Major Triterpenoid Saponins from *Saponaria officinalis*

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Two major triterpenoid saponins, named saponariosides A and B, were isolated from the whole plants of *Saponaria officinalis* and were respectively defined to be 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-*β*-D-4-*O*-acetylquinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**1**); 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-4-*O*-acetylquinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**2**). Their structures were established on the basis of extensive NMR (DEPT, DQF−COSY, HOHAHA, HETCOR, HMBC, and NOESY) and MS studies as well as chemical degradation.

Saponaria officinalis L. (Caryophyllaceae), commonly called fuller's herb or soapwort, is native to Europe and western to central Asia and is nowadays cultivated in many countries throughout the world. *S. officinalis* was well-known for its detersive 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] Early chemical investigation in the 1970s on this plant established two main triterpenoid saponins, saponasids A and D, with the latter containing up to 10 sugar units.[2] Chemical efforts to determine the sapogenin composition have been carried out as early as 1930s, and gypsogenin was proposed as the sapogenin.[3] Some confusion concerning the structures of the sapogenins lasted until the 1980s[4,5] when Kubota et al. found only quillaic acid and gypsogenic acid existing in *S. officinalis*, and most recently Henry et al. confirmed the presence of quillaic acid in the rhizomes using ^{13}C NMR techniques. The medicinal and commercial importance attached to the plant and our continuing interest in the chemistry of triterpenoid saponins of the Caryophyllaceae family[6−8] prompted us to reinvestigate the saponin components of *S. officinalis*. In this paper, we wish to report the isolation and structural study of two major saponins, saponariosides A and B 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 water soluble fraction, on chromatographic purification over Diaion HP-20, followed by repeated MPLC and HPLC purification, afforded two novel triterpenoid saponins, saponariosides A (**1**) and B (**2**) (Chart 1).

Saponarioside A (**1**), an amorphous solid, had a molecular formula of $C_{82}H_{128}O_{45}$ determined from its negative ion HRFABMS (at *m*/*z* 1831.7649 [M − H]$^-$) and from ^{13}C, DEPT NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. Of the 82 carbons, 30 were assigned to the aglycone part, 50 to the oligosaccharide moiety, and the remaining two to an acetyl group. The IR spectrum showed absorptions at 3406 cm^{-1} (OH) and at 1728 cm^{-1} (ester carbonyl). The six sp^3 carbons at *δ* 11.1, 15.8, 17.4, 24.5, 27.0, 33.2, and the two sp^2 carbons at *δ* 122.2 (d) and 144.4 (s), coupled with the information from ^1H NMR (six methyl proton singlets and a broad triplet vinyl proton at *δ* 5.54), indicated that the aglycone possessed an olean-12-ene skeleton. After an extensive 2D-NMR study, the aglycone was identified as quillaic acid (Tables 1 and 2), a common aglycone of triterpenoid glycosides.[9,10] The chemical shifts of C-3 (*δ* 84.4) and C-28 (*δ* 175.9) indicated that **1** was a bisdesmosidic glycoside. The ^1H and ^{13}C NMR of **1** displayed nine sugar anomeric protons at [*δ* 4.89 d (*J* = 7.3 Hz), 4.99 d (2H, *J* = 7.6 Hz), 5.13 d (2H, *J* = 7.1 Hz), 5.32 d (*J* = 7.7 Hz), 5.55 d (*J* = 7.3 Hz), 5.93 d (*J* = 8.2 Hz), 6.29 s] and carbons (*δ* 94.4, 100.9, 103.8, 104.2, 104.9, 105.5, 105.8, 106.2, 106.9), respectively (Tables 1 and 2). Alkaline hydrolysis of **1** furnished a prosapogenin (**1a**) (Chart 2), identified as quillaic acid 3-*O*-*β*-D-galactopyranosyl-(1→2)[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranoside from its spectral data (Tables 1 and 2). Acid hydrolysis afforded quillaic acid (**3**) (Chart 2), and the monosaccharide components were identified as fucose, galactose, xylose, quinovose, and rhamnose (1:1:4:1:1) based on GLC analysis. The remaining monosaccharide was identified as glucuronic acid by co-TLC analysis (both compound **1** and the authentic sugar were applied to the TLC plate and then hydrolyzed under HCl vapor at 65 °C for 1 h, developing solvent: $CHCl_3$−MeOH−H_2O, 10:5:1). From the above evidence, it was concluded that **1** was a bisdesmosidic triterpenoid glycoside with glucuronic acid, galactose, and xylose linked to the C-3 position of the aglycone, and the other six monosaccharides were linked to the C-28 of the aglycone through an ester bond.

The sequence of the oligosaccharide chains was established by a combination of DQF−COSY, HOHAHA, DEPT, HETCOR, HMBC, and phase-sensitive NOESY experiments. To facilitate proton assignments, the nine anomeric protons were consecutively labeled by the letters A to I as shown in Figure 1. Unfortunately, the anomeric protons labeled with E and F as well as G and H were overlapped, thus making the proton assignment formidable. The individual spin-systems can be discerned from the subspectra corresponding to the anomeric protons or the methyl groups (for the deoxy sugars) in the HOHAHA

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Chart 1

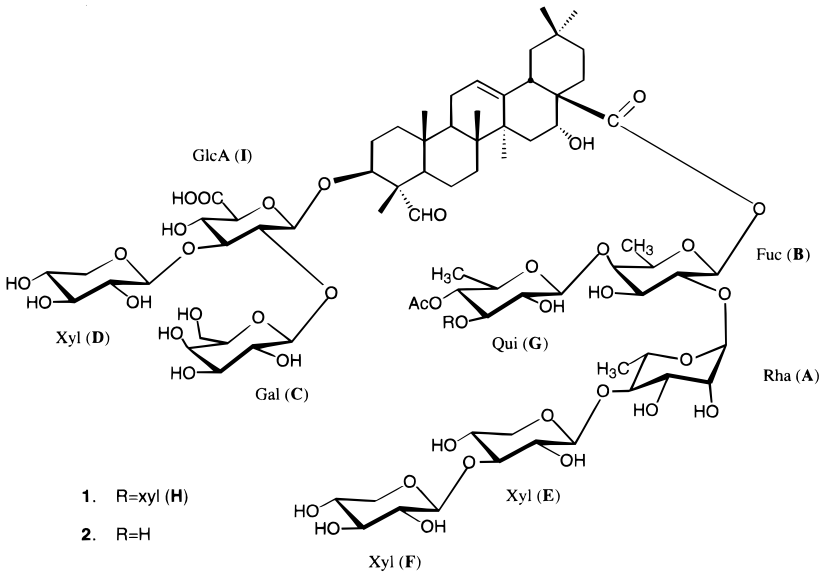


Table 1. ^{13}C NMR Data for the Sugar Moieties (125 MHz in pyridine-d_5)[a]

	1	2	1a		1	2
3-*O*-GlcA (I)				Rham (A)		
1	103.8	103.9	103.8	1	100.9 (171)	101.3
	($J_{C1,H1}$156 Hz)			2	71.7	71.7
2	78.4	78.5	78.6	3	72.4	72.3
3	86.1	86.1	86.0	4	83.7	83.5
4	71.4	71.3	71.3	5	68.3	68.3
5	77.3	77.3	77.2	6	18.5	18.6
6	172.0	171.8	171.7	Xyl′ (E)		
Gal (C)				1	106.2 (150)	106.2
1	104.2 (163)	104.2	104.2	2	75.3[b]	73.7
2	73.7	73.7	73.7	3	87.2	87.2
3	75.4	75.5	75.4	4	68.9	69.0
4	70.2	70.2	70.2	5	66.8	66.8
5	76.7	76.6	76.7	Xyl″ (F)		
6	61.7	61.6	61.7	1	105.8 (156)	105.9
Xyl (D)				2	75.1	75.1
1	104.9 (152)	105.0	104.9	3	78.1	78.1
2	75.2[b]	75.3	75.2	4	70.8[c]	70.7
3	78.5	78.6	78.5	5	67.3	67.3
4	70.7[c]	70.8	70.7	Qui (G)		
5	67.3	67.3	67.3	1	105.5 (162)	106.2
				2	75.0	75.0
				3	84.7	74.4
28-*O*-Fuc (B)				4	74.2	79.2
1	94.4 (167)	94.5		5	70.4	73.1
2	73.9	74.4		6	17.8	18.3
3	76.6	76.4		Xyl‴ (H)		
4	83.3	83.8		1	106.9 (151)	
5	71.4	71.5		2	75.7	
6	17.0	17.1		3	78.3	
				4	70.9[c]	
				5	67.5	

[a] The assignment was based upon DQF-COSY, HOHAHA, HETCOR, NOESY, and HMBC experiments. The data with the same labels *b*,*c* are interchangeable.

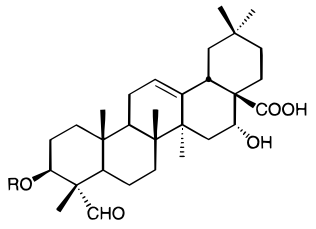
experiment (Figure 1, τ = 150 ms). Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were delineated using DQF−COSY with the aid of 2D-HOHAHA and NOESY spectra. A NOESY experiment (τ = 600 ms), in addition to the NOEs across the glycosidic bonds, also revealed the 1,3- and 1,5-diaxial relationship for the sugars of the pyranosyl rings, thus greatly simplifying the mapping of these spin systems. Information from COSY, HOHAHA, and NOESY furnished most of the assignments. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by HETCOR and further confirmed by HMBC. Interpretation of the COSY and 2D-HOHAHA spectra revealed the presence of nine monosaccharide units. As shown in the HOHAHA spectrum (Figure 1), three of monosaccharide units (A, B, and G) belonged to 6-deoxy sugars. Spin-system A displayed two broad singlet protons (H-1: δ, 6.29 s and H-2: 4.68 s), a double doublet proton (H-3: δ 4.60, dd, 8.5, 3.2 Hz), two overlapped protons (H-4 and H-5), and a terminal doublet methyl group (δ 1.53, d, 5.8 Hz) (Figure 1b). Furthermore, the large $^1J_{CH}$ (171 Hz) and three-bond strong HMBC correlations from the anomeric proton to C-3 and C-5 (the dihedral angles between H-1 and C-3, H-1 and C-5 about 180°), indicating that the

Table 2. ^{1}H NMR Data for the Sugar Moieties of **1**, **1a**, and **2** (500 MHz in pyridine-d_5)[a]

	1	2	1a		1	2
3-*O*-GlcA (I)				Rham (A)		
1	4.89 d (7.3)	4.89 d (7.3)	4.91 d (7.6)	1	6.29 br s	6.23 br s
2	4.32	4.33	4.35 dd	2	4.68 br s	4.69 br s
			(7.9, 7.6)	3	4.60 dd	4.61 dd
3	4.25	4.26	4.27		(8.5, 3.2)	(9.1, 3.1)
4	4.42	4.44	4.45	4	4.32	4.34
5	4.47	4.51	4.52	5	4.35	4.39
6	–	–	–	6	1.53 d (5.8)	1.56 d (6.4)
Gal (C)				Xyl′ (E)		
1	5.55 d (7.6)	5.55 d (7.6)	5.54 d (7.6)	1	5.13 d (7.1)	5.15 d (7.3)
2	4.46	4.47	4.45	2	3.94	3.98
3	4.14	4.14	4.15 dd	3	3.97	4.01
			(9.8, 3.0)	4	3.98	4.01
4	4.55	4.56 d (3.1)	4.56 d (3.0)	5	3.40, 4.13	3.42 t (11.3)
5	3.93	3.99	4.03 d (6.1)			4.13
6	4.36, 4.51	4.40, 4.50	4.44, 4.50	Xyl″ (F)		
Xyl (D)				1	5.13 d (7.1)	5.14 d (7.6)
1	5.32 d (7.7)	5.32 d (7.9)	5.31 d (8.0)	2	4.02	4.03
2	3.95	3.95	3.95 dd	3	4.09	4.11
			(8.0, 8.3)	4	4.14	4.14
3	4.10	4.10	4.09	5	3.63, 4.24	3.65, 4.28
4	4.10	4.10	4.09	Qui (G)		
5	3.64, 4.24	3.65, 4.23	3.64 t (10.3)	1	4.99 d (7.6)	4.99 d (7.9)
			4.23	2	3.99	4.00
28-*O*-Fuc (B)				3	4.08	3.64
1	5.93 d (8.2)	5.94 d (7.9)		4	5.08 dd	5.65 dd
2	4.50	4.50			(9.4, 9.8)	(9.1, 7.2)
3	4.17	4.18		5	3.66 m	3.64
4	3.95	3.98		6	1.26 d (6.1)	1.53 d (5.2)
5	3.91 m	3.62		Xyl‴ (H)		
6	1.48 d (6.4)	1.55 d (6.7)		1	4.99 d (7.6)	
				2	3.91	
				3	4.12	
				4	4.12	
				5	3.65, 4.28	

[a] The assignment was based upon DQF-COSY, HOHAHA, HETECOR, NOESY, and HMBC experiments.

Chart 2



1a. R=gal(1→2)[xyl(1→3)]glcA

3. R=H

anomeric proton was equatorial, thus possessed an α configuration. Combining this with ^{13}C NMR data, the spin-system A was identified as α-rhamnopyranose. For the B-system, only three cross-peaks can be traced from the subspectrum corresponding to the anomeric proton (δ 5.93, d, 8.2 Hz). However, by examining the row corresponding to the doublet methyl group at δ 1.48 (d, 6.4 Hz), it was clear that this system consisted of a terminal methyl and five methine protons. As indicated in Figure 1 (1a and 1b), high values of the coupling constants existed between H-5 and the methyl group, but the coherence transfer was barely propagated through H-5 and H-4 due to the small $^3J_{H4,H5}$ coupling. This combined with NOE relationships (between H-1 and H-3, H-1 and H-5) in the NOESY spectrum as well as ^{13}C NMR data identified the B-system as β-fucopyranose (6-deoxy-β-galactopyranose). The other deoxy system (G) showed couplings from the anomeric proton (δ, 4.99, d, 7.6 Hz) through the system including the terminal methyl group (δ, 1.26, d, 6.1 Hz). An acetyl group attached to the C-4 of the system was evident since H-4 (5.08, t, 9.4, 9.8 Hz) was well isolated from other methine protons due to the acetylation effect. Further supporting information came from the long-range HMBC coupling between H-4 and the acetyl carbonyl carbon (170.2 ppm). The coupling patterns of H-4 (dd, 9.4, 9.8 Hz) suggested a trans-diaxial relationship between H-3 and H-4, H-4 and H-5. NOESY showed significant through-space interaction between H-1 and H-3 as well as H-1 and H-5. The above information suggested the G-system was β-6-deoxy glucose, that is β-quinovopyranose. Proceeding in the same way, the other six sugar units were identified as a β-glucopyranuronic acid (I), a β-galactopyranose (C), and four β-xylopyranoses (D, E, F, H). It should be pointed out that in addition to furnishing the information concerning the intersugar linkages as discussed below, HMBC has also found significant application in discerning some closely related protons and carbons, especially in the case of the systems E and F, G and H in which the anomeric protons were overlapped.

The linkage of the sugar units at C-3 was established from the following HMBC correlations: H-1 of C spin-system (galactose) with C-2 of I (glucuronic acid); H-1 of D (xylose) with C-3 of I (glucuronic acid). The attachment of the trisaccharide moiety to that of C-3 of the aglycone was confirmed by the long-range correlation between H-1 of glucuronic acid with C-3 of the aglycone. The sugar chain at C-28 was established from the following HMBC correlation: H-1 of F (terminal xylose) with C-3 of E (inner xylose); H-1 of E with C-4 of A (rhamnose); H-1 of A with C-2 of B (fucose); H-1 of H (terminal xylose) with C-3 of G (quinovose); H-1 of G with C-4 of B (fucose), while the attachment of the hexasaccharide chain to C-28 of the aglycone was based on a correlation between H-1 of fucose (B) and the C-28 of the aglycone. The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiment. However, due to the highly overlapped nature

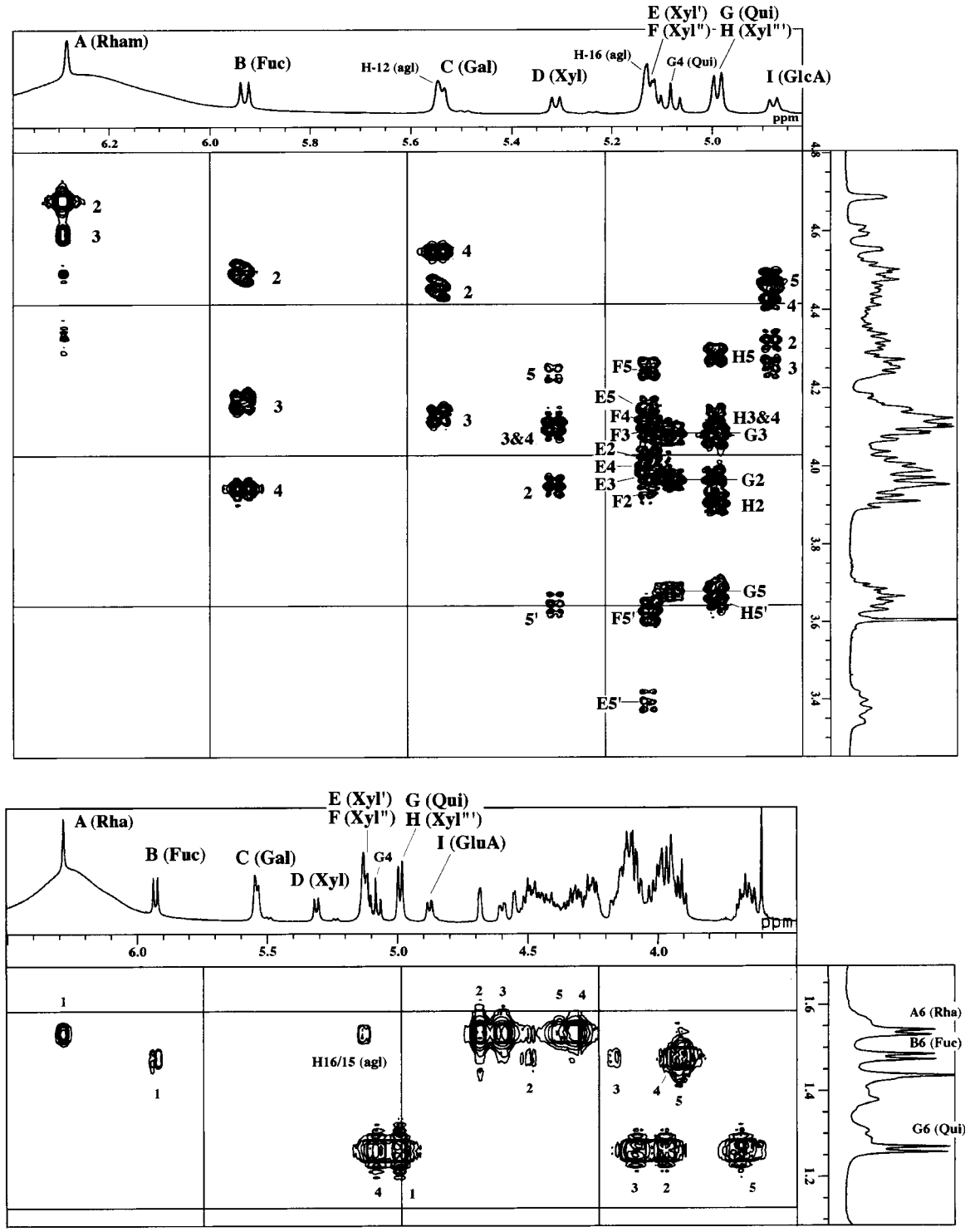


Figure 1. HOHAHA (τ_m = 150 ms) spectra (part) of saponarioside A (**1**) (500 MHz in pyridine-d_5) showing the subspectra corresponding to each anomeric proton (1a, upper) and methyl group (1b, lower). Note the resonances labeled E and F, G and H were overlapped.

of the proton spectrum, NOEs could not be used as the sole source of evidence for the intersugar linkage. The linkage was also supported from the fragmentation patterns observed in the ESI-MS/MS experiment. MS/MS analysis of the deprotonated molecular ion $[M - H]^-$ (*m*/*z* 1831) gave a daughter ion at *m*/*z* 1699 $[(M - H)^- - 132]$ by the loss of one of the terminal pentoses (xylose). The most prominent fragment observed at *m*/*z* 955 was due to the loss of the hexasaccharide chain linked to C-28 of saponarioside A (**1**). Other ions from losing 1 mol of water (*m*/*z* 1813) or an acetyl group (*m*/*z* 1789) from the deprotonated molecular ion were also observed.

All monosaccharides were in the pyranose form as determined from their ^{13}C NMR data. The β anomeric configurations for the fucose, galactose, glucuronic acid, quinovose, and xylose were evident from their $^3J_{H1,\,H2}$ (7−8 Hz) and $^1J_{C1,H1}$ coupling constants (Tables 1 and 2) as well as from NOE information. The broad singlet anomeric proton, large $^1J_{C1,H1}$ (171.7 Hz) and the three-bond HMBC couplings between the anomeric proton with C-3 and C-5 of the rhamnose indicated α orientation. The absolute configurations of these sugars were chosen in keeping with those mostly encountered among other plant glycosides. Thus, the structure of saponarioside A is established as

3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-*β*-D-4-*O*-acetylquinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**1**).

Saponarioside B (**2**), an amorphous solid, had a molecular formula of $C_{77}H_{120}O_{41}$ determined from its negative ion HRFABMS (at *m/z* 1699.7227 [M − H]$^-$) and from ^{13}C, DEPT NMR data. Its ^{1}H and ^{13}C NMR spectra indicated that compound **2** possessed the same aglycone as that of **1** but differed in the sugar part (Tables 1 and 2). The presence of eight sugars in **2** was indicated from the eight anomeric protons [*δ* 4.89 d (*J* = 7.3 Hz), 4.99 d (*J* = 7.9 Hz), 5.14 d (*J* = 7.6 Hz), 5.15 d (*J* = 7.3 Hz), 5.32 d (*J* = 7.9 Hz), 5.55 d (*J* = 7.6 Hz), 5.94 d (*J* = 7.9 Hz), 6.23 s] and carbons [*δ* 94.5, 101.3, 103.9, 104.2, 105.0, 105.9, 106.2 (×2)], respectively (Tables 1 and 2). From the assigned aglycone, it was apparent that the eight sugars were present in two saccharide units, one attached to C-3 and the other at C-28. Alkaline hydrolysis resulted in the same prosapogenin, quillaic acid 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranoside (**1a**), as that of compound **1**, indicating that the remaining five sugars were connected to C-28. The overall structural assignment was accomplished using the same protocol as in **1**. Extensive NMR (DQF−COSY, HOHAHA, HETCOR, NOESY, and HMBC) studies permitted the full assignment of the protons and carbons. The exact linkage positions for the sugar units were established using the HMBC and NOESY correlations as described for **1**. The sugar arrangement was further supported by the fragmentation patterns observed in the negative ESI-MS/MS experiment. Compound **2** gave the same fragmentation patterns as observed in **1**. The stereochemistry of each anomeric carbon was determined from the same observations as that of **1**. Thus, saponarioside B is established as 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-4-*O*-acetylquinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**2**).

Due to its medicinal and commercial importance, numerous chemical investigations have been carried out on *S. officinalis*. Early chemical study in the 1970s on this plant led to the isolation of several triterpenoid saponins, and the most polar saponin named saponasid D was chemically established to be based upon gypsogenin with up to 10 sugar units.[2] Later, it was proven that the sapogenin was quillaic acid.[4,5] In the present study, we confirmed that the sapogenin for the major saponins was quillaic acid. In addition, we also found that saponins from the *n*-BuOH soluble portion were based upon gypsogenic acid and 16-α OH gypsogenic acid. These saponins are relatively small with four or five sugar units, and their structures are under study. Triterpenoid saponins with oligosaccharide structures similar to that of **1** and **2** have been isolated from the commercial Merck saponin extract (*Gypsophyla paniculata*).[11]

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. FABMS and ESIMS were conducted using JEOL JMS-700 and Finnigan LCQ mass spectrometers, respectively. ^{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. Diaion HP-20 (Mitsubishi Chemical), silica gel (Silica 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. The fresh whole plants of *S. officinalis* were collected in the botanical garden of Toho University in July 1997 and were identified by one of the authors (T. Nikaido). 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 water, and then partitioned successively with EtOAc (53.7 g) and *n*-BuOH (124.0 g). The water soluble fraction (320 g) was applied to a column of Diaion HP-20 (2000 mL) and washed with 30, 50, 70, and 100% MeOH. The 70% MeOH fractions were combined and repeatedly chromatographed over silica gel and ODS columns to give several saponin fractions. HPLC purification on the high polar fraction (70% MeOH−0.06% TFA in H_2O, 1.0 mL/min., UV detector, 210 nm) afforded saponariosides A (100 mg) and B (40 mg), respectively.

Saponarioside A (1): an amorphous solid; mp 243−245 °C (dec); $[\alpha]^{20}_D$ −25.5° (*c* 0.90, pyridine); IR ν^{KBr}max: 3406, 2928, 1727, 1633, 1373, 1246, 1048 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz): *δ* 9.91 (1H, s, H-23), 5.54 (1H, br.t, H-12), 5.14 (1H, br.s, H-16), 4.02 (1H, m, H-3), 3.36 (1H, dd, *J* = 13.8, 3.7 Hz, H-18), 2.72 (1H, t, *J* = 13.5 Hz, H-19), 2.16 (3H, s, COCH_3), 1.73, 1.44, 1.03, 0.97, 0.94, 0.81 (each 3H, s, H_3-27, 24, 26, 30, 29, 25); ^{13}C NMR (pyridine-d_5, 125 MHz): *δ* 210.2 (d, C-23), 175.9 (s, C-28), 170.2 (s, *C*OCH$_3$), 144.4 (s, C-13), 122.2 (d, C-12), 84.4 (d, C-3), 74.1 (d, C-16), 55.1 (s, C-4), 49.3 (s, C-17), 48.8 (d, C-5), 47.4 (t, C-19), 46.9 (d, C-9), 42.1 (s, C-14), 41.6 (d, C-18), 40.3 (s, C-8), 38.1 (t, C-1), 36.3 (s, C-10), 35.9 (t, C-21), 33.2 (q, C-29), 32.8 (t, C-7), 32.0 (t, C-22), 30.7 (s, C-20), 27.0 (q, C-27), 25.3 (t, C-2), 24.5 (q, C-30), 23.7 (t, C-11), 21.1 (q, CO*C*H$_3$), 20.6 (t, C-6), 17.4 (q, C-26), 15.8 (q, C-25), 11.1 (q, C-24), other NMR data see, Tables 1 and 2; ESI-MS (negative ion mode) *m/z*: 1831 [M−H]$^-$, 915 [M − 2H]$^{2-}$, 1813 [M − H − H_2O]$^-$, 1789 [M − H − CH_3CO]$^-$, 1669 [M − H-xyl]$^-$, 955 [M − H-hexasaccharide at C_{28}]$^-$; HRFABMS (negative ion mode) *m/z* 1831.7649 [M−H]$^-$ (calcd for $C_{82}H_{127}O_{45}$).

Saponarioside B (2): an amorphous solid, mp 236−238 °C (dec), $[\alpha]^{20}_D$ −15.0° (*c* 1.1, pyridine); IR ν^{KBr}max: 3400, 2933, 1726, 1632, 1374, 1250, 1047 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz): *δ* 5.53 (1H, br t, H-12), 5.13 (1H, br s, H-16), 4.06 (1H, m, H-3), 1.96 (3H, s, COCH_3), 1.74, 1.56, 1.44, 1.03, 0.98, 0.94, 0.82 (each 3H, s, H_3 of C-27, C-24, C-26, C-30, C-29, C-25); ^{13}C NMR (pyridine-d_5, 125 MHz): *δ* 210.2 (d, C-23), 176.0 (s, C-28), 170.2 (s, *C*OCH$_3$), 144.4 (s, C-13), 122.2 (d, C-12), 84.5 (d, C-3), 74.3 (d, C-16), 55.1 (s, C-4), 49.2 (s, C-17), 48.8 (d, C-5), 47.4 (t, C-19), 46.9 (d, C-9), 42.1 (s, C-14), 41.6 (d, C-18), 40.3 (s, C-8), 38.1 (t, C-1), 36.2 (s, C-10), 35.9 (t, C-21), 33.2 (q, C-29), 32.8 (t, C-7), 31.9 (t, C-22), 30.7 (s, C-20), 27.0 (q, C-27), 25.3 (t, C-2), 24.7 (q, C-30), 23.7 (t, C-11), 21.1 (q, CO*C*H$_3$), 20.6 (t, C-6), 17.4 (q, C-26), 15.8 (q, C-25), 11.1 (q, C-24), other NMR data see, Tables 1 and 2; ESI-MS (negative ion mode) *m/z*: 1699 [M − H]$^-$, 1681 [M − H − H_2O]$^-$, 1657 [M−H−CH_3CO]$^-$, 1567 [M−H-xyl]$^-$, 955 [M−H-pentasaccharide]$^-$; HRFABMS (negative ion mode) *m/z* 1699.7227 [M − H]$^-$ (calcd for $C_{77}H_{119}O_{41}$).

Acid Hydrolysis of Saponariosides A (1) and B (2). Compound **1** (30 mg) was heated in 1 mL of 1 N 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 extraction was washed with water and then concentrated to give an amorphous powder (**3**, 8 mg). The monosaccharide portion was neutralized by passing through an exchange resin (Amberlite MB-3) column, concentrated (dried overnight), and then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After the excess reagent was decomposed with

water, the reaction product was extracted with *n*-hexane (1 mL × 2). The TMSi derivatives of the monosaccharides were identified to be fucose, galactose, rhamnose, quinovose, and xylose (1:1:1:1:4) by co-GLC analyses with standard monosaccharides. The glucuronic acid was detected by co-TLC analysis with standard sample (solvent: $CHCl_3$−MeOH−H_2O, 10:5:1). By the same method, **2** (20 mg) afforded **3** (5 mg). GLC analyses showed the monosaccharides of **2** were to be fucose, galactose, rhamnose, quinovose, and xylose (1:1:1:1:3).

Alkaline Hydrolysis of Saponariosides A (1) and B (2). Saponarioside A (**1**, 30 mg) in 2 mL of 1 N KOH was heated at 80 °C for 2 h. After cooling, the reaction mixture was neutralized with 1 N HCl and then extracted with *n*-BuOH (three times). The organic layers were combined and then evaporated to dryness in a vacuum. The residue was subjected to column purification (ODS, 70% MeOH−H_2O) affording prosapogenin **1a** (8 mg). By the same method, **2** (10 mg) afforded **1a** (4 mg).

Prosapogenin 1a: an amorphous solid; mp 208−210 °C (dec); $[\alpha]^{23}_D$ +13.3° (*c* 1.4, MeOH); IR ν^{KBr}max: 3416, 2940, 1681, 1145, 1045 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz): δ 9.92 (1H, s, H-23), 5.60 (1H, br t, H-12), 5.22 (1H, br s, H-16), 4.09 (1H, dd, J = 11.9, 4.4, H-3), 3.59 (1H, dd, J = 13.1, 4.0 Hz, H-18), 2.82 (1H, t, J = 13.4, H-19), 1.82, 1.41, 1.18, 1.06, 0.97, 0.82 (each 3H, s, H_3 of C-27, C-24, C-26, C-30, C-29, C-25); ^{13}C NMR (pyridine-d_5, 125 MHz): δ 209.7 (d, C-23), 179.9 (s, C-28), 145.1 (s, C-13), 122.0 (d, C-12), 84.3 (d, C-3), 74.6 (d, C-16), 55.1 (s, C-4), 48.8 (s, C-17), 48.5 (d, C-5), 47.2 (t, C-19), 46.9 (d, C-9), 42.1 (s, C-14), 41.4 (d, C-18), 40.1 (s, C-8), 38.0 (t, C-1), 36.2 (s, C-10), 36.2 (t, C-21), 33.3 (q, C-29), 32.7 (t, C-7), 32.7 (t, C-22), 31.0 (s, C-20), 27.1 (q, C-27), 25.1 (t, C-2), 24.7 (q, C-30), 23.7 (t, C-11), 20.4 (t, C-6), 17.3 (q, C-26), 15.6 (q, C-25), 10.9 (q, C-24); other NMR data, see Tables 1 and 2; ESI-MS (negative ion mode) *m*/*z*: 955 $[M - H]^-$.

Quillaic acid (3): an amorphous solid; mp 206−208 °C; $[\alpha]^{23}_D$ +30.0 (*c* 0.2, MeOH); IR ν^{KBr}max: 3427, 2924, 2855, 1725, 1260 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz): δ 9.62 (1H, s, H-23), 5.66 (1H, br t, H-12), 5.24 (1H, br s, H-16), 4.11 (1H, t, J = 8.5, H-3), 3.63 (1H, dd, J = 14.5, 4.8 Hz, H-18), 2.85 (1H, t, J = 14.0, H-19) 1.84, 1.36, 1.19, 1.07, 1.03, 0.94 (each 3H, s, H_3 of C-27, C-24, C-26, C-30, C-29, C-25); ^{13}C NMR (pyridine-d_5, 125 MHz): δ 207.3 (d, C-23), 179.9 (s, C-28), 145.2 (s, C-13), 122.1 (d, C-12), 74.6 (d, C-16), 71.6 (d, C-3), 56.3 (s, C-4), 48.8 (d, C-5), 47.3 (s, C-17), 47.2 (d, C-9), 47.1 (t, C-19), 42.2 (s, C-14), 41.5 (d, C-18), 40.2 (s, C-8), 38.0 (t, C-1), 36.2 (s, C-10), 36.1 (t, C-21), 33.2 (q, C-29), 32.8 (t, C-7), 32.1 (t, C-22), 31.1 (s, C-20), 27.2 (q, C-27), 24.7 (t, C-2), 24.7 (q, C-30), 23.8 (t, C-11), 21.1 (t, C-6), 17.4 (q, C-26), 14.3 (q, C-25), 9.7 (q, C-24); EI-MS *m*/*z*: 487 $[M + H]^+$, 469, 425, 355.

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