TRITERPENE GLYCOSIDES FROM *Astragalus* AND THEIR GENINS. LXXVI. GLYCOSIDES FROM *A. sieversianus*[**]

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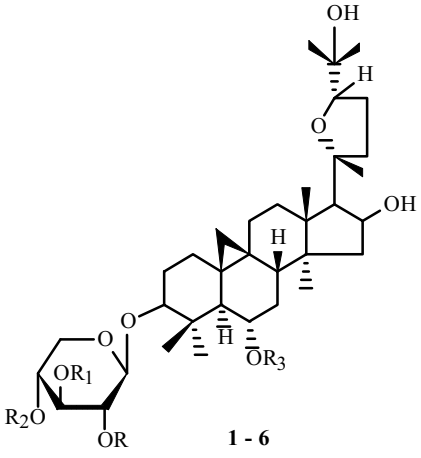
Nine compounds including six cycloartane glycosides cyclosieversiosides A, B, F, G, and H and astrasieversianin IX; β-*sitosterol,* β-*sitosterol* β-*D-glucopyranoside, and D-3-*O-*methyl-chiro-inositol were isolated and identified from roots of* Astragalus sieversianus *Pall. (Leguminosae) growing in the Republic of Kyrgyzstan.*

Key words: cycloartane glycosides, sterols, inositol, *Astragalus*, Leguminosae, PMR and ^{13}C NMR spectra, DEPT.

In continuation of research on triterpene glycosides in plants of the genus *Astragalus* (Leguminosae), we studied *A. sieversianus* Pall. growing in the Republic of Kyrgyzstan [1] and indigenous to the Republic of Uzbekistan and the People's Republic of China. The chemistry of this species is well studied [2-10]. We isolated 16 cyclosieversigenin glycosides from this plant [11].

Total triterpene glycosides from roots of *A. sieversianus*, called astragaloside, possess hypocholesterolemic activity, enhance lipid metabolism, and improve cardiac activity of test animals with experimental endogenous hypercholesterolemia [12]. The extract of this plant exhibits diuretic and hypotensive activity and prevents the appearance of experimental stomach ulcer [13]. Cyclosieversioside D (**7**) and other analogs of cyclosieversioside F (**4**) exhibit antiviral and antitumor activity and low toxicity in oral and parenteral administration. The studied glycosides are interferon inductors [14].



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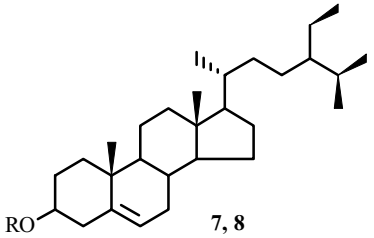

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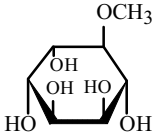

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1: R = R_1 = Ac, R_2 = H, R_3 = *β*-D-Xyl*p*; **2:** R = R_1 = Ac, R_2 = H, R_3 = *β*-D-Glc*p*
3: R = *α*-L-Rha*p*, R_1 = Ac, R_2 = H, R_3 = *β*-D-Xyl*p*; **4:** R = R_1 = R_2 = H, R_3 = *β*-D-Glc*p*
5: R = *α*-L-Rha*p*, R_1 = R_2 = H, R_3 = *β*-D-Xyl*p*; **6:** R = *α*-L-Rha*p*, R_1 = R_2 = H, R_3 = *β*-D-Glc*p*
7: R = H; **8:** R = *β*-D-Glc*p*

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TABLE 1. Chemical Shifts of C Atoms in **1-6** (C_5D_5N,δ, ppm)

C atom	Compound					
	1	**2**	**3**	**4**	**5**	**6**
1	31.36	31.93	31.73	32.14	31.43	
2	29.33	29.74	29.85	30.16	29.70	
3	88.58	89.02	87.79	88.46	87.14	87.45
4	41.78	42.14	42.41	42.59	42.16[a]	42.23
5	51.64	52.36	51.79	52.46	51.53	51.98
6	78.32	79.26	78.52	79.23	79.19	79.14
7	33.39	34.82	33.42*	34.56	32.04*	*
8	44.03	45.85	42.80	45.64	42.16[a]	43.69
9	20.86	21.11[a]	21.15	21.03[a]	20.83	20.76
10	27.99	29.06	27.76	28.90	27.41	
11	26.76	26.41	26.10	26.09	26.13**	
12	33.04	33.28	32.58	33.30	33.12*	33.69*
13	44.77	46.14[b]*	46.07[a]	44.96	44.86	44.78
14	45.78	46.14[b]	46.07[a]	46.14	45.75	45.84
15	45.54	44.96*	45.15	46.14	44.88	45.32
16	73.07	73.35	73.36	73.34	73.05	73.06
17	57.77	58.17	57.91	58.13	57.58	57.71
18	20.47[a]	20.77[c]	19.89	19.76	19.39	20.05
19	25.87	28.84	25-26	28.92	25.89	25.83
20	86.94	87.19	87.27	87.19	86.99	86.96
21	27.82	28.25	28.10	28.12	27.81	27.85
22	34.52	34.82	34.81	34.81	34.49	34.55
23	26.11	26.06	26.42	26.40	24.26**	
24	81.28	81.60	81.54	81.58	81.22	81.26
25	70.92	71.23	71.22	71.23[b]	70.93	70.96
26	26.77	27.02	27.03	27.00	26.73	26.71
27	27.51	28.14	27.40	28.52[c]	26.98	
28	19.33	19.81	19.40	21.03[a]	19.04	19.26
29	28.25	28.52	28.56	28.52[c]	28.27	28.25
30	16.17	16.49	16.59	16.57	16.53	16.60
			3-*O*-*β*-D-Xyl*p*			
1	103.63	103.96	105.13	107.63	105.33	105.03
2	72.70	73.01	77.07	75.53[d]	77.98	77.75**
3	76.42	76.73	78.36	78.48	76.66	77.59
4	68.44	68.73	70.47	71.23[b]	71.15	71.15
5	66.34	66.63	66.26	66.99	66.54[b]	66.39
	β-D-Xyl*p*	*β*-D-Glc*p*	*β*-D-Xyl*p*	*β*-D-Glc*p*	*β*-D-Xyl*p*	*β*-D-Glc*p*
1	105.35	105.19	105.75	105.19	105.49	105.43
2	75.09	75.54	75.42	75.53[d]	75.13	75.38
3	77.51	79.14	78.22	79.10	77.44	78.51
4	70.74	71.76	71.01	71.73	70.73	71.60
5	66.70	78.14	66.85	78.09	66.54[b]	78.06**
6		62.98		62.98		62.98

TABLE 1. (continued)

C atom	Compound					
	1	**2**	**3**	**4**	**5**	**6**
			3-*O*-β-D-Xyl*p*			
			α-L-Rha*p*		α-L-Rha*p*	α-L-Rha*p*
1			102.27		101.59	101.62
2			72.38		72.11[c]	72.17[a]
3			71.93		72.11[c]	72.17[a]
4			73.69		73.80	73.90
5			68.95		69.31	69.34
6			18.60		18.40	18.45
OAc	170.52	170.50	170.65			
	169.86	169.87	21.15			
	20.47[a]	21.11[a]				
	20.26	20.77[c]				

Resonances marked with the same letter are mutually interchangeable within a column; with asterisks, ambiguously assigned. The resonance for C-19 in the spectrum of **3** was observed at δ 25-26, the chemical shift of which was omited. Chemical shifts of resonances missing in column 6 were also omited.

Careful separation of total extracted substances from roots of the plant growing in the Republic of Kyrgyzstan isolated nine pure compounds. The PMR and ^{13}C NMR spectra of the isolated compounds indicated they were cycloartane triterpenoids (**1**-**6**), sterols (**7** and **8**), and cyclitols (**9**). The PMR and ^{13}C NMR spectra and a direct comparison with authentic samples identified the isolated compounds as cyclosieversiosides A (**1**), B (**2**), F (**4**), G (**5**), H (**6**), astrasieversianin IX (**3**), β-sitosterol (**7**), β-sitosterol β-D-glycopyranoside (**8**), and D-3-*O*-methyl-*chiro*-inositol (**9**).

Portions of the PMR and ^{13}C NMR spectra of the isolated glycosides that were obtained at low operating frequency were used previously [2-10]. Therefore, we interpreted fully ^{13}C NMR spectra (Table 1) and PMR spectra (see Experimental) of the isolated compounds.

DEPT experiments and literature data on analogous compounds were used to interpret the ^{13}C NMR spectra. It should be noted that the resonance for C-19 is shifted noticeably to high field at δ 25.83 in the ^{13}C NMR spectra of **1**, **3**, **5**, and **6** compared with other cyclosieversigenin glycosides. This resonance was assigned in the spectra of these glycosides in analogy with askendosides B and D, the spectra of which were interpreted by using also heteronuclear correlation spectra [15]. The slight high-field shift of the resonance for C-8 to δ 42.16 in the spectra of **3**, **5**, and **6** is also somewhat unusual.

Although all 16 glycosides were found in the Chinese plant, 8 glycosides, cyclosieversiosides A, B, C, D, E, F, G, and H, were isolated from the Uzbek plant [2-8]. The plant from the Republic of Kyrgyzstan contained 6 cycloartane glycosides (**1**-**6**); β-sitosterol (**7**), β-sitosterol β-D-glucopyranoside (**8**), and D-3-*O*-methyl-*chiro*-inositol (**9**). The last three compounds were observed in this plant for the first time.

It can be seen that the chemical compositions of *A. sieversianus* from the three habitats differ significantly from each other.

EXPERIMENTAL

General comments have been published [16]. We used the following solvent systems: $CHCl_3$:CH_3OH (10:1, 1; 20:1, 4), $CHCl_3$:CH_3OH:H_2O (70:12:1, 2; 70:23:4, 3; 140:14:1, 5), EtOAc:CH_3OH (15:1, 6).

NMR spectra in C_5D_5N (δ, ppm) were recorded on Bruker AM-300 and UNITYplus-400 spectrometers. ^{13}C NMR spectra were obtained with full C–H decoupling and using DEPT. The internal standard in spectra of **7**-**9** was HMDS or TMS. Spectra of other compounds were recorded without an internal standard. Chemical shifts of protons were placed relative to the resonance of residual β-protons of C_5D_5N with a chemical shift of δ 7.19 vs. TMS. Chemical shifts of C atoms in ^{13}C NMR

spectra of these compounds were placed relative the resonance of the β-C atoms of C_5D_5N with a chemical shift of δ 123.493 vs. TMS.

Isolation and Separation of Glycosides from *A. sieversianus*. Air-dried ground roots of *A. sieversianus* (3.6 kg) that were collected on June 9, 2003, near Kara-Jygach, Kara-Kuljin Region, Osh District, Republic of Kyrgyzstan, were extracted exhaustively with CH_3OH (18 L × 5). The CH_3OH extract was evaporated to dryness to produce total extracted substances (490 g, 13.6 mass % of air-dried raw material). TLC using various solvent systems identified nine triterpene and sterol compounds in them.

Total extracted substances (100 g) were chromatographed over a column of silica gel (L) with elution successively by $CHCl_3$ and solvent systems 1-3. Isolated compounds are given in the order of increasing polarity. They were also identified by direct comparison with authentic samples.

β-Sitosterol (7). Identical fractions obtained by elution with $CHCl_3$ were combined and recrystallized from CH_3OH to afford **7** (11 mg, 0.0015%, here and henceforth, yield is calculated for air-dried raw material), mp 136-139°C, identified as β-sitosterol [17]. Mass, PMR, and ^{13}C NMR spectra corresponded with those published [17].

Elution of the column with system 1 isolated **8**, **1**, and **2**. These and subsequent fractions contained a pigment that gave the solutions a pinkish-violet color. Fractions containing pure glycosides were rechromatographed in order to remove the pigment. Compound **8** was rechromatographed over a column by elution with system 4; **1**, system 5; **2**, system 6.

β-Sitosterol β-D-Glucopyranoside (8). Yield 0.01 g, 0.0013%; mp 276-279°C (CH_3OH); identified as β-sitosterol β-D-glucopyranoside [17].

PMR and ^{13}C NMR spectra agreed with those published [17].

Cyclosieversioside A (1). Yield 2.523 g, 0.34%; mp 219-221°C (CH_3OH); identified as cyclosieversioside A [5, 11].

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 0.11 (d, ^2J = 3.9, H-19), 0.53 (d, ^2J = 3.2, H-19), 1.05, 1.18, 1.28, 1.28, 1.36, 1.56, 1.65 (s, $7CH_3$), 1.83 (d, ^3J = 8, H-5), 1.94 (s, CH_3COO on C-3 of D-xylose situated on C-3), 2.02 (s, CH_3COO on C-2 of D-xylose situated on C-3), 2.54 (d, ^3J = 7.7, H-17), 3.10 (q, $^2J = {}^3J_1 = {}^3J_2$ = 9, H-22), 3.30 (dd, $^3J_1$ = 11.5, $^3J_2$ = 4, H-3), 4.76 (d, ^3J = 7.8, H-1 of D-xylose on C-3), 4.84 (d, ^3J = 7.2, H-1 of D-xylose on C-6), 5.03 (m, H-16), 5.41 (dd, $^3J_1$ = 9.3, $^3J_2$ = 8, H-2 of D-xylose on C-3), 5.63 (t, $^3J_1 = {}^3J_2$ = 9.2, H-3 of D-xylose on C-3).

Table 1 gives the ^{13}C NMR spectrum.

Cyclosieversioside B (2). Yield 2.640 g, 0.36%; mp 221-223°C (CH_3OH), identified as cyclosieversioside B [8, 11].

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 0.17 and 0.53 (d, ^2J = 4, 2H-19), 0.90, 1.23, 1.28, 1.28, 1.38, 1.56, 1.76 (s, $7CH_3$), 1.85 (d, ^3J = 8.6, H-5), 1.94 (s, CH_3COO on C-3 of D-xylose), 2.01 (s, CH_3COO on C-2 of D-xylose), 2.49 (d, ^3J = 7.7, H-17), 3.10 (q, $^2J = {}^3J_1 = {}^3J_2$ = 10, H-22), 3.36 (dd, $^3J_1$ = 12, $^3J_2$ = 4, H-3), 4.77 (d, ^3J = 7.8, H-1 of D-xylose), 4.90 (d, ^3J = 7.7, H-1 of D-glucose), 4.96 (m, H-16), 5.42 (dd, $^3J_1$ = 9.6, $^3J_2$ = 7.9, H-2 of D-xylose), 5.62 (t, $^3J_1 = {}^3J_2$ = 9.3, H-3 of D-xylose).

Table 1 gives the ^{13}C NMR spectrum.

Elution of the column with systems 2 and 3 produced fractions containing pure **3**, **4**, **9**, **5**, and **6** in the given order.

Astrasieversianin IX (3). Yield 3.640 g, 0.49%; mp 212-214°C (CH_3OH, identified as astrasieversianin IX [9, 18].

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 0.07 and 0.58 (d, ^2J = 3.6, 2H-19), 1.14, 1.27, 1.27, 1.27, 1.34, 1.56, 1.71 (s, $7CH_3$), 1.66 (d, ^3J = 6, CH_3 of L-rhamnose), 2.05 (s, CH_3COO), 2.57 (d, ^3J = 7.5, H-17), 3.10 (q, $^2J = {}^3J_1 = {}^3J_2$ = 10.7, H-22), 3.32 (dd, $^3J_1$ = 11.9, $^3J_2$ = 4, H-3), 4.73 (d, ^3J = 6.8, H-1 of D-xylose on C-3), 4.78 (d, ^3J = 7.2, H-1 of D-xylose on C-6), 5.63 (t, $^3J_1 = {}^3J_2$ = 8.8, H-3 of D-xylose on C-3), 5.73 (s, H-1 of L-rhamnose).

Table 1 gives the ^{13}C NMR spectrum.

Cyclosieversioside F (4) Yield 0.590 g, 0.08%; mp 255-257°C (CH_3OH), identified as cyclosieversioside F [4, 11].

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 0.17 and 0.60 (d, ^2J = 3.6, 2H-19), 0.91, 1.27, 1.27, 1.34, 1.38, 1.56, 2.06 (s, $7CH_3$), 2.50 (d, ^3J = 7.5, H-17), 3.10 (q, $^2J = {}^3J_1 = {}^3J_2$ = 10, H-22), 3.49 (dd, $^3J_1$ = 11.5, $^3J_2$ = 4, H-3), 4.82 (d, ^3J = 7.1, H-1 of D-xylose), 4.87 (d, ^3J = 7.7, H-1 of D-glucose), 4.96 (q, $^3J_1 = {}^3J_2 = {}^3J_3$ = 7, H-16).

Table 1 gives the ^{13}C NMR spectrum.

D-3-*O*-Methyl-*chiro*-inositol (9). The fraction with **9** was dissolved in $CHCl_3$:CH_3OH (1:1). A compound that crystallized on standing (0.220 g, 0.03%, mp 189-191°C) was identified as D-3-*O*-methyl-*chiro*-inositol [19, 20].

PMR spectrum (200 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = TMS): 3.84 (s, CH_3O), 4.06 (1H, t, $^3J_1 = {}^3J_2$ = 8), 4.52 (1H, t, $^3J_1 = {}^3J_2$ = 8), 4.60-4.70 (4H, m).

^{13}C NMR spectrum (50 MHz, C_5D_5N, δ, ppm, 0 = TMS): 85.78 (d), 74.65 (d), 74.15 (d), 73.71 (d), 73.06 (d), 72.25 (d), 60.71 (q).

Cyclosieversioside G (5). Yield 0.948 g, 0.13%; mp 199-200°C; identified as cyclosieversioside G [6, 11, 18].

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 0.02 (d, ^2J = 4.4, H-19), 0.58 (d, ^2J = 3.6, H-19), 1.15, 1.26, 1.27, 1.33, 1.39, 1.55, 1.75 (s, 7CH_3), 1.71 (d, ^3J = 6, CH_3 of L-rhamnose), 1.81 (d, ^3J = 7.3, H-5), 2.57 (d, ^3J = 7.8, H-17), 3.10 (q, $^2J = {}^3J_1 = {}^3J_2$ = 10, H-22), 3.35 (dd, $^3J_1$ = 11.3, $^3J_2$ = 4, H-3), 4.73 (d, ^3J = 7.7, H-1 of D-xylose), 4.75 (d, ^3J = 7.5, H-1 of D-xylose), 5.04 (q, $^3J_1 = {}^3J_2 = {}^3J_3$ = 7.4, H-16), 6.53 (s, H-1 of L-rhamnose).

Table 1 gives the ^{13}C NMR spectrum.

Cyclosieversioside H (6). Yield 0.590 g, 0.08%; mp 264-266°C (CH_3OH); identified as cyclosieversioside H [7, 11].

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 0.08 and 0.56 (d, ^2J = 4, 2H-19), 1.01, 1.26, 1.27, 1.35, 1.40, 1.56, 1.86 (s, 7CH_3), 1.72 (d, ^3J = 6.2, CH_3 on L-rhamnose), 2.53 (d, ^3J = 7.7, H-17), 3.10 (q, $^2J = {}^3J_1 = {}^3J_2$ = 9, H-22), 3.40 (dd, $^3J_1$ = 11.4, $^3J_2$ = 3.9, H-3), 4.78 (d, ^3J = 7.2, H-1 of D-xylose), 4.85 (d, ^3J = 7.9, H-1 of D-glucose), 5.05 (m, H-16), 6.54 (s, H-1 of L-rhamnose).

Table 1 gives the ^{13}C NMR spectrum.

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