyl at C-28 (Table 1). The existence of a CHO group at C-23 (δ 210.4 for **1**, 210.1 for **2**) was deduced from the upfield chemical shifts of Me-24 (δ 11.3 for **1**, 11.1 for **2**). Thus, the aglycone was identified as quillaic acid methyl ester for **1** and gypsogenin in **2** when compared with the literature (2–5). In addition, the ^{13}C-nmr spectra showed they had the same sugar units and similar linkages. Acid hydrolysis of the saponins **1** and **2** gave glucuronic acid, galactose, and xylose as sugar components as detected by gc after silylation. The positions of connectivity of the sugars were determined by comparing the spectral data with those reported (2–5), and were confirmed by HETCOR (6), COSY (7), and ROESY (8) nmr experiments. Galactose and xylose were found to be terminal

TABLE 1. ^{13}C-Nmr Spectral Data of **1–3** in Pyridine-d_5 at 125 MHz.

Carbon	**1** (mult.)	**2** (mult.)	**3** (mult.)	Carbon	**1** (mult.)	**2** (mult.)	**3** (mult.)
1	38.2 (t)	38.1 (t)	37.8 (t)	5	76.7 (d)	76.9 (d)	76.7 (d)
2	25.4 (t)	23.7 (t)	23.5 (t)	6	170.2 (s)	170.0 (s)	170.0 (s)
3	84.8 (d)	84.6 (d)	84.4 (d)	xyl			
4	55.3 (s)	55.1 (s)	54.9 (s)	1	105.2 (d)	105.1 (d)	104.8 (d)
5	48.1 (d)	47.8 (d)	47.7 (d)	2	75.1 (d)	75.4 (d)	75.1 (d)
6	20.7 (t)	20.5 (t)	20.3 (t)	3	77.0 (d)	77.2 (d)	76.9 (d)
7	32.7 (t)	32.7 (t)	32.3 (t)	4	71.3 (d)	71.4 (d)	71.1 (d)
8	40.2 (s)	40.1 (s)	39.8 (s)	5	67.6 (t)	67.4 (t)	67.1 (t)
9	48.8 (d)	48.7 (d)	48.4 (d)	gal			
10	36.4 (s)	36.3 (s)	36.1 (s)	1	104.5 (d)	104.4 (d)	104.1 (d)
11	23.9 (t)	23.9 (t)	23.5 (t)	2	73.9 (d)	73.8 (d)	73.5 (d)
12	122.3 (d)	122.3 (d)	122.1 (d)	3	75.5 (d)	75.4 (d)	75.2 (d)
13	145.2 (s)	144.9 (s)	144.7 (s)	4	71.0 (d)	71.1 (d)	70.7 (d)
14	40.2 (s)	42.3 (s)	42.0 (s)	5	75.7 (d)	75.7 (d)	75.3 (d)
15	34.5 (t)	28.4 (t)	35.6 (t)	6	62.0 (t)	61.9 (t)	61.6 (t)
16	73.9 (d)	23.8 (t)	73.5 (t)	28-*O*-rha			
17	46.9 (s)	46.6 (s)	46.3 (s)	1			94.0 (d)
18	42.4 (d)	42.1 (d)	41.8 (d)	2			70.0 (d)
19	46.7 (t)	46.7 (t)	46.5 (t)	3			81.9 (d)
20	31.2 (s)	31.0 (s)	30.8 (s)	4			77.1 (d)
21	34.5 (t)	34.3 (t)	34.1 (t)	5			69.9 (d)
22	33.6 (t)	33.3 (t)	33.0 (t)	6			17.2 (q)
23	210.4 (s)	210.1 (s)	210.0 (s)	fuc			
24	11.3 (q)	11.1 (q)	10.9 (q)	1			98.7 (d)
25	15.8 (q)	15.7 (q)	15.4 (q)	2			70.8 (d)
26	17.6 (q)	17.6 (q)	17.2 (q)	3			73.5 (d)
27	26.4 (q)	26.2 (q)	26.0 (q)	4			72.4 (d)
28	180.2 (s)	180.2 (s)	172.2 (s)	5			71.9 (d)
29	33.6 (q)	33.3 (q)	33.1 (q)	6			15.6 (q)
30	23.9 (q)	23.9 (q)	23.6 (q)	glc			
28-OMe	52.5 (q)	52.3 (q)		1			106.1 (d)
3-*O*-glcA				2			74.3 (d)
1	104.1 (d)	104.0 (d)	103.7 (d)	3			78.5 (d)
2	78.8 (d)	78.7 (d)	78.1 (d)	4			70.7 (d)
3	85.9 (d)	86.1 (d)	86.0 (d)	5			78.4 (d)
4	70.4 (d)	70.3 (d)	70.0 (d)	6			61.6 (t)

and bound to C-2 and C-3 of the glucuronic acid, respectively, in both compounds. In the ROESY spectrum of **1**, for example, connectivities were observed between H-1 of galactose at δ 5.53 and H-2 of glucuronic acid at δ 4.28, H-1 of xylose at δ 5.28 and H-3 of glucuronic acid at δ 4.23, and H-1 of glucuronic acid at δ 4.86 and H-3 of the aglycone at δ 4.09. The structures of **1** and **2** were concluded to be 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid methyl ester and 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl gypsogenin methyl ester, respectively.

The ^{13}C-nmr and DEPT spectra of **3** showed its aglycone to be quillaic acid. The anomeric ^{13}C-nmr sugar signals indicated the presence of two groups: one was similar to that in **1** linked at C-3 of the aglycone (δ 103.7, 104.1, and 104.8), while the other was attached at C-28 (δ 94.0, 98.7, and 106.1) (Table 1). Acid hydrolysis afforded rhamnose, fucose, and glucose in addition to xylose, galactose, and glucuronic acid, all of which were detected by gc after silylation. In the ^{1}H-nmr spectrum, the proton signals were assigned by means of the HETCOR experiment, and showed six sugar anomeric protons at δ 4.90 (d, J=8 Hz, glucuronic acid), 5.53 (d, J=7.5 Hz, galactose), 5.30 (d, J=7.5 Hz, xylose), 5.79 (br s, rhamnose), 5.21 (d, J=7.5 Hz, fucose), and 5.17 (d, J=8 Hz, glucose). The positions of connectivity of the sugars were deter-

mined by comparing the spectral data with those reported in the literature (2–5), and were confirmed by HMBC (9) and HETCOR experiments (Table 2). For the sugar group at C-28, rhamnose was found to be the inner unit due to its ^{13}C-nmr chemical shifts caused by glycosylation, and glucose and fucose were attached at the C-3 and C-4 of rhamnose, respectively. Based on the above results, the structure of **3** was concluded to be 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-{*O*-β-D-fucopyranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl} ester.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr measurements were performed on a Varian VXR-500 spectrometer. Samples were dissolved in 0.7 ml pyridine-d_5. The fabms was recorded on a Kratos triple-analyzer MS-50 instrument. Prep. and analytical hplc were carried out using a Gilson instrument equipped with Dynamax-60A (21.4 mm×25 cm) and Dynamax-60A (4.6 mm×25 cm) columns.

PLANT MATERIAL.—Dried roots of *Gypsophila oldhamiana* were collected in September, 1992 in Gansu Province, People's Republic of China. The plant was identified by Prof. Runeng Zhao, Department of Pharmacognosy, Lanzhou Medical College, and a voucher specimen has been deposited in the Herbarium of the Institute of Organic Chemistry, Lanzhou University, People's Republic of China.

EXTRACTION AND ISOLATION.—Air-dried roots (3.5 kg) were extracted twice with 95% EtOH at room temperature. The extract was concentrated under reduced pressure, hot H_2O was added, and the resulting mixture was filtered. The aqueous solution was extracted with petroleum ether (60–90°), EtOAc, and *n*-BuOH, yielding three corresponding residues. The *n*-BuOH portion was chromatographed over Si gel to obtain the crude saponins, which were purified by hplc on a Dynamax-60A RP-18 column with MeCN 0.1% TFA-H_2O 0.1% TFA (linear gradient 2:8–7:3 and uv detection at 220 nm) to afford **1** (30 mg), **2** (40 mg), and **3** (15 mg).

Saponin **1**.—Amorphous white powder, $C_{48}H_{74}O_{20}$; ^{1}H nmr (pyridine-d_5, 500 MHz) δ 9.93 (1H, s, H-23), 5.53 (1H, d, J=8 Hz, H-1 of galactose), 5.41 (1H, br s, H-12), 5.28 (1H, d, J=8 Hz, H-1 of xylose), 4.86 (1H, d, J=7.5 Hz, H-1 of glucuronic acid), 4.28 (1H, t, J=8 Hz, H-2 of glucuronic acid), 4.23 (1H, t, J=8 Hz, H-3 of glucuronic acid), 4.09 (1H, m, H-3), 1.41, 1.21, 0.98, 0.93, 0.89, 0.86 (3H each, s, 6×Me); ^{13}C-nmr data, see Table 1; fabms *m/z* 993 $[M+Na]^+$, 977 $[M+Li]^+$, 861 $[M+Na-xylose]^+$, 845 $[M+Li-xylose]^+$, 699 $[M+Na-xylose-galactose]^+$.

Saponin **2**.—Light yellow powder, $C_{48}H_{74}O_{19}$; ^{1}H nmr (pyridine-d_5, 500 MHz) δ 9.93 (1H, s, H-23), 5.54 (1H, d, J=8.5 Hz, H-1 of galactose), 5.41 (1H, br s, H-12), 5.31 (1H, d, J=7.5 Hz, H-1 of xylose), 4.89 (1H, d, J=8 Hz, H-1 of glucuronic acid), 4.28 (1H, t, J=8 Hz, H-2 of glucuronic acid), 4.23 (1H, t, J=8 Hz, H-3 of glucu-

TABLE 2. ^{1}H-Nmr and HMBC Spectral Data of **3** in Pyridine-d_5 at 500 MHz.

Proton	δ_H	HMBC	Proton	δ_H	HMBC
Aglycone ...			H-4	4.42 (m)	170.0
H-3	4.06 (m)	103.7	H-5	4.42 (m)	76.7
H-12	5.42 (br s)	42.0, 48.4	xyl		
H-16	4.50 (m)	172.2	H-1	5.30 (d, J=7.5 Hz)	86.0
H-23	9.97 (s)		gal		
H-24	1.41 (s)	210.0, 54.9, 84.4	H-1	5.53 (d, J=7.5 Hz)	78.1
H-25	0.99 (s)	36.1, 47.7	28-*O*-rha		
H-26	0.89 (s)	32.3, 39.8, 42.0, 48.4	H-1	5.79 (br s)	
			H-2	5.12 (d, J=2 Hz)	
H-27	1.24 (s)	26.0, 42.0, 35.6	H-3	4.47 (m)	106.1
H-29	0.87 (s)	34.1	H-4	4.13 (m)	98.7
H-30	0.94 (s)	33.1, 23.6, 46.5	H-5	4.41 (m)	
Sugars			H-6	1.66 (d, J=6 Hz)	
3-*O*-glcA ...			fuc		
H-1	4.90 (d, J=8 Hz)	84.4	H-1	5.21 (d, J=7.5 Hz)	81.9
H-2	4.17 (m)	104.1	glc		
H-3	4.22 (t, J=8.5 Hz)	104.8	H-1	5.17 (d, J=8 Hz)	77.1

ronic acid), 4.09 (1H, m, H-3), 1.41, 1.23, 0.99, 0.94, 0.90, 0.87 (3H each, s, 6×Me); for ^{13}C-nmr data, see Table 1; fabms *m/z* 977 $[M+Na]^+$, 961 $[M+Li]^+$, 845 $[M+Na-xylose]^+$, and 829 $[M+Li-xylose]^+$.

Saponin **3**.—Light yellow powder, $C_{65}H_{102}O_{32}$; ^{13}C- and ^{1}H-nmr data, see Tables 1 and 2, respectively.

ACID HYDROLYSIS AND SUGAR DETECTION OF **1–3**.—A mixture containing each compound (2 mg), 1 ml MeOH-H_2O (1:1), and 0.5 ml 3 N HCl was refluxed in a sealed tube at 105° for 4 h. The solution was neutralized with 0.2 N NaOH, filtered and then taken to dryness. To this was added 0.2 ml DMF and 0.2 ml BSTFA which was silylated at 75° for 15 min. Glucuronic acid, galactose, xylose, rhamnose, fucose, and glucose were identified by comparison with standard samples, respectively, using gc: column: RTX-1 (30 m×0.32 mm i.d.); injection temperature: 230°; column temperature: 145° (1 min), 5°/min 178° (5 min), 1°/min 190°, 15°/min 275° (5.74 min); flow rate (splitless) He 30 ml/min; H_2 30 ml/min; air 300 ml/min, instrument HP-5890. For saponin **3**, the R_f values (min) were as follows: 7.63 (rha), 8.65, 10.06 (fuc), 9.94, 11.34 (xyl), 13.37, 14.75, 16.52 (gal), 17.84, 19.10 (glcA), 15.88, 20.36 (glc).

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