New Triterpenoid Saponins and Sapogenins from *Saponaria officinalis*

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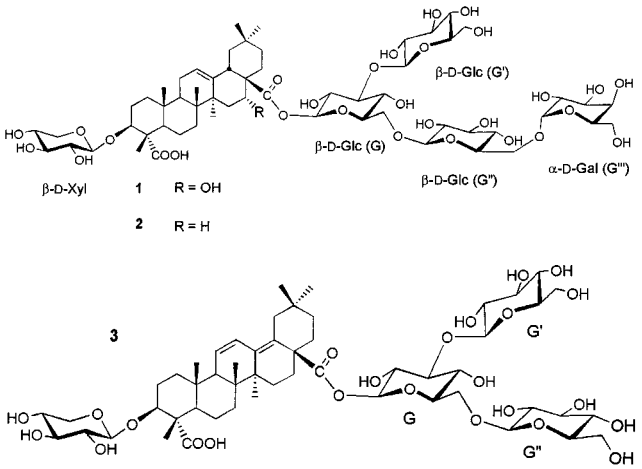
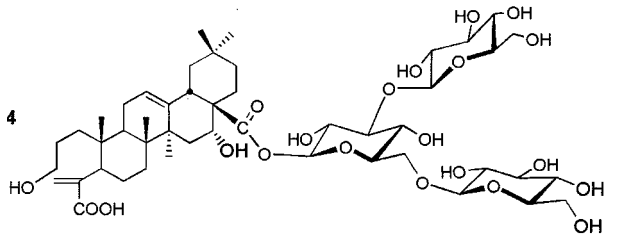
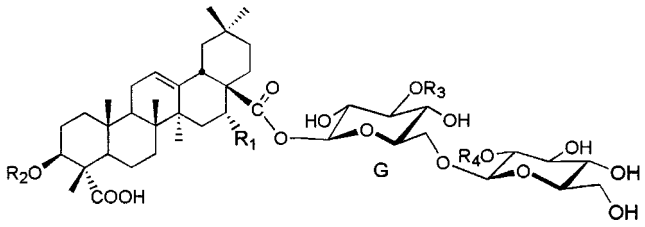
Received June 21, 1999

Five new triterpenoid saponins, named saponariosides I−M, were isolated from the whole plants of *Saponaria officinalis*. Their structures were established as saponarioside I (**1**) [3-*O*-*β*-D-xylopyranosyl-16α-hydroxygypsogenic acid 28-*O*-α-D-galactopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside], saponarioside J (**3**) [3-*O*-*β*-D-xylopyranosylolean-11,13(18)-diene-23,28-dioic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside], saponarioside K (**4**) [3,4-*seco*-16α-hydroxygypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside], saponarioside L (**5**) [3-*O*-*β*-D-xylopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside], and saponarioside M (**6**) [3-*O*-*β*-D-glucopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] by NMR studies and chemical degradations. The aglycons of saponariosides J (**3**) and K (**4**) are new sapogenins.

Saponaria officinalis L. (Caryophyllaceae), commonly called fuller's herb or soapwort, is native to Europe and western to central Asia and is cultivated in many countries throughout the world. This species is well-known for its detergent properties and was used as a soap in ancient times. Medicinally, it has been used as an expectorant in bronchitis, and in folk medicine it is still used for skin complaints and in rheumatic disorders.[1] The isolation and structure elucidation of eight triterpenoid saponins, saponariosides A−H, from the whole plant of *S. officinalis* was reported earlier.[2,3] Further investigation of the plant material collected from another location, has led to the isolation of nine triterpenoid saponins, including five new compounds and two new aglycons. In this paper, we report the isolation and structure study of these saponins, saponariosides I−M (**1**, **3**−**6**).







	R_1	R_2	R_3	R_4
5	H	Xyl	Glc (G')	H
6	H	Xyl	H	Glc (G")
7	H	Glc	Glc	Glc
8	OH	Xyl	Glc	Glc
9	OH	Xyl	Glc	H

Results and Discussion

A methanolic extract of the whole plant of *S. officinalis* was suspended in water and then partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction, on chromatographic purification over Diaion HP-20 followed by repeated MPLC and HPLC purification, afforded five new triterpenoid saponins, saponariosides I (**1**), J (**3**), K (**4**), L (**5**), and M (**6**), along with four previously isolated compounds, saponariosides C (**2**), E (**7**), F (**8**), and G (**9**).[3]

Saponarioside I (**1**) was assigned a molecular formula of $C_{59}H_{94}O_{30}$ as determined from its MALDI-TOF MS (*m*/*z* 1305 $[M + Na]^+$, 1321 $[M + K]^+$) and from its DEPT ^{13}C NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. Of the 59 carbons, 30 were assigned to the aglycon and 29 to the oligosaccharide moieties. Detailed NMR analysis identified the aglycon as 16α-hydroxygypsogenic acid (Table 1).[3] The chemical shifts of C-3 (*δ* 85.0) and C-28 (*δ* 175.7) revealed that **1** was a bisdesmosidic glycoside. The pentasaccharide nature of compound **1** was evident in its ^1H [*δ* 4.89 d (*J* = 7.7 Hz), 4.99 d (*J* = 7.4), 5.22 d (*J* = 7.8), 5.46 d (*J* = 3.7),

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10.1021/np990311r CCC: $18.00
Published on Web 11/11/1999

Table 1. ^{13}C NMR Data for the Aglycon Parts of Saponariosides I (**1**), J (**3**), K (**4**), L (**5**), and M (**6**) (125 MHz, pyridine-d_5)[a]

position	**1**	**3**	**4**	**5** and **6**
1	38.9	38.3	37.2	38.8
2	26.4	26.7	27.6	26.3
3	85.0	85.1	63.3	85.1
4	53.4	53.4	146.3	53.3
5	52.2	51.7	43.5	52.1
6	21.4	21.4	26.0	23.2
7	33.1	32.8	32.2	33.9
8	40.4	41.5	39.7	40.2
9	47.4	54.8	37.5	48.3
10	36.7	36.4	39.8	36.7
11	23.8	127.1	24.4	23.8
12	122.5	125.9	122.8	122.7
13	144.4	137.1	144.3	144.0
14	42.0	42.4	42.7	42.1
15	36.1	37.0	36.1	28.2
16	74.1	25.3	74.2	21.3
17	49.1	48.7	49.2	46.9
18	41.2	132.1	41.4	41.7
19	47.1	40.8	47.3	46.1
20	30.8	32.7	30.8	30.7
21	32.2	37.1	32.4	32.8
22	35.8	32.0	35.9	32.4
23	180.4	180.2	171.6	180.6
24	12.7	12.1	124.4	12.7
25	16.2	18.8	19.1	16.0
26	17.4	16.7	17.7	17.3
27	27.1	19.9	27.2	26.0
28	175.7	175.5	175.9	176.3
29	33.1	32.2	33.2	33.1
30	24.6	24.3	24.6	23.7

[a] The assignments are based upon ^1H NMR, ^{13}C NMR, COSY, HOHAHA, DEPT, HMQC, HMBC, and NOESY spectra.

Table 2. ^{13}C NMR Data for the Sugar Moieties of Compounds **1** and **3**−**6** (125 MHz in pyridine-d_5)[a]

sugar units	**1**	**3**	**4**	**5**	**6**
3-*O*-sugar					
1	106.3	106.4		106.3	106.3
2	75.3	75.4		75.3	75.3
3	78.1	78.2		78.1	78.1
4	71.1	71.1		71.1	71.1
5	67.1	67.1		67.1	67.1
28-*O*-sugar					
1	95.0	95.6	95.3	95.1	95.6
2	72.8	72.8	72.7	72.7	74.1
3	88.3	88.4	88.4	88.4	78.8
4	69.0	69.4	68.8	68.7	71.0
5	77.6	77.9	77.6	77.6	77.1
6	69.2	69.3	68.9	68.8	69.3
1′→3					
1′	105.7	105.8	105.7	105.7	
2′	75.5	75.5	75.4	75.4	
3′	78.3	78.3	78.3	78.3	
4′	71.6	71.5	71.5	71.5	
5′	78.5	78.4	78.4	78.4	
6′	62.5	62.6	62.6	62.6	
1″→6					1′→6
1″	105.3	105.3	105.4	105.3	102.8
2″	74.9	75.2	75.2	75.2	84.6
3″	78.2	78.7	78.6	78.6	78.3
4″	71.9	71.6	71.6	71.6	70.3
5″	76.2	78.5	78.4	78.4	78.4
6″	68.2	62.4	62.4	62.4	62.1
1‴→6					1″→2
1‴	100.6				106.4
2‴	70.6				76.4
3‴	71.6				78.2
4‴	71.1				70.6
5‴	72.5				78.6
6‴	62.7				62.1

[a] The assignments are based upon ^1H NMR, ^{13}C NMR, COSY, HOHAHA, DEPT, HMQC, HMBC, and NOESY spectra.

6.20 d (J = 8.2)] and ^{13}C [δ 95.0, 100.6, 105.3, 105.7, 106.3] NMR spectra, respectively (Tables 2 and 3). The identity of the monosaccharides and the determination of the oligosaccharide chain sequence were carried out by a combination of DQF-COSY, HOHAHA, DEPT, HMQC, HMBC, and phase-sensitive NOESY NMR experiments. Among the five sugar units in the molecule, three were identified as glucose, one as xylose, and the remaining one as galactose. The β anomeric configurations for the glucose and xylose units were determined from their $^3J_{H1,\ H2}$ coupling constants (7−8 Hz), and the galactose unit was determined to be of an α configuration based on the $^3J_{H1,\ H2}$ (3.7 Hz) and $^1J_{CH}$ (167 Hz; 163 Hz for the β-anomer[2]) values observed.

The detailed sugar arrangement and the linkages between the sugar and the aglycon were determined from an HMBC experiment with the same conclusions with regard to the sugar sequence also drawn from the NOESY experiment. The absolute configurations of the sugars were determined by HPLC analysis after conversion to 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives.[4,5] Thus, saponarioside I was elucidated as 3-*O*-β-D-xylopyranosyl-16α-hydroxygypsogenic acid 28-*O*-α-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**1**). In a previous communication,[3] we have reported an α-D-galactose-containing saponin, saponarioside C, possessing the same sugar arrangement but differing in the aglycon, also isolated from *S. officinalis* collected at the Toho University botanical garden. However, at that time we were unable to detect compound **1**, found as a relatively abundant compound in the present study.

Saponarioside J (**3**) had a molecular formula of $C_{52}H_{82}O_{24}$ determined from its MALDI-TOF MS (*m*/*z* 1125 $[M + Na]^+$, 1141 $[M + K]^+$) and from its DEPT ^{13}C NMR data. The UV spectrum indicated the presence of a conjugated diene system in the molecule (λ_{max} 242, 251, and 261 nm). The ^1H NMR spectrum exhibited two olefinic proton signals at δ 5.73 (1H, d, J = 11.2 Hz) and 6.59 (1H, dd, J = 11.2, 3.1 Hz), while the ^{13}C NMR spectrum showed four olefinic carbons at δ 125.9 (d), 127.1 (d), 132.1 (s), and 137.1 (s), respectively. Six sp^3 carbons were assigned to the methyl group at δ 12.1, 16.7, 18.8, 19.9, 24.3, and 32.2, and the corresponding methyl protons were identified by an HMQC experiment. Two carboxyl carbons and a methine carbon bearing oxygen were found at δ 175.5 (assigned to C-28), 180.2 (C-23), and 85.1 (C-3) ppm, respectively. The structure assignment for the aglycon was initiated from the long-range coupling networks observed between the methyl and olefinic protons with the adjacent carbons in an HMBC experiment (Figure 1). After extensive NMR analysis, the aglycon was established to be olean-11,13(18)-diene-23,-28-dioic acid (Table 1), a new triterpenoid sapogenin. The chemical shifts of C-3 (δ 85.1) and C-28 (δ 175.5) revealed that **3** was a bisdesmosidic glycoside. The tetrasaccharide nature of compound **3** was evident in its ^1H [δ 5.00 d (J = 7.7 Hz), 5.05 d (J = 7.3 Hz), 5.27 d (J = 7.9 Hz), 6.23 d (J = 8.2 Hz)] and ^{13}C [δ 95.6, 105.3, 105.8, 106.4] NMR spectra, respectively (Tables 2 and 3). The overall structure assignment was accomplished using the same protocol as for **1**. Among the four sugar units in the molecule, three were identified as glucose and the other as xylose. The β-anomeric configurations for these sugars were determined from their $^3J_{H1,H2}$ coupling constants (7−8 Hz). The exact sugar arrangement at C-28 was established from the following HMBC correlations: H-1′ with C-3, H-1″ with C-6. The attachment of the trisaccharide chain to C-28 of

Table 3. ^{1}H NMR Data for the Sugar Moieties of Saponariosides I (**1**), J (**3**), and M (**6**) (500 MHz in pyridine-d_5)[a]

sugar units	**1**	**3**	**6**
3-*O*-sugar			
1	4.99 d ($^3J_{H1,H2}$7.4 Hz)	5.05 d (7.3)	5.01 d (7.3)
2	3.96	4.00	3.95
3	4.04	4.11	4.03
4	4.19	4.22	4.20
5	3.69 dd (11.0, 10.5)	4.40	4.34
	4.35 dd (11.0, 5.2)	3.73 t (11.0)	3.67 t (11.0)
28-*O*-sugar			
1	6.20 d (8.2)	6.23 d (8.2)	6.24 d (7.3)
2	4.11	4.10	4.25
3	4.22	4.20	4.08
4	4.23	4.20	4.19
5	4.06	4.10	4.05
6	4.28, 4.63	4.65 d (11.4), 4.21	4.51, 4.33
1′→3			
1′	5.22 d (7.8)	5.27 d (7.9)	
2′	4.00	4.02	
3′	4.13	4.21	
4′	4.14	4.21	
5′	3.98	3.98	
6′	4.25, 4.50	4.28, 4.53	
1″→6			1′→6
1″	4.89 d (7.7)	5.00 d (7.7)	4.95 d (7.7)
2″	3.94	3.99	4.03
3″	4.13	4.21	4.22
4″	4.04	4.21	4.22
5″	3.62	3.98 m	3.78
6″	4.28, 4.48	4.36	4.36 (2H)
		4.49 dd (11.0, 2.4)	
1‴→6″			1″→2′
1‴	5.46 d (3.7)		5.30 d (7.7)
2‴	4.64 dd (3.7, 9.8)		4.04
3‴	4.55 dd (9.8, 3.2)		4.08
4‴	4.59 d (3.2)		4.19
5‴	4.60 m		3.91 m
6‴	4.40 (2H) d (6.4)		4.36 (2H)

[a] The assignments are based upon ^{1}H NMR, ^{13}C NMR, COSY, HOHAHA, DEPT, HETCOR, HMBC, and NOESY spectra.

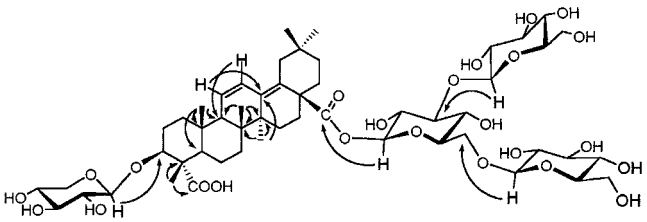

Figure 1. Selected HMBC correlations for saponarioside J (**3**).

the aglycon was based on a correlation between H-1 and C-28 of the aglycon. The remaining xylose unit was attached to C-3 of the aglycon as determined from the HMBC correlation between H-1 of xylose and C-3 of the aglycon (Figure 1). Thus, saponarioside J was elucidated as 3-*O*-*β*-D-xylopyranosylolean-11,13(18)-diene-23,28-dioic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (**3**).

Saponarioside K (**4**) had a molecular composition $C_{48}H_{76}O_{21}$ as determined from MALDI-TOF MS analysis (molecular ions at *m*/*z* 1011 $[M + Na]^+$, 1027 $[M + K]^+$) and the DEPT ^{13}C NMR data. The spectral features indicated that saponarioside K (**4**) possesses the same sugar chain attached to C-28 of the aglycon as in **3**. The aglycon part of **4**, unlike the aglycon of **1** (16α-hydroxygypsogenic acid), showed five methyl proton singlets (^{1}H NMR, *δ* 1.00, 1.01, 1.05, 1.23, 1.79) and two vinyl singlets (*δ* 5.59, 6.57) besides a triplet-like vinyl proton (*δ* 5.63). The characteristic *t*-like signal at *δ* 5.63 coupled with the two sp^2 hybrid carbons (*δ* 122.8, d and 144.3, s) indicated that the aglycon was of an olean-12-ene skeleton. Detailed

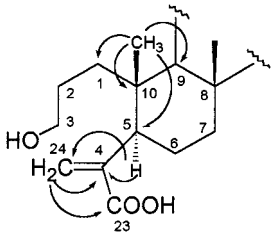

Figure 2. Key HMBC correlations for the aglycon part of saponarioside K (**4**).

analysis of the COSY, HOHAHA, HMQC, HMBC, and NOESY NMR spectra indicated that the aglycon in **4** possesses the same partial structure in the C, D, and E rings as that of 16α-hydroxygypsogenic acid. However, a $-CH_2CH_2CH_2OH$ unit and an α-substituted α,*β*-unsaturated carbonyl group, which originated from the A ring of 16α-hydroxygypsogenic acid, was mapped out from the one-bond and long-range couplings (Figure 2). The above information suggested that the aglycon in **3** was the 3,4-*seco* derivative of 16α-hydroxygypsogenic acid. The 3,4-*seco* A ring has a significant influence on the NMR chemical shifts of the B ring. The C-5 and C-9 signals were shifted to higher field, while C-6 and C-10 occurred at lower field. Also, C-9 moved to *δ* 37.5, 10 ppm different from its counterpart (*δ* 47.4) in saponarioside I (**1**). Based upon the above information, saponarioside K was elucidated as 3,4-*seco*-16α-hydroxygypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (**4**), with 3,4-*seco*-16α-hydroxygypsogenic acid being a novel aglycon. Its analogue, 3,4-*seco*-gypsogenic acid has been isolated as an aglycon of triterpenoid saponins from *Dianthus superbus* L. var. *longicalycinus* Williams[6] and *Vaccaria segetalis* (Neck) Garcke[7] (both Caryophyllaceae).

Saponarioside L (**5**) had a molecular composition $C_{53}H_{84}O_{24}$ as determined from MALDI-TOF MS analysis (molecular ion at *m*/*z* 1127 $[M + Na]^+$, 1143 $[M + K]^+$) and the DEPT ^{13}C NMR data. The chemical shifts of C-3 (*δ* 85.1) and C-28 (*δ* 176.3) indicated that **5** was a bisdesmosidic glycoside. Its ^{1}H and ^{13}C NMR data displayed four sugar anomeric protons [*δ* 4.99 (× 2) d (*J* = 7.7 Hz), 5.23 d (*J* = 7.9), 6.18 d (*J* = 8.2)] (Experimental Section) and carbons [*δ* 95.1, 105.3, 105.7, 106.3] (Table 2). The ^{13}C NMR data for the aglycon portion were assignable to gypsogenic acid[3] (Table 1), and the sugar units were the same as those of **3**. Acid hydrolysis yielded gypsogenic acid, and the component sugars were identified as glucose and xylose (3:1) from GLC analysis. The *β* configuration for the sugars was determined from their $^3J_{H1,H2}$ (7−8 Hz) values. Based on the above information, saponarioside L was established as 3-*O*-*β*-D-xylopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (**5**).

Saponarioside M (**6**) was assigned the same molecular composition $C_{53}H_{84}O_{24}$ as **5** from MALDI-TOF MS analysis (molecular ions at *m*/*z* 1127 $[M + Na]^+$, 1143 $[M + K]^+$) and its DEPT ^{13}C NMR data. ^{1}H and ^{13}C NMR data for the aglycon part were superimposable with those of **5**, suggesting that both compounds had the same aglycon, gypsogenic acid.[3] The presence of four sugars in **6** was indicated from the four anomeric protons [*δ* 4.95 d (*J* = 7.7 Hz), 5.01 d (*J* = 7.3 Hz), 5.30 d (*J* = 7.7 Hz), 6.24 d (*J* = 7.3 Hz)] and carbons [*δ* 95.6, 102.8, 106.3, 106.4], respectively (Tables 2 and 3). Acid hydrolysis afforded gypsogenic acid, and the component sugars were identified as glucose and xylose (3:1) from GLC analysis. Detailed NMR analysis indicated the sugar sequence at C-28 to be of a linear structure, glc(1→2)glc(1→6)glc (Tables 2 and 3).

The remaining xylose was determined to be attached to C-3 of the aglycon. Thus, saponarioside M was established as 3-*O*-*β*-D-glucopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**6**).

In addition, the previously reported compounds,[3] saponariosides C (**2**), E (**7**), F (**8**), and G (**9**) were also isolated and identified by comparison with the authentic samples. However, saponarioside D, 3-*O*-*β*-D-xylopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside, the main compound in the *n*-BuOH-soluble fraction in our previous investigation,[3] was not found in the present study.

Experimental Section

General Experimental Procedures. All melting points were measured using a Yanaco microscope apparatus and are uncorrected. IR spectra were determined using a JASCO D-300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. ^{1}H and ^{13}C NMR were recorded using a JEOL α-500 or a JEOL EX-400 FT-NMR spectrometer. Chemical shifts were expressed in δ (ppm) referring to TMS. MALDI-TOF MS were conducted using a PerSeptive Biosystems Voyager DE-STR mass spectrometer, respectively. Diaion HP-20 (Mitsubishi Chemical), Si gel (Si gel 60, Merck), and ODS (Chromatorex, 100−200 mesh, Fujisylisia) were used for column chromatography. Preparative HPLC was performed using an ODS column (PEGASIL ODS, Senshu Pak, 10 mm i.d. × 250 mm, detector, UV 210 nm). GLC, Shimadzu GC-7A; column, silicone OV-17 on Uniport HP (80−100 mesh), 3 mm i.d. × 2.1 m; column temperature, 160 °C; carrier gas, N_2, flow rate 30 mL/min.

Plant Material. *Saponaria officinalis* was collected in the botanical garden of Tokyo University of Pharmacy and Life Sciences, in July 1997. A voucher sample of the plant has been deposited at the Department of Pharmacognosy, Toho University.

Extraction and Isolation. The air-dried and powdered whole plants of *S. officinalis* (800 g) were extracted with MeOH three times under reflux for 2 h. The combined MeOH extract was concentrated (280 g), suspended in H_2O, and then partitioned successively between EtOAc (16 g) and *n*-BuOH (105 g). The *n*-BuOH-soluble fraction was applied to a column of Diaion HP-20 (2000 mL) and eluted with 30, 50, 70, and 100% MeOH. The fractions eluted with 70% MeOH were combined and repeatedly chromatographed over Si gel and ODS columns to give several saponin fractions. Further HPLC purification (70−75% MeOH−0.06% TFA in H_2O, 1.0 mL/min, UV detector, 210 nm) afforded **1** (70 mg), **2** (15 mg), **3** (4 mg), **4** (6 mg), **5** (40 mg), **6** (38 mg), **7** (12 mg), **8** (10 mg), and **9** (8 mg), respectively.

Saponarioside I (1): an amorphous solid; $[\alpha]^{22}_D$ +10.0° (*c* 0.4, MeOH); IR (KBr) ν_{max} 3415, 2926, 1711, 1071 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.58 (1H, br t, H-12), 5.20 (1H, br s, H-16), 4.65 (1H, dd, J = 10.1, 3.7 Hz, H-3), 3.45 (1H, dd, J = 13.8, 3.9 Hz, H-18), 1.76, 1.57, 1.10, 1.01, 1.00, 0.94 (each 3H, s, H_3 of C-27, C-24, C-26, C-30, C-25, C-29); other NMR data, see Tables 1−3; MALDI-TOF MS (positive ion mode) *m*/*z* 1305 [M + Na]$^+$, 1321 [M + K]$^+$.

Saponarioside J (3): an amorphous solid; $[\alpha]^{22}_D$ −33.7° (*c* 0.35, MeOH); IR (KBr) ν_{max} 3399, 2928, 1685, 1457, 1368, 1069 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.59 (1H, dd, J = 11.2, 3.1 Hz, H-12), 5.73 (1H, d, J = 11.2 Hz, H-11), 4.07 (1H, dd, J = 12.1, 4.8 Hz, H-3), 2.62, 2.09 (each 1H, d, J = 14.4 Hz, H-19), 1.58, 1.00, 0.97, 0.94, 0.85, 0.83 (each 3H, s, H_3 of C-24, C-26, C-27, C-25, C-29, C-30); other NMR data, see Tables 1−3; MALDI-TOF MS (positive ion mode) *m*/*z* 1125 [M + Na]$^+$, 1141 [M + K]$^+$.

Saponarioside K (4): an amorphous solid; $[\alpha]^{22}_D$ +6.0° (*c* 0.60, MeOH); IR (KBr) ν_{max} 3409, 2934, 1679, 1198, 1069 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.57, 5.59 (each 1H, s, H_2-24), 6.21 (1H, d, J = 8.3 Hz, H_1 of Glc), 5.63 (1H, br t, H-12), 5.26 (1H, d, J = 7.7 Hz, H_1 of G′lc), 5.25 (1H, br s, H-16), 5.01 (1H, d, J = 7.2 Hz, H_1 of G″lc), 3.94, 3.81 (each, 1H, m, H_2-3), 3.52 (1H, dd, J = 14.3, 4.1 Hz, H-18), 2.76 (1H, t, J = 13.5, H-19), 1.79, 1.23, 1.05, 1.01, 1.00 (each 3H, s, H_3 of C-27, C-26, C-30, C-25, C-29); other NMR data, see Tables 1 and 2; MALDI-TOF MS (positive ion mode) *m*/*z* 1011 [M + Na]$^+$, 1027 [M + K]$^+$.

Saponarioside L (5): an amorphous solid; $[\alpha]^{22}_D$ +3.6° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3409, 2930, 1711, 1646, 1456, 1072 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.18 (1H, d, J = 8.2 Hz, H_1 of Glc), 5.41 (1H, br t, H-12), 5.23 (1H, d, J = 7.9 Hz, H_1 of G′lc), 4.99 (2H, d, J = 7.7 Hz, H_1 of G″lc and Xyl), 4.61 (1H, dd, J = 11.9, 4.3 Hz, H-3), 3.14 (1H, dd, J = 13.8, 4.3 Hz, H-18), 1.55, 1.18, 1.06, 1.97, 0.86, 0.85 (each 3H, s, H_3 of C-24, C-27, C-26, C-25, C-30, C-29); other NMR data, see Tables 1 and 2; MALDI-TOF MS (positive ion mode) *m*/*z* 1127 [M + Na]$^+$, 1143 [M + K]$^+$.

Saponarioside M (6): an amorphous solid; $[\alpha]^{22}_D$ +2.8° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3409, 2938, 1678, 1072 cm^{-1}; ^{1}H NMR data for the aglycon were the same as those reported for **5**; other NMR data, see Tables 1−3; MALDI-TOF MS (positive ion mode) *m*/*z* 1127 [M + Na]$^+$, 1143 [M + K]$^+$.

Acid Hydrolysis of Saponariosides. Compound **1** (10 mg) was heated in 1 mL 1 M HCl (dioxane−H_2O, 1:1) at 80 °C for 2 h in a water bath. After dioxane was removed, the solution was extracted with EtOAc (1 mL × 3). The extract was washed with H_2O and then concentrated to give an amorphous powder (16α-hydroxygypsogenic acid, 3 mg). The monosaccharide portion was neutralized by passing it through an ion-exchange resin (Amberlite MB-3) column, concentrated (dried overnight), then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After excess reagent was decomposed with H_2O, the reaction product was extracted with hexane (1 mL × 2). The TMSi derivatives of the monosaccharides were identified to be glucose, galactose, and xylose (3:1:1) by co-GLC analysis with standard monosaccharides. Sugars in compounds **3**−**6** were also identified by the same method.

Determination of the Absolute Configuration of the Carbohydrate Subunits.[4,5] A solution of **1** (8 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 100 °C for 2 h. After extracting with EtOAc, the H_2O layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, and concentrated to furnish the monosaccharide residue. After dissolving in H_2O (1 mL), solutions of l-(−)-α-methylbenzylamine (5 mg) and Na[BH_3CN] (8 mg) in EtOH (1 mL) were added. The mixture was allowed to stand overnight and was then acidified by addition of glacial HOAc (0.2 mL) and evaporated to dryness. The resulting solid was acetylated with $(Ac)_2O$ (0.3 mL) in pyridine (0.3 mL) at 100 °C for 1 h. After co-distillation with toluene, H_2O (1 mL) was added to the residue, and the crude mixture was passed through a Sep-pak C_{18} cartridge and washed with H_2O−MeCN (4:1; 1:1, each 5 mL). The H_2O−MeCN (1:1) eluate contained a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which were identified by co-HPLC analysis with standard sugars prepared under the same conditions. HPLC conditions: column, Waters Puresil C_{18}, 4.6 × 150 mm; solvent, MeCN−H_2O (2:3); flow rate, 0.8 mL min^{-1}; detection, UV 230 nm. The derivatives of D-glucose, D-galactose, and D-xylose were detected with t_R (min) of 12.40, 11.25, and 8.66, respectively. Using the same method, the xylose subunits and glucose subunits in compounds **3**−**6** were also determined to be of the D type.

16α-Hydroxygypsogenic acid: an amorphous solid; mp 294−296 °C; $[\alpha]^{22}_D$ +54.7° (*c* 0.3; MeOH); spectral data consistent with literature values.[3]

Gypsogenic acid: an amorphous solid; mp 250−252 °C; $[\alpha]^{22}_D$ + 64.5° (*c* 0.4, MeOH); spectral data consistent with literature values.[3]

Acknowledgment. The authors are grateful to Professor Y. Sashida, Tokyo University of Pharmacy and Life Sciences, for kindly providing and identifying the plant materials and

Dr. T. Kato, Japan Electron Optics Laboratory (JEOL), Tokyo, Japan, for some NMR measurements.

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NP990311R