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CYCLOSIEVERSIGENIN 3-O-β-*D*-GLUCOPYRANOSIDE FROM *Astragalus kuhitangi*

R. Karimov, R. U. Umarova, Z. Saatov, M. G. Levkovich, and N. D. Abdullaev

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*Cyclosieversigenin, cyclosieversigenin 3-O-β-*D*-xylopyranoside, cyclosieversioside F, astragaloside VII, and, for the first time in native form, cyclosieversigenin 6-O-β-*D*-glucopyranoside have been isolated from the roots of* Astragalus kuhitangi *(Nevski) Sirj.*

In an investigation of the roots of *Astragalus kuhitangi* (Nevski) Sirj (fam. Leguminosae) gathered in the Kukhitang mountains, Republic of Tadzhikistan, in 1988, in addition to the known cyclosieversigenin (**1**), cyclosieversigenin 3-O-β-*D*-xylopyranoside (**2**), cyclosieversioside F (**3**), and astragaloside VII (**4**) [1—6], we have isolated the cycloartane (**5**).

In the strong-field region of the PMR spectrum of compound (**5**), at 0.63 and 0.28 ppm, there are the two one-proton doublets that are characteristic for the methylene group of a cyclopropane ring and also the signals of seven tertiary methyls (see the Experimental part). These resonance lines permit compound (**5**) to be assigned to the methylsteroids of the cycloartane series.

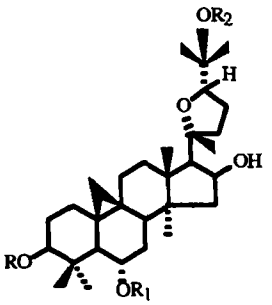
Acid hydrolysis of compound (**5**) led to a genin identified as cyclosieversigenin (**1**). *D*-Glucose was identified in the hydrolysate by paper chromatography (PC).

In the ^{13}C NMR spectrum of compound (**5**) the signal of the C-6 carbon atom appeared at 79.77 ppm, and, in comparison with the corresponding signal in the spectrum of cyclosieversigenin (**1**) at 68.57 ppm, was shifted downfield by 11.20 ppm.

The results presented unambiguously show the attachment of the *D*-glucose residue to the hydroxy group at C-6. The anomeric proton of compound (**5**) resonated at 4.94 ppm in the form of a doublet with $^3J = 7.9$ Hz while the corresponding C-1′ carbon atom resonated at 105.11 ppm. Taken together, these facts show the β-configuration of the glycosidic center.

Thus, compound (**5**) is cyclosieversigenin 6-O-β-*D*-glucopyranoside.

Cyclosieversigenin 6-O-β-*D*-glucopyranoside (5) has been obtained previously in the acid hydrolysis of cyclosieversioside F [4]. Its isolation from *Astragalus kuhitangi* is the first time that it has been obtained in native form.



1. R=R$_1$=H
2. R=β-*D*-Xyl, R$_1$=R$_2$=H
3. R=β-*D*-Xyl, R$_1$=β-*D*-Glc, R$_2$=H
4. R=β-*D*-Xyl, R$_1$=R$_2$=β-*D*-Glc
5. R=R$_2$=H, R$_1$=β-*D*-Glc

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (371) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 751—755, November-December, 1998. Original article submitted December 29, 1997.

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TABLE 1. Chemical Shifts (ppm) of the ^{13}C Carbon Nuclei of Cyclosieversigenin (1), Cyclosieversioside F (3), Astragaloside VII (4), and Cyclosieversigenin 6-O-β-*D*-Glucopyranoside (5) in C_5D_5N

C	Compound			
	1	3	4	5
1	35.16	32.53	32.04	34.94
2	33.01	29.18	28.83	34.87
3	78.53	88.82	88.44	78.22
4	42.69	42.91	42.58	42.34
5	54.20	52.82	52.36	52.52
6	68.57	79.40	79.10	79.77
7	39.08	34.90	33.99	33.37
8	47.50	46.02	45.12	46.20
9	21.19	21.41	20.91	21.02
10	30.11	29.31	28.83	29.45
11	26.51	26.50	25.99	26.40
12	33.65	33.70	33.31	31.20
13	45.28	45.39	45.12	45.01
14	46.41	46.53	46.01	46.20
15	46.99	46.53	45.34	46.34
16	73.69	73.67	73.50	73.35
17	58.64	58.53	57.89	58.23
18	21.87	21.41	20.91	21.26
19	31.70	30.46	30.10	29.57
20	87.48	87.52	87.10	87.19
21	27.38	28.86	27.64	27.02
22	31.21	35.24	34.96	32.51
23	26.69	26.75	25.99	26.19
24	81.93	82.00	82.02	81.64
25	71.48	71.55	78.58	71.21
26	28.44	22.36	22.78	28.12
27	28.81	28.45	28.46	28.52
28	29.67	20.16	19.64	29.06
29	16.38	16.92	16.51	16.08
30	20.47	20.15	20.91	19.88
		3-O-β-*D*-Xyl	3-O-β-*D*-Xyl	6-O-β-*D*-Glc
1′		105.46	107.56	105.11
2′		75.84	75.07	75.49
3′		79.84	78.95	78.01
4′		71.55	71.71	71.81
5′		67.25	66.93	78.01
6′				63.03
		6-O-β-*D*-Glc	6-O-β-*D*-Glc	
1″		107.83	104.95	
2″		75.84	75.52	
3″		78.70	78.43	
4″		72.31	71.18	
5″		78.29	78.06	
6″		63.44	62.89	
			25-O-β-*D*-Glc	
1‴			98.82	
2‴			75.52	
3‴			78.43	
4‴			71.18	
5‴			77.91	
6‴			62.59	

EXPERIMENTAL

General Observations. For thin-layer chromatography (TLC) we used silica gel containing 10% of gypsum that had been passed through a sieve with 0.08 mm apertures, and also Silufol plates. For column chromatography we used type KSK silica gel with particle sizes of 0.1—0.08 and 0.16—0.1 mm. The cycloartanes and their derivatives were revealed in TLC with a 25% solution of tungstophosphoric acid, followed by heating for 5—10 min. IR spectra were taken on a UR-20 spectrometer and a Perkin-Elmer System 2000 FT-IR Fourier spectrometer in anhydrous KBr. ^1H and ^{13}C NMR spectra were recorded in C_5D_5N on BS-567A (Tesla), CFT-20, G-200, and UNITY-400 Plus (Varian) instruments.

Paper chromatography was conducted on type FN-11 paper. The following solvent systems were used: 1) chloroform—methanol (7:1); 2) chloroform—methanol—water (70:23:4); and 3) butan-1-ol—pyridine—water (4:6:3).

Isolation of the Cycloartanes. Air-dry comminuted roots of *A. kuhitangi* (2.5 kg) were extracted with methanol (5 × 8 liters). The combined methanolic extracts were concentrated to a syrupy mass, which was diluted with two volumes of water and extracted with butanol. The butanolic extract was evaporated to dryness, giving 80 g of cycloartane triterpenoids. From this total, 30 g was chromatographed on a column of silica gel. Elution of the column with system 2 yielded several fractions. Identical fractions were combined and rechromatographed on a column. Five substances of genin and glycosidic natures were isolated.

Cyclosieversigenin (1). Compound (1), of which 250 mg was isolated, had the composition $C_{30}H_{50}O_5$, mp 240—242°C (from methanol), $[\alpha]_D^{20}$ +49.0±2° (*c* 1.37; methanol). IR spectrum (KBr, ν, cm^{-1}): 3396 (OH), 3035 (cyclopropane group).

PMR spectrum (C_5D_5N, TMS, δ ppm, 400 MHz): 1.03, 1.31, 1.39, 1.46, 1.59, and 1.92 (s, each 3H, tertiary methyl groups), 0.35 and 0.63 (each 1H, d, ^2J=3.9 Hz, 2H-19), 2.56 (1H, d, ^3J=7.6 Hz, H-17), 3.86 (1H, dd, ^3J=11.4 and 3.4 Hz, H-3), 3.81 (1H, ddd, ^3J=9.5 Hz, 9.5 and 3.4 Hz, H-6), 3.90 (1H, dd, ^3J=8.9 and 5.4 Hz, H-24), 5.05 (1H, ddd, 1:3:3:1, Σ^3J=22.5 Hz or ^3J each 7.5-7.6 Hz, H-16).

On the basis of a comparison of the given spectral results with the literature [1—3], and also by a direct comparison with an authentic specimen (TLC, system 2), compound (1) was characterized as cyclosieversigenin.

Cyclosieversigenin 3-O-β-*D*-Xylopyranoside (2). Compound (2), of which 250 mg was isolated, had the composition $C_{35}H_{58}O_9$, mp 259—260°C (from methanol), $[\alpha]_D^{22}$ +40.0±2° (*c* 0.60; methanol).

Acid Hydrolysis. Substance (2) (50 mg) was hydrolyzed by heating with 10 ml of 0.3% methanolic sulfuric acid in the boiling water bath for 4 h. After cooling, 20 ml of water was added to the reaction mixture, and the excess of methanol was distilled off. The precipitate that had deposited was filtered off, washed with water, and chromatographed on a column of silica gel. Elution with system 1 yielded 17 mg of cyclosieversigenin (1).

The aqueous part of the hydrolysate was neutralized with barium carbonate, the solid was filtered off, and the filtrate was evaporated. The hydrolysate was chromatographed on paper in system 3, and *D*-xylose was detected.

The physicochemical constants and spectral characteristics of compound (2), and also a direct comparison with an authentic specimen (TLC, system 2) enabled it to be identified as cyclosieversigenin 3-O-β-*D*-xylopyranoside [4, 5].

Cyclosieversioside F (3). Compound (3) was isolated in an amount of 10 g, with the composition $C_{41}H_{68}O_{14}$, mp 286—289°C (from methanol), $[\alpha]_D^{20}$ +37.5±2° (*c* 0.50; methanol).

IR spectrum (KBr, cm^{-1}): 3400—3500(OH), 3040 (cyclopropane group).

PMR spectrum (C_5D_5N, TMS, δ ppm, 200 MHz): 0.93, 1.29, 1.29, 1.35, 1.40, 1.57, 2.01 (s, tertiary methyl groups), 0.20 and 0.58 (each 1H, d, ^2J = 4.0 Hz, 2H-19), 2.51 (1H, d, ^3J = 7.7 Hz, H-17), 3.40—5.00 (multiplets of the protons of the carbohydrate residues and of the hydrogen atoms located geminally to the oxygen functions of the genin) including 4.83 and 4.88 (each 1H, d, ^3J = 7.04 and 7.5 Hz, H-1′ and H″, respectively).

Acid Hydrolysis. Compound (3) (150 mg) was hydrolyzed as described above. The reaction mixture was chromatographed on a column of silica gel. Elution with system 1 gave 31 mg of cyclosieversigenin (1). Further elution of the column with the same system led to the isolation of 47 mg of cyclosieversigenin 3-O-β-*D*-xylopyranoside (2). On continuing the elution of the column with the same mixture of solvents, we obtained 58 mg of cyclosieversigenin 6-O-β-*D*-glucopyranoside (5). *D*-Xylose and *D*-glucose were detected in the aqueous part of the hydrolysate by paper chromatography in system 5.

From the physicochemical constants and spectral characteristics given above, compound (3) was identified as cyclosieversioside F [4].

Astragaloside VII (4). Compound (4) was isolated in an amount of 1 g, with the composition $C_{47}H_{78}O_{19}$, mp 268—272°C (from methanol—water), $[\alpha]_D^{20}$ +65±2° (*c* 0.66; pyridine).

Stepwise Hydrolysis of (4). Compound (4) (600 mg) was dissolved in 200 ml of 1:1 aqueous methanol and was hydrolyzed with 0.25% sulfuric acid at 70°C for 3.5 h. The reaction mixture was diluted with water, and the methanol was distilled off. The reaction products were extracted from the aqueous residue with butanol. After evaporation of the butanolic extract, the residue was chromatographed on a column of silica gel, with elution by system 1. This permitted the isolation of 4 mg of the genin (1), identified as cyclosieversigenin, and 7 mg of a monoside identified as cyclosieversigenin 3-O-β-*D*-xylopyranoside (2).

On continuing the elution of the column with the same solvent system, we isolated 20 mg of a glycoside that was identified as cyclosieversigenin 6-O-β-*D*-glucopyranoside (5). In addition to compound (5), we also found 45 mg of the initial cyclosieversioside F (3).

Thus, on the basis of its physicochemical constants and spectral characteristics, compound (4) was identified as astragaloside VII [6].

Cyclosieversigenin 6-O-β-*D*-Glucopyranoside (5). Compound (5) was isolated in an amount of 200 mg, with the composition $C_{36}H_{60}O_{10}$, mp 288—289°C (from methanol), $[\alpha]_D^{20}$ +375±2° (*c* 0.50; methanol). IR spectrum (KBr, cm^{-1}): 3395 (OH), 2966 (cyclopropane group).

PMR spectrum (C_5D_5N, TMS, δ ppm, 400 MHz): 0.93, 1.30, 1.30, 1.41, 1.44, 1.58, 1.96 (each 3H, s, tertiary methyl groups), 0.28 and 0.63 (each 1H, d, ^2J = 3.9 Hz, 2H-19), 2.52 (1H, d, ^3J = 7.6 Hz, H-17), 3.5—4.5 (signals of the protons of the carbohydrate residue and of the hydrogen atoms of the genin located geminally to oxygen functions), 4.94 (1H, d, ^3J = 7.9 Hz, H-1′), 4.99 (1H, ddd, H-16).

Smith Degradation. An aqueous methanolic (1:1) solution of 100 mg of compound (5) was treated with 0.5 g of sodium periodate, and the mixture was stirred at room temperature for 6 h. The unconsumed oxidant was decomposed with ethylene glycol, the residue obtained after evaporation of the methanol was treated with 15 ml of water, and the mixture was extracted with chloroform. The chloroform was distilled off, the residue was treated with 30 ml of methanol, and 0.3 g of sodium tetrahydroborate was added in portions. The reaction mixture was heated at 80°C for 7 h, after which it was acidified to pH 2.0 and left at room temperature for 17 h. The reaction products were extracted with chloroform, the solvent was evaporated, and the residue was chromatographed on a column of silica gel. Elution with ethyl acetate yielded 78 mg of cyclosieversigenin (1).

Acid Hydrolysis. A solution of 50 mg of compound (5) in 20 ml of methanol was treated with 20 ml of 0.5% aqueous sulfuric acid, and the reaction mixture was heated in the boiling water bath for 4 h. After cooling, the reaction products were extracted with chloroform and chromatographed on a column of silica gel. By elution with system 1, 11 mg of cyclosieversigenin (1) was isolated.

The aqueous solution after the separation of the aglycon was neutralized by the method given above and was chromatographed in system 3. After the chromatogram had been treated with aniline phthalate, *D*-glucose was detected.

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