Pancreatic Lipase-Inhibiting Triterpenoid Saponins from *Gypsophila oldhamiana*

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Three new triterpenoid saponins, gypsosaponins A—C (1—3), were isolated from the roots of *Gypsophila oldhamiana* (Caryophyllaceae). Their structures were established as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-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)]-methyl-β-D-glucuronopyranosyl gypsogenin 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (2), and 23-*O*-β-D-glucopyranosyl gypsogenic acid 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (3), on the basis of various spectroscopic analyses and chemical degradations. The biological activities of 1—3 were examined inhibitory activity against pancreatic lipase, which showed inhibition of 58.2%, 99.2% and 50.3% at concentration of 1 mg/ml, respectively.

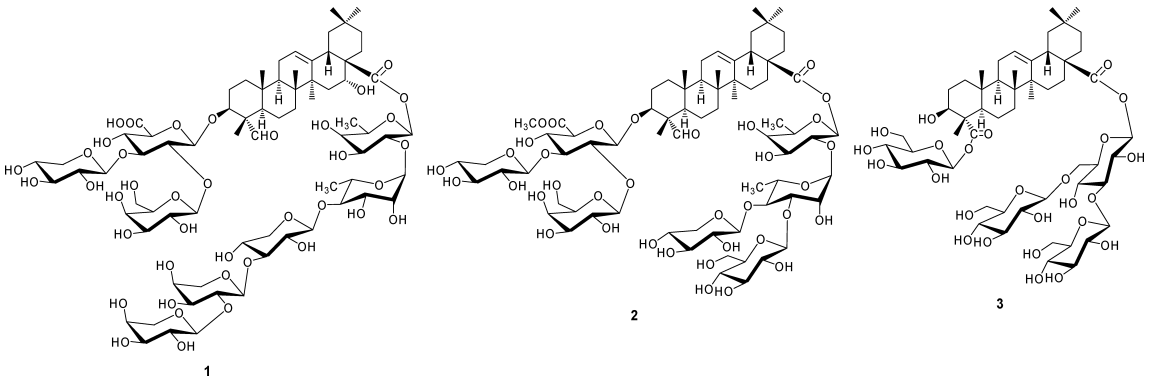
Key words *Gypsophila oldhamiana*; Caryophyllaceae; triterpenoid saponin; pancreatic lipase

Gypsophila oldhamiana (MIQ.) (Caryophyllaceae) is widely distributed in the north of China. Its roots have been used as a traditional Chinese medicine for the treatment of fever, consumptive disease, and infantile malnutrition syndrome.[1)] Previous chemical investigation on the roots of *G. oldhamiana* has demonstrated the occurrence of saponins, sterols, and fatty acids.[2)] As a part of our continuous investigation on bioactive triterpenoid saponins[3—5)] and our current interest in the natural lipase inhibitors,[6,7)] we initiated a chemical investigation of this plant. In this paper, we report the isolation and structure elucidation of three new triterpenoid saponins, named gypsosaponins A—C (**1**—**3**) from the roots of *G. oldhamiana*, along with their pancreatic lipase inhibitory activities.

Results and Discussion

A 95% EtOH extract of the roots of *G. oldhamiana* was suspended in H_2O and then partitioned successively with EtOAc. The water-soluble fraction, on chromatographic purification over Diaion HP-20 column chromatography, followed by repeated medium-pressure liquid chromatography (MPLC) and HPLC purification, afforded three new compounds, gypsosaponins A—C (**1**—**3**).

Gypsosaponins A (**1**) was obtained as an amorphous powder, $[\alpha]_D^{24}$ −5.0° (c=1.0, MeOH). Its molecular formula $C_{74}H_{116}O_{40}$ was determined from data of the positive-ion high-resolution (HR)-FAB-MS (m/z 1667.6956 $[M+Na]^+$). The ^{13}C-NMR spectrum showed 74 carbon signals, of which 30 were assigned to the aglycon, and 44 to the sugar moieties. The ^{1}H-NMR spectrum of **1** showed six tertiary methyl proton singlets at δ 0.82, 0.94, 0.99, 1.04, 1.41 and 1.74, an olefinic proton at δ 5.54 (br s), an aldehyde proton at δ 9.84 (s), and a broad singlet for the hydroxymethylene proton at δ 5.14 characteristic of H-16β, suggesting the aglycon to be quillaic acid.[8)] On acid hydrolysis of **1**, in addition to quillaic acid, it also afforded D-glucuronic acid, D-galactose, D-xylose, D-fucose, L-rhamnose and L-arabinose in the ratio of 1 : 1 : 2 : 1 : 1 : 2 by GC-MS analysis following conversion to the trimethylsilyl thiazolidine derivatives.[9)] In the ^{13}C-NMR spectrum, the chemical shifts of C-3 at δ 84.4 and C-28 at δ 176.0 revealed that **1** was a bisdesmosidic glycoside.[10)] The ^{1}H-NMR data showed eight anomeric proton signals at δ 6.33 (br s, Rha-H-1), 5.95 (d, J=8.0 Hz, Fuc-H-1), 5.51 (d, J=7.6 Hz, Gal-H-1), 5.28 (d, J=7.6 Hz, Xyl I-H-1), 5.13 (d, J=6.6 Hz, Ara I-H-1), 5.13 (d, J=7.1 Hz, Xyl II-H-1), 5.00 (d, J=7.4 Hz, Ara II-H-1), and 4.88 (d, J=7.3 Hz, GlcA-H-1), and the corresponding anomeric carbon signals at δ 101.1, 94.8, 104.1, 105.0, 105.4, 106.2, 106.9 and 103.9 in



1 **2** **3**

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the ^{13}C-NMR spectrum, respectively. All proton signals due to sugars were assigned by careful analyses of the DQF-COSY, TOCSY, HMQC, HMQC-TOCSY and HMBC correlations. The β anomeric configurations for the glucuronopyranosyl, galactopyranosyl, xylopyranosyl and fucopyranosyl moieties were determined from their large $^{3}J_{H1,H2}$ coupling constants (7.1—8.0 Hz). For the rhamnopyranosyl moiety, a broad singlet of H-1 and the three bond strong HMBC correlations from the anomeric proton to Rha-C-3 and Rha-C-5, indicated that the anomeric proton was equatorial, thus possessing a configuration in the $^{1}C_{4}$ form.[11] Two arabinopyranosyl moieties were both determined as α-configuration on the basis of the $^{3}J_{H1,H2}$ values (6.6, 7.4 Hz) and the NOESY correlation of H-1/H-3 and H-1/H-5 in the $^{4}C_{1}$ form.[12] The linkage of the sugar units at C-3 of the aglycon was established from the following HMBC correlations: δ_H 5.51 (Gal-H-1) with δ_C 78.5 (GlcA-C-2), δ_H 5.28 (Xyl I-H-1) with δ_C 86.2 (GlcA-C-3), and δ_H 4.88 (GlcA-H-1) with δ_C 84.4 (C-3). Similarly, the sugar chain at C-28 was established from the following HMBC correlations: δ_H 6.33 (Rha-H-1) with δ_C 73.9 (Fuc-C-2), δ_H 5.13 (Xyl II-H-1) with δ_C 83.8 (Rha-C-4), δ_H 5.13 (Ara I-H-1) with δ_C 86.8 (Xyl II-C-3), δ_H 5.00 (Ara II-H-1) with δ_C 78.6 (Ara I-C-2), and δ_H 5.95 (Fuc-H-1) with δ_C 176.0 (C-28). The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiment. Therefore, the structure of gypsosaponins A (**1**) was elucidated as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside.

Table 1. ^{13}C-NMR Spectroscopic Data (δ) for the Aglycon Moieties of **1**—**3** (125 MHz in pyridine-d_5)

Position	**1**	**2**	**3**
1	38.2	38.1	38.9
2	25.2	25.2	27.5
3	84.4	84.6	75.1
4	55.2	55.0	55.1
5	48.6	48.9	52.2
6	20.6	20.6	23.3
7	32.9	32.5	32.8
8	40.3	40.2	40.2
9	47.0	47.8	48.4
10	36.3	36.3	37.0
11	23.8	23.7	23.8
12	122.1	122.4	123.1
13	144.6	144.1	144.2
14	42.1	42.2	42.1
15	36.3	28.1	28.2
16	74.0	23.5	23.3
17	49.3	47.0	47.0
18	41.6	41.9	41.7
19	47.4	46.3	46.2
20	30.8	30.8	30.7
21	36.0	33.9	33.9
22	31.9	32.4	32.5
23	209.8	210.2	177.6
24	11.0	11.2	12.0
25	15.8	15.8	16.2
26	17.5	17.4	17.4
27	27.1	25.9	26.0
28	176.0	176.4	176.3
29	33.2	33.2	33.1
30	24.6	23.8	23.7

Gypsosaponins B (**2**) was obtained as an amorphous powder, $[\alpha]_D^{21}$ −1.6° (c=1.0, MeOH). The molecular formula $C_{71}H_{112}O_{36}$ was determined by the positive-ion HR-FAB-MS (m/z 1563.6850 $[M+Na]^+$). The ^{1}H- and ^{3}C-NMR spectra data assignable to both the aglycon and the sugar moieties showed different signal patterns compared to **1**. The six tertiary methyl groups at δ 0.83, 0.88, 0.92, 1.07, 1.21 and 1.47, an olefinic proton at δ 5.36 (br s) and an aldehyde proton at δ 9.94 (s) were observed in the ^{1}H-NMR spectrum, coupled with the information from the ^{13}C-NMR spectrum (six sp^3 carbons at δ 15.8, 33.2, 23.8, 17.4, 25.9 and 11.2, and two sp^2 olefinic carbons at δ 122.4 and 144.1, and an sp^2 aldehyde carbon at δ 210.2), indicating the aglycon to be gypsogenin.[13] Acid hydrolysis of **2** yielded gypsogenin and monosaccharides as D-glucuronic acid, D-galactose, D-xylose, D-fucose, L-rhamnose and D-glucose in the ratio of 1 : 1 : 2 : 1 : 1 : 1. The chemical shifts of C-3 and C-28 were observed at δ_C 84.4 and 176.0, suggesting that **2** was a bisdesmosidic glycoside. The ^{1}H- and ^{13}C-NMR spectra showed six anomeric proton signals at δ 5.99 (d, J=8.0 Hz, Fuc-H-1), 5.89 (br s, Rha-H-1), 5.52 (d, J=7.6 Hz, Gal-H-1), 5.46 (d, J=7.8 Hz, Xyl II-H-1), 5.40 (d, J=7.6 Hz, Glc-H-1), 5.27 (d, J=7.8 Hz, Xyl I-H-1), and 4.86 (d, J=7.3 Hz, GlcA-H-1), and the corresponding anomeric carbon signals at δ 95.0, 102.1, 104.2, 105.2, 105.4, 105.0, and 103.9, respectively. Carefully analyses of the ^{1}H- and ^{13}C-NMR and 2D extensive NMR data, suggested the presence of a β-galactopyranosyl, two β-xylopyranosyl, a β-glucuronopyranosyl, a β-glucopyranosyl, an α-rhamnopyranosyl, and a β-fucopyranosyl moieties. From its ^{1}H- and ^{13}C-NMR data, it was evident that the sugar structure at C-3 was the same as that in **1**, except that one methyl group was attached the carboxyl group of C-6 in the glucuronic acid moiety, which was confirmed by the HMBC correlation between the methyl proton (δ 3.73) and the carboxyl carbon of glucuronic acid (δ 169.9). The linkage of the remaining four sugars at C-28 was determined from the following HMBC correlations: δ_H 5.89 (Rha-H-1) with δ_C 75.7 (Fuc-C-2), δ_H 5.40 (Glc-H-1) with δ_C 78.8 (Rha-C-3), δ_H 5.46 (Xyl II-H-1) with δ_C 82.4 (Rha-C-4), and δ_H 5.99 (Fuc-H-1) with δ_C 176.4 (C-28). Thus, the structure of gypsosaponins B (**2**) was established as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-methyl-β-D-glucuronopyranosyl gypsogenin 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside. Since **2** was not detectable in the 95% EtOH extract by HPLC analysis, it was considered as an artifact during the isolation and purification.

Gypsosaponins C (**3**) was isolated as an amorphous powder, $[\alpha]_D^{21}$ +14.9° (c=0.9, MeOH). The molecular formula $C_{54}H_{86}O_{25}$ was determined by the positive-ion HR-FAB-MS (m/z 1157.5372 $[M+Na]^+$). In the ^{1}H-NMR spectrum of **3**, six tertiary methyl groups at δ 0.85, 0.86, 0.96, 1.03, 1.10 and 1.57, and one olefinic proton at δ 5.39 (br s) were observed, coupled with the information from the ^{13}C-NMR spectrum (six sp^3 carbons at δ 33.1, 23.7, 16.2, 17.4, 26.0 and 12.0, and two sp^2 olefinic carbons at δ 123.1 and 144.2, and an sp^2 carbonyl carbon signal at δ 177.6), indicating that the aglycon was gypsogenic acid.[14] Acid hydrolysis of **3** yielded gypsogenic acid and only D-glucose as component sugars. The ^{1}H- and ^{13}C-NMR spectra showed four anomeric

Table 2. ^{13}C- and ^{1}H-NMR Spectroscopic Data (δ) for the Sugar Moieties of **1**—**3** in Pyridine-d_5

	1		**2**		**3**		
Position	δ_C	δ_H	δ_C	δ_H	Position	δ_C	δ_H
3-*O*-Sugar					23-*O*-Sugar		
Glc A					Glc I		
1	103.9	4.88 (d, 7.3)	103.9	4.86 (d, 7.3)	1	96.6	6.40 (d, 8.0)
2	78.5	4.29 (dd, 8.7, 7.3)	78.5	4.28 (dd, 8.7, 7.3)	2	74.5	4.15
3	86.2	4.23 (t, 9.0)	85.7	4.19 (dd, 9.0, 8.0)	3	78.7	4.23
4	71.3	4.40 (t, 9.6)	71.0	4.25 (t, 9.6)	4	71.3	4.28
5	77.3	4.48	76.4	4.38	5	79.4	3.97
6	171.7		169.9		6	62.3	4.28 (dd, 11.0, 5.0)
CH_3			52.2	3.73 (s)			4.33 (dd, 11.0, 2.2)
Gal					28-*O*-Sugar		
1	104.1	5.51 (d, 7.6)	104.2	5.52 (d, 7.6)	Glc II		
2	73.7	4.38 (dd, 8.7, 7.6)	73.7	4.44 (dd, 8.7, 7.6)	1	95.1	6.14 (d, 8.3)
3	75.4	4.09	75.4	4.13	2	72.7	4.08
4	70.2	4.51 (t, 3.5)	70.2	4.54	3	88.5	4.17
5	76.6	3.95	76.7	4.00	4	69.0	4.21
6	61.7	4.33 (dd, 13.3, 5.7)	61.7	4.42	5	77.6	3.99
		4.46		4.49	6	69.0	4.22
Xyl I							4.56 (br d, 10.1)
1	105.0	5.28 (d, 7.6)	105.0	5.27 (d, 7.8)	Glc III		
2	75.3	3.91 (t, 7.6)	75.2	3.93 (t, 7.8)	1	105.7	5.22 (d, 7.8)
3	78.5	4.06 (t, 8.0)	78.5	4.08	2	75.5	3.98
4	70.8	4.07	70.8	4.11	3	78.3	4.12 (t, 8.5)
5	67.3	3.62 (t, 10.6)	67.3	3.62 (t, 10.3)	4	71.7	4.08
		4.21 (dd, 10.6, 3.0)		4.22	5	78.6	3.93
28-*O*-Sugar					6	62.6	4.22
Fuc							4.47 (dd, 11.5, 2.3)
1	94.8	5.95 (d, 8.0)	95.0	5.99 (d, 8.0)	Glc IV		
2	73.9	4.57 (t, 9.6)	75.7	4.43	1	105.3	4.97 (d, 7.7)
3	76.5	4.10	72.2	3.85 (dd, 9.0,3.5)	2	75.2	3.95
4	73.1	3.91	75.3	4.09	3	78.4	4.15
5	72.4	3.88	72.9	3.94 (q, 6.0)	4	71.7	4.14
6	16.9	1.45 (d, 6.2)	16.9	1.45 (d, 6.0)	5	78.3	3.82
Rha					6	62.8	4.27
1	101.1	6.33 (br s)	102.1	5.89 (br s)			4.42 (dd, 11.6, 2.1)
2	71.9	4.71 (br s)	70.9	5.22 (br s)			
3	72.4	4.60 (dd, 8.7, 3.0)	78.8	4.50			
4	83.8	4.28 (dd, 9.0, 8.7)	82.4	4.90			
5	68.2	4.39	69.0	4.46			
6	18.5	1.56 (d, 5.9)	18.9	1.70 (d, 5.7)			
Glc							
1			105.4	5.40 (d, 7.6)			
2			75.4	4.00 (t, 8.0)			
3			78.6	4.13			
4			72.0	4.04			
5			78.3	3.92			
6			63.0	4.19			
				4.52			
Xyl II							
1	106.2	5.13 (d, 7.1)	105.2	5.46 (d, 7.8)			
2	75.3	3.91	75.9	3.92 (t, 7.8)			
3	86.8	3.96	79.4	4.08			
4	68.9	3.98	71.3	4.15			
5	66.9	3.36 (t, 11.0)	67.1	3.37 (t, 12.5)			
		4.13 (dd, 11.0, 5.0)		4.14			
Ara I							
1	105.4	5.13 (d, 6.6)					
2	78.6	4.31 (dd, 8.7, 6.6)					
3	74.3	4.16					
4	73.2	4.35					
5	66.5	3.74 (br d, 11.4)					
		4.43 (dd, 11.4, 3.5)					
Ara II							
1	106.9	5.00 (d, 7.4)					
2	73.0	4.40 (dd, 8.0, 6.6)					
3	74.5	4.07					
4	69.6	4.20					
5	67.2	3.70 (br d, 11.4)					
		4.21 (dd, 11.4, 3.5)					

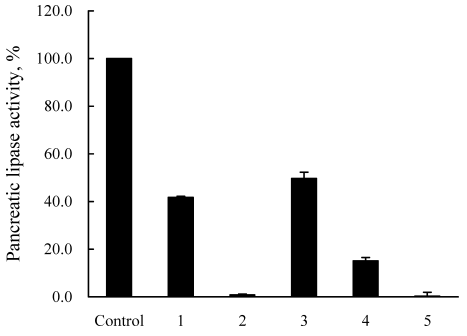

Fig. 1. Effects of Various Saponins Isolated from *Gypsophila oldhamiana* on Pancreatic Lipase Activity *in Vitro*

Results are expressed as mean±S.E.M., $n=4$. (**1**—**3**: gypsosaponins A—C, final concentration: 1 mg/ml; **4**, **5**: olistat, final concentration: 0.01 and 0.1 mg/ml respectively).

proton signals at δ 6.40 (d, $J=8.0$ Hz, Glc I-H-1), 6.14 (d, $J=8.3$ Hz, Glc II-H-1), 5.22 (d, $J=7.8$ Hz, Glc III-H-1) and 4.97 (d, $J=7.7$ Hz, Glc IV-H-1) and the corresponding anomeric carbon signals at δ 96.6, 95.1, 105.7 and 105.3, respectively. The trisaccharide moiety attached to C-28 was established by the HMBC correlations of δ_H 5.22 (Glc III-H-1) with δ_C 88.5 (Glc II-C-3), δ_H 4.97 (Glc IV-H-1) with δ_C 69.0 (Glc II-C-6) and δ_H 6.14 (Glc I-H-1) with δ_C 176.3 (C-28). The remaining glucopyranosyl moiety attached to C-23 was determined from the HMBC correlations between δ_H 6.40 (Glc I-H-1) and δ_C 177.6 (C-23). Thus, the structure of gypsosaponins C (**3**) was determined to be 23-*O*-β-D-glucopyranosyl gypsogenic acid 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

It is known that reduction of dietary fat absorption through pancreatic lipase inhibition is an effective way to manage obesity.[15] It has been reported that oleanane-type triterpenoid saponins exhibited inhibitory effects on pancreatic lipase and suppressed the increase of body weight due to a high-fat diet.[4] Therefore, we decided to conduct the pancreatic lipase inhibitory test on the extract of *G. oldhamiana* and gypsosaponins A—C (**1**—**3**). The water-soluble fraction of the roots of *G. oldhamiana* showed potent inhibitory activity against pancreatic lipase dose-dependently with an IC_{50} value of 0.54 mg/ml. As shown in Fig. 1, gypsosaponins B (**2**) showed inhibitory activity against pancreatic lipase with inhibition of 99.2% and gypsosaponins A (**1**) and C (**3**) with inhibition of 58.2% and 50.3% in the concentration of 1 mg/ml.

Experimental

General Experimental Procedures The IR spectra were determined with a JASCO FT/IR-300E (by a KBr disk method) spectrophotometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter in a 0.5 dm length cell. ^{1}H- and ^{13}C-NMR were recorded using a JEOL ECP-500 NMR spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). MALDI-TOF MS was conducted using a PerSeptive Biosystems Voyager DE-STR, and HR-FAB-MS was taken on a JEOL JMS-700 MStation. Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (silica gel 60, Kanto Chemical Co., Inc., Tokyo, Japan) and ODS (Chromatorex DM1020TM, 100—200 mesh, Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for column chromatography. Preparative HPLC was performed on a JASCO model PU-2080 HPLC system, equipped with a Shodex RI-101 differential refractometer detector and a YMC-Pack RP-C_{18} column (150×20 mm i.d.). TLC was conducted with Kieselgel 60 F_{254} plates (E. Merck). GLC was carried out on a Perkin-Elmer Clarus 500 GC-MS instrument.

Plant Material *Gypsophila oldhamiana* was collected from Dalian, Liaoning Province, People's Republic of China, in October 1999, and identified by Professor Chen Chen (Liaoning Normal University).

Extraction and Isolation The air-dried roots of *G. oldhamiana* (18 kg) were refluxed three times with 95% aqueous EtOH. The combined EtOH extracts were concentrated (1200 g), suspended in H_2O, and then partitioned with EtOAc successfully. The water-soluble fraction (890 g) was applied to a column of Diaion HP-20 and eluted with 40, 70 and 100% MeOH. The fractions eluted with 70% MeOH were combined and repeatedly chromatographed over silica gel, ODS open columns, MPLC, and further HPLC purification (62—70% MeOH in H_2O containing 0.05% trifluoroacetic acid, UV detector, 210 nm) to afford **1** (1400 mg), **2** (30 mg), and **3** (9 mg), respectively.

Gypsosaponin A (**1**): An amorphous powder; $[\alpha]_D^{24}$ −5.0° ($c=1.0$, MeOH); IR (KBr) ν_{max} cm^{-1}: 3423, 1846, 1637. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 9.84 (s, H-23), 5.54 (1H, br s, H-12), 4.03 (1H, m, H-3), 5.14 (1H, s, H-16), 0.82, 0.94, 0.99, 1.04, 1.41, 1.74 (3H each, s, H-25, 29, 23, 26, 30, 24, 27); other NMR data, see Tables 1—3; MALDI-TOF MS (positive) *m/z* 1667 $[M+Na]^+$; HR-FAB-MS (positive) *m/z* 1667.6956, (Calcd for $C_{74}H_{116}O_{40}Na$, 1667.6941).

Gypsosaponin B (**2**): An amorphous powder; $[\alpha]_D^{21}$ −1.6° ($c=1.0$, MeOH); IR (KBr) ν_{max} cm^{-1}: 3419, 1738, 1639. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 9.94 (s, H-23), 5.36 (1H, br s, H-12), 4.03 (1H, m, H-3), 3.10 (1H, br s, H-18), 0.83, 0.88, 0.92, 1.07, 1.21, 1.47 (3H each, s, H-25, 29, 30, 23, 26, 27, 24); other NMR data, see Tables 1—3; MALDI-TOF MS (positive) *m/z* 1563 $[M+Na]^+$; HR-FAB-MS (positive) *m/z* 1563.6850 $[M+Na]^+$ (Calcd for $C_{71}H_{112}O_{36}Na$, 1563.6831).

Gypsosaponin C (**3**): An amorphous powder; $[\alpha]_D^{21}$ +14.9° ($c=0.9$, MeOH); IR (KBr) ν_{max} cm^{-1}: 3393, 1731, 1645. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 5.39 (1H, br s, H-12), 3.13 (1H, dd, $J=13.7$, 3.9 Hz, H-18), 4.62 (1H, dd, $J=11.0$, 5.3 Hz, H-3), 0.85, 0.86, 0.96, 1.03, 1.10, 1.57 (3H each, s, H-29, 30, 25, 26, 27, 24); other NMR data, see Tables 1—3; MALDI-TOF MS (positive) *m/z* 1157 $[M+Na]^+$; HR-FAB-MS (positive) *m/z* 1157.5372 $[M+Na]^+$ (Calcd for $C_{54}H_{86}O_{25}Na$, 1157.5356).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides A solution of **1** (10 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was heated at 100 °C for 2 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (2 ml×3).The extract was washed with H_2O, dried over $MgSO_4$, and evaporated to give quillaic acid (2 mg), which was identified by comparison of their $[\alpha]_D$ and ^{1}H-NMR data with the standard sample. The H_2O layer was concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 ml), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 ml) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 ml each) and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, EQUITY^{TM}-1 (30 m×0.25 mm×0.25 μm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas; detection in EI mode, ionization potential, 70 eV; ion-source temperature, 280 °C. In the acid hydrolysate of **1**, D-galactose, D-glucuronic acid, L-rhamnose, D-fucose, L-arabinose and D-xylose were confirmed by comparison of the retention times of their derivatives with those of D-galactose, D-glucuronic acid, L-rhamnose, D-fucose, L-arabinose and D-xylose derivatives prepared in the same way, which showed retention times of 11.40, 8.60, 7.51, 7.74, 6.31 and 6.30 min, respectively. It was very difficult to distinguish D-xylose and L-arabinose because their retention times were very close by using L-cysteine methyl ester hydrochloride. Therefore, D-cysteine methyl ester hydrochloride was used to prepare the trimethylsilyl thiazolidine derivatives of L-arabinose and D-xylose, which showed retention times of 13.20 and 13.05 min, respectively. In a similar way, **2** (10 mg) and **3** (5 mg) afforded the aglycone as gypsogenin (2 mg) and gypsogenic acid (1 mg), respectively. The component sugars in **2** and **3** were also determined by the same method as **1**.

Measurement of Pancreatic Lipase Activity Lipase activity was determined by measuring the rate of release of oleic acid from triolein. A suspension of triolein (80 mg), lecithin (10 mg) and taurocholic acid (5 mg) in 9 ml of 0.1 M *N*-tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid buffer (pH 7.0) containing 0.1 M NaCl was sonicated for 5 min. The sonicated substrate suspension (0.05 ml) was incubated with 0.05 ml of pancreatic lipase and 0.1 ml of various concentrations of the sample solution for 30 min at 37 °C in a final volume of 0.2 ml. The amount of release of oleic acid produced was determined based on the method described by Zapf *et al.*[16] with a minor modification.[17] The absorbance was then measured at 480 nm. The li-

pase activity was expressed as moles of oleic acid released per liter of reaction mixture per hour.

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