Solakhasoside, a Novel Steroidal Saponin from *Solanum khasianum*

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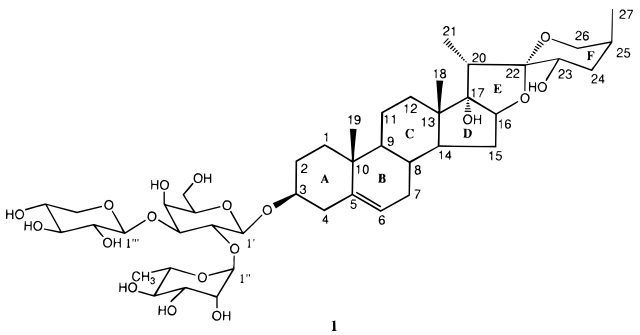
Solakhasoside (**1**), a novel steroidal saponin, was isolated from the fruit of *Solanum khasianum*. Its structure was determined as (23*S*,25*S*)-spirot-5-en-3β,17α,23-triol-3-*O*-[α-L-rhamnopyranosyl-(1→2)[β-D-xylopyranosyl-(1→3)]-β-D-galactopyranoside] (**1**) by spectroscopic analysis.

Solanum khasianum C.B. Clarke (Solanaceae) has gained importance because of its high content of solasodine-type glycoalkaloids, which have been found to be useful as starting materials for the production of steroidal hormones, to substitute for diosgenin.[1,2] In addition to steroidal alkaloid glycosides, such as solamargine,[3] solasonine, and khasianine,[4] steroidal saponins are also abundant in *Solanum* species.[5] In the course of our screening of solasodine-type glycoalkaloids using a monoclonal antibody (MAb) against solamargine,[6,7] a steroid saponin containing a novel aglycon was obtained from fruits of *S. khasianum*, and its structure elucidation is reported herein.

After column chromatography over MCI gel, Cosmosil ODS, and silica gel, sequentially, a novel steroidal saponin, solakhasoside (**1**), was obtained as a colorless amorphous powder from the MeOH extract of the fruits of *S. khasianum*, along with solamargine and solasonine.[3]



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A positive reaction with Liebermann's reagent was observed along with a negative reaction to Dragendorff's reagent, indicating that **1** was a saponin rather than an alkaloid. Furthermore, no reactivity to MAb against solamargine by either competitive ELISA or western blotting supported **1** having a different aglycon moiety from solamargine.[6,7] FABMS also confirmed the absence of nitrogen. Its molecular formula was identified as $C_{44}H_{69}O_{18}$ according to negative FABMS and ^{13}C NMR spectral data. The molecular ion peak at *m*/*z* 885 [M − H]$^-$ and the fragmentation at *m*/*z* 753 [M − H − pentose]$^-$ and 739 [M − deoxyhexose − H]$^-$ indicated the existence of one deoxyhexose and one pentose in a branched sugar moiety. Complete hydrolysis with HCl yielded galactose, rhamnose, and xylose by comparison to the authentic samples on high-performance TLC (HPTLC).

Table 1 shows the ^1H NMR and ^{13}C NMR spectral data of **1**. All signals were assigned unequivocally according to $^1H-^1H$ COSY, HMQC, HMBC, and NOESY analysis. The ^1H NMR spectrum showed diagnostic signals of two tertiary methyl groups (δ 0.85, 1.04, s) and two secondary methyl groups (δ 1.02, d, J = 7.3 Hz; 0.77, d, J = 6.6 Hz) corresponding to the angular methyl groups of a steroid

Table 1. ^1H NMR and ^{13}C NMR Spectral Data for Solakhasoside (**1**)[a]

carbon	δ_C (125 MHz, CD_3OD)	δ_H (500 MHz, CD_3OD)
1	38.6	1.09, m; 1.87, m
2	30.8	1.89, m; 1.59, m
3	79.0	3.62, m
4	39.4	2.30, m; 2.45, m
5	142.0	
6	122.5	5.37, d (5.3)
7	33.2	1.55, m; 2.00, m
8	33.4	1.60, m
9	51.5	1.60, m
10	38.0	
11	21.6	1.55, m; 1.62, m
12	32.7	1.30, m; 1.65, m
13	46.2	
14	53.9	1.73, m
15	32.3	1.24, m; 2.02, m
16	90.1	4.04, dd (5.7, 7.8)
17	91.8	
18	17.4	0.85, s
19	10.9	1.04, s
20	44.5	2.41, q (7.3)
21	19.8	1.02, d (7.3)
22	110.5	
23	70.9	3.50, m
24	37.4	1.62, m; 1.66, m
25	24.9	2.02, m
26	67.4	3.36, m; 3.48, m
27	17.4	0.77, d (6.6)
1′	100.8	4.48 d (7.6)
2′	75.8	3.77, dd (7.6, 9.4)
3′	85.2	3.70, m
4′	70.4	4.00, d (3.0)
5′	76.0	3.49, m
6′	62.3	3.70, m
1″	102.3	5.19, d (1.6)
2″	72.1	3.93, dd (1.6, 3.2)
3″	72.4	3.64, m
4″	74.0	3.38, m
5″	69.8	4.14, dt (9.4, 6.2)
6″	18.0	1.23, d (6.2)
1‴	106.5	4.42, d (7.1)
2‴	74.8	3.28, m
3‴	77.9	3.30, m
4‴	71.1	3.47, m
5‴	66.9	3.20, m; 3.86, dd (5.3, 11.4)

[a] Chemical shifts are reported in ppm. Proton signals are followed by multiplicity and coupling constants (Hz) in parentheses, with assignments determined by $^1H-^1H$ COSY, HMQC, HMBC, and NOESY measurements.

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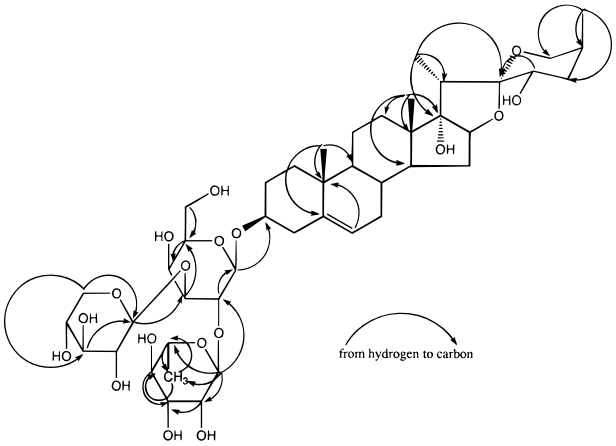

Figure 1. HMBC correlations of solakhasoside.

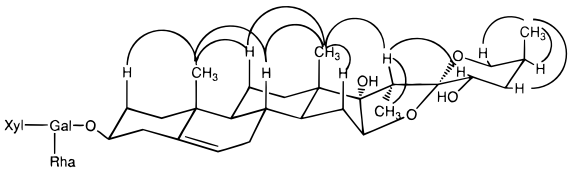

Figure 2. NOE correlations of solakhasoside.

sapogenin. An olefinic proton at δ 5.37 (d, $J = 5.3$ Hz) could be attributed to 5,6-unsaturation. In addition, three anomeric hydrogens (δ 4.48, d, $J = 7.6$ Hz; 4.42, d, $J = 7.1$ Hz; 5.19, d, $J = 1.6$ Hz) were consistent with the three monosacharides yielded from acid hydrolysis. These conclusions were supported by the ^{13}C NMR spectral data of **1**. In addition to the signals of the angular methyl groups (δ 10.9, 19.8, 17.4 × 2), olefinic carbons (δ 122.5, 142.0), and anomeric carbons (δ 100.8, 102.3, 106.5), a spiroketal carbon (δ 110.5) suggested **1** to be a spirostene triglycoside.

Calculated from the FABMS, the molecular weight of the aglycon moiety was 446, 32 more than that of diosgenin.[3] In comparison to diosgenin, there were one more quaternary carbon and one less secondary carbon in the ^{13}C NMR spectrum by DEPT measurements. From the HMBC correlations shown in Figure 1, the signal at δ 91.8 could be assigned to C-17 according to its long-range coupled cross peak with that of an angular methyl group (δ_H CH_3-18, 0.85, s; CH_3-21, 1.02, d, $J = 7.3$ Hz). Owing to this tertiary hydroxyl group, the signals of C-13 (δ 46.2) and C-16 (δ 90.1) were shifted downfield compared with those of diosgenin. In the same way, another hydroxyl group could be placed at C-23 (δ_H 3.50, m; δ_C 70.9) because of the long-range correlation of the resonance with that of the spiroketal carbon at C-22 (δ 110.5).

The stereochemistry of the aglycon moiety was determined by NOESY measurements, as shown in Figure 2. From the angular methyl groups CH_3-18 (δ 0.85, s) and CH_3-19 (δ 1.04, s), NOE correlations with axial protons on rings A−D confirmed the stereostructure of **1** to be identical to that of diosgenin. The orientation of CH_3-21 (δ_H1.02, d, $J = 7.3$ Hz; δ_C19.8) was assigned as α according to the cross peak between CH_3-18 (δ 0.85, s) and H-20β (δ 2.41, q, $J =$ 7.3 Hz). Accordingly, C-20 was in the *S* configuration, the same as diosgenin. The NOE correlation between H-20β and H-23 (δ 3.50, m) corresponded to the 22*S*,23*S* configuration because of the γ-gauche conformation. The lack of an NOE between H-23 and H-25 suggested an axial CH_3-27 and *S* configuration of C-25 (δ 24.9). The high-field chemical shift of C-25 also suggested that the configuration at this position was different from that of diosgenin. Consequently, the aglycon of **1** could be determined as (23*S*,25*S*)-spirot-5-en-3β,17α,23-triol.

As described above, the sugar moiety of **1** consisted of galactose, rhamnose, and xylose in a branched chain. With the assumption of D configuration for galactose and xylose and L for rhamnose, the configurations of the anomeric carbons were determined as β, α, and β, respectively, according to the coupling constants of anomeric protons ($J_{1',2'} = 7.6$ Hz, $J_{1'',2''} = 1.6$ Hz, $J_{1''',2'''} = 7.1$ Hz), along with the chemical shifts of the anomeric carbons (δ C-1′, 100.8; C-1‴, 102.3; C-1‴, 106.5). The 3β-hydroxy group was glycosidated by a β-D-galactose unit according to the long-range coupling between the anomeric proton of galactose (δ 4.48, d, $J = 7.6$ Hz) and C-3 (δ 79.0) measured by HMBC. NOE correlations of H-1′ (δ 4.48, d, $J = 7.6$ Hz) and H-3α (δ 3.62, m) indicated these two hydrogens were in the γ-gauche conformation. In the same way, β-D-xylose was attached to OH-3′, while α-L-rhamnose was connected with OH-2′ of galactose according to HMBC correlations between the anomeric protons and the glycosidated carbons, as shown in Figure 1.

In conclusion, **1** was identified as (23*S*,25*S*)-spirot-5-en-3β,17α,23-triol-3-*O*-[α-L-rhamnopyranosyl-(1→2)[β-D-xylopyranosyl-(1→3)]-β-D-galactopyranoside](**1**). All the signals of carbons and protons were assigned unequivocally as a result of complete spectroscopic analysis. To our knowledge, this is the first isolation of a spirostanol glycoside having a 17α-hydroxyl group.

Experimental Section

General Experimental Procedures. The melting point was measured on a Vanaco micromelting point apparatus. The optical rotation was determined on a JASCO/DIP-4 digital polarimeter. The IR spectrum was obtained on a JASCO FT/IR-410 spectrometer. ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC were all measured by a Varian Unity-500P spectrometer. The negative FABMS was analyzed by a JEOL JMS-SX102 spectrometer using glycerin as matrix. TLC was carried out on precoated silica gel 60 F_{254} (0.2 mm, Merck). Column chromatography was performed with MCI gel CHP-20P (75−150 μm, Mitsubishi Chemical Institutes, Ltd., Tokyo, Japan), Cosmosil 75 C_{18}-OPN (42−105 μm, Nacalai Tesque, Inc., Kyoto, Japan), and silica gel 60 (70−230 μm, Merck). All chemical reagents were standard commercial products of analytical grade.

Plant Material. The fruits of *S. khasianum* was obtained in September 1997 from the herbal garden of the Faculty of Pharmaceutical Sciences, Kyushu University, Japan. A voucher specimen of the plant is deposited (No. 970925) at the herbarium of Faculty of Pharmaceutical Sciences, Kyushu University, Japan.

Extraction and Isolation. Dry fruits of *S. khasianum* (300 g) were extracted with MeOH. After removal of the solvent by evaporation, the combined extract (9 g) was subjected to column chromatography on MCI gel CHP-20P eluted with 40−100% MeOH in a gradient isolation. The 60% MeOH effluent was chromatographed on Cosmosil 75 C_{18}-OPN (40−70% MeOH) and then on a silica gel column ($CHCl_3$−MeOH−H_2O 7:3:1) to give **1** (4 mg).

Solakhasoside (**1**) was obtained as a colorless amorphous powder (MeOH): mp 250−252 °C; $[\alpha]^{28}_D$ −50.0° ($c = 0.1$, MeOH); IR (KBr) ν_{max} 3368, 2934, 1650, 1050-975 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; FABMS *m*/*z* 885 [M − H]$^-$, 753 [M − H − pentose]$^-$, 739 [M − deoxyhexose − H]$^-$.

Acidic Hydrolysis of 1. **1** was dissolved in 1 M HCl and then heated at 80 °C in a water bath for 2 h. After extraction with $CHCl_3$, the aqueous residue was evaporated to dryness. Sugar components were identified on TLC by comparison of authentic sugar samples, with *n*-BuOH−AcOH−H_2O (4:1:5, upper layer) as the developing solvent.

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