Four New Triterpene Glycosides from *Thalictrum squarrosum*

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Four new triterpene glycosides were isolated from the dried aerial parts of *Thalictrum squarrosum* (Ranunculaceae). They were designated as squarroside I, being a cycloartane-type glycoside, and squarrosides II, III and IV, being oleanene-type glycosides. Their structures were established by using two dimensional (2D) NMR techniques.

Key words *Thalictrum squarrosum*; squarroside; Ranunculaceae; cycloartane glycoside; oleanene glycoside

Thalictrum squarrosum 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.

As regards its constituents, cycloartane-type glycosides named squarrosides A1, A2, B1, B2, B3 and B4 have been isolated.[1)] As part of our continuing investigation on the chemical constituents in the genus *Thalictrum* plants,[2)] this paper deals with structural elucidation of four new triterpene glycosides, named squarrosides I (**1**), II (**2**), III (**3**) and IV (**4**).

The methanolic extract of the dried aerial parts of *Thalictrum squarrosum* STEPH. collected in Heilungkiang province of China was partitioned into a benzene–water solvent system. A styrene polymer MCI gel CHP20P column chromatography of the water soluble portion provided the glycosidic constituents, which were purified by using a combination of silica gel and octadecyl silica gel (ODS) column chromatography to furnish four new glycosides, designated squarrosides I (**1**), II (**2**), III (**3**) and IV (**4**), together with a known cycloartane-type glycoside, named thalictoside C (**5**).[2a)]

Squarroside I (**1**) was obtained as a white powder, $[\alpha]_D$ −2.7° (MeOH), and displayed a peak at *m/z* 911 $[M-H]^-$ in the neg. FAB-MS. The ^{1}H-NMR spectrum of **1** showed a couple of doublet signals at δ 0.24 and 0.55, which is characteristic of a cyclopropane methylene, four tertiary methyls at δ 0.91, 1.03, 1.23 and 1.29, an olefinic methyl at δ 2.01, three secondary methyls at δ 1.15 (d, J=6.7 Hz), 1.60 (d, J=6.1 Hz) and 1.63 (d, J=6.1 Hz), an olefinic proton at δ 5.66 (1H, dd, J=6.7, 7.3 Hz) and three anomeric protons at δ 4.70 (1H, d, J=7.9 Hz), 5.06 (1H, d, J=7.9 Hz) and 6.37 (1H, br s). The above ^{1}H-NMR data of **1** were similar to those of thalictoside C (**5**). In the ^{13}C-NMR spectrum of **1**, the signals due to the aglycone moiety were in good agreement with those of **5**, although the signals due to the sugar moiety were not identical. Furthermore, a nuclear Overhauser effect (NOE) was observed between the olefinic methyl proton at δ 2.01 and the olefinic proton at δ 5.66. On acid hydrolysis, **1** afforded D-glucose, D-fucose and L-rhamnose, together with several unidentified artificial sapogenols.[3)] The ^{1}H- and ^{13}C-NMR spectrum of **1**, which could be assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple quantum coherence (HMQC) techniques, showed that the signals due to the trisaccharide moiety consisted of one glucopyranosyl moiety, one fucopyranosyl moiety and one rhamnopyranosyl moiety. In the heteronuclear multiple bond correlation spectroscopy (HMBC) experiment, the anomeric proton signals at δ 4.70 (2,4-di-substituted fucosyl moiety), 5.06 (terminal glucosyl moiety) and 6.37 (terminal rhamnosyl moiety) showed long-range correlations with the ^{13}C signals at δ 88.4 (C-3 of aglycone), 84.8 (C-4 of 2,4-di-substituted fucosyl moiety) and 76.5 (C-2 of 2,4-di-substituted fucosyl moiety), respectively. From the above evidence, the structure of **1** was concluded to be (22*S*,24*Z*)-cycloart-24-en-3β,22,26-triol 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-fucopyranoside.

Squarroside II (**2**) was obtained as a white powder, $[\alpha]_D$ −4.8° (MeOH), and exhibited a $[M-H]^-$ peak at *m/z* 1057 in the neg. FAB-MS. The ^{1}H-NMR spectrum of **2** displayed seven tertiary methyls at δ 0.87, 0.89, 0.92, 1.08, 1.08, 1.16 and 1.25, an olefinic proton at δ 5.43 (1H, br s) and four anomeric protons at δ 4.76 (1H, d, J=6.1 Hz), 5.12 (1H, d, J=7.9 Hz), 6.12 (1H, br s) and 6.24 (1H, d, J=7.9 Hz). The ^{13}C-NMR spectrum revealed the presence of six quaternary carbon signals at δ 30.8, 37.0, 39.5, 39.9, 42.1 and 47.0, a set of olefinic carbon signals at δ 122.9 and 144.1, one ester carbonyl carbon signal at δ 176.4 and four anomeric carbon signals at δ 95.7, 101.7, 104.9 and 106.3. These spectral data indicated that **2** was the 3,28-bisdesmoside of oleanolic acid (**6**),[4)] having four monosaccharide units. On acid hydrolysis, **2** afforded oleanolic acid, D-glucose, D-xylose and L-rhamnose.[3)] The ^{1}H- and ^{13}C-NMR spectrum of the sugar moiety were assigned by the ^{1}H–^{1}H COSY and HMQC techniques. In the HMBC experiment, long-range correlations were observed between the anomeric proton [δ 4.76 (d, J=6.1 Hz)] of 2,4-di-substituted xylosyl moiety and the C-3 (δ 88.7) of aglycone, the anomeric proton [δ 5.12 (d, J=7.9 Hz) H-1′] of terminal glucosyl moiety and the C-4 (δ 79.4) of 2,4-di-substituted xylosyl moiety, the anomeric proton [δ 6.12 (br s)] of terminal rhamnosyl moiety and the C-2 (δ 76.4) of 2,4-di-substituted xylosyl moiety and the anomeric proton [δ 6.24 (d, J=7.9 Hz) H-1″] of terminal glucosyl moiety and the C-28 (δ 176.4) of aglycone. Based on the above evidence, the structure of **2** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-xylopyranosyl oleanolic acid 28-β-D-glucopyranosyl ester.

Squarroside III (**3**) was obtained as a white powder, $[\alpha]_D$ −14.1° (MeOH), and exhibited a $[M-H]^-$ peak at *m/z* 1219 in the neg. FAB-MS. The ^{1}H-NMR spectrum of **3** displayed seven tertiary methyls at δ 0.89, 0.89, 0.89, 1.09, 1.09, 1.16

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and 1.25, an olefinic proton at δ 5.42 (1H, br s) and five anomeric protons at δ 4.76 (1H, d, J=6.1 Hz), 5.01 (1H, d, J=7.9 Hz), 5.12 (1H, d, J=7.9 Hz), 6.12 (1H, br s) and 6.24 (1H, d, J=7.9 Hz). The above ^{1}H-NMR data of **3** were similar to those of **2** except for the new additional anomeric proton at δ 5.01. In the ^{13}C-NMR spectrum of **3**, the signals due to the aglycone moiety were in good agreement with those of **2**, although the signals due to the sugar moiety were not identical. On acid hydrolysis, **3** afforded oleanolic acid, D-glucose, D-xylose and L-rhamnose.[3] Meanwhile, the neg. FAB-MS of **3** gave a quasi-molecular peak at *m/z* 1219, which was higher by 162 mass units than that of **2**. Furthermore, a comparative study of the ^{13}C-NMR spectrum of **3** with that of **2** indicated the presence of an additional glucosyl unit in **3**, which was linked to the C-6 hydroxy group of the ester linked glucopyranosyl moiety according to the glycosylation shifts.[5] The ^{1}H- and ^{13}C-NMR spectrum of **3**, which could be assigned with the aid of ^{1}H–^{1}H COSY, HMQC and total correlation spectroscopy (TOCSY) techniques, showed that the signals due to the pentasaccharide moiety consisted of three glucopyranosyl moieties, one xylopyranosyl moiety and one rhamnopyranosyl moiety. In the HMBC experiment, long-range correlations were observed between the anomeric proton [δ 4.76 (d, J=6.1 Hz)] of 2,4-di-substituted xylosyl moiety and the C-3 (δ 88.7) of aglycone, the anomeric proton [δ 5.01 (d, J=7.9 Hz) H-1‴] of terminal glucosyl moiety and the C-6 (δ 69.4) of 6-substituted glucosyl moiety, the anomeric proton [δ 5.12 (d, J=7.9 Hz) H-1′] of terminal glucosyl moiety and the C-4 (δ 79.5) of 2,4-di-substituted xylosyl moiety, the anomeric proton [δ 6.12 (br s)] of terminal rhamnosyl moiety and the C-2 (δ 76.4) of 2,4-di-substituted xylosyl moiety and the anomeric proton [δ 6.24 (d, J=7.9 Hz) H-1″] of 6-substituted glucosyl moiety and the C-28 (δ 176.5) of aglycone. Based on the above data, **3** was formulated as 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-xylopyranosyl oleanolic acid 28-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Squarroside IV (**4**) was obtained as a white powder, $[\alpha]_D$ −4.2° (MeOH), and showed the same ion peak at *m/z* 1219 due to $[M-H]^-$ as that of **3** in the neg. FAB-MS. The ^{1}H-NMR spectrum of **4** displayed seven tertiary methyls at δ 0.87, 0.90, 0.92, 1.10, 1.15, 1.27 and 1.33, an olefinic proton at δ 5.44 (1H, br s) and five anomeric protons at δ 4.71 (1H, d, J=6.7 Hz), 5.13 (1H, d, J=7.9 Hz), 5.45 (1H, d, J=7.9 Hz), 6.14 (1H, br s) and 6.33 (1H, d, J=7.9 Hz). The above ^{1}H-NMR data of **4** were similar to those of **2** except for the new additional anomeric proton at δ 5.45. In the ^{13}C-NMR spectrum of **4**, the signals due to the aglycone moiety were in good agreement with those of **3**, although the signals due to the sugar moiety were not identical. On acid hydrolysis, **4** afforded oleanolic acid, D-glucose, D-xylose and L-rhamnose.[3] Furthermore, a comparative study of the ^{13}C-NMR spectrum of **4** with that of **2** indicated the presence of an additional glucosyl unit in **4**, which was linked to the trisaccharide moiety. The ^{1}H- and ^{13}C-NMR spectrum of **4**, which could be assigned by using 2D NMR techniques (^{1}H–^{1}H COSY, HMQC, TOCSY), showed that the signals due to the pentasaccharide moiety consisted of three glucopyranosyl moieties, one xylopyranosyl moiety and one rhamnopyranosyl moiety. In the HMBC experiment, long-range correlations were observed between the anomeric proton [δ 4.71 (d, J=6.7 Hz)] of 2,4-di-substituted xylosyl moiety and the C-3 (δ 88.7) of aglycone, the anomeric proton [δ 5.13 (d, J=7.9 Hz) H-1′] of terminal glucosyl moiety and the C-4 (δ 80.1) of 2,4-di-substituted xylosyl moiety, the anomeric proton [δ 5.45 (d, J=7.9 Hz) H-1‴] of terminal glucosyl moiety and the C-3 (δ 83.2) of 3-substituted rhamnosyl moiety, the anomeric proton [δ 6.14 (br s)] of 3-substituted rhamnosyl moiety and the C-2 (δ 76.2) of 2,4-di-substituted xylosyl moiety and the anomeric proton [δ 6.33 (d, J=7.9 Hz) H-1″] of terminal glucosyl moiety and the C-28 (δ 176.4) of aglycone. Consequently, the structure of **4** was determined to be 3-*O*-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)]-β-D-xylopyranosyl oleanolic acid 28-β-D-glucopyranosyl ester.

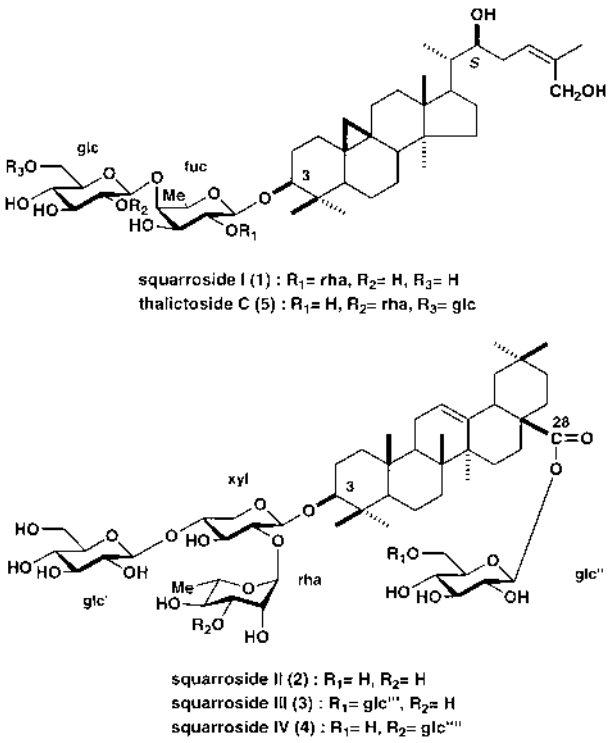

Fig. 1

Experimental

Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL alpha 500 NMR spectrometer and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard. The FAB-MS was measured with a JEOL DX-303 HF spectrometer and taken in a 3-nitrobenzyl alcohol matrix. HPLC was carried out using a TSK gel-120A (7.8 mm i.d.×30 cm) column with a Tosoh CCPM pump and Tosoh RI-8010 differential refractometer as detector. TLC was performed on pre-coated Kiesel gel 60 F_{254} (Merck) and detection was achieved by spraying with 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (230—400 mesh, Merck), ODS (PrePAK-500/C_{18}, Waters) and MCI gel CHP20P (Mitsubishi Chemical Ind.)

Extraction and Separation The fresh aerial parts of *Thalictrum squarrosum* STEPH. were collected in Heilungkiang province of China. The dried aerial parts (4.1 kg) of this plant were extracted with MeOH under reflux, and the extract (511 g) was partitioned in benzene and water (1 : 1). The water soluble portion (402 g) was subjected to MCI gel CHP-20P column chromatography with MeOH–H_2O (30→40→50→60→70→80→90%) to afford nine fractions (fr. 1—fr. 9). Fraction 7 (6.5 g) was further separated by ODS column chromatography with MeOH–H_2O (40→50→60→70→80→90%) to afford four fractions (fr. 10—fr. 13). Fraction 11 (533 mg) was

Table 1. ^{13}C-NMR Data for **1** and **5** (Pyridine-d_5)

	1	**5**		**1**	**5**
C- 1	32.3	32.0	3-*O*-		
C- 2	30.0	29.7	Fuc-1	105.1	107.0
C- 3	88.4	89.1	Fuc-2	76.5	74.9
C- 4	41.2	41.1	Fuc-3	77.1	75.6
C- 5	47.9	47.5	Fuc-4	84.8	77.8
C- 6	21.1	20.6	Fuc-5	70.3	72.7
C- 7	26.1	27.8	Fuc-6	17.4	17.8
C- 8	47.7	47.8	Rha-1	101.9	101.7
C- 9	19.9	19.8	Rha-2	72.3	72.1
C-10	26.3	26.1	Rha-3	72.4	72.3
C-11	26.7	26.0	Rha-4	74.1	74.1
C-12	33.4	35.6	Rha-5	69.5	70.4
C-13	45.4	45.2	Rha-6	18.7	18.5
C-14	49.1	48.9	Glc-1	107.0	102.7
C-15	35.8	33.2	Glc-2	75.7	78.5
C-16	28.0	26.5	Glc-3	78.7	78.2
C-17	49.0	48.9	Glc-4	71.5	71.4
C-18	18.2	19.4	Glc-5	78.5	76.6
C-19	29.6	29.5	Glc-6	62.8	69.8
C-20	41.7	41.4	Glc-1		105.3
C-21	12.1	11.9	Glc-2		75.6
C-22	72.7	72.6	Glc-3		78.2
C-23	34.8	34.6	Glc-4		71.7
C-24	125.2	124.9	Glc-5		78.2
C-25	137.7	137.4	Glc-6		62.5
C-26	61.1	60.8			
C-27	22.7	22.0			
C-28	19.5	18.1			
C-29	25.6	25.6			
C-30	15.5	15.1			

separated by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (6 : 4 : 1), followed by HPLC with MeOH–H_2O (7 : 3), to furnish squarroside III (**3**) (34 mg). Fraction 12 (814 mg) was separated by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (12 : 8 : 1), followed by HPLC with MeOH–H_2O (7 : 3), to furnish squarroside II (**2**) (311 mg). Fraction 13 (1.6 g) was separated by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (6 : 4 : 1), followed by HPLC with MeOH–H_2O (7 : 3), to furnish squarroside IV (**4**) (655 mg). Fraction 9 (2.7 g) was further separated by ODS column chromatography with MeOH–H_2O (40→50→60→70→80→90%) to afford four fractions (fr. 14—fr. 17). Fraction 15 (393 mg) was separated by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (12 : 8 : 1), followed by HPLC with MeOH–H_2O (4 : 1), to furnish thalictoside C (**5**) (135 mg). Fraction 16 (165 mg) was separated by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (14 : 6 : 1), followed by HPLC with MeOH–H_2O (4 : 1), to furnish squarroside I (**1**) (43 mg).

Squarroside I (**1**): A white powder, $[\alpha]_D^{25}$ −2.7° (c=0.90, MeOH). Neg. FAB-MS (*m/z*): 911 [M−H]$^-$, 749 [M−H−hexose]$^-$. ^{1}H-NMR (pyridine-d_5): δ 0.24, 0.55 (each 1H, d, J=3.7 Hz, H2-19), 0.91 (3H, s, H_3-28), 1.03 (3H, s, H_3-18), 1.15 (3H, d, J=6.7 Hz, H_3-21), 1.23 (3H, s, H_3-30), 1.29 (3H, s, H_3-29), 2.01 (3H, s, H_3-27), 3.38 (1H, dd, J=4.3, 11.6 Hz, H-3), 4.01 (1H, br s, H-22), 5.66 (1H, dd, J=6.7, 7.3 Hz, H-24); fuc-1 to fuc-6, 4.70 (1H, d, J=7.9 Hz), 4.46 (1H, overlapped), 4.10 (1H, br d, J=9.2 Hz), 3.98 (1H, overlapped), 3.74 (1H, m), 1.60 (3H, d, J=6.1 Hz); rha-1 to rha-6, 6.37 (1H, br s), 4.78 (1H, br d, J=3.7 Hz), 4.62 (1H, dd, J=3.7, 9.2 Hz), 4.27 (1H, dd, J=9.8, 9.2 Hz), 4.75 (1H, m), 1.63 (3H, d, J=6.1 Hz); glc-1 to glc-6, 5.06 (1H, d, J=7.9 Hz), 3.98 (1H, dd, J=7.9, 9.2 Hz), 4.14 (1H, dd, J=9.2, 8.6 Hz), 4.18 (1H, dd, J=8.6, 8.6 Hz), 4.00 (1H, m), 4.33 (1H, dd, J=4.9, 11.6 Hz), 4.48 (1H, overlapped). ^{13}C-NMR (pyridine-d_5): Table 1.

Squarroside II (**2**): A white powder, $[\alpha]_D^{25}$ −4.8° (c=0.90, MeOH). Neg. FAB-MS (*m/z*): 1057 [M−H]$^-$, 895 [M−H−hexose]$^-$, 733 [M−H−hexose−hexose]$^-$, 587 [M−H−hexose−hexose−deoxyhexose]$^-$, 455 [M−H−hexose−hexose−deoxyhexose−pentose]$^-$. ^{1}H-NMR (pyridine-d_5): δ 0.87(3H, s, H_3-25), 0.89 (3H, s, H_3-30), 0.92 (3H, s, H_3-29), 1.08 (3H, s, H_3-24), 1.08 (3H, s, H_3-26), 1.16 (3H, s, H_3-23), 1.25 (3H, s, H_3-27), 3.19 (1H, dd, J=11.4, 4.6 Hz, H-3), 5.43 (1H, br s, H-12); xyl-1 to xyl-5, 4.76 (1H, d, J=6.1 Hz), 4.47 (1H, overlapped), 4.25 (1H, overlapped), 4.27 (1H, m), 3.80 (1H, br d, J=10.4 Hz), 4.18 (1H, overlapped); rha-1 to rha-6, 6.12 (1H, br s), 4.70 (1H, br s), 4.57 (1H, br d, J=9.2 Hz), 4.27 (1H, dd, J=9.2, 9.2 Hz), 4.59 (1H, m), 1.63 (3H, d, J=6.1 Hz); glc-1′ to glc-6′, 5.12 (1H, d, J=7.9 Hz), 4.10 (1H, dd, J=7.9, 8.6 Hz), 4.19 (1H, overlapped), 4.21 (1H, overlapped), 3.89 (1H, m), 4.37 (1H, overlapped), 4.48 (1H, overlapped); glc-1″ to glc-6″, 6.24 (1H, d, J=7.9 Hz), 4.18 (1H, dd, J=7.9, 8.5 Hz), 4.34 (1H, overlapped), 4.37 (1H, overlapped), 4.01 (1H, m), 4.37 (1H, overlapped), 4.48 (1H, overlapped). ^{13}C-NMR (pyridine-d_5): Table 2.

Squarroside III (**3**): A white powder, $[\alpha]_D^{25}$ −14.1° (c=0.55, MeOH). Neg. FAB-MS (*m/z*): 1219 [M−H]$^-$, 1057 [M−H−hexose]$^-$, 895 [M−H−hexose−hexose]$^-$, 733 [M−H−hexose−hexose−hexose]$^-$, 587 [M−H−hexose−hexose−hexose−deoxyhexose]$^-$, 455 [M−H−hexose−hexose−hexose−deoxyhexose−pentose]$^-$. ^{1}H-NMR (pyridine-d_5): δ 0.89(3H, s, H_3-25), 0.89 (3H, s, H_3-30), 0.89 (3H, s, H_3-29), 1.09 (3H, s, H_3-24), 1.09 (3H, s, H_3-26), 1.16 (3H, s, H_3-23), 1.25 (3H, s, H_3-27), 3.20 (1H, br d, J=15.2 Hz, H-3), 5.42 (1H, br s, H-12); xyl-1 to xyl-5, 4.76 (1H, d, J=6.1 Hz), 4.47 (1H, overlapped), 4.25 (1H, overlapped), 4.27 (1H, m), 3.80 (1H, br d, J=10.4 Hz), 4.39 (1H, br d, J=11.6 Hz); rha-1 to rha-6, 6.12 (1H, br s), 4.70 (1H, br s), 4.57 (1H, br d, J=9.2 Hz), 4.27 (1H, overlapped), 4.58 (1H, m), 1.63 (3H, d, J=6.1 Hz); glc-1′ to glc-6′, 5.12 (1H, d, J=7.9 Hz), 4.01 (1H, dd, J=7.9, 8.5 Hz), 4.18 (1H, overlapped), 4.17 (1H, overlapped), 3.87 (1H, m), 4.34 (1H, overlapped), 4.50 (1H, br d, J=11.6 Hz); glc-1″ to glc-6″, 6.24 (1H, d, J=7.9 Hz), 4.13 (1H, dd, J=7.9, 8.6 Hz), 4.25 (1H, overlapped), 4.27 (1H, overlapped), 4.10 (1H, m), 4.35 (1H, overlapped), 4.69 (1H, br d, J=9.2 Hz); glc-1‴ to glc-6‴, 5.01 (1H, d, J=7.9 Hz), 3.98 (1H, dd, J=7.9, 8.6 Hz), 4.16 (1H, overlapped), 4.17 (1H, overlapped), 3.88 (1H, m), 4.33 (1H, overlapped), 4.46 (1H, overlapped). ^{13}C-NMR (pyridine-d_5): Table 2.

Squarroside IV (**4**): A white powder, $[\alpha]_D^{25}$ −4.2° (c=0.85, MeOH). Neg. FAB-MS (*m/z*): 1219 [M−H]$^-$, 1057 [M−H−hexose]$^-$, 895 [M−H−hexose−hexose]$^-$, 733 [M−H−hexose−hexose−hexose]$^-$, 587 [M−H−hexose−hexose−hexose−deoxyhexose]$^-$, 455 [M−H−hexose−hexose−hexose−deoxyhexose−pentose]$^-$. ^{1}H-NMR (pyridine-d_5): δ 0.87(3H, s, H_3-25), 0.90 (3H, s, H_3-30), 0.92 (3H, s, H_3-29), 1.10 (3H, s, H_3-26), 1.15 (3H, s, H_3-24), 1.27 (3H, s, H_3-27), 1.33 (3H, s, H_3-23), 3.25 (1H, br d, J=15.8 Hz, H-3), 5.44 (1H, br s, H-12); xyl-1 to xyl-5, 4.71 (1H, d, J=6.7 Hz), 4.42 (1H, dd, J=6.7, 8.6 Hz), 4.18 (1H, overlapped), 4.23 (1H, m), 3.74 (1H, br d, J=11.0 Hz), 4.41 (1H, overlapped); rha-1 to rha-6, 6.14 (1H, br s), 4.97 (1H, br s), 4.81 (1H, dd, J=3.7, 9.2 Hz), 4.48 (1H, overlapped), 4.64 (1H, m), 1.56 (3H, d, J=6.1 Hz); glc-1′ to glc-6′, 5.13 (1H, d, J=7.9 Hz), 4.03 (1H, dd, J=7.9, 9.2 Hz), 4.22 (1H, overlapped), 4.21 (1H, overlapped), 3.90 (1H, m), 4.36 (1H, overlapped), 4.50 (1H, overlapped); glc-1″ to glc-6″, 6.33 (1H, d, J=7.9 Hz), 4.22 (1H, overlapped), 4.29 (1H, dd, J=9.2, 9.2 Hz), 4.36 (1H, overlapped), 4.03 (1H, m), 4.42 (1H, overlapped), 4.48 (1H, overlapped); glc-1⁗ to glc-6⁗, 5.45 (1H, d, J=7.9 Hz), 4.09 (1H, dd, J=7.9, 8.5 Hz), 4.22 (1H, overlapped), 4.21 (1H, overlapped), 3.97 (1H, m), 4.36 (1H, overlapped), 4.50 (1H, overlapped). ^{13}C-NMR (pyridine-d_5): Table 2.

Sugar Analysis of 1, 2, 3 and 4 A solution of each compound (**1**, **2**, **3** or **4**) (2 mg) in 2 N HCl–dioxane (1 : 1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C_{18} cartridge to give the sugar fraction. A solution of the sugar fraction analyzed by TLC [CH_3CN–MeOH–H_2O (6 : 4 : 1), **1**: glucose, *Rf* 0.29; rhamnose, *Rf* 0.54; fucose, *Rf* 0.38; **2**: rhamnose, *Rf* 0.54; xylose, *Rf* 0.40; glucose, *Rf* 0.29; **3**: rhamnose, *Rf* 0.54; xylose, *Rf* 0.40; glucose, Rf 0.29; **4**: rhamnose, *Rf* 0.54; xylose, *Rf* 0.40; glucose, *Rf* 0.29]. A solution of the sugar fraction in 1 ml of H_2O was treated with a solution of L-(−)-α-methylbenzylamine (150 ml) and $NaBH_3CN$ (8 mg) in 1 ml of EtOH, and the mixture was kept at 40 °C for 3 h. Then several drops of acetic acid were added and the mixture was concentrated to dryness. The residue dissolved in Ac_2O–C_5H_5N (1 : 1, 2 ml) was treated with 4-dimethylaminopyridine (DMAP) (20 mg), and the whole mixture was left at room temperature overnight. After removal of excess Ac_2O and C_5H_5N, the residue dissolved in 20% CH_3CN was loaded into a SEP-PAK C_{18} cartridge and eluted with 20% CH_3CN (total 7 ml) and 100% CH_3CN. The fraction eluted with 100% CH_3CN was analyzed by normal-phase HPLC. Conditions of HPLC : column, Develosil 60-3, 3 μm (4.6 mm i.d.×150 mm); solvent, *n*-hexane–EtOH (19 : 1); flow rate, 1.20 ml/min; detection, ultraviolet (UV) (230 nm). t_R (min) of 1-(*N*-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetates were as follows. **1**: L-rhamnose 16.9, D-fucose 17.5, D-glucose 30.8. **2**: L-rhamnose 17.1, D-xylose 30.6, D-glucose 30.6. **3**: L-rhamnose 17.2, D-xylose 30.8, D-glucose 30.8. **4**: L-rhamnose 16.8, D-xylose 30.8, D-glucose 30.8 (reference: L-rhamnose 17.0, D-fucose 17.8, D-xylose 30.7, D-glucose 30.8).

Table 2. ^{13}C-NMR Data for **2**—**4** and **6** (Pyridine-d_5)

	2	**3**	**4**	**6**		**2**	**3**	**4**
C- 1	38.9	38.9	38.9	39.0	3-*O*-			
C- 2	26.6	26.6	26.7	28.1	Xyl-1	104.9	104.9	105.4
C- 3	88.7	88.7	88.7	78.2	Xyl-2	76.4	76.4	76.2
C- 4	39.5	39.5	39.6	39.4	Xyl-3	73.9	73.8	74.4
C- 5	56.0	56.0	56.0	55.9	Xyl-4	79.4	79.5	80.1
C- 6	18.5	18.5	18.5	18.8	Xyl-5	64.4	64.3	65.4
C- 7	32.5	32.6	32.5	33.3	Rha-1	101.7	101.8	101.5
C- 8	39.9	39.9	39.9	39.8	Rha-2	72.2	72.2	71.6
C- 9	48.1	48.1	48.0	48.2	Rha-3	72.5	72.5	83.2
C-10	37.0	37.0	37.0	37.5	Rha-4	74.1	73.9	62.9
C-11	23.8	23.8	23.8	23.8	Rha-5	69.8	69.8	69.8
C-12	122.9	122.9	122.9	122.7	Rha-6	18.6	18.6	18.6
C-13	144.1	144.1	144.1	145.0	Glc-1′	106.3	106.3	106.6
C-14	42.1	42.1	42.1	42.2	Glc-2′	75.4	75.4	75.5
C-15	28.2	28.3	28.2	28.4	Glc-3′	78.8	78.5	78.9
C-16	23.4	23.4	23.4	23.8	Glc-4′	71.3	71.3	71.3
C-17	47.0	47.0	47.0	46.7	Glc-5′	78.7	78.7	78.7
C-18	41.7	41.7	41.7	42.1	Glc-6′	62.5	62.5	62.5
C-19	46.2	46.3	46.2	46.6	Glc-1′′′′			106.9
C-20	30.8	30.8	30.8	31.0	Glc-2′′′′			75.9
C-21	34.0	34.0	34.0	34.3	Glc-3′′′′			78.5
C-22	33.1	33.1	33.1	33.3	Glc-4′′′′			71.5
C-23	28.1	28.1	28.1	28.8	Glc-5′′′′			78.5
C-24	17.0	17.0	17.2	16.6	Glc-6′′′′			62.6
C-25	15.6	15.7	15.6	15.6	28-*O*-			
C-26	17.5	17.5	17.5	17.4	Glc-1″	95.7	95.7	95.8
C-27	26.1	26.1	26.1	26.2	Glc-2″	74.0	7.40	74.1
C-28	176.4	176.5	176.4	180.3	Glc-3″	78.5	78.4	78.4
C-29	33.1	33.1	33.1	33.3	Glc-4″	71.1	71.0	71.1
C-30	23.7	23.7	23.7	23.8	Glc-5″	79.3	78.0	79.3
					Glc-6″	62.2	69.4	62.2
					Glc-1‴		105.3	
					Glc-2‴		75.1	
					Glc-3‴		78.4	
					Glc-4‴		71.5	
					Glc-5‴		78.7	
					Glc-6‴		62.6	

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