Note

Two New Triterpene Glycosides in the Roots of *Uraria crinita*

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Two new triterpene glycosides, 24-deoxyoxytrogenin 3-*O*-*α*-L-rhamnopyranosyl (1→2)[*β*-D-glucopyranosyl]-*β*-D-galactopyranosyl (1→2)-*β*-D-glucuronopyranoside and sophoradiol 3-*O*-*α*-L-rhamnopyranosyl (1→2)-*β*-D-glucuronopyranosyl (1→2)-*β*-D-glucuronopyranoside with four known glycosides were isolated from a Chinese natural medicine, the roots of *Uraria crinita* (L.) Desv. Their structures were determined by chemical and spectral methods.

Key words triterpene; saponin; *Uraria crinita*; Leguminoseae; Fabaceae

Introduction

Uraria crinita (L.) Desv. is distributed in southern Japan, Taiwan, South China, and India. Its aerial parts and roots have been used in traditional Chinese medicine for the treatment of kidney disease as well as its anti-inflammatory and antitoxic effects. Several flavone *C*-glycosides were previously isolated from the aerial parts of this species,[1] while some triterpenes, isoflavonoids, and flavonolignans were found in its roots.[2] In our search for bioactive substances in leguminous plants,[3,4] we have studied the constituents of this plant. This paper deals with the isolation and structural elucidation of triterpene saponins from its roots. Specifically, the methanol extract of the roots of *U. crinita* was separated by normal and reverse-phase column chromatography to give two new triterpene glycosides (**1** and **2**) along with four known ones (**3**–**6**) (Chart 1). Compounds **3**–**6** were identified as abrisaponin So_1, abrisaponin F, phaseoside IV, and kaikasaponin III, respectively, according to the reported saponin data.[5,6]

Compound **1** was obtained as a brown amorphous powder showing $[\alpha]_D$ −128.4° [$c = 0.5$, pyridine–H_2O = (1 : 1)]. The negative FAB-MS exhibited a peak at *m*/*z* 1117 due to $[M-H]^-$. The exact measurement under high-resolution (HR) conditions showed that the composition is $C_{54}H_{85}O_{24}$ at *m*/*z* 1117.5413 $[M-H]^-$ in the HR/negative FAB-MS. The monosaccharide mixture obtained by acid hydrolysis of **1** revealed the presence of D-glucuronic acid, D-galactose, D-glucose, and L-rhamnose by the following analytical method of Tanaka *et al.*[7] The ^{1}H-NMR spectrum showed seven tertiary methyl signals between δ 0.88 and 1.76. Moreover, the ^{1}H-signals displayed the presence of four anomeric protons at δ 4.98 (1H, d, $J = 7.9$ Hz, glcA H-1), 5.00 (1H, d, $J = 7.6$ Hz, glc H-1), 5.59 (1H, d, $J = 7.9$ Hz, gal H-1) and 6.17 (1H, d, $J = 1.5$ Hz, rha H-1). The ^{13}C-NMR spectrum of **1** exhibited 54 signals indicating a triterpene moiety, including two oxygen-bearing carbons (δ 75.7 and δ 90.8) and a carboxylic carbon (δ 182.0). The carbon signals due to the sugar part were in agreement with those of arbisaponin F.[6] The structure of the sapogenol part was verified by various two-dimensional (2D) NMR (^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC)) techniques. The data of the sapogenol part were identical to those of 24-deoxyoxytrogenin[5,8] except for C-2 and -3 signals due to glycosylation.[9,10] Moreover, the H-1 (d, $J = 7.9$ Hz at δ 4.98) of glucuronic acid correlated with the C-3 (δ 90.8) of that in the HMBC (Fig. 1). Thus, **1** was characterized as 24-deoxyoxytrogenin 3-*O*-*α*-L-rhamnopyranosyl (1→2)[*β*-D-glucopyranosyl]-*β*-D-galactopyranosyl (1→2)-*β*-D-glucuronopyranoside.

Compound **2** was obtained as an amorphous powder showing $[\alpha]_D$ −44.8° [pyridine–H_2O = (1 : 1)]. The negative FAB-MS exhibited a peak at *m*/*z* 939 due to $[M-H]^-$ and fragment peaks at *m*/*z* 793 [M−H−Rha] and 599 [M−H−Rha−GlcA]. The exact measurement under HR conditions showed that the composition is $C_{48}H_{75}O_{18}$ at *m*/*z* 939.4976 $[M-H]^-$ in the HR/negative FAB-MS. The monosaccharide mixture obtained by acid hydrolysis of **2** revealed the presence of D-glucuronic acid and L-rhamnose.[7] The ^{1}H-NMR spectrum showed eight tertiary methyl signals between δ 0.84 and 1.42. Moreover the ^{1}H-signals displayed the presence of three anomeric protons at δ 5.07 (1H, d, $J = 7.6$ Hz), δ 5.82 (1H, d, $J = 7.3$ Hz), and δ 6.31 (1H, d, $J = 1.5$ Hz). The ^{13}C-NMR spectrum of **2** exhibited 48 signals consisting of a triterpene moiety and three sugar moieties. The data of the sapogenol part, including two oxygen-bearing carbons (δ 90.7 and 75.9), were superimposable on those of abrisaponin So_1.[6] Moreover, the signals due to sugar signals at C-3 were identical to those of yunganoside N2.[11]

In the HMBC, the correlations were observed between the H-1 (d, $J = 7.6$ Hz, δ 5.07) of glucuronic acid and the C-3 (δ 90.7) of the aglycone, between C-2 (δ 79.3) of glucuronic acid and H-1 (d, $J = 7.3$ Hz, δ 5.82) of the inner glucuronic acid, and between C-2 (δ 78.6) of the inner glucuronic acid and H-1 (d, $J = 1.5$ Hz, δ 6.31) of the terminal rhamnose (Fig. 2). Thus, **2** was characterized as sophoradiol 3-*O*-*α*-L-rhamnopyranosyl (1→2)-*β*-D-glucuronopyranosyl (1→2)-*β*-D-glucuronopyranoside.

These saponins were characteristic glycosides obtained from Leguminoseae plants.

Experimental

General Procedures Optical rotations were determined

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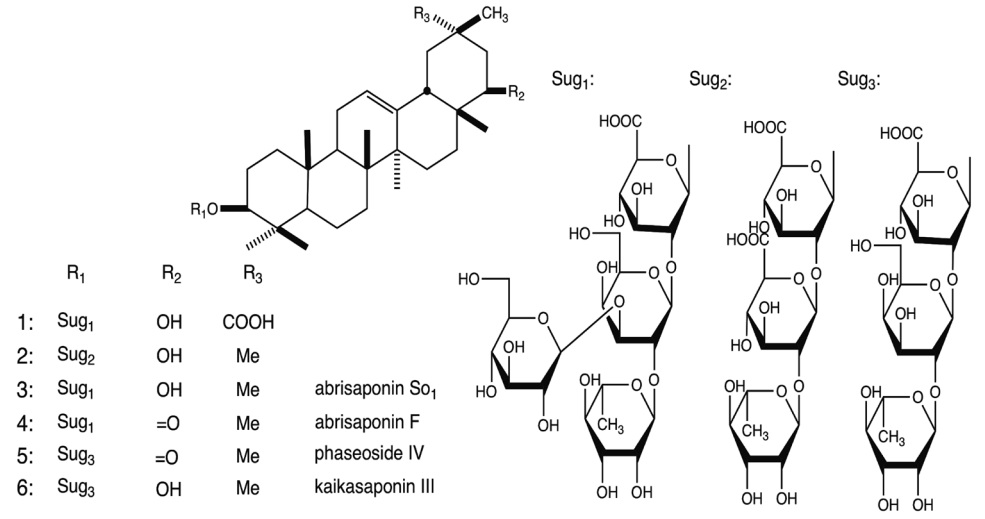

	R_1	R_2	R_3	
1:	Sug_1	OH	COOH	
2:	Sug_2	OH	Me	
3:	Sug_1	OH	Me	abrisaponin So_1
4:	Sug_1	=O	Me	abrisaponin F
5:	Sug_3	=O	Me	phaseoside IV
6:	Sug_3	OH	Me	kaikasaponin III

Chart 1

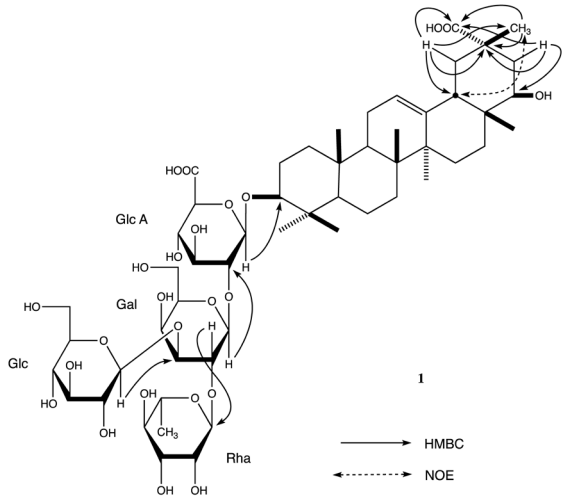

Fig. 1. NOE and Key HMBC Correlations of Compound **1**

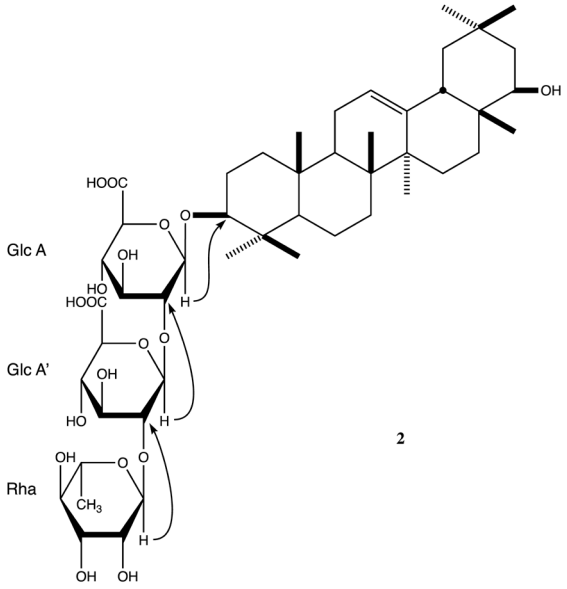

Fig. 2. Key HMBC Correlations of Compound **2**

on a JASCO DIP-360 polarimeter ($l = 0.5$). FAB-MS data were obtained in a glycerol matrix in positive ion mode using a JEOL JMS-HX110 mass spectrometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained with a JEOL JNM-A500 spectrometer. Column chromatography was carried out with silica gel 60 (230–400 mesh and ASTM; Kanto Kagaku, Japan), Diaion HP-20 (Mitsubishi Kasei, Japan), Sephadex LH-20 (25–100 nm; Pharmacia Fine Chemicals, Japan), MCI gel CHP 20P (75–150 nm; Mitsubishi Kasei), and COSMOSIL ($75C_{18}$-OPN and $150C_{18}$-OPN; Nacalai Tesque Inc., Japan). TLC was conducted on a precoated silica-gel $60F_{254}$ plate (0.2 mm; Merck, Germany), and detection was achieved by spraying it with 10% H_2SO_4, followed by heating.

Plant Material *Uraria crinita* (L.) Desv. (Leguminosae) was cultured at the Botanical Garden of Fukuoka University in 2013, and its roots were collected. These seeds were purchased from Yoshioka Seed Bank (Nagano, Japan).

Extraction and Isolation The fresh roots (485 g) of *Uraria crinita* were extracted with MeOH. This MeOH extract (15.2 g) was subjected to Diaion HP-20 to remove the sugar part, eluting first with water and then with MeOH. The MeOH portion (5.5 g) was applied to Sephadex LH-20 to collect a saponin fraction (2.4 g) by eluting with 80% MeOH. This saponin fraction was then subjected to MCI gel CHP 20P column chromatography using 60% MeOH→MeOH to give five fractions. Fraction 1 (129 mg) was further chromatographed on octadecyl silica (ODS) (70% MeOH +0.2% AcOH) and silica gel (BuOH–AcOH–water = 4 : 1 : 2) to provide compound **1** (11.9 mg). Fraction 2 (139 mg) was also chromatographed on ODS (70% MeOH +0.2% AcOH) and silica gel ($CHCl_3$–MeOH–water = 7 : 3 : 0.5 + 0.2% AcOH) to afford compound **2** (16.2 mg). Fractions 3 (313 mg) and 4 (320 mg) were chromatographed on ODS (30% CH_3CN) and silica gel ($CHCl_3$–MeOH–water = 7 : 3 : 0.5 + 0.2% AcOH/BuOH–AcOH–water = 4 : 1 : 5) to provide compounds **3** (176.3 mg), **4** (18.7 mg), **5** (7.4 mg), and **6** (17.7 mg).

Compound 1 An amorphous powder. $[\alpha]_D^{20}$ −128.4° [$c = 0.5$, pyridine–H_2O: (1 : 1)]. HR FAB-MS *m/z* 1117.5413 (Calcd. for $C_{54}H_{85}O_{24}$: 1117.5431). Negative FAB-MS *m/z* 1117 $[M-H]^-$.

^{1}H-NMR (pyridine-d_5) δ: Table 1.

^{13}C-NMR: Tables 2, 3.

Identification of Sugars for 1 A solution of **1** (5.0 mg) in 0.5 M HCl (1 mL) was heated at 95°C in screw-capped vials for two hours. The mixture was evaporated to dryness *in vacuo*, dissolved in 100 μL of L-cysteine solution (10 mg/mL pyridine), and reacted at 60°C for one hour. A solution (1 μL) of *o*-toryl isothiocyanate was added to the mixture, followed by heating at 60°C for one hour. The final mixture was directly analyzed by HPLC [COSMOSIL 5C_{18} AR II (250 × 4.6 mm i.d.; Nacalai Tesque Inc.); mobile phase CH_3CN in 50 mM H_3PO_4 (4–30% linear gradient at 0–39 min and 30%–75% linear gradient at 39–54 min); flow rate, 0.8 mL/min; and column temperature, 35°C ; detection, 250 nm]. The t_R values of the peaks of D-galactose (16.008 min), L-galactose (16.561 min), D-glucose (18.564 min), L-glucose (16.960 min), D-glucuronic acid (18.958 min), and L-rhamnose (31.294) were confirmed in those of comprised sugars of **1** at 16.215 (D-galactose), 18.602 (D-glucose), 19.353 (D-glucuronic acid), and 31.596 (L-rhamnose) minutes.

Compound 2 An amorphous powder. $[\alpha]_D^{20}$ −44.8°[*c* = 0.5, pyridine–H_2O (1 : 1)]. HR FAB-MS *m/z* 939.497 (Calcd for $C_{48}H_{75}O_{18}$: 939.4953). Neg. FAB-MS *m/z* 939 $[M-H]^-$, 793 $[M-H-Rha]^-$, 599 $[M-H-Rha-GlcA]^-$.

^{1}H-NMR (pyridine-d_5) δ: Table 1.

^{13}C-NMR: Tables 2, 3.

Identification of Sugars for 2 In the same manner as described above for **1**, the t_R values of the peaks of D-glucuronic acid (18.958 min) and L-rhamnose (31.294 min) were confirmed in those of comprised sugar of **2** at 18.659 (D-glucuronic acid) min and 31.744 (L-rhamnose) min.

Compound 3 (Abrisaponin So_1) A white amorphous powder. $[\alpha]_D^{20}$ −2.7° [*c* = 0.5, pyridine–H_2O (1 : 1)]. Neg. FAB-MS *m/z* 1087 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.87, 0.99, 1.00, 1.22, 1.24, 1.27, 1.28, 1.40 (each 3H, s, *tert*-Me × 8), 1.78 (3H, d, *J* = 6.1 Hz, rha H-6), 5.01 (1H, d, *J* = 7.6 Hz, glcA H-1), 5.02 (1H, d, *J* = 7.3 Hz, glc H-1), 5.32 (1H, br s, H-12), 5.64 (1H, d, *J* = 7.6 Hz, gal H-1), 6.21 (1H, s, rha H-1).

^{13}C-NMR: Tables 2, 3.

Table 1. ^{1}H-NMR Data for Compounds **1** and **2**

	1	**2**			**1**	**2**
1	α1.04 (o)	α0.88 (t-like, 13.7)	Glc A	1	5.00 (d, 7.6)	5.07 (d, 7.6)
	β1.46 (o)	β1.44 (o)		2	4.52 (t, 9.2)	4.50 (o)
2	α1.94 (o)	α1.86 (o)		3	4.55 (o)	4.35 (o)
	β2.22 (br d 9.8)	β2.17 (o)		4	4.40 (o)	4.43 (t, 9.7)
3	3.32 (dd, 11.6, 4.6)	3.34 (dd, 11.6, 4.3)		5	4.59 (d, 9.2)	4.63 (o)
4				6		
5	0.97 (br d, 11.3)	0.8.1 (br d, 11.3)	Glc A’	1		5.82 (d, 7.3)
6	α1.60 (o)	α1.33 (o)		2		4.34 (o)
	β1.80 (o)	β1.54 (o)		3		4.27 (t, 8.9)
7	α1.34 (br d, 11.3)	α1.34 (o)		4		4.50 (o)
	β1.54 (br d, 11.3)	β1.54 (o)		5		4.63 (o)
8				6		
9	1.61 (o)	1.61 (o)	Gal	1	5.59 (d, 7.9)	
10				2	4.57 (o)	
11	1.85 (o)	1.83 (o)		3	4.08 (dd, 8.5, 5.2)	
12	5.37 (br s)	5.31 (brs)		4	4.71 (o)	
13				5	3.94 (t, 7.9)	
14				6	4.18 (o)	
15	α1.02 (o)	α1.03 (dd, 12.5, 5.8)			4.26 (o)	
	β1.80 (o)	β1.85 (o)	Glc	1	4.98 (d, 7.6)	
16	α1.22 (o)	α1.43 (o)		2	3.93 (o)	
	β1.36 (o)	β1.87 (o)		3	3.89 (m)	
17				4	4.03 (t, 9.5)	
18	2.46 (br d, 13.3)	2.35 (br d, 13.1)		5	4.19 (o)	
19	α1.62 (o)	α1.18 (br d, 12.2)		6	4.30 (t, 9.5)	
	β2.73 (t, 13.3)	β1.88 (o)			4.42 (o)	
20			Rha	1	6.16 (d, 1.5)	6.31 (br s)
21	α2.53 (br d, 13.3)	α1.77 (t-like, 7.9)		2	4.82 (dd, 3.4, 1.5)	4.73 (br s)
	β2.11 (dd, 13.4, 10.4)	β1.63 (o)		3	4.71(o)	4.70 (dd, 9.4, 3.2)
22	3.99 (o)	3.3.75 (dd, 7.2, 3.6)		4	4.28 (td, 9.5, 4.6)	4.36 (o)
23	1.37 (s)	1.42 (s)		5	5.00 (o)	5.00 (dd, 9.4, 6.4)
24	1.20 (s)	1.21 (s)		6	1.75 (d, 6.4)	1.81 (m)
25	0.88 (s)	0.84 (s)				
26	0.99 (s)	0.98 (s)				
27	1.26 (s)	1.26 (s)				
28	1.25 (s)	1.21 (s)				
29		0.98 (s)				
30	1.76 (s)	1.24(s)				

Chemical sifts (δ) are shown in ppm, (o) means overlapping signal.

Table 2. ^{13}C-NMR Data for Compounds **1**–**6** (Aglycone Moieties)

	1	2	3	4	5	6
C-1	39.3	39.2	39.1	39.2	39.2	39.1
2	26.6	26.8	26.6	27.7	26.7	26.5
3	90.8	90.7	90.6	90.8	90.6	90.6
4	40.1	40.0	40.0	40.1	40.0	39.9
5	56.3	56.3	56.1	56.3	56.2	56.1
6	18.9	18.8	18.7	18.8	18.7	18.6
7	33.6	33.6	33.4	33.3	33.2	33.4
8	40.3	40.3	40.2	40.3	40.2	40.1
9	48.2	48.3	48.1	48.3	48.3	48.1
10	37.2	37.2	37.0	37.2	37.1	37.0
11	24.2	24.1	24.0	24.1	24.1	24.0
12	123.7	122.9	122.8	124.3	124.3	122.7
13	144.5	145.1	145.0	142.2	142.1	144.9
14	42.8	42.8	42.6	42.4	42.7	42.6
15	26.8	26.7	26.7	27.7	26.6	26.5
16	29.2	29.2	29.0	27.7	27.7	29.0
17	38.2	38.3	38.2	48.3	48.2	38.1
18	45.0	45.8	45.7	48.2	48.1	45.6
19	41.7	47.1	46.9	47.0	46.9	46.9
20	42.9	31.1	31.0	34.5	34.4	31.0
21	37.7	42.3	42.3	51.3	51.2	42.3
22	75.7	75.9	75.8	217.4	217.1	75.7
23	28.7	28.7	28.6	28.7	28.6	28.8
24	17.1	17.2	17.0	17.1	17.0	16.9
25	16.0	16.0	15.9	15.9	15.9	15.8
26	17.5	17.5	17.4	17.3	17.2	17.3
27	25.8	25.9	25.8	25.8	25.8	25.8
28	21.1	21.2	21.2	21.3	21.2	21.2
29	182.2	33.2	33.3	32.2	32.1	33.2
30	25.2	29.0	28.9	25.7	25.6	28.5

Table 3. ^{13}C-NMR Data for Compounds **1**–**6** (Sugar Moieties)

		1	2	3	4	5	6
Glc A	C-1	105.6	105.4	105.7	105.7	105.6	105.2
	2	78.7	79.3	78.6	78.6	79.1	79.0
	3	78.7	77.4	76.3	76.3	76.7	76.6
	4	73.8	73.6	73.7	73.4	73.6	73.9
	5	77.2	77.4	77.3	78.4	77.2	76.6
	6	173.7	173.1	173.3	172.4	173.4	—
Glc A′	C-1		102.8				
	2		78.6				
	3		79.0				
	4		73.6				
	5		77.2				
	6		172.8				
Gal	C-1	102.6		102.6	102.2	102.2	101.9
	2	76.4		76.3	77.0	78.7	78.8
	3	85.4		85.2	85.4	75.8	76.5
	4	70.0		70.0	69.5	70.7	70.7
	5	75.7		75.8	75.6	76.2	76.1
	6	62.5		62.4	62.4	62.4	62.4
Glc	C-1	105.7		105.7	105.8		
	2	75.7		75.8	75.0		
	3	78.4		78.3	78.3		
	4	71.7		71.6	71.6		
	5	78.3		78.3	78.3		
	6	62.6		62.5	62.6		
Rha	C-1	102.2	102.3	102.2	102.6	102.9	102.7
	2	72.6	72.4	72.6	72.4	72.5	72.4
	3	72.4	72.6	72.4	72.6	72.6	72.4
	4	74.5	74.4	74.4	74.4	74.4	74.3
	5	69.5	69.7	69.4	69.9	69.6	69.4
	6	19.1	19.1	19.1	19.1	19.0	18.9

Compound 4 (Abrisaponin F) A white amorphous powder. $[\alpha]_D^{20}$ −32.1° ($c = 0.5$, pyridine). Neg. FAB-MS *m/z* 1085 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.88 (6H, s, *tert.*-Me × 2), 0.93, 1.00, 1.17, 1.18, 1.31, 1.40 (each 3H, s, *tert.*-Me × 6), 1.76 (3H, d, $J = 6.1$ Hz, rha H-6), 4.99 (1H, d, $J = 7.6$ Hz, glcA H-1), 5.02 (1H, d, $J = 7.3$ Hz, glc H-1), 5.45 (1H, brs, H-12), 5.61 (1H, d, $J = 7.6$ Hz, gal H-1), 6.18 (1H, d, $J = 1.5$ Hz, rha H-1).

^{13}C-NMR: Tables 2, 3.

Compound 5 (Phaseoside IV) A white amorphous powder. $[\alpha]_D^{20}$ −28.6° ($c = 0.5$, pyridine) Neg. FAB-MS *m/z* 923 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.86, 0.86, 0.92, 0.96, 1.17, 1.18, 1.28, 1.42 (each 3H, s, *tert.*-Me × 8), 1.76 (3H, d, $J = 6.1$ Hz, rha H-6), 5.06 (1H, d, $J = 7.0$ Hz, glcA H-1), 5.70 (1H, d, $J = 7.3$ Hz, gal H-1), 6.29 (1H, s, rha H-1).

^{13}C-NMR: Tables 2, 3.

Compound 6 (Kaikasaponin III) A white amorphous powder. $[\alpha]_D^{20}$ −14.9° ($c = 0.5$, pyridine). Neg. FAB-MS *m/z* 925 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.87, 1.00, 1.00, 1.20, 1.23, 1.28, 1.28, 1.37 (each 3H, s, *tert.*-Me × 8), 1.74 (3H, d, $J = 5.5$ Hz, rha H-6), 5.57 (1H, d, $J = 5.7$ Hz, gal H-1), 6.20 (1H, s, rha H-1).

^{13}C-NMR: Tables 2, 3.

Conflict of Interest The authors declare no conflict of interest.

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