

Dolabellane Diterpene and Three Cycloartane Glycosides from *Thalictrum squarrosom*

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A new dolabellane diterpene glycoside, named squoside A, and three new cycloartane glycosides, named squarrosides V, VI, and VII, have been isolated from the dried aerial parts of *Thalictrum squarrosom* (Ranunculaceae). Their structures were determined by two dimensional (2D) NMR spectroscopic analysis and chemical evidence.

Key words *Thalictrum squarrosom*; dolabellane diterpene glycoside; squoside; cycloartane glycoside; squarroside; Ranunculaceae

Thalictrum squarrosom STEPH. is a ranunculaceous plant distributed from the northern area of China to east Siberia. Its dried whole plant has been used as an antacid in traditional Chinese medicine. Previously, we have reported on the isolation and structural elucidation of four triterpene glycosides, squarrosides I, II, III, and IV from the aerial parts of *T. squarrosom*.¹⁾ In a continuation of our study on the same plant, we have now isolated a new dolabellane diterpene glycoside, squoside A (**1**), and three new cycloartane glycosides, squarrosides V (**2**), VI (**3**), and VII (**4**). In this paper, we describe the isolation and structural elucidation based on 2D NMR spectroscopic analysis and hydrolysis.

Results and Discussion

The methanolic extract of the dried aerial parts of *T. squarrosom* was partitioned into a benzene–water solvent system. The water-soluble portion was subjected to MCI gel CHP20P, Sephadex-LH20, octadecyl silica gel (ODS), and silica gel column chromatographies and finally HPLC to give squoside A (**1**) and squarrosides V (**2**), VI (**3**), and VII (**4**).

Squoside A (**1**) was obtained as an amorphous powder. The molecular formula of **1**, C₃₁H₅₀O₁₁, was established by the high-resolution (HR)-electrospray ionization (ESI)-MS [*m/z* 621.3238, (M+Na)⁺]. The ¹H- and ¹³C-NMR (methanol-*d*₄) spectrum revealed 31 carbon signals due to four quaternary methyls [δ_C 11.6; δ_H 1.57, δ_C 18.6; δ_H 1.29, δ_C 21.7; δ_H 1.27, and δ_C 24.8; δ_H 1.72], a secondary methyl [δ_C 18.9; δ_H 1.30 (d, *J*=6.3 Hz)], six methylenes [δ_C 25.4; δ_H 2.16 and 2.43, δ_C 28.7; δ_H 1.55 and 1.77, δ_C 32.3; δ_H 1.37 and 1.58, δ_C 39.1; δ_H 1.39 and 1.83, δ_C 40.6; δ_H 1.25

and 2.11, and δ_C 113.1; δ_H 4.71 and 4.92], an oxygen-bearing methylene [δ_C 68.3; δ_H 3.54 and 3.88], three methines [δ_C 43.2; δ_H 1.65, δ_C 53.6; δ_H 2.56, and δ_C 129.5; δ_H 5.25], ten oxygen-bearing methines [δ_C 68.4; δ_H 2.88, δ_C 69.7; δ_H 3.89, δ_C 70.8; δ_H 3.79, δ_C 72.9; δ_H 3.90, δ_C 73.4; δ_H 3.92, δ_C 74.4; δ_H 3.57, δ_C 75.4; δ_H 3.52, δ_C 79.0; δ_H 4.42, δ_C 84.6; δ_H 3.55, and δ_C 87.5; δ_H 3.12], two acetal methines [δ_C 104.2; δ_H 4.76 (br s) and δ_C 107.4; δ_H 4.48 (d, *J*=7.5 Hz)], three quaternary carbons [δ_C 51.7, 138.3, and 147.3], and an oxygen-bearing quaternary carbon [δ_C 64.3] (Table 1). The structural assignment was achieved by the ¹H–¹H correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments. The acid hydrolysis of **1** with 2.0 M hydrochloric acid afforded L-rhamnose and L-arabinose, the structure of which was confirmed by the ¹H-NMR coupling pattern and optical rotation using chiral detection in the HPLC analysis. The aglycon decomposed under an acid condition.

Five partial structures A (2-C–3-C), B (5-C–7-C), C (9-C–14-C), D (1'-C–6'-C), and E (1''-C–5''-C) were deduced from detailed analyses of 2D NMR data (¹H–¹H COSY, TOCSY, and HMQC) of **1** (Fig. 1). The connectivities of A, B, and C and three quaternary methyls (15-C, 16-C, 17-C) through three quaternary carbons (1-C, 4-C, 8-C) were showed by elucidation of HMBC spectrum. Furthermore, the long-range correlations between 15-H₃ (δ_H 1.27) and 11-C (δ_C 43.2), and between 20-H₃ (δ_H 1.72) and 12-C (δ_C 53.6), 18-C (δ_C 147.3), and 19-C (δ_C 113.1) resulted in the formations of eleven- and five-membered rings composed of the C–C bond between 1-C and 11-C, and the isopropenyl group attached to 12-C, respectively.

Meanwhile, the HMBC correlations were observed between 1'-H (δ_H 4.76) of the L-rhamnose unit in D and 2-C (δ_C 87.5), and 1''-H (δ_H 4.48) of the L-arabinose unit in E and 4'-C (δ_C 84.6) (Fig. 1). The anomeric centers of the L-arabinose and L-rhamnose units were determined to be both α -configuration in comparison with the ¹³C-NMR data of reference methyl glycosides.^{2,3)} These data proved the sugar sequence as 2-O- α -L-arabinopyranosyl-(1→4)- α -L-rhamnopyranosyl.

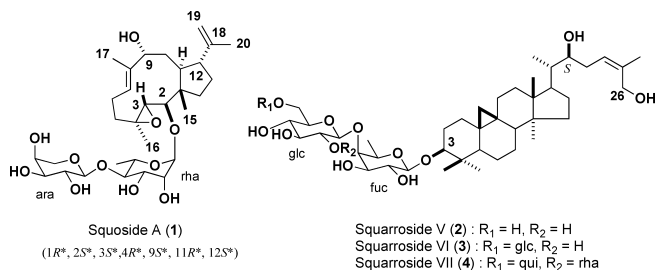
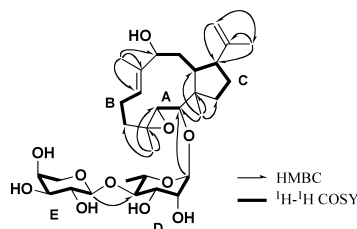
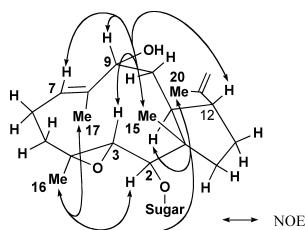


Chart 1

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Table 1. ^1H - and ^{13}C -NMR Data for **1** in Methanol- d_4

	δ_{C}	δ_{H}		δ_{C}	δ_{H}
1	51.7			rha	
2	87.5	3.12 (d, 9.2)	1'	104.2	4.76 (br s)
3	68.4	2.88 (d, 9.2)	2'	72.9	3.90 (br d, 3.5)
4	64.3		3'	73.4	3.92 (dd, 3.5, 8.6)
5	40.6	1.25 (dt, 5.7, 13.8) 2.11 (ddd, 2.9, 4.6, 13.8)	4'	84.6	3.55 (dd, 8.6, 8.6)
6	25.4	2.16 (m) 2.43 (m)	5'	69.7	3.89 (m)
7	129.5	5.25 (br d, 11.5)	6'	18.9	1.30 (d, 6.3)
8	138.3			ara	
9	79.0	4.42 (dd, 5.7, 11.5)	1''	107.4	4.48 (d, 7.5)
10	32.3	1.37 (td, 11.5, 13.8) 1.58 (br dd, 5.8, 13.8)	2''	74.4	3.57 (dd, 7.5, 8.6)
11	43.2	1.65 (br dd, 6.9, 11.5)	3''	75.4	3.52 (dd, 3.5, 8.6)
12	53.6	2.56 (td, 6.3, 12.0)	4''	70.8	3.79 (br s)
13	28.7	1.55 (m) 1.77 (br dt, 2.3, 12.0)	5''	68.4	3.54 (br d, 10.3)
14	39.1	1.39 (m) 1.83 (m)			3.88 (dd, 2.3, 11.0)
15	21.7	1.27 (s)			
16	18.6	1.29 (s)			
17	11.6	1.57 (s)			
18	147.3				
19	113.1	4.71 (br s) 4.92 (br s)			
20	24.8	1.72 (s)			

Fig. 1. ^1H - ^1H COSY and HMBC Correlations of **1**Fig. 2. NOE Correlations of **1**

The relative stereochemistry of **1** was characterized by a nuclear Overhauser and exchange spectroscopy (NOESY) experiment, which showed nuclear Overhauser effect (NOE) correlations between the following proton pairs (2-H and 16-H₃; 3-H and 15-H₃; 7-H and 15-H₃; 9-H and 15-H₃; 11-H and 20-H₃; 12-H and 15-H₃; 16-H₃ and 17-H₃; 16-H₃ and 20-H₃) (Fig. 2). The NOESY spectrum revealed that 3-H, 7-H, 9-H, 12-H, and 15-H₃ were β -oriented, while H-2, 11-H, 16-H₃, 17-H₃, and the isopropenyl group linked to 12-C were α -oriented, and 7-C—8-C double bond had *trans*-geometry (*E*-configuration). Therefore, the structure of **1** was elucidated to be (1*R**,2*S**,3*S**,4*R**,7*E*,9*S**,11*R**,12*S**)-3,4-epoxy-dolabella-7,18-diene-2,9-diol 2-*O*- α -L-arabinopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranoside.

The molecular formula of squarroside V (**2**) was determined as C₄₂H₇₀O₁₂ by the HR-ESI-MS showing a [C₄₂H₇₀O₁₂Na]⁺ ion at *m/z* 789.4744. The ^1H -NMR (pyridine-*d*₅) spectrum contained signals for a cyclopropane methylene proton at δ_{H} 0.23 (1H, d, *J*=4.0 Hz) and 0.48 (1H, d, *J*=4.0 Hz), four quaternary methyl protons at δ_{H} 0.88, 1.03, 1.03, and 1.31, an olefinic methyl proton at δ 1.99, two secondary methyl protons at δ_{H} 1.15 (d, *J*=6.9 Hz) and 1.61 (d, *J*=6.3 Hz), two anomeric protons at δ_{H} 4.72 (d, *J*=7.5 Hz) and 5.16 (d, *J*=7.5 Hz), and an olefinic proton at δ_{H} 5.65 (1H, t, *J*=7.5 Hz). The ^1H -NMR spectrum of **2** was similar to that of squarroside I.¹⁾ The molecular formula of **2** was lower by C₆H₁₀O₄ than that of squarroside I. In the ^{13}C -NMR (pyridine-*d*₅) spectrum of **2**, the chemical shifts showed coincidence with those of squarroside I, with the exception of the signals owing to the sugar moiety. On acid hydrolysis, **2** afforded D-fucose and D-glucose, together with several unidentified artificial sapogenols. The anomeric centers of the D-fucose and D-glucose units were determined to be both β -configurations from the $^3J_{\text{H1-H2}}$ value. In the HMBC experiment, long-range correlations were observed between the anomeric proton (δ_{H} 4.72) of 4-substituted D-fucose unit and the 3-C (δ_{C} 88.6), and the anomeric proton (δ_{H} 5.16) of terminal D-glucose unit and the 4'-C (δ_{C} 83.6) of 4-substituted D-fucose unit. These data proved the sugar sequence as 3-*O*- β -D-glcopyranosyl-(1 \rightarrow 4)- β -D-fucopyranosyl. From the above evidence, the structure of **2** was elucidated as a cycloartan glycoside with a rhamnose unit absent from squarroside I.

The molecular formula of squarroside VI (**3**) was higher by C₆H₁₀O₅ than that of **2**. A comparative study of the ^1H -NMR (pyridine-*d*₅) spectrum of **3** with that of **2** revealed them to be identical except for appearance of the new additional anomeric proton at δ_{H} 5.08 (d, *J*=8.1 Hz). Meanwhile, a detailed comparison of the ^{13}C -NMR (pyridine-*d*₅) spectrum of **3** with that of **2** showed the signal due to 6''-C of glucose unit, which was shifted remarkably downfield by 7.5 ppm, and additional six carbon signals (δ_{C} 105.4, 78.2, 78.0, 75.0, 71.2, 62.5). On acid hydrolysis, **3** afforded D-fucose and D-glucose. The foregoing evidence indicated the presence of an additional glucose unit, which was linked to the hydroxy group at 6''-C of the glucose unit, in **3**. The anomeric proton signals at δ_{H} 5.08 (1''-H) showed long-range correlation with carbon signals at δ_{C} 70.1 (6''-C) in the HMBC experiment. Consequently, these data proved the sugar sequence as 3-*O*- β -D-glcopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-fucopyranosyl.

The HR-ESI-MS of squarroside VII (**4**) showed a peak at 1081.5912 corresponding to the molecular formula [C₅₄H₉₀O₂₀Na]⁺ (Calcd for 1081.5923). The ^1H -NMR (pyridine-*d*₅) spectrum of **4** was similar to that of thalictoside C.⁴⁾ In the ^{13}C -NMR (pyridine-*d*₅) spectrum of **4**, the chemical shifts showed coincidence with those of thalictoside C except for the signals owing to the terminal glucose unit, which was linked to the hydroxy group at 6-C of the inner glucose unit. On acid hydrolysis, **4** afforded D-fucose, D-glucose, D-quinovose, and L-rhamnose. The anomeric center of the D-quinovose unit was determined to be β -configuration from the $^3J_{\text{H1-H2}}$ value. These data indicated the replacement of D-quinovose instead of D-glucose attached to the hydroxy group at 6-C of the inner glucose unit in thalictoside C. In the

Table 2. ¹H- and ¹³C-NMR Data for **2**, **3**, and **4** in Pyridine-*d*₅

	2		3		4	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	32.1		32.1		32.2	
2	29.9		29.9		29.9	
3	88.6	3.45 (dd, 4.6, 12.1)	88.5	3.44 (dd, 4.6, 12.0)	89.2	3.42 (dd, 4.0, 12.0)
4	41.2		41.2		41.2	
5	47.9		47.9		47.9	
6	21.0		21.1		21.1	
7	26.1		26.1		26.1	
8	47.5		47.6		47.6	
9	20.0		19.7		20.0	
10	26.2		26.2		26.2	
11	26.6		26.6		26.2	
12	33.3		33.3		33.3	
13	45.3		45.4		45.4	
14	49.0		49.0		49.0	
15	35.7		35.7		35.8	
16	27.9		27.9		27.9	
17	49.0		49.0		49.0	
18	18.2	1.03 (s)	18.2	1.03 (s)	18.2	1.01 (s)
19	29.6	0.23 (d, 4.0) 0.48 (d, 4.0)	29.6	0.24 (d, 4.1) 0.48 (d, 4.1)	29.6	0.20 (d, 4.1) 0.45 (d, 4.1)
20	41.5		41.5		41.5	
21	12.0	1.15 (d, 6.9)	12.0	1.15 (d, 6.3)	12.0	1.15 (s)
22	72.6	4.02 (br s)	72.7	4.03 ^{a)}	72.7	4.02 ^{a)}
23	34.6		34.6		34.6	
24	125.1	5.65 (t, 7.5)	125.1	5.65 (dd, 6.9, 7.5)	125.2	5.65 (dd, 6.9, 7.5)
25	137.4		137.4		137.4	
26	60.9	4.45 (d, 12.1) 4.52 (d, 12.1)	60.9	4.45 (d, 12.1) 4.52 (d, 12.1)	60.9	4.45 (d, 12.1) 4.52 (d, 12.1)
27	22.1	1.99 (s)	22.1	2.00 (s)	22.1	2.00 (s)
28	19.5	0.88 (s)	19.5	0.89 (s)	19.5	0.91 (s)
29	25.7	1.31 (s)	25.7	1.31 (s)	25.7	1.29 (s)
30	15.3	1.03 (s)	15.3	1.02 (s)	15.3	1.00 (s)
		fuc		fuc		fuc
1'	106.6	4.72 (d, 7.5)	106.7	4.69 (d, 7.5)	107.1	4.63 (d, 7.5)
2'	73.1	4.33 (dd, 7.5, 9.2)	73.2	4.32 (dd, 7.5, 9.2)	72.7	4.44 (dd, 7.5, 9.2)
3'	75.4	4.09 (dd, 3.5, 9.2)	75.5	4.03 (dd, 3.2, 9.2)	75.5	4.03 (dd, 3.1, 9.2)
4'	83.0	4.12 (dd, 1.1, 3.5)	82.6	4.09 (dd, 1.2, 3.5)	78.1	4.36 (br d, 2.9)
5'	70.3	3.78 (dq, 1.1, 6.3)	70.3	3.74 (dq, 1.2, 6.3)	70.5	3.73 ^{a)}
6'	17.6	1.61 (d, 6.3)	17.8	1.70 (d, 6.3)	18.0	1.65 (d, 6.3)
		glc		glc		glc
1''	106.8	5.16 (d, 7.5)	106.2	5.14 (d, 8.0)	103.2	5.43 (d, 7.4)
2''	75.9	4.00 (dd, 7.5, 8.6)	75.6	3.93 (dd, 8.0, 8.6)	78.4	4.10 ^{a)}
3''	78.2	4.19 (t, 8.6)	78.2	4.11 (t, 8.6)	78.5	4.09 (t, 8.6)
4''	71.4	4.16 (t, 8.6)	71.5	4.07 (t, 8.6)	72.0	3.99 (t, 8.6)
5''	78.4	3.89 (m)	77.3	3.99 (m)	76.7	3.96 (m)
6''	62.6	4.31 (dd, 5.2, 11.5) 4.47 (dd, 2.3, 11.5)	70.1	4.29 (dd, 5.7, 11.5) 4.74 (dd, 1.2, 11.5)	70.1	4.19 (dd, 6.3, 11.5) 4.74 (dd, 1.2, 11.5)
				glc		qui
1'''			105.4	5.08 (d, 8.1)	105.1	4.96 (d, 7.4)
2'''			75.0	4.04 (dd, 8.1, 8.6)	75.1	4.01 (dd, 7.4, 8.6)
3'''			78.0	4.20 (t, 8.6)	77.8	4.12 (t, 8.6)
4'''			71.2	4.17 (t, 8.6)	76.5	3.69 (t, 8.6)
5'''			78.2	3.89 (m)	72.9	3.73 ^{a)}
6'''			62.5	4.30 (dd, 5.7, 11.5) 4.47 (dd, 2.3, 11.5)	18.7	1.58 (d, 5.7)
						rha
1''''					101.9	6.24 (d, 1.8)
2''''					71.8	4.68 (dd, 1.8, 3.5)
3''''					72.3	4.64 (dd, 3.5, 9.2)
4''''					74.1	4.21 (t, 9.2)
5''''					70.1	4.84 (m)
6''''					18.6	1.72 (d, 6.3)

a) Submerged by other signals.

HMBC experiment, the anomeric proton signals at δ_{H} 4.63 (fuc 1'-H), 4.96 (qui 1''-H), 5.43 (glc 1''-H), and 6.24 (rha 1'''-H) showed long-range correlations with the carbon signals at δ_{C} 89.2 (3-C), 70.1 (glc 6''-C), 78.1 (fuc 4'-C), and 78.4 (glc 2''-C), respectively. Thus, sugar sequence determined as 3-*O*- β -D-quinovopyranosyl-(1 \rightarrow 6)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-fucopyranosyl.

At the present time, the absolute configuration of squoside A (**1**) except for the sugar moiety has not yet been established. Although a number of dolabellane diterpenoids have been isolated from marine sources, this was the second report of dolabellane diterpene glycoside isolation from a higher plant.^{5,6)}

Meanwhile, in spite of the same ¹H-NMR coupling pattern in D-fucopyranose units of **3** and **4**, the ¹³C signal due to fuc 4'-C (δ_{C} 78.1) in **4** was shifted to upfield by 4.5 ppm, as compared with fuc 4'-C (δ_{C} 82.6) in **3**. This upfield shift suggested to be caused by L-rhamnopyranosyl group attached to glc 2''-C. The strange shift pattern was observed in thalicoside C.⁴⁾

Experimental

General Procedure Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. Both ¹H- and ¹³C-NMR spectra were recorded in methanol-*d*₄ and pyridine-*d*₅ solutions on a JEOL ECA 500 (500, 125 MHz, respectively) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) and *J* values in Hz, using pyridine-*d*₅ for ¹H-NMR (7.20 ppm) and ¹³C-NMR (123.5 ppm), and methanol-*d*₄ for ¹H-NMR (3.30 ppm) and ¹³C-NMR (49.0 ppm) as an internal standard. The HR-ESI-MS was recorded with a JEOL JMS-T100LP spectrometer. HPLC was carried out using the Mightysil RP-18 (10.0 mm i.d. \times 250 mm, Kanto Chemical Co., Ltd., Tokyo, Japan) column with a Tosoh CCPM pump, Tosoh RI-8010 detector, and JASCO OR-2090 detector. TLC was performed on pre-coated silica gel 60 F₂₅₄ (Merck Ltd., Tokyo, Japan), and detection was achieved by spraying with 10% H₂SO₄ followed by heating. Column chromatography was carried out on silica gel 60 (230–400 mesh, Merck Ltd., Tokyo, Japan), Sephadex-LH20 (Pharmacia Fine Chemicals), ODS (Bondapak C₁₈ Cartridge 15–20 mm, Waters Corp.), and MCI gel CHP20P (Mitsubishi Chemical Ind.).

Plant Material The fresh aerial parts of *Thalictrum squarrosom* STEPH. were collected in Heilungkiang province of China. A voucher specimen was deposited in the Herbarium of Kumamoto University.

Extraction and Isolation The dried aerial parts of *Thalictrum squarrosom* (4.1 kg) was extracted with hot methanol. The MeOH extract (511 g) was partitioned between water-soluble (402 g) and benzene-soluble (109 g) portions. The water-soluble portion subjected to MCI gel CHP20P [MeOH–H₂O (30:70 \rightarrow 40:60 \rightarrow 50:50 \rightarrow 60:40 \rightarrow 70:30 \rightarrow 80:20 \rightarrow 90:10, v/v) \rightarrow MeOH] column chromatography to afford four fractions [Fractions 1 (14.5 g), 2 (6.5 g), 3 (7.3 g), and 4 (2.7 g)]. Fraction 1 (14.5 g) was further separated by ODS [MeOH–H₂O (40:60 \rightarrow 50:50 \rightarrow 60:40, v/v)] and silica gel [CHCl₃–MeOH–H₂O (8:2:0.2, v/v/v)] column chromatographies, followed by HPLC [MeOH–H₂O (60:40, v/v)] to furnish squoside A (**1**,

14 mg). Fraction 4 (2.7 g) subjected to ODS [MeOH–H₂O (70:30 \rightarrow 80:20 \rightarrow 90:10, v/v)] column chromatography to afford five fractions [Fractions 5 (393 mg), 6 (165 mg), 7 (124 mg), 8 (187 mg), and 9 (129 mg)]. Fraction 6 was further separated by Sephadex-LH20 [MeOH] and silica gel [CHCl₃–MeOH–H₂O (7:3:0.5, v/v/v)] column chromatographies, followed by HPLC [MeOH–H₂O (85:15, v/v)] to furnish squarrosides VII (**4**, 19 mg). Fraction 7 was further separated by silica gel [CHCl₃–MeOH–H₂O (7:3:0.5, v/v/v)] column chromatography, followed by HPLC [MeOH–H₂O (85:15, v/v)] to furnish squarrosides VI (**3**, 10 mg). Fraction 8 was further separated by Sephadex-LH20 [MeOH] and silica gel [CHCl₃–MeOH–H₂O (7:3:0.5, v/v/v)] column chromatographies, followed by HPLC [MeOH–H₂O (85:15, v/v)] to furnish squarrosides V (**2**, 8 mg).

Squoside A (**1**): Amorphous powder; [α]_D –27.4° (*c*=0.71, MeOH); HR-ESI-MS *m/z* 621.3238 [M+Na]⁺; (Calcd for C₃₁H₅₀O₁₁Na, 621.3251); ¹H- and ¹³C-NMR data (Table 1).

Squarroside V (**2**): Amorphous powder; [α]_D +17.0° (*c*=0.38, MeOH); HR-ESI-MS *m/z* 789.4744 [M+Na]⁺; (Calcd for C₄₂H₇₀O₁₂Na, 789.4765); ¹H- and ¹³C-NMR data (Table 2).

Squarroside VI (**3**): Amorphous powder; [α]_D +5.3° (*c*=0.50, MeOH); HR-ESI-MS *m/z* 951.5271 [M+Na]⁺; (Calcd for C₄₈H₈₀O₁₇Na, 951.5293); ¹H- and ¹³C-NMR data (Table 2).

Squarroside VII (**4**): Amorphous powder; [α]_D –17.9° (*c*=0.94, MeOH); HR-ESI-MS *m/z* 1081.5912 [M+Na]⁺; (Calcd for C₅₄H₉₀O₂₀Na, 1081.5923); ¹H- and ¹³C-NMR data (Table 2).

Sugar Analysis A solution of each compound (**1**, **2**, **3**, or **4**) (1.0 mg) in 2 M HCl/dioxane (1:1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H₂O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C₁₈ cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH₃CN/H₂O (3:1, 250 μ l). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pak DC-613 (6.0 mm i.d. \times 150 mm, Showa Denko, Tokyo, Japan); solvent, CH₃CN/H₂O (3:1); flow rate, 1.0 ml/min; column temperature, 70 °C; detection, optical rotation (OR). The *t*_R (min) of the sugars was as follows. **1**: L-rhamnose 4.7 (–), L-arabinose 6.2 (+), **2**: D-fucose 4.6 (+), D-glucose 7.2 (+), **3**: D-fucose 4.6 (+), D-glucose 7.2 (+), **4**: D-fucose 4.6 (+), D-quinovose 4.9 (+), D-glucose 7.2 (+). [Reference: D-fucose 4.6 (positive optical rotation: +), L-rhamnose 4.7 (negative optical rotation: –), D-quinovose 4.9 (positive optical rotation: +), L-arabinose 6.2 (positive optical rotation: +), D-glucose 7.4 (positive optical rotation: +)].

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