Three New Acylated Triterpene Saponins from *Acanthophyllum squarrosum*

Ghezala Gaidi,[†] Tomofumi Miyamoto,[‡] Abdolhossein Rustaiyan,[§] and Marie-Aleth Lacaille-Dubois*[,†]

Laboratoire de Pharmacognosie, Unité MIB, JE 2244, Faculté de Pharmacie, Université de Bourgogne, 7, Bd. Jeanne d'Arc, BP 87900, 21079 Dijon, Cedex France, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan, and Department of Chemistry, Shahid Beheshty University, Eveen, Tehran, Iran

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Three new triterpenoid saponins, **1**−**3**, were isolated from the roots of *Acanthophyllum squarrosum*. Their structures were established mainly by 2D NMR techniques as 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl-gypsogenin-28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*β*-D-xylopyranosyl-(1→4)-3-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-*β*-D-fucopyranoside (**1**), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl-gypsogenin-28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[5-*O*-acetyl-α-L-arabinofuranosyl-(1→3)]-4-*O*-acetyl-*β*-D-fucopyranoside (**2**), and 3-*O*-*β*-D-glucopyranosyl-quillaic acid-28-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (**3**).

Our previous phytochemical studies on the MeOH extract of the roots of *Acanthophyllum squarrosum* Boiss. [syn.: *A. pungens* (Bunge) Boiss. var. *squarrosum* Golenk.]-(Caryophyllaceae) have led to the isolation of three new gypsogenin glycosides.[2,3] A further detailed investigation of the roots of this plant has resulted in the isolation of three additional major triterpene saponins (**1**−**3**). Their structures were elucidated mainly by 1D and 2D NMR experiments (COSY, TOCSY, NOESY, HSQC, and HMBC). This paper deals with the isolation and structure elucidation of **1**−**3** (Chart 1).

Results and Discussion

A crude saponin mixture was obtained from the MeOH extract of the roots of *A. squarrosum* according to a previously described procedure.[3] From this saponin fraction, saponins **1**−**3** were isolated following repeated chromatographic steps.[3]

The FABMS of compound **1** showed a quasimolecular ion peak at *m*/*z* 1751 $[M - H]^-$, indicating a molecular weight of 1752, compatible with a molecular formula of $C_{75}H_{100}O_{47}$. Other significant ion peaks appeared at *m*/*z* 1487 $[(M - H) - 132 - 132]^-$ and 1445 $[(M - H) - 132 - 132 - 42]^-$, corresponding to the loss of two pentosyl moieties and one acetyl group. Moreover, fragment ion peaks at *m*/*z* 939 and 469 corresponded to a hexosyl-pentosyl-hexosyluronic acid-aglycon fragment and to the aglycon moiety, respectively. Its MALDITOFMS showed a $[M + Na]^+$ ion at *m*/*z* 1775, which confirmed the proposed molecular weight.

Hydrolysis of **1** with 2 N TFA at 100 °C afforded galactose, xylose, rhamnose, and fucose in a molar ratio of 1:4:1:1 (estimated by GLC analysis after conversion into their alditol acetates) and glucuronic acid (co-TLC), and the aglycon gypsogenin, which was identified from its spectral features by comparison with literature data.[3,4] The spectroscopic NMR data of the prosapogenin of **1** obtained by alkaline hydrolysis were in good agreement with those of the prosapogenin of squarroside A.[2]

The ^{1}H NMR spectrum of **1** displayed signals for eight anomeric protons at *δ* 5.54 (d, *J* = 7.9 Hz), 4.87 (s), 4.57 (d, *J* = 7.9 Hz), 4.52 (d, *J* = 7.1 Hz), 4.40 (d, *J* = 7.4 Hz), 4.39 (d, *J* = 7.4 Hz), 4.30 (d, *J* = 7.8 Hz), and 4.13 (d, *J* = 7.5 Hz), which correlated with the carbon signals at *δ* 92.7, 100.5, 102.6, 102.3, 104.1, 104.7, 105.3, and 101.6, respectively, in the HSQC spectrum. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the COSY, TOCSY, HSQC, and HMBC spectra (Table 3). Evaluation of spin−spin couplings and chemical shifts allowed the identification of one *β*-fucopyranosyl (Fuc), one α-rhamnopyranosyl (Rha), one *β*-galactopyranosyl (Gal), one *β*-glucuronopyranosyl (GlcA), and four *β*-xylopyranosyl (Xyl) units. The common D-configuration for Fuc, Gal, Xyl, and GlcA and the L-configuration for Rha were assumed, according to those most often encountered among the plant glycosides in each case.

The sequence of the oligosaccharide chains in **1** was determined from the NOESY and HMBC spectra. The cross-peaks in the HMBC spectrum between the ^{1}H NMR signals at δ_H (GlcA-1) 4.13 and the ^{13}C NMR signals at δ_C (Agly-3) 82.6, δ_H (Xyl-1) 4.57, and δ_C (GlcA-3) 82.6, and at δ_H (GlcA-2) 3.38 and δ_C (Gal-1) 102.3, showed that the trisaccharide moiety 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl was linked to the gypsogenin unit at C-3. These sugar linkages were confirmed by NOESY correlations between signals at δ_H (GlcA-1) 4.13 and δ_H (Agly-3) 3.67, and at δ_H (Xyl-1) 4.57 and δ_H (GlcA-3) 3.54, as well as between signals at δ_H (Gal-1) 4.52 and δ_H (GlcA-3) 3.38. The correlation in the HMBC spectrum between the ^{1}H NMR signal at δ_H (Fuc-1) 5.54 (d, *J* = 7.9 Hz) and the ^{13}C NMR signal at δ_C (Agly-28) 175.3 proved the fucose to be attached at C-28 of the aglycon. The location of the two acetyl groups at Fuc-3 and Fuc-4 (δ_H 5.12 and δ_H 5.02) was determined by the TOCSY and COSY spectra, starting from the anomeric ^{1}H NMR signal of fucose at *δ* 5.54 (d, *J* = 7.9 Hz). The downfield shifts observed in the HSQC spectrum for the Fuc H-3/Fuc C-3 and Fuc H-4/Fuc C-4 resonances at δ_H 5.12/δ_C 71.4 and at δ_H 5.02/δ_C 70.0 proved the secondary alcoholic functions Fuc-3-OH and Fuc-4-OH to be acetylated. A cross-peak in the HMBC spectrum between the ^{1}H NMR signals at δ_H (Fuc-2) 3.70 and the ^{13}C NMR signals at δ_C (Rha-1) 100.5 indicated that fucose was glycosylated by the rhamnose unit at Fuc C-2. This linkage was confirmed by the NOESY correlation between δ_H (Rha-1) 4.87 and δ_H (Fuc-

* To whom correspondence should be addressed. Tel: 0033-3-80 39 32 29. Fax: 0033-3-80 39 33 00. E-mail: malacd@u-bourgogne.fr.
[†] Université de Bourgogne.
[‡] Kyushu University.
[§] Shahid Beheshty University.

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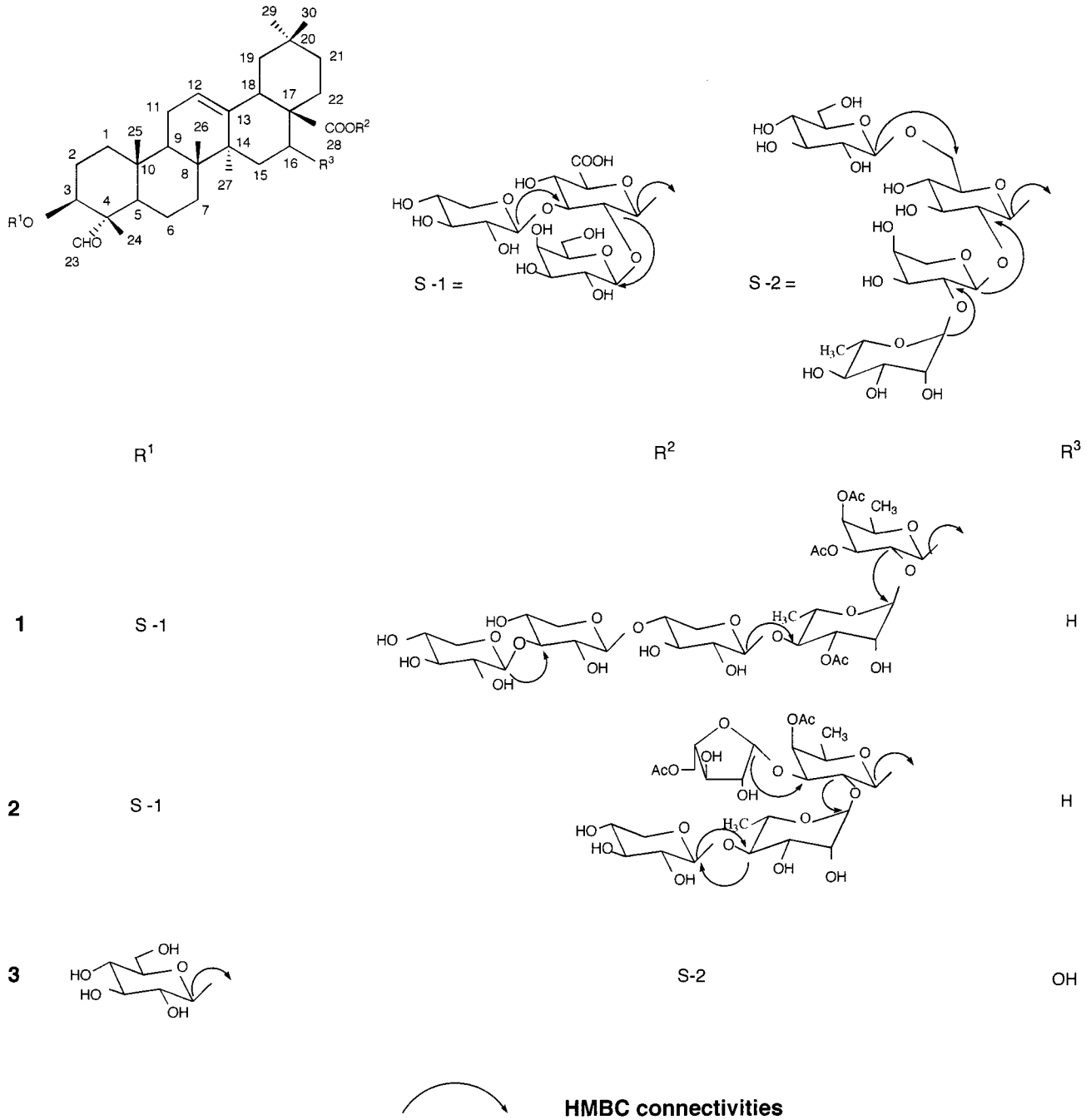
Chart 1



2) 3.70. All these results were confirmed by the observation of glycosidation and acylation-induced shifts reported in the literature.[5]

In the TOCSY and $^1H-^1H$ COSY spectra of **1**, the ^1H NMR signal of Rha H-1 at δ 4.87 (brs) showed a set of correlations to H-2 and to the deshielded H-3, H-4, and H-5 protons at δ 3.60, 5.10, 3.34, and 3.52, respectively. The downfield signal of Rha-3 at δ 5.10 gave a cross-peak with the downfield signal of C-3 at δ 72.8 in the HSQC spectrum and revealed the location of the third acetyl group at this position. The HMBC experiment showed long-range couplings between the ^1H NMR signal at δ_H (Rha-6) 1.12 (d, J = 6.1 Hz) and the ^{13}C NMR signals at δ_C (Rha-5) 67.4 (^2J) and δ_C (Rha-4) 82.3 (^3J) and proved the Rha C-4 position to be glycosylated.

Assignments of the ^1H NMR and ^{13}C NMR signals of **1** from the TOCSY, HSQC, and HMBC spectra showed that the three remaining sugars were two disubstituted xylose units (Xyl-1,4; Xyl-1,3) and a terminal xylose unit (T-Xyl). All the carbon signals due to the two last-mentioned sugar moieties were in good agreement with literature data for similarly linked sugar moieties.[6] In the HMBC spectrum, long-range $^1H-^{13}C$ correlations were observed between signals at δ_{H-1} (T-Xyl) 4.40 and δ_{C-3} (Xyl-1,3) 85.3, at δ_{H-1} (Xyl-1,4) 4.30 and δ_{C-4} (Rha) 82.3, and at δ_{H-2} (Fuc) 3.70 and δ_{C-1} (Rha) 100.5, allowing the sequencing of the glycosidic ester chain at C-28. On the basis of the above results, the structure of **1** was determined as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-3-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-β-D-fucopyranoside, a new natural compound.[2-18]

Table 1. ^{13}C NMR and ^{1}H NMR Data of the Aglycons of **1**−**3** (DMSO-d_6)[a,b]

position	1 mult[a]	1 δ ^{13}C	1 δ ^{1}H	2 δ ^{13}C	2 δ ^{1}H	3 mult[a]	3 δ ^{1}H	3 δ ^{13}C
1	CH_2	37.5	0.96,1.54	37.2	0.96,1.54	CH_2	1.56, 1.04	37.6
2	CH_2	24.2	nd,1.92	24.2	0.90,1.70	CH_2	1.65, 1.78	24.2
3	CH	82.6	3.67	82.7	3.67	CH	3.82	78.6
4	C	53.9		53.9		C		54.4
5	CH	47.2	1.24	47.3	1.26	CH	1.28	47.0
6	CH_2	19.0	nd, nd	19.8	1.49, 1.82	CH_2	nd	19.7
7	CH_2	31.6	1.46,1.60	31.6	1.42,1.62	CH_2	nd, 1.56	31.7
8	C	41.3		41.1		C		41.0
9	CH	46.7	1.54	46.9	1.54	CH	1.64	45.9
10	C	35.4		35.4		C		nd
11	CH_2	23.8	nd, 1.92	23.8	1.58, 1.95	CH_2	1.82, nd	22.7
12	CH	121.6	5.19 (br s)	121.6	5.18 (br s)	CH	5.21	121.2
13	C	143.0		143.1		C		143.4
14	C	nd		nd		C		
15	CH_2	27.0	1.05, 1.49	27.0	1.05, nd	CH_2	1.72, 1.18	34.7
16	CH_2	23.7	1.92, nd	23.7	1.95, nd	CH	4.42	72.4
17	C	46.1		46.0	-	CH		47.6
18	CH	40.9	2.70	41.4	2.70	CH	2.89	40.0
19	CH_2	45.4	1.07, 1.64	45.4	1.05, 1.65	CH_2	0.99, 2.24	46.2
20	C	30.2		30.2		C		30.0
21	CH_2	33.2	1.14, 1.33	33.2	1.12, 1.32	CH_2	1.08, 1.88	34.9
22	CH_2	28.9	nd	28.0	nd	CH_2	nd	
23	CHO	210.0	9.44 (s)	210.0	9.45 (s)	CHO	9.39 (s)	207.3
24	CH_3	10.2	1.07 (s)	10.2	1.07 (s)	CH_3	0.99 (s)	9.8
25	CH_3	15.3	0.89 (s)	15.3	0.90 (s)	CH_3	0.89 (s)	15.2
26	CH_3	16.7	0.69 (s)	16.8	0.68 (s)	CH_3	0.64 (s)	16.6
27	CH_3	25.3	1.10 (s)	25.2	1.10 (s)	CH_3	1.32 (s)	26.3
28	C	175.3		175.3		C		174.2
29	CH_3	32.6	0.87 (s)	32.6	0.86 (s)	CH_3	0.82 (s)	32.7
30	CH_3	23.4	0.86 (s)	23.4	0.85 (s)	CH_3	0.88 (s)	24.1

[a] Multiplicities were assigned from DEPT spectra. [b] The assignments were based on the HMBC, HSQC, and DEPT experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR).

Table 2. ^{13}C NMR Data of the Sugar Moieties of Compounds **1**−**3** (DMSO-d_6)[a,b]

1		2		3	
3-*O*-sugars		3-*O*-sugars		3-*O*-sugars	
GlcA1	*101.6*	GlcA1	*101.0*	Glc1	*104.0*
2	*77.5*	2	*77.9*	2	73.7
3	*82.6*	3	*82.7*	3	76.8
4	70.6	4	70.6	4	69.9
5	76.3	5	76.3	5	76.7
6	171.9	6	172.1	6	60.9
Xyl 1	*102.6*	Xyl 1	*102.6*		
2	73.6	2	73.4		
3	76.3	3	76.5		
4	69.3	4	69.3		
5	65.7	5	65.7		
Gal 1	*102.3*	Gal 1	*102.3*		
2	73.6	2	71.5		
3	73.4	3	74.8		
4	69.4	4	69.3		
5	73.6	5	73.5		
6	59.7	6	59.7		
28-*O*-sugars		28-*O*-sugars		28-*O*-sugars	
Fuc 1	*92.7*	Fuc 1	*92.9*	Glc 1	*93.3*
2	*71.4*	2	*72.6*	2	78.6
3	*72.8*	3	*79.3*	3	75.9
4	*70.0*	4	*72.6*	4	70.9
5	68.3	5	68.9	5	75.9
6	15.5	6	16.0	6	*67.4*
Ac at C-3	20.4, 169.5	Ac at C-4	20.6, 170.1		
Ac at C-4	20.3, 170.2				
Rha 1	*100.5*	Rha 1	*100.4*	T-Glc 1	*103.1*
2	69.3	2	69.6	2	73.4
3	*72.8*	3	70.6	3	76.6
4	*82.3*	4	*82.3*	4	70.0
5	67.4	5	67.2	5	76.7
6	17.6	6	17.7	6	61.0
Ac at C-3	20.4, 169.3				
Xyl (1,4) 1	*105.3*	Ara f 1	*110.2*	Ara 1	*100.3*
2	73.6	2	80.7	2	*73.7*
3	76.5	3	77.2	3	71.9
4	*74.7*	4	85.4	4	67.0
5	65.8	5	*63.2*	5	63.6
		Ac at C-5	20.6, 169.8		
Xyl (1,3) 1	*104.7*	T-Xyl 1	*105.5*	T-Rha 1	*99.6*
2	73.2	2	74.4	2	70.3
3	*85.3*	3	76.5	3	70.3
4	67.6	4	70.6	4	72.0
5	65.8	5	67.9	5	68.0
T-Xyl 1	*104.1*			6	17.6
2	73.2				
3	76.3				
4	69.6				
5	65.7				

[a] ^{13}C chemical shifts of substituted residues are italicized. [b] The assignments were based on the COSY, TOCSY, NOESY, HSQC, and HMBC experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR). Multiplicities were assigned from DEPT spectra.

The FABMS of compound **2** showed a quasimolecular ion peak at *m*/*z* 1579 $[M - H]^-$, compatible with the molecular formula $C_{72}H_{108}O_{38}$. Other significant ion peaks observed at *m*/*z* 1447 $[(M - H) - 132]^-$ and 1405 $[(M - H) - 132 - 42]^-$ corresponded to the loss of one pentosyl moiety and one acetyl group. Additionally, fragment ion peaks at *m*/*z* 939 and 469 corresponded to a hexosyl-pentosyl-hexosyluronic acid-aglycon fragment and to the aglycon moiety, respectively. Acid hydrolysis of **2** afforded gypsogenin and galactose, arabinose, xylose, rhamnose, and fucose in a molar ratio of 1:1:2:1:1, as well as glucuronic acid, which were identified by the same procedures as described for **1**.

Compound **2** was shown to contain two acetoxy methyl groups resonating at δ 2.01 and 2.06 (s) and seven sugar residues, from the HSQC spectrum. The anomeric ^{1}H NMR signals at δ 5.40 (d, $J = 7.9$ Hz), 4.95 (s), 4.90 (brs), 4.57 (d, $J = 7.9$ Hz), 4.52 (d, $J = 7.1$ Hz), 4.29 (d, $J = 7.6$ Hz), and 4.13 (d, $J = 7.5$ Hz) gave correlations with the ^{13}C NMR signals at δ 92.9, 100.4, 110.2, 102.6, 102.3, 105.5, and 101.0, respectively.

The ^{1}H and ^{13}C NMR data of **2** determined by TOCSY, HSQC, and HMBC experiments were similar to those of squarroside A[2] except for the appearance of an additional acetyl group. The site of linkage of the second acetyl was determinated by the HMBC experiment, which showed a long-range correlation between δ_H (Araf-5) 3.94 and δ_C 169.8. These results confirmed that the Araf residue was substituted by an acetyl group at position C-5. On the basis of the above results, the structure of **2** was established as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin-28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[5-*O*-acetyl-α-L-arabinofuranosyl-(1→3)]-4-*O*-acetyl-β-D-fucopyranoside, a new natural compound.[2−18]

The FABMS of compound **3** showed a quasimolecular ion peak at *m*/*z* 1249 $[M - H]^-$, indicating a molecular weight of 1250, compatible with the molecular formula $C_{59}H_{94}O_{28}$. Other fragment ion peaks visible at *m*/*z* 1233 $[(M - H) - 16]^-$, 1087 $[(M - H) - 162]^-$, 763 $[(M - H) - 16 - 162 - 162 - 146]^-$, and 485 $[(M - H) - 162 - 162 - 146 - 132 - 162]^-$ indicated the successive loss of one hexosyl, one hexosyl, one deoxyhexosyl, and one pentosyl-hexosyl moiety, respectively. The fragment ion at *m*/*z* 485 corresponded to the pseudomolecular ion of the aglycon, quillaic acid.[4] The MALDITOFMS of **3** showed a $[M + Na]^+$ ion at *m*/*z* 1273, which confirmed the proposed molecular weight. Acid hydrolysis of **3** afforded glucose, arabinose, and rhamnose (in a molar ratio of 3:1:1) and quillaic acid.[4]

Compound **3** was investigated by TOCSY, COSY, NOESY, HSQC, and HMBC experiments, and full assignments of all ^{1}H and ^{13}C NMR resonances were obtained (Tables 1 and 2). The anomeric proton signals at δ_H 5.30 (d, $J = 7.7$ Hz), 4.91 (s), 4.29 (d, $J = 7.5$ Hz), 4.16 (d, $J = 7.9$ Hz), and 4.07 (d, $J = 5.2$ Hz) gave correlations with anomeric carbon signals at δ_C 93.3, 99.6, 104.0, 103.1, and 100.3, respectively. The NMR data of **3** were similar to those of 3-*O*-β-D-glucopyranosyl-gypsogenin-28-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2) [β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside[3] except for the presence of signals due to the hydroxylation at position C-16 of

Table 3. ^{1}H NMR Data of the Sugar Moieties of Compounds **1**−**3** (DMSO-d_6)[a,b]

1		**2**		**3**	
3-*O*-sugars (*J* Hz)		3-*O*-sugars (*J* Hz)		3-*O*-sugars (*J* Hz)	
GlcA1	*4.13 (d, J* = 7.5)	GlcA1	*4.13 (d, J* = 7.5)	Glc1	*4.29 (d, J* = 7.5)
2	*3.38*	2	*3.38*	2	3.06
3	*3.54*	3	*3.56*	3	3.20
4	3.19	4	3.19	4	3.04
5	3.15	5	3.15	5	3.02
6	-	6	-	6	3.36, 3.68
Xyl 1	*4.57 (d, J* = 7.9)	Xyl 1	*4.57 (d, J* = 7.9)		
2	2.99	2	2.99		
3	3.15	3	3.09		
4	3.28	4	3.26		
5	3.08, 3.67	5	3.06, 3.67		
Gal 1	*4.52 (d, J* = 7.1)	Gal 1	*4.52 (d, J* = 7.1)		
2	3.24	2	3.22		
3	3.20	3	3.26		
4	3.50	4	3.30		
5	3.19	5	3.19		
6	3.45, 3.58	6	3.54, 3.58		
28-*O*-sugars		28-*O*-sugars		28-*O*-sugars	
Fuc 1	*5.54 (d, J* = 7.9)	Fuc 1	*5.40 (d, J* = 7.9)	Glc 1	*5.30 (d, J* = 7.7)
2	*3.70*	2	*3.63*	2	*3.80*
3	*5.12*	3	*3.96*	3	3.48
4	*5.02 (d, J* = 3.3)	4	*5.13*	4	3.31
5	4.07	5	3.89	5	3.18
6	0.97 (d, *J* = 6.4)	6	0.91 (d, *J* = 6.4)	6	*3.38*, *3.90*
Ac-3	1.97 (s)	Ac at C-4	2.06 (s)		
Ac-4	2.11 (s)				
Rha 1	*4.87(bs)*	Rha 1	*4.95 (bs)*	T-Glc 1	*4.16 (d, J* = 7.9)
2	3.60	2	3.73	2	2.94
3	*5.10*	3	3.59	3	3.09
4	*3.34*	4	*3.36*	4	3.40
5	3.52	5	3.59	5	3.02
6	1.12 (d, *J* = 6.1)	6	1.12 (d, *J* = 6.1)	6	3.43, 3.72
Ac-3	1.96 (s)				
Xyl (1,4) 1	*4.30 (d, J* = 7.8)	Ara f 1	*4.90 (brs)*	Ara 1	*4.07 (d, J* = 5.2)
2	2.95	2	3.81	2	*3.40*
3	3.08	3	3.62	3	3.43
4	*3.22*	4	3.34	4	3.54
5	3.00, 3.68	5	*4.16, 3.94*	5	3.26, 3.64
		Ac at C-5	2.01 (s)		
Xyl (1,3) 1	*4.39 (d, J* = 7.4)	T-Xyl 1	*4.29 (d, J* = 7.6)	T-Rha1	*4.91 (bs)*
2	3.07	2	2.93	2	3.59
3	*3.35*	3	3.09	3	3.40
4	3.34	4	3.19	4	3.16
5	3.07, 3.72	5	3.32, 3.65	5	3.62
T-Xyl 1	*4.40 (d, J* = 7.4)			6	1.08 (d, *J* = 6.4)
2	3.07				
3	3.15				
4	3.28				
5	3.08, 3.73				

[a] ^{1}H NMR chemical shifts of substituted residues are italicized. [b] The assignments were based on the COSY, TOCSY, NOESY, HSQC, and HMBC experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR). Multiplicities were assigned from DEPT spectra.

gypsogenin (quillaic acid, δ_{C-16} 72.4, δ_{H-16} 4.42). On the basis of the above results, the structure of compound **3** was determined as 3-*O*-*β*-D-glucopyranosyl-quillaic acid-28-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside, a new natural compound.[2−18]

Experimental Section

General Experimental Procedures. All physical data of the isolated compounds were obtained on the same instruments as those used in a previous paper.[3] In addition, NOESY NMR and MALDITOFMS were used. The mixing time in the NOESY experiment was set to 500 ms. MALDITOFMS was conducted using a PerSeptive Biosystems Voyager DE-STR mass spectrometer.

Plant Material. As previously described.[3]

Extraction and Isolation. The saponin mixture (2 g) obtained fom the roots of *A. squarrosum*[2,3] was submitted to column chromatography on Sephadex LH-20 (MeOH) and further fractionated by Si gel 60 [$CHCl_3$−MeOH−H_2O (8:5:1)] and medium-pressure liquid chromatography on Lichroprep RP-18 (40−63 μm) (MeOH−H_2O linear gradient 50−70%) to give compounds **1** (11 mg), **2** (15 mg), and **3** (16 mg). Final purification of **1**−**3** was carried out on a Sephadex LH-20 column eluted with MeOH.

Compound 1: white amorphous powder; $[\alpha]_D^{20}$ +4° (*c* 0.10, MeOH); IR ν_{max} 3500−3300 (OH), 2930 (CH), 1740 (C=O ester), 1710 (CO carboxylic acid), 1610, 1450, 1390, 1300 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Tables 1−3; negative FABMS (thioglycerol matrix) *m*/*z* 1751 [M − H]$^-$, 1487 [(M − H) − 132 − 132]$^-$, 1445 [(M − H) − 132 − 132 − 42]$^-$; MALDITOFMS [M + Na]$^+$ *m*/*z* 1775; TLC R_f 0.6 ($CHCl_3$−MeOH−AcOH−H_2O (15:8:3:2; system a); blue spot by spraying with Komarowsky reagent.

Compound 2: white amorphous powder; $[\alpha]_D^{20}$ −20° (*c* 0.10, MeOH); IR ν_{max} 3400 (OH), 2930 (CH), 1735 (C=O ester) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Tables 1−3; negative FABMS (thioglycerol matrix) *m*/*z* 1579 [M − H]$^-$, 1447 [(M − H) − 132]$^-$, 1405 [(M − H) − 132 − 42]$^-$; TLC R_f 0.48 (system a); blue spot by spraying with Komarowsky reagent.

Compound 3: white amorphous powder; $[\alpha]_D^{20}$ −2° (*c* 0.10, MeOH); IR ν_{max} 3400 (OH), 2930 (CH), 1735 (C=O ester) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Tables 1−3; negative FABMS (thioglycerol matrix) *m*/*z* 1249 [M − H]$^-$, 1233 [(M − H) − 16]$^-$, 1087 [(M − H) − 162]$^-$, 763 [(M − H) − 16 − 162 − 162 − 146]$^-$, 485 [(M − H) − 162 − 162 − 146 − 132 − 162]$^-$, MALDITOFMS [M + Na]$^+$ *m*/*z* 1273; TLC R_f 0.30 (system a); brown-violet spot by spraying with Komarowsky reagent.

Acid Hydrolysis. A solution of each saponin (3 mg) in 2 N aqueous CF_3COOH (5 mL) was refluxed on a water bath for 3 h. After this period, workup by liquid−liquid partitioning was performed.[3] The organic layer (CH_2Cl_2) gave gypsogenin[3] for **1** and **2** and quillaic acid[4] for **3** (co-TLC with an authentic sample). The sugars in the aqueous layer were analyzed by Si gel TLC in comparison with standard sugars. A 2 mg quantity of saponin was refluxed in 2 N aqueous CF_3COOH (2 mL) in a sealed serum vial at 100 °C for 3 h. After this period, sugars in the hydrolysate were converted into the alditol acetates and then subjected to GLC analysis according to a method previously described.[19]

Alkaline Hydrolysis. Each saponin (7 mg) was refluxed with 5% aqueous KOH (10 mL) for 1 h. The reaction mixture was adjusted to pH 6 with dilute HCl and then extracted with H_2O-saturated *n*-BuOH (3 × 10 mL). The combined *n*-BuOH extracts were washed (H_2O) and concentrated to dryness, yielding a prosapogenin. The prosapogenin of **1** and **2** was identified by comparison with that of squarroside A.[2] The acidic hydrolysis of this prosapogenin in 2 N aqueous CF_3-COOH for 2 h at 120 °C furnished gypsogenin and glucoronic acid, galactose, and xylose (co-TLC with authentic samples). The acidic hydrolysis of the prosapogenin of **3** in 2 N aqueous CF_3COOH for 2 h at 120 °C furnished quillaic acid and glucose (co-TLC with authentic samples).

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