Saponarioside C, the First α-D-Galactose Containing Triterpenoid Saponin, and Five Related Compounds from *Saponaria officinalis*

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Six novel triterpenoid saponins, named saponariosides C−H, were isolated from the whole plants of *Saponaria officinalis*. Their structures were established as saponarioside C (**1**), 3-*O*-*β*-D-xylopyranosyl-gypsogenic acid-28-*O*-α-D-galactopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside; saponarioside D (**2**), 3-*O*-*β*-D-xylopyranosyl-gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside; saponarioside E (**3**), 3-*O*-*β*-D-glucopyranosyl-gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside; saponarioside F (**4**), 3-*O*-*β*-D-xylopyranosyl-16α-hydroxygypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside; saponarioside G (**5**), 3-*O*-*β*-D-xylopyranosyl-16α-hydroxygypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside; and saponarioside H (**6**), 3-*O*-*β*-D-xylopyranosyl-gypsogenic acid-28-*O*-*β*-D-glucopyranoside, by a combination of extensive NMR (DEPT, COSY, HOHAHA, HETCOR, HMBC, and NOESY) studies and chemical degradation.

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. *S. officinalis* was well known for its detergent property 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 two major triterpenoid saponins, saponariosides A and B, based on quillaic acid from the water-soluble fraction of the whole plant of *S. officinalis*, has been reported.[2] Investigation of the *n*-BuOH-soluble portion has led to the isolation of six novel triterpenoid saponins based upon gypsogenic acid or 16α-hydroxygypsogenic acid. In this paper, we report the isolation and structure study of these saponins, saponariosides C−H, from the whole plants of *S. officinalis*.

Results and Discussion

A MeOH extract of the freshly collected 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 six novel triterpenoid saponins, saponariosides C−H (**1**−**6**, respectively, Chart 1). Compounds **1**, **2**, **3**, and **6** were based upon the aglycon gypsogenic acid, while the aglycon for **4** and **5** was 16α-hydroxygypsogenic acid.

Saponarioside C (**1**) had a molecular formula of $C_{59}H_{94}O_{29}$ determined from its matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS (*m*/*z* 1289 $[M + Na]^+$, 1305 $[M + K]^+$) and from ^{13}C, DEPT 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 moiety. The six sp^3 carbons at *δ* 12.6, 16.0, 17.4, 23.7, 26.0, and 33.1 and the two sp^2 carbons at *δ* 122.7 (d) and 144.1 (s), coupled with information from ^1H NMR (six methyl proton singlets and a broad triplet-like vinyl proton signal at *δ* 5.41), indicated that the aglycon had an olean-12-ene skeleton. Detailed NMR analysis identified the aglycon as gypsogenic acid.[3] The chemical shifts of C-3 (*δ* 85.0) and C-28 (*δ* 176.4) revealed that **1** was a bisdesmosidic glycoside. The pentasaccharide nature of compound **1** was evident in its ^1H [*δ* 4.90 d (*J* = 7.7 Hz), 4.99 d (*J* = 7.3), 5.24 d (*J* = 7.9), 5.47 d (*J* = 3.6), 6.19 d (*J* = 8.2)] and ^{13}C [*δ* 95.0, 100.6, 105.3, 105.7, 106.3] spectra, respectively (Table 1). The identity of the monosaccharides and the oligosaccharide chain sequence was determined by a combination of DQF-COSY, HOHAHA, DEPT, HETCOR, HMBC, and phase-sensitive NOESY experiments. Starting from the anomeric protons of each sugar unit, all hydrogens within each spin system were assigned using COSY with the aid of 2D HOHAHA and NOESY spectra. A NOESY experiment, in addition to the NOEs across glycosidic bonds, also revealed the 1,3- and 1,5-diaxial relationships for the *β*-anomers of the pyranosyl rings, thus facilitating the mapping of the spin systems. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by HETCOR and further confirmed by HMBC experiments. Among the five sugar units in the molecule, three were identified as glucose and another one as xylose. The *β*-anomeric configurations for the glucose and xylose units were determined from their $^3J_{H1,H2}$ coupling constants (7−8 Hz). Only four protons were traced from the subspectrum corresponding to the remaining anomeric proton [*δ* 5.47 d (*J* = 3.6 Hz)]. Such was typical for the galactosyl residue in which the distribution of the scalar coupling around the system was impeded by the small coupling between H-4 and H-5. A small $^3J_{H1,H2}$ coupling constant (3.6 Hz) indicated the gauche orientation, or rather equatorial (H-1)/axial (H-2) relationship between the two vicinal protons. Further evidence showing the α configuration came from the long-range HMBC correlation from the anomeric proton to C-3 and C-5 (the dihedral angles between H-1 and C-3, H-1 and C-5 were about 180°), and NOE existed only between H-1 and H-2. Additionally, the one-bond $^{13}C-^1H$ coupling ($^1J_{CH}$) of 167 Hz for the anomeric carbon (163 for the *β*-anomer[2]), as well as the

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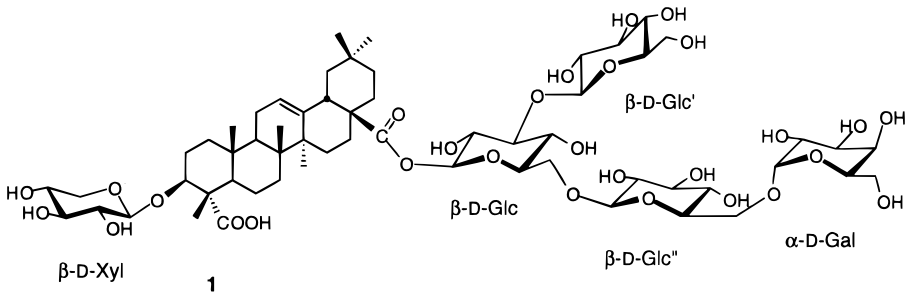
Chart 1



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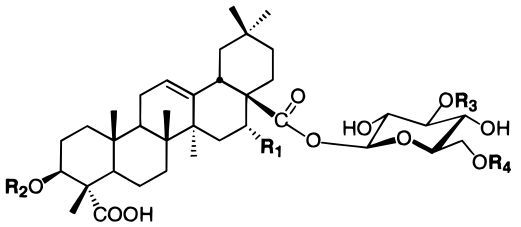

	R_1	R_2	R_3	R_4
2	H	Xyl	Glc'	Glc"2-1Glc"'
3	H	Glc""	Glc'	Glc"2-1Glc"'
4	OH	Xyl	Glc'	Glc"2-1Glc"'
5	OH	Xyl	Glc'	Glc"
6	H	Xyl	H	H

relative upfield shifts of C-1, C-2, C-3, and C-5 as compared to that of the *β*-anomer, also indicated the α-anomer.

From the completely assigned ^{13}C NMR data, the branched nature of the sugar moiety was evident, and the noticeable ^{13}C chemical shift differences between inner sugars and terminal ones indicated that the glucose directly connected to C-28 was glycosylated at C-3 (Δ 10.0 ppm) and C-6 (Δ 6.7 ppm). Also, the glucose linked to C-6 was glycosylated at C-6″ (Δ 5.7 ppm). The detailed sugar arrangement at C-28 was established from the following HMBC correlation: H-1‴ (galactose) with C-6″, H-1″ with C-6, and H-1′ with C-3, while attachment of the tetrasaccharide chain to C-28 of the aglycon was based on a correlation between H-1 and C-28 of the aglycon. The remaining xylose 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). The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiment. The absolute configurations of these sugars were determined by HPLC analysis following conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives.[4,5] Thus, saponarioside C was elucidated to be 3-*O*-*β*-D-xylopyranosyl-gypsogenic acid-28-*O*-α-D-galactopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside (**1**).

Saponarioside C (**1**) contains an α-D-galactopyranosyl moiety, which is a common component for glycolipids,[6,7] but rare for triterpenoid saponins. *β*-D-galactose is commonly seen as a component sugar for triterpenoid saponins.[2] To the best of our knowledge, this is the first example of a triterpenoid saponin containing an α-D-galactose unit.

Saponarioside D (**2**) had the same molecular formula ($C_{59}H_{94}O_{29}$) as **1** from its negative ion ESIMS (at *m*/*z* 1265 $[M - H]^-$) and from ^{13}C, DEPT NMR data. Its ^1H and ^{13}C NMR spectra indicated that **2** possessed the same aglycon as **1** but differed in the sugar part (Table 1). Five sugar units in **2** were indicated, as there were five anomeric protons and carbons (Table 1). It was apparent that the five sugars were present in two saccharide units, one attached to C-3 and the other at C-28. The overall structure assignment was accomplished using the same protocol as in **1**, which permitted the full assignment of the protons and carbons, and the sugar components were identified as glucose and xylose (4:1). The linkage positions for the sugar units were established using the HMBC and NOESY correlations. Compound **2** had nearly the same sugar arrangement as **1** except that the terminal galactose at the C-6″ in **1** was replaced by a glucose and linked to the C-2″ instead. The linkage was also supported from fragmentation patterns observed in the ESIMS−MS experiment. MS−MS analysis of the deprotonated molecular ion $[M - H]^-$ (*m*/*z* 1265) gave a daughter ion at *m*/*z* 1085 $[(M - H) - H_2O - 162]^-$ by the loss of one of the terminal hexose units. Further subjected to MS−MS analysis, the resulting fragment (*m*/*z* 1085) afforded a prominent fragment at *m*/*z* 617 from loss of the tetrasaccharide chain linked to C-28 of saponarioside D. Further MS−MS analysis on *m*/*z* 617 yielded a major fragment corresponding to the aglycon part from losing the xylose at C-3. The *β* configuration for the sugars was determined from their $^3J_{H1,H2}$ (Table 1). Based on the above information, saponarioside D is 3-*O*-*β*-D-xylopyranosyl-gypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside (**2**).

Saponarioside E (**3**) had molecular composition $C_{60}H_{96}O_{30}$ as determined from MALDI-TOF analysis (molecular ion at 1319 $[M + Na]^+$) and ^{13}C, DEPT NMR data. Detailed

Table 1. ^{1}H and ^{13}C NMR Data for the Sugar Parts for **1** and **2** (in pyridine-d_5)[a]

sugar units	**1**		**2**	
3-*O*-sugar				
1	106.3 ($^1J_{CH}$159 Hz)	4.99 d (7.3 Hz)	106.3	4.98 d (7.4 Hz)
2	75.3	3.96	75.3	3.96 dd (7.4, 8.8)
3	78.1[b]	4.04	78.1[b]	4.04
4	71.1	4.19	71.1	4.19
5	67.1	3.69 dd (10.7, 10.5) 4.35 dd (11.3, 5.2)	67.1	3.68 dd (11.0, 10.4) 4.34
28-*O*-sugars				
1	95.0 (163)	6.19 d (8.2)	94.8	6.19 d (7.9)
2	72.8	4.11	73.2	4.21
3	88.3	4.22	88.1	4.26
4	69.0	4.23	69.2	4.30
5	77.6	4.06	76.8	4.10
6	69.2	4.28, 4.63	68.9	4.49 dd (9.2, 2.0) 4.26
1′	105.7 (158)	5.24 d (7.9)	105.8	5.30 d (8.2)
2′	75.5	4.00	75.7	4.05
3′	78.3[b]	4.13	78.0[b]	4.15
4′	71.6	4.14	71.3	4.13
5′	78.5	3.98	78.5	3.91 m
6′	62.5	4.25, 4.50	62.3	4.44 dd (9.5, 2.0) 4.25
1″	105.3 (160)	4.90 d (7.7)	102.6	4.99 d (7.6)
2″	74.9	3.94	83.7	4.04
3″	78.2[b]	4.13	78.0[b]	4.26
4″	71.9	4.04	70.8	4.17
5″	76.2	3.62	78.3	3.78 m
6″	68.2	4.28, 4.48	62.2	4.04 dd (10.1, 2.1) 4.35
1‴	100.6 (167)	5.47 d (3.6)	105.8	5.28 d (8.2)
2‴	70.6	4.64 dd (3.6, 9.8)	76.4	4.05
3‴	71.6	4.55 dd (9.8, 3.2)	78.1[b]	4.13
4‴	71.1	4.59 d (3.2)	71.1	4.17
5‴	72.5	4.60 m	78.4	3.91 m
6‴	62.7	4.40 (2H) d (6.4)	62.5	4.53 dd (9.7, 1.9) 4.33

[a] Assignments were based upon DQF-COSY, HOHAHA, DEPT, HETCOR, NOESY, and HMBC experiments (500 MHz for ^{1}H and 125 MHz for ^{13}C). [b] Data are interchangeable.

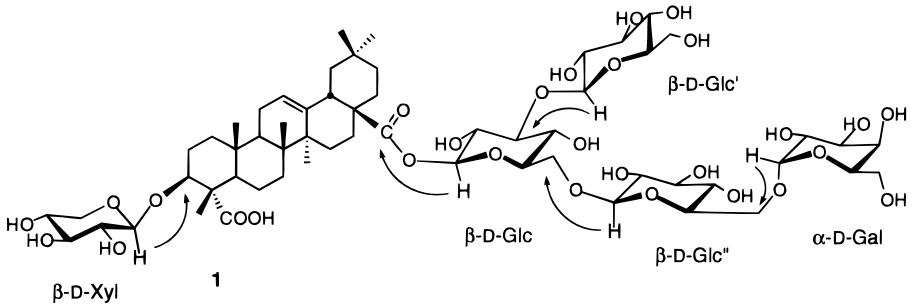

Figure 1. Key long-range HMBC correlations for **1**.

analysis of the ^{1}H and ^{13}C NMR data showed that **3** had five sugar units, and the aglycon was the same as compound **1** (gypsogenic acid). Acid hydrolysis afforded gypsogenic acid and glucose as the only component monosaccharide. Comparison of its ^{13}C and ^{1}H NMR data and COSY, HOHAHA patterns with those of **2** indicated that they had the same sugar sequence at C-28 (Table 2). The C-3 of the aglycon resonated at δ 85.0, indicating that the remaining glucose was connected to this carbon. Thus, saponarioside E (**3**) was established to be 3-*O*-β-D-glucopyranosyl-gypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside.

Saponarioside F (**4**) had molecular composition $C_{59}H_{94}O_{30}$, as inferred from its MALDI-TOF analysis (molecular ions *m*/*z* 1305 $[M + Na]^+$, 1321 $[M + K]^+$) and ^{13}C, DEPT NMR data. The spectra indicated that **4** had the same sugar arrangement at both C-3 and C-28 as that of **2** (Table 2). Detailed NMR analysis established the aglycon to be 16α-hydroxygypsogenic acid.[8] Acid hydrolysis afforded the aglycon, 16-OH gypsogenic acid, and the component sugars were determined to be glucose and xylose (4:1) from GLC analysis. Thus, saponarioside F (**4**) was identified as 3-*O*-β-D-xylopyranosyl-16α-hydroxygypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside.

Saponarioside G (**5**) was assigned a molecular formula of $C_{53}H_{84}O_{25}$ from its MALDI-TOF (molecular ions *m*/*z* 1143 $[M + Na]^+$, 1159 $[M + K]^+$) and ^{13}C, DEPT NMR data. The chemical shifts of C-3 (δ 85.0) and C-28 (δ 176.4) indicated that **5** was a bisdesmosidic glycoside. Its ^{1}H and ^{13}C NMR displayed four sugar anomeric protons at [δ 4.99 d (J = 7.8 Hz), 5.00 d (J = 7.3), 5.23 d (J = 8.0), and 6.19 d (J = 8.3) and carbons at δ 95.2, 105.4, 105.7, and 106.3 (Table

2). The ^{13}C data for the aglycon part was the same as for compound **4**, and most of the sugar parts were the same except that the signals attributed to the terminal glucose (Glc‴) disappeared. Also, C-2″ (δ 83.6) in compound **4** shifted upfield to δ 75.2 in **5**. Acid hydrolysis yielded 16-OH gypsogenic acid, and the component sugars were identified as glucose and xylose (3:1) from GLC analysis. Thus, saponarioside G (**5**) was elucidated to be 3-*O*-β-D-xylopyranosyl-16α-hydroxygypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside.

A minor compound, saponarioside H (**6**), was also isolated from the *n*-BuOH-soluble fraction. Its molecular formula was determined to be $C_{41}H_{64}O_{14}$ (MALDI-TOF MS molecular ion at *m/z* 803 $[M + Na]^+$). The ^1H [anomeric δ 5.01, d (J = 7.3 Hz), 6.32 d (J = 8.2)] and ^{13}C NMR (δ 95.8, 106.3) data indicated the presence of two sugar units in the molecule (Table 2). The sugars were identified as β-D-xylopyranose and β-D-glucopyranose from their NMR data. The aglycon was identified as gypsogenic acid, and the chemical shifts of C-3 (δ 85.1) and C-28 (δ 176.5) indicated that **6** was a bisdesmosidic glycoside with xylose linked to C-3 and glucose to C-28, as indicated from their ^{13}C NMR data (Table 2). Accordingly, **6** was identified as 3-*O*-β-D-xylopyranosyl-gypsogenic acid-28-*O*-β-D-glucopyranoside.

Triterpenoid saponins with similar structures have also been isolated from the seeds of *Vaccaria segetalis*[3] and the aerial parts of *Dianthus chinensis*.[9] Saponins of this type display a distinct color (sky blue) that develops on Si gel TLC plates when they are sprayed with 5% H_2SO_4 and heated.

Table 2. ^{13}C NMR Data of the Sugar Moieties for **3**, **4**, **5**, and **6** (125 or 100 MHz in pyridine-d_5)

sugar units	3	4	5	6
3-*O*-sugar				
1	105.4	106.3	106.3	106.3
2	75.6	75.3	75.3	75.3
3	78.5	78.1[a]	78.1	78.1
4	71.6	71.1	71.1	71.1
5	78.6	67.1	67.1	67.1
6	62.9			
28-*O*-sugars				
1	94.9	95.0	95.2	95.8
2	73.2	73.2	72.7	74.1
3	88.1	87.9	88.4	78.9
4	69.3	69.3	68.8	71.1
5	76.8	76.9	77.6	79.4
6	68.9	68.9	68.9	62.2
1′	105.9	105.8	105.7	
2′	75.7	75.7	75.4	
3′	78.1[a]	78.1[a]	78.1	
4′	71.3	71.3	71.5	
5′	78.4	78.4	78.4	
6′	62.3	62.3	62.6	
1″	102.6	102.6	105.4	
2″	83.7	83.6	75.2	
3″	78.0[a]	78.0[a]	78.6	
4″	70.8	70.8	71.6	
5″	78.4	78.4	78.3	
6″	62.2	62.2	62.4	
1‴	105.9	105.8		
2‴	76.4	76.4		
3‴	78.2[a]	78.2[a]		
4‴	71.2	71.1		
5‴	78.5	78.5		
6‴	62.4	62.5		

[a] Data are interchangeable.

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 300E FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. ESIMS and MALDI-TOF MS were conducted using Finnigan LCQ and PerSeptive Biosystems Voyager DE-STR mass spectrometer, respectively. ^1H 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. 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.

Extraction and Isolation. Fresh whole plants of *Saponaria officinalis* were collected in the botanical garden of Toho University in July 1997. A voucher sample of the plant is deposited at the Department of Pharmacognosy, Toho University. The finely cut, whole plants of *S. officinalis* (12 kg) were extracted with MeOH three times under reflux for 2 h. The combined MeOH extract was concentrated (510 g), suspended in H_2O, and then partitioned successively with EtOAc (53.7 g) and *n*-BuOH (124.0 g). The *n*-BuOH-soluble fraction was applied to a column of Diaion HP-20 (2000 mL) and washed 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** (28 mg), **2** (50 mg), **3** (10 mg), **4** (26 mg), **5** (30 mg), and **6** (8 mg), respectively.

Saponarioside C (1): an amorphous solid; mp 234 °C (dec); $[\alpha]^{22}_D$ +8.9° (*c* 0.63, MeOH); IR (KBr) ν_{max} 3403, 2941, 1678, 1072, 1040 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 5.41 (1H, br t, H-12), 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, 0.97, 0.86, 0.85 (each 3H, s, H_3 of C-24, C-27, C-26, C-25, C-30, C-29); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 180.4 (s, C-23), 176.4 (s, C-28), 144.1 (s, C-13), 122.7 (d, C-12), 85.0 (d, C-3), 53.3 (s, C-4), 52.1 (d, C-5), 48.3 (d, C-9), 47.0 (s, C-17), 46.1 (t, C-19), 42.1 (s, C-14), 41.7 (d, C-18), 40.2 (s, C-8), 38.8 (t, C-1), 36.7 (s, C-10), 33.9 (t, C-7), 33.1 (q, C-29), 32.8 (t, C-21), 32.3 (t, C-22), 30.7 (s, C-20), 28.2 (t, C-15), 26.3 (t, C-2), 26.0 (q, C-27), 23.8 (t, C-11), 23.7 (q, C-30), 23.2 (t, C-6), 21.3 (t, C-16), 17.4 (q, C-26), 16.0 (q, C-25), 12.6 (q, C-24); other NMR data, see Table 1; MALDI-TOF MS (positive ion mode) *m/z* 1289 $[M + Na]^+$, 1305 $[M + K]^+$.

Saponarioside D (2): an amorphous solid; mp 237 °C (dec); $[\alpha]^{22}_D$ −3.4° (*c* 0.53, MeOH); IR (KBr) ν_{max} 3413, 2937, 1711, 1072 cm^{-1}; ^1H and ^{13}C NMR data of the aglycon were the same as those reported for **1**; other NMR data, see Table 1; ESIMS (negative ion mode) *m/z* 1265 $[M - H]^-$, 1085 $[(M - H) - H_2O - 162]^-$, 617 $[(M - H) - 162 \times 4]^-$, 485 $[(M - H) - 162 \times 4 - 132]^-$, 423.

Saponarioside E (3): an amorphous solid; mp 210 °C (dec); $[\alpha]^{22}_D$ −6.9° (*c* 0.23, MeOH); IR (KBr) ν_{max} 3409, 2938, 1678, 1072 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 6.20 (1H, d, J = 7.9 Hz, H-1), 5.34 (1H, d, J = 8.1 Hz, H-1′), 5.32 (1H, d, J = 7.8 Hz, H-1‴), 5.09 (1H, d, J = 7.8 Hz, H-1⁗), 5.00 (1H, d, J = 7.9 Hz, H-1″); ^1H and ^{13}C NMR data of the aglycon were the same as those reported for **1**; other NMR see data, Table 2; MALDI-TOF MS (positive ion mode) *m/z* 1319 $[M + Na]^+$.

Saponarioside F (4): an amorphous solid; mp 232 °C (dec); $[\alpha]^{22}_D$ −19.6° (*c* 0.56, MeOH); IR (KBr) ν_{max} 3404, 2926, 1710, 1073 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 6.19 (1H, d, J = 7.9 Hz, H-1), δ 5.59 (1H, br t, H-12), 5.30 (1H, d, J = 7.4 Hz, H-1′), 5.28 (1H, d, J = 7.6 Hz, H-1‴), 5.19 (1H, br s, H-16), 4.99 (1H, d, J = 7.9 Hz, H-1″), 4.98 (1H, d, J = 7.4 Hz, H-1 of Xyl), 4.65 (1H, dd, J = 11.9, 4.3 Hz, H-3), 3.46 (1H, dd, J = 14.0, 3.9 Hz, H-18), 1.76, 1.57, 1.10, 1.02, 1.01, 0.94 (each 3H, s, H_3 of C-27, C-24, C-26, C-30, C-25, C-29); ^{13}C NMR (pyridine-

d_5, 125 MHz) δ 180.4 (s, C-23), 175.8 (s, C-28), 144.4 (s, C-13), 122.5 (d, C-12), 85.0 (d, C-3), 74.1 (d, C-16), 53.4 (s, C-4), 52.2 (d, C-5), 49.1 (s, C-17), 47.4 (d, C-9), 47.1 (t, C-19), 42.0 (s, C-14), 41.2 (d, C-18), 40.4 (s, C-8), 38.9 (t, C-1), 36.7 (s, C-10), 36.1 (t, C-15), 35.8 (t, C-21), 33.1 (t, C-7), 33.1 (q, C-29), 32.1 (t, C-22), 30.8 (s, C-20), 26.4 (t, C-2), 27.1 (q, C-27), 24.6 (q, C-30), 23.8 (t, C-11), 21.4 (t, C-6), 17.4 (q, C-26), 16.2 (q, C-25), 12.6 (q, C-24), other NMR data, see Table 2; MALDI-TOF MS (positive ion mode) *m*/*z* 1305 $[M + Na]^+$, 1321 $[M + K]^+$.

Saponarioside G (5): an amorphous solid; mp 233 °C (dec); $[\alpha]^{22}_D$ −12.6° (*c* 0.57, MeOH); IR (KBr) ν_{max} 3402, 2937, 1681, 1071 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 6.19 d (1H, J = 8.3 Hz, H-1), 5.23 d (1H, J = 8.0 Hz, H-1′), 5.00 d (1H, J = 7.3 Hz, H-1″), 4.99 d (1H, J = 7.8 Hz, H-1 of Xyl); ^1H and ^{13}C NMR data of the aglycon were the same as those reported for **4**; other NMR data, see Table 2; MALDI-TOF MS (positive ion mode) *m*/*z* 1143 $[M + Na]^+$, 1159 $[M + K]^+$.

Saponarioside H (6): an amorphous solid; mp 244 °C (dec); $[\alpha]^{22}_D$ +15.2° (*c* 0.29, MeOH); IR (KBr) ν_{max} 3413, 2944, 1678, 1072 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 6.32 (1H, d, J = 8.2 Hz, H-1 of Glc), 5.01 (1H, d, J = 7.3 Hz, H-1 of Xyl); ^1H and ^{13}C NMR data of the aglycon were the same as those reported for **1**; other NMR data, see Table 2; MALDI-TOF MS (positive ion mode) *m*/*z* 803 $[M + Na]^+$.

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 H_2O 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 (gypsogenic acid, 3 mg). The monosaccharide portion was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, concentrated (dried overnight), then treated with 1-(trimethylsilyl)imidazole (TMSi) at room temperature for 2 h. After excess reagent was decomposed with H_2O, the reaction product was extracted with hexane (1 mL × 2 times). The TMSi derivatives of the monosaccharides were identified to be glucose, galactose, and xylose (3:1:1) by co-GLC analyses with standard monosaccharides. Sugars in compounds **2**−**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), the 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, then was acidified by addition of glacial HOAc acid (0.2 mL) and evaporated to dryness. The resulting solid was acetylated with Ac_2O anhydride (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 **2**−**6** were also determined to be of D type.

References and Notes

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