Squarroside C, a New Cycloartene Bisdesmoside from *Thalictrum squarrosum*

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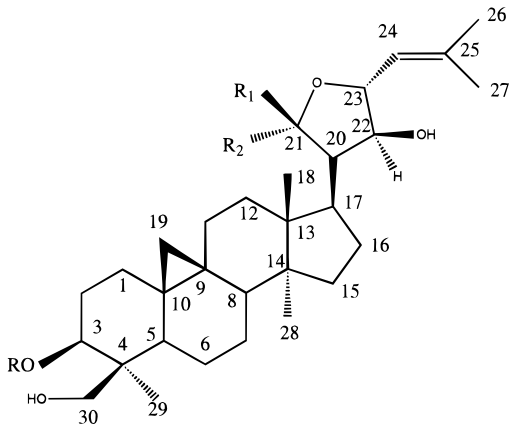
Squarroside C (**1**), a new cycloartane 3,21-bisdesmoside, was isolated from the above-ground parts of *Thalictrum squarrosum*. The structure of **1** was established as 3-*O*-[*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl]-21-*O*-*β*-D-glucopyranosyl-21(*S*),22(*S*),23(*R*),3*β*,21α,22*β*,30-tetrahydroxy-21,23-epoxy-cycloart-24-ene by 2D NMR spectroscopy and FABMS.

Thalictrum squarrosum Stephan ex Willd. (Ranunculaceae) is a grassy plant, that occurs in East Siberia and is used in traditional folk medicine for the treatment of hypertension. Previous chemical studies on *T. squarrosum* have resulted in the isolation of six triterpenoid saponins[1,2] and their genins,[3] and also an uncommon dialloside of apigenin.[4] In this communication we report the isolation and structure determination of a new cycloartane saponin, squarroside C (**1**). Structure **1** was determined using a variety of NMR techniques including DEPT, HETCOR, COSY, HMBC, and ROESY. Saponin **1** is a bisdesmoside with sugar chains made up of two monosaccharide units linked to C-3 and a third sugar unit linked to the C-21 hemiacetal hydroxyl group.



1 R= - Glc^6-Rha R_1= H; R_2= Glc

Rha : α-L-rhamnopyranosyl

Glc : β-D-glucopyranosyl

A concentrated methanol extract of the above-ground part of the plant was partitioned between water and chloroform, and then between water and *n*-BuOH. The *n*-BuOH-soluble portion was subjected to flash chromatography using Si gel to afford a crude saponin fraction that was further separated by droplet countercurrent chromatography [$CHCl_3$−MeOH−H_2O (6:5:3.5)] yielding the pure compound **1**.

A molecular weight of 981.5026 daltons was determined by HRFABMS, corresponding to the molecular formula, $C_{48}H_{78}O_{19}Na$. In addition to the molecular ion, the following fragments were found in the FABMS: $[M + Na - 162]^+$ at *m/z* 819, corresponding to the loss of 1 mole of hexose from the parent molecular ion; $[M + Na - 162 - 146]^+$ at *m/z* 673, representing the loss of 1 mole of hexose and 1 mole of deoxyhexose; and $[M + Na - 162 - 146 - 162]^+$ at *m/z* 511, representing the loss of two hexose units together with one deoxyhexose unit. Thus, **1** was found to consist of a genin and three sugar units.

The ^1H and ^{13}C NMR spectral data of **1** showed three anomeric signals (*δ* 5.64, 5.56, 5.06 and 96.87, 102.93, 106.58 ppm, respectively) and a hemiacetal center on the triterpene moiety (H-21, C-21; *δ* 5.96 and 99.10, respectively). Also in the ^1H NMR spectrum of **1**, two doublets were clearly seen at *δ* 0.10 (J = 3.9 Hz) and *δ* 0.42 (J = 3.9 Hz), which is characteristic of a cyclopropane methylene group. In addition, an olefin proton signal of a trisubstituted double bond was observed at *δ* 6.22 (dd, J = 8.9, 1.2 Hz), and signals occurred due to three tertiary methyl groups (*δ* 0.81 s, 1.03 s, 1.58 s) and two methyl groups attached to the double bond (*δ* 1.67 s, 1.77 s). Also, there were signals due to methine protons attached to a carbon bearing an oxygen function (other than the signals of the anomeric protons) [*δ* 3.80 (dd, J = 12.0; 4.9 Hz), 4.25 m, 5.04 (dd, J = 8.9; 4.0 Hz)] and a methylene group (*δ* 3.86 d; 4.56 d, J = 10.5 Hz) of a primary alcohol linked to the tertiary carbon atom C-4. Therefore, **1** was concluded to be a triglycoside of a triterpenoid of the cycloartane series.

Most of the NMR signals of this saponin were assigned using COSY, HMBC, and ROESY 2D NMR spectra. The carbon resonances were associated with the corresponding proton signals using the $^1H-^{13}C$ HETCOR experiment, and the results are shown in Tables 1 and 2.

A careful comparison of the ^1H and ^{13}C NMR spectra for squarroside C (**1**) showed that the NMR data of the polycyclic fragments (C-1−C-19, C-28−C-30) were the same as those for squarrogenins 3 and 4, isolated from *T. squarrosum* earlier.[2] Therefore, the secondary hydroxyl group was assigned at the 3*β*-position (signals at *δ* 3.80 and 89.84) and the hydroxymethyl at the 4*β*-position (signals at *δ* 3.86 d and 4.56 d in the ^1H NMR spectrum typical of an AB system, and at *δ* 63.75 in ^{13}C NMR spectrum).

The bond sequence of atoms in the tetrahydrofuran ring of squarroside C (**1**) was established by the $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC techniques (Table 1) and proved to be the same as found in squarrogenins 3 and 4.[2] The relative configurations of the substituents in the tetrahydrofuran ring were established by NOE experiments. The H-23

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Table 1. NMR Data for the Aglycone Portion of Squarroside C (**1**) (125 and 500 MHz, C_5D_5N)

carbon	δ_C ppm	δ_H, ppm *J* (Hz)	HMBC (C→H)
1	32.33	1.09 m, 1.54 m	H_2−19, H-2
2	30.71	2.11 ddt (12.0,10.0, 10.0,4.0) 2.53 m	
3	89.84	3.80 dd (12.0, 4.9)	H-1, H-2, Me-29, 1Glc-H-1
4	45.44		Me-29
5	48.14	1.40 m	Me-29, H-19, H-7, H-1
6	22.44	0.96 m, 1.67 m	H-8, H-7, H-5
7	27.19	0.92 m, 1.25 m	H-8, H-6, H-5
8	48.97	1.40 m	Me-28, H-19, H-15, H-11, H-6
9	21.59		H-7
10	26.09		H-19, H-1
11	27.76	1.53 m, 2.32 m	H-8, H-12
12	36.59	H_2 1.40 m	H-11
13	45.70		Me-28, Me-18, H-17, H-16, H-11
14	48.97		Me-28, Me-18
15	26.73	0.78 m, 1.66 m	
16	31.68	1.64 m, 1.98 br t(11.5)	H-15
17	41.04	2.92 q (11.5)	H-20, Me-18, H-16
18	20.40	1.03 s	H-16, H-17
19	30.52	0.10 br d (3.9), 0.42 d (3.9)	H-8, H-5
20	53.22	2.21 dt (11.5, 4.0, 4.0)	H_2−16
21	99.10	5.96 d (4.0)	2Glc-H-1
22	75.69	4.25 m	H-21
23	81.71	5.04 dd (8.9, 4.0)	Me-27, Me-26, H-21
24	123.75	6.22 dq (8.9, 1.2)	Me-27, Me-26
25	136.26		Me-27, Me-26
26	18. 73	1.77 d (1.2)	Me-27
27	26.37	1.67 d (1.2)	Me-26
28	20.16	0.81 s	H-17, H-15
29	21.66	1.58 s	H-5
30	63.75	3.86, 4.56 dd (10.5)	Me-29

Table 2. ^1H and ^{13}C NMR Data for the Sugar Portion of Squarroside C (**1**)

sugar	carbon	δ_C	δ_H (J_{HH} in Hz)	HMBC (C→H)
Glc-1	1	106.58	5.06 d (8.0)	H-2
	2	75.87	4.00[a]	H-3
	3	79.18	4.22[a]	H-5
	4	72.41	3.99[a]	H-3, H-5, H-6
	5	77.46	4.13[a]	H-6
	6	68.59	4.21[a]	H-5
			4.68 d (11.5)	H-4
Glc-2	1	96.87	5.64 d (8.0)	H-2
	2	75.29	4.09 dd (9.2,8.0)	H-3
	3	79.05	4.26[a]	H-2
	4	72.41	4.15[a]	H-3, H-6
	5	79.18	3.92[a]	H-4
	6	63.27	4.32 dd (11.2,5.2)	H-4
			4.52[a]	
Rha	1	102.93	5.60 br s	2H-6 Glc-1
	2	72.79	4.64 br s	H-1
	3	73.25	4.56[a]	H-1, H-2, H-4
	4	74.43	4.29[a]	H-2, H-3, H-5
	5	70.14	4.41[a]	H-1, H-4
	6	19.14	1.62 d (6.1)	

[a] Signal pattern unclear due to overlapping.

signal was observed to have a ROESY cross-peak with the signal of H-20. It is known that H-20 has a *β*-configuration in lanostane triterpenoids; therefore, it was concluded that H-23 also has a *β*-configuration regarding the tetrahydrofuran ring, and that the bulky CH═CMe_2 substituent is in the α-configuration.

A *β*-configuration for H-21 was established on the basis of the presence of ROESY cross-peaks between H-21 and CH_3-18, H-20, and H-23. The signal of H-22 exhibits a cross-peak with H-24 and H-16*β* (1.64 ppm), but not with H-20 or H-21. Therefore, the configurations were assigned as H-22α and OH-22*β*, respectively (Figure 1). This aglycon is very similar to that isolated by Yashimitsu et al. from "Thalictrum herba",[5] but they claim an α orientation for OH-22, whereas we find the opposite configuration.

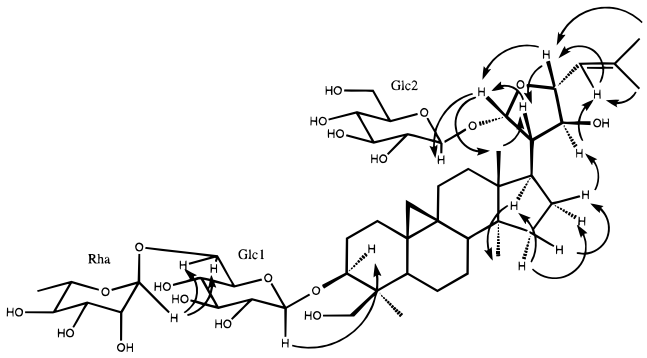

Figure 1. ROESY correlations observed for squarroside C(**1**).

The ^1H NMR signals of each monosaccharide unit in **1** were connected by $^1H-^1H$ COSY data, and HETCOR data were used to associate the protons with the corresponding carbon resonances. On comparison, the ^{13}C NMR signals of each sugar residue were found to coincide with those for α-L-rhamnopyranoside and *β*-D-glucopyranoside.[6] The *β*-configuration at the anomeric centers of the D-glucopyranosides was confirmed by the observation of large *J* coupling (8.0 Hz for H-1 of both glucose substituents). The attachment sites of the glucoside residues were obtained from HMBC experiments, which showed correlations between H-1 of the inner glucose and the C-3 atom of the aglycon, and between H-1 of the terminal glucose and the C-21 atom of the aglycon. There was a cross-peak between H-1 of the rhamnose and C-6 of the inner glucose, and so it was deduced that the rhamnose unit was bound to C-6 of the inner glucose. Furthermore, there were interactions between H-3 of the genin and H-1 of the inner glucose, between H-6 of the inner glucose and H-1 of the rhamnose, and between H-21 of the genin and H-1 of the terminal glucose in the ROESY spectra (Figure 1). On the basis of the foregoing data, the structure 3-*O*-[*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl]-21-*O*-*β*-D-glucopyranosyl-21(*S*),22(*S*),23(*R*),3*β*,21α,22*β*,30-tetrahydroxy-21,23-epoxycycloart-24-ene was established for compound **1**.

Experimental Section

General Experimental Procedures. Melting points were measured on a Boetus apparatus and are uncorrected. Optical rotation data were obtained using a Perkin−Elmer 241 polarimeter. IR spectra were obtained with a Mattson Polaris spectrometer. All NMR spectra were obtained using a Varian VXR-500 spectrometer (^1H, 500 MHz; ^{13}C, 125 MHz) equipped with a SUN SPARC 20 workstation. Samples were run at 26 °C in 5-mm MNR tubes at a concentration of about 12 mg/0.7 mL of pyridine-d_5 with a trace of TMS as a reference. FABMS with a thioglycerol matrix was conducted on a JEOL SX102A instrument. The target was bombardment with 6 keV Xe atoms.

TLC experiments were conducted using Si gel plates [L 5/40 (Lachema)]. The following TLC solvent systems were used: $CHCl_3$−MeOH−H_2O (70:23:1) and $CHCl_3$−MeOH−H_2O (70:23:4). The spray reagent used for the saponins was 0.5% vanillin in 50% H_3PO_4.

Plant Materials. The whole plant of *T. squarrosum* was collected at Buryatia, Russia, in July 1996. A herbarium sample is deposited in the M. G. Popov Herbarium of the

Siberian Institute of Plant Physiology and Biochemistry of the Russian Academy of Sciences, Irkutsk.

Extraction and Isolation. The above-ground parts of *T. squarrosum* (2.5 kg) were extracted using 80% MeOH. The concentrated extract was partitioned between H_2O and $CHCl_3$ and then between H_2O and *n*-BuOH. The *n*-BuOH-soluble fraction (30 g) was subjected to flash chromatography over Si gel eluted by $CHCl_3$−MeOH−H_2O (70:23:4) to afford 5.6 g of a crude saponin fraction. This fraction was further separated by repeated droplet countercurrent chromatography ($CHCl_3$−MeOH−H_2O, 5:6:3.5), yielding 18 mg of squarroside C (**1**).

Squarroside C (1): obtained as white needles (MeOH−$CHCl_3$−H_2O); mp 211−213 °C; $[\alpha]^{25}_D$ −46.9 (*c* 1.1, MeOH); UV, no absorptions above 210 nm; IR (KBr disk) ν_{max} 3345 (OH), 2933 (CH), 1448, 1079, 1043 cm^{-1}; ^1H and ^{13}C NMR spectra, see Tables 1 and 2; FABMS *m*/*z* 981 $[M + Na]^+$, 819 $[M + Na - 162]^+$, 673 $[M + Na - 162 - 146]^+$, 511 $[M + Na - 2 \times 162 - 146]^+$; HRFABMS *m*/*z* 981.5026 (calcd for $C_{48}H_{78}O_{19}Na$, 981.5034).

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