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STRUCTURE OF CYCLOGLOBICEPOSIDE A FROM *Astragalus globiceps*

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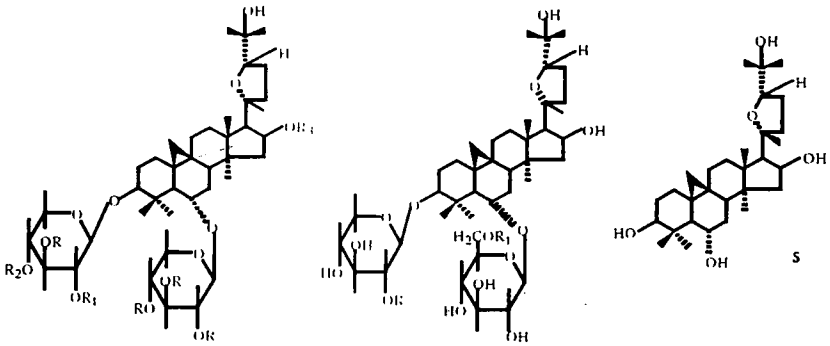
Four compounds of cycloartane nature have been isolated from the roots of Astragalus globiceps *Bunge. Their structures have been established on the basis of spectral characteristics and chemical transformations. Cyclosieversiosides E and F and astrasieversianin III have been described previously. Cycloglobiceposide A has now been investigated for the first time and found to be 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-(2'-O-acetyl-β-D-xylopyranoside) 6-O-(6'-O-acetyl-β-D-glucopyranoside).*

Eleven substances of glycosidic nature have been detected by TLC in a methanolic extract of the roots of *Astragalus globiceps* Bunge (fam. Leguminosae) [1]. By column chromatography of the total glycosides, in addition to known substances — cyclosieversiosides E (**1**) and F (**2**) and astrasieversianin III (**3**) [2-4] — a new glycoside has been isolated that has been called cycloglobiceposide A (**4**).

In the strong-field region of the PMR spectrum of glycoside (**4**) at 0.14 and 0.57 ppm (^2J = 4.2 Hz) there were one-proton doublets split in an AB system and unambiguously assigned to the methylene hydrogen atoms of a cyclopropane ring. This permitted the assumption that glycoside (**4**) belonged to the cycloartane series.

Acid hydrolysis of cycloglobiceposide A (**4**) led to the genin (**5**), identified as cyclosieversigenin [5-7].

The IR spectrum of compound (**4**) contained absorption bands at 1725, 1742, and 1250 cm^{-1}, which are characteristic for an ester group. The ^1H and ^{13}C NMR spectra contained two three-proton singlets at 1.96 and 2.04 ppm and the signals of carbon atoms at 19.73, 20.77 and 169.84, 170.50 ppm. Consequently, cycloglobiceposide A has two acetyl groups.



1. R = R_1 = R_2 = R_3 = H.
3. R = R_3 = H, R_1 = R_2 = Ac.
6. R = R_1 = R_2 = R_3 = Ac.
7. R_3 = H, R = R_1 = R_2 = Ac.

2. R = R_1 = H.
4. R = R_1 = Ac.

5

The alkaline hydrolysis of cycloglobiceposide A led to cyclosieversioside F (**2**), while acid hydrolysis formed cyclosieversigenin (**7**) as the saponification product. In the hydrolysate, D-xylose and D-glucose were detected by TLC in comparison with authentic specimens.

The positions of the acetyl residues were found by a comparative study of the ^{13}C NMR spectra of glycosides (**2**) and (**4**) (Tables 1 and 2).

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TABLE 1. Chemical Shifts (ppm) of the Carbon Nuclei in the ^{13}C NMR Spectra of Cyclosieversioside E (**1**), Cyclosieversioside F (**2**), Astrasieversianin III (**3**), and Its Acetyl Derivatives (**6**) and (**7**), Cycloglobiceposide A (**4**), and Cyclosieversigenin (**5**) in C_5D_5N

C	Compound						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1	34.86	34.66	33.83	33.46	35.16	33.95	34.93
2	26.68	29.03	31.77	27.14	33.01	31.56	31.55
3	88.27	42.67	88.84	88.97	78.53	88.76	88.80
4	42.55	42.67	42.18	42.15	42.69	41.72	41.73
5	52.07	52.56	52.08	52.04	52.20	51.58	51.71
6	78.53	79.20	78.61	78.63	68.57	79.17	79.31
7	34.86	34.92	34.94	34.93	39.08	36.86	34.93
8	44.11	46.24	45.19	44.49	47.50	45.46	45.09
9	21.15	21.13	21.22	21.52	21.19	21.63	21.14
10	28.38	28.89	29.98	29.99	30.11	30.11	29.63
11	26.24	26.49	26.47	26.48	26.51	26.12	26.48
12	33.43	33.41	33.38	33.78	33.65	33.95	34.23
13	45.15	45.08	44.53	45.18	45.28	46.40	46.07
14	46.13	46.24	46.18	46.17	46.41	46.55	46.21
15	45.18	45.75	46.02	44.99	46.99	45.39	45.48
16	73.40	73.41	73.44	73.43	73.69	75.98	73.44
17	58.08	58.23	58.20	58.18	58.64	57.59	58.27
18	20.44	21.13	21.08	20.82	21.87	21.63	21.14
19	30.05	30.23	29.72	29.70	31.70	29.53	30.01
20	87.27	87.27	87.31	87.30	87.48	85.73	87.26
21	27.10	28.60	27.15	28.18	27.38	27.83	27.21
22	31.89	32.24	33.46	31.37	31.21	32.69	33.31
23	26.44	26.20	26.26	26.27	26.69	26.80	26.48
24	81.60	81.69	81.70	81.69	81.93	82.91	81.69
25	71.23	71.29	71.26	71.26	71.48	70.81	71.27
26	28.13	28.21	28.46	28.43	28.44	28.72	28.61
27	28.57	28.60	28.60	28.61	28.81	27.93	28.20
28	28.38	27.10	28.18	27.91	29.67	27.83	27.81
29	16.64	16.65	16.51	16.53	16.38	16.48	16.48
30	20.44	19.87	20.66	20.82	20.47	20.92	20.84
				3-O-β-*D*-Xyl			
1	107.59	107.56	104.33	103.99		103.52	103.50
2	75.57	75.61	72.42	76.84		72.42	72.42
3	78.53	78.15	72.80	75.41		72.69	72.70
4	71.05	71.28	73.44	71.10		69.86	69.86
5	67.04	67.07	63.06	67.03		62.48	62.48
	6-O-β-*D*-Xyl	6-O-β-*D*-Glc	6-O-β-*D*-Xyl	6-O-β-*D*-Glc		6-O-β-*D*-Xyl	6-O-β-*D*-Xyl
1	105.70	105.46	105.66	105.69		101.54	101.76
2	75.37	75.61	75.36	75.41		73.05	73.11
3	77.77	79.31	77.98	77.93		72.31	72.35
4	71.25	71.29	71.12	71.10		69.61	69.78
5	66.95	78.55	67.02	73.10		62.39	62.44
6		63.1		66.73			

The signals of the anomeric carbon atoms in the ^{13}C NMR spectrum of compound (**4**) were present at 103.99 and 105.69 ppm. A comparison of the chemical shifts of these carbon atoms for compounds (**4**) and (**2**) showed that in the first case a diamagnetic shift by 3.57 ppm (see Table 1) had been undergone by the signal of the C-1 atom of xylose. This indicated that an acetyl residue was present in the xylose molecule. The very magnitude of the upfield shift of the signal of the C-1′ carbon showed the position of the acetyl group on the neighboring carbon atom (C-2′). This conclusion was confirmed by a 2.74 ppm upfield shift of the C-3′ signal (see Table 1) [8, 9]. What has been said above enabled us to conclude that one of the acetyl groups in glycoside (**4**) was undoubtedly present at C-2′ of the xylose residue.

The position of the other acetyl group was found in the following way. The signals of the C-5 and C-6 carbon atoms of the β-D-glucose residue in the spectrum of cyclosieversioside F (**2**) appeared in the 78.55 and 63.11 regions (see Table 1). In the spectrum of compound (**4**) the same signals appeared in the 73.10 and 66.73 regions. The changes in the chemical shifts of these carbon atoms of the glucose residue showed that the second acetyl group had substituted the OH group at C-6 of this monosaccharide.

Thus, cycloglobiceposide is 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-(2′-O-acetyl-β-D-xylopyranoside) 6-O-(6′-O-acetyl-β-D-glucopyranoside).

TABLE 2. Chemical Shifts of the Carbon Nuclei in the ^{13}C NMR Spectra of the Acetyl Groups of Compounds (3), (4), (6), and (7) (δ, ppm, 0 — TMS, C_5D_5N)

C	3	4	6	7
>C=O	169.91	169.84	169.66;169,69;169,96	169.65;169.70;170.07
	170.39	170.50	170.07;170.24;170,34	170.07;170.25;170.33
OCH_3	19.74	19.73	19.94;20.54; 20.61	19.82;20.55;20.58
	20.66	20.77	20.84;21.01;26.69; 26.80	20.61;27.21;27.81

EXPERIMENTAL

For thin-layer chromatography (TLC) we used silica gel containing 10% of gypsum and Silufol plates, and for column chromatography type KSK silica gels with particle sizes of 0.1-0.08 mm and 0.16-0.1 mm. The cycloartanes and their derivatives were detected in TLC with a 25% methanolic solution of tungstophosphoric acid followed by heating at 120°C for 5-10 min. IR spectra were taken on a Perkin-Elmer System 2000 FT-IR Fourier spectrometer in KBr tablets. PMR and ^{13}C NMR spectra were taken in C_5D_5N on a Unity-400 Plus instrument (Varian). 0 — TMS.

Paper chromatography was conducted on type FN-11 paper.

The following solvent systems were used: 1) chloroform–methanol–water (70:23:3); 2) (9:1:0.05); 3) butan-1-ol–pyridine–water (6:4:3); and 4) chloroform–methanol (70:1).

Isolation of the Cycloartanes. The air-dry ground roots (1.2 kg) of *Astragalus globiceps* Bunge gathered in May, 1997, in the village of Koplanbek, Sary-Agaskaii region, Chimkent oblast, Republic of Kazakhstan, were extracted with 5 liters of methanol 5 times. The extract was concentrated, the residue was diluted with water, and the resulting precipitate was eliminated. The methanol was distilled off, and the aqueous residue was extracted first with ethyl acetate and then with butanol. After evaporation of the solvents in vacuum, 16.63 g of ethyl acetate fraction and 5.5 g of butanol fraction were obtained.

Separation of the Ethyl Acetate Fraction. The ethyl acetate fraction was chromatographed on a column of silica gel. Elution with system 1 yielded the following compounds: (1) — 2.5 g (0.208%) (here and below the yields are calculated on the air-dry raw material); (2) — 2.0 g (0.16%); (3) — 0.2 g (0.01%); and (4) 1 g (0.08%).

Cyclosieversioside E (1). $C_{40}H_{66}O_{13}$, mp 257-258°C (from methanol).

IR spectrum (KBr, ν, cm^{-1}): 3394 (OH), 2970 (cyclopropane group).

PMR spectrum: 0.15 and 0.60 (each 1H, d, ^2J = 4.3 Hz, 2H-19), 1.11, 1.31 (2 × CH_3), 1.32, 1.40, 1.59, 1.94 (s, each 3H, tertiary methyl groups), 3.45 (1H, dd, J = 11.7 and 4.5 Hz, H-3), 3.80 (1H, ddd, ^3J = 8.4; 8.4 and 5.0 Hz, H-6), 2.52 (1H, d, ^3J = 7.8 Hz, H-17), 3.15 (2H, q, ^3J = 11.0 Hz, H-22), 3.90 (1H, dd, ^3J = 9.2 and 5.2 Hz, H-24), 4.82 and 4.86 (each 1H, d, ^3J = 7.3 and 7.6 Hz, respectively H-1′ and H-1″).

For the ^{13}C NMR spectrum, see Table 1.

According to the literature, for cyclosieversioside E [2]: mp 218-220°C (from methanol); $[\alpha]_D^{20}$ +29.9° (c 0.67; methanol).

Acid Hydrolysis. Substance (1) (100 g) was hydrolyzed in 15 ml of 0.5% methanolic sulfuric acid at 70°C for 4 h. After cooling, 25 ml of water was added to the reaction mixture, and the methanol was distilled off. The precipitate that had formed was filtered off, washed with water, and, after drying, chromatographed on a column of silica gel. Elution with system 2 gave 23 mg of cyclosieversigenin (5), $C_{30}H_{50}O_5$, mp 240-242°C (from methanol).

IR spectrum (KBr, ν, cm^{-1}): 3396 (OH), 3037 (cyclopropane group).

D-Xylose was detected in the hydrolysate, after its neutralization with barium acetate and evaporation, by a PC comparison with authentic specimens in system 3. According to the literature, for cyclosieversigenin [5]: mp 229-231°C (ethyl acetate), $[\alpha]_D^{20}$ +67.1 (c 1.92; methanol).

Cyclosieversioside F (2). $C_{41}H_{68}O_{14}$, mp 260-261°C (from methanol). IR spectrum (KBr, ν, cm^{-1}): 3382 (OH), 2941 (cyclopropane group).

PMR spectrum: 0.21 and 0.60 (each 1H, d ^2J = 4.2 Hz, 2H-19), 0.94, 1.30, 1.31, 1.39, 1.42, 1.60, 2.06 (s, each 3H, tertiary methyl groups), 2.53 (1H, d ^3J = 7.8 Hz, H-17), 3.54 (1H, dd, ^3J = 11.7 and 4.6 Hz, H-3), 3.81 (1H, ddd, ^3J =

8.4, 8.4 and 4.2 Hz, H6), 3.15 (2H, q, J = 11.5 Hz, H-22), 3.90 (1H, dd, ^3J = 9.1 and 6.5 Hz, H-24), 5.05 (1H, q, ^3J = 7.4 Hz, H-16), 4.86 and 4.92 (each 1H, d, ^3J = 7.4 and 7.7 Hz, respectively H-1′ and H-1″).

For the ^{13}C NMR spectrum, see Table 1.

According to the literature, for cyclosieversioside F [3]; mp 247-249°C (from methanol), $[\alpha]_D^{20}$ +36.6° (c 0.47; methanol).

Acid Hydrolysis. Compound (**2**) (100 mg) was hydrolyzed as described above. This gave 17 mg of cyclosieversigenin (**5**). D-Xylose and D-glucose were detected in the hydrolysate by PC in comparison with authentic specimens.

Astrasieversianin III (3). $C_{44}H_{70}O_{15}$, mp 252-254°C (from methanol).

IR spectrum (KBr, ν, cm^{-1}): 3414 (OH), 2972 (cyclopropane group), 1737 (broad band), 1250 (ester group).

PMR spectrum: 0.16 and 0.46 (each 1H, q, ^2J = 4.5 Hz, 2H-19), 1.08, 1.22, 1.31 (2×CH_3), 1.39, 1.59, 1.71 (s, each 3H, tertiary methyl groups), 2.58 (1H, d, ^3J = 7.7 Hz, H-17), 3.13 (2H, q, ^3J = 11.0 Hz, H-22), 3.32 (1H, dd, ^3J = 9.4, 4.5 Hz, H-3), 3.80 (1H, ddd, 8.4, 8.4 and 5.4 Hz, H-6), 3.90 (1H, dd, ^3J = 9.2 and 5.2 Hz, H-24), 1.96 and 2.03 (6H, Hz, 2×OAc), 4.78 and 4.90 (each 1H, d, ^3J = 7.8 and 7.5 Hz, respectively H-1′ and H-1″).

For the ^{13}C NMR spectrum, see Tables 1 and 2.

According to the literature, for astrasieversianin III [4]: mp 250-254°C (from methanol), $[\alpha]_D^{24}$ +15.7° (c 0.49; methanol).

Acid Hydrolysis. Compound (**3**) (100 mg) was hydrolyzed as described above, giving 20 mg of cyclosieversigenin (**5**).

Alkaline Hydrolysis. Compound (**3**) (50 mg) was saponified with 20 ml of a 0.5% methanolic solution of potassium hydroxide. The reaction mixture was left at room temperature for a day and was then diluted with 25 ml of water, and neutralized with acetic acid, after which the methanol was evaporated off and the residue was extracted with butanol. The residue from the distillation of the butanol was chromatographed on a column of silica gel. Elution with system 1 yielded 41 mg of cyclosieversioside E (**1**), mp 257-258°C (from methanol) [2].

Acid Hydrolysis of the Saponification Product from Compound (3). The acid hydrolysis of the saponification product (41 mg) led to 27 mg of genin (**5**). D-Xylose was detected in the hydrolysate by PC in comparison with authentic specimens.

Acetylation of Astrasieversianin III (3). The acetylation of 55 mg of compound (**3**) in 2 ml of pyridine was carried out with 2.0 ml of acetic anhydride at room temperature for 24 h. After elimination of the solvent, the residue was chromatographed on a column of silica gel. Elution with system 4 yielded 20 mg of the heptaacetate (**6**), $C_{54}H_{80}O_{20}$, mp 227-230°C (from methanol). IR spectrum (KBr, ν, cm^{-1}): 3401 (OH), 1750, 1249 (ester group).

PMR spectrum: 0.21 and 0.50 (each 1H, d, ^2J = 4.4 Hz, 2H-19), 0.95, 1.12, 1.33, 1.36, 1.39, 1.41, 1.43 (s, each 3H, tertiary methyl groups), 2.55 (1H, d, ^3J = 8.0 Hz, H-17), 3.32 (1H, dd, ^3J = 11.6 and 4.5 Hz, H-3), 1.98, 1.99, 2.05, 2.06, 2.13, 2.16, 2.22 (7×OAc), 4.92 and 4.96 (each 1H, d, ^3J = 7.4 and 7.1 Hz, respectively H-1′ and H-1″).

For the ^{13}C NMR spectrum, see Tables 1 and 2.

The further elution of the column with the same mixture of solvents led to 15 mg of the hexaacetate (**7**), $C_{52}H_{78}O_{19}$, mp 220-222°C (from methanol), IR spectrum (KBr, ν, cm^{-1}): 3422 (OH), 1756, 1251 (ester group).

PMR spectrum: 0.20 and 0.42 (each 1H d, ^2J = 4.4 Hz, 2H-19), 1.00, 1.11, 1.31-1.32 (3×CH_3), 1.41, 1.60 (s, each 3H, tertiary methyl groups), 2.58 (1H, d, ^3J = 7.7 Hz, H-17), 3.45 (1H, dd, ^3J = 11.4 and 4.6 Hz, H-3), 1.98, 2.00, 2.05, 2.06, 2.16, 2.21 (6×OAc), 4.85 and 4.86 (each 1H, d, ^3J = 7.5 and 7.4 Hz, respectively H-1′ and H-1″).

For the ^{13}C NMR spectrum, see Tables 1 and 2.

Cycloglobiceposide A (4), $C_{45}H_{72}O_{16}$, mp 248-251°C (from methanol), IR spectrum (KBr, ν, cm^{-1}): 3420 (OH), 2980 (cyclopropane group), 1725, 1742, 1250 (ester group).

PMR spectrum: 0.14 and 0.57 (each 1H, d, ^2J = 4.2 Hz, 2H-19), 1.09, 1.21, 1.30, (2×CH_3), 1.39, 1.52, 1.68 (s, each 3H, tertiary methyl groups), 2.58 (1H, d, ^3J = 7.7 Hz, H-17), 3.15 (2H, q, ^3J = 11.0 Hz, H-22), 3.34 (1H, dd, ^3J = 11.7 and 4.6 Hz, H-3), 3.80 (1H, ddd, ^3J = 8.5, 8.5 and 3.4 Hz, H-6), 3.90 (1H, dd, ^3J = 8.7 and 5.4 Hz, H-24), 1.96 and 2.04 (6H, s, 2×OAc), 4.79 and 4.88 (each 1H, d, ^3J = 7.9 and 7.3 Hz, respectively, H-1′ and H-1″).

For the ^{13}C NMR spectrum, see Tables 1 and 2.

Acid Hydrolysis. Compound (**4**) (100 mg) was hydrolyzed as described above, giving 17 mg of cyclosieversigenin (**5**).

Alkaline Hydrolysis. Compound (**4**) (100 mg) was saponified with 25 ml of a 0.5% solution of potassium hydroxide. For the working up of the reaction mixture and isolation of the product, see above. This yielded 73 mg of cyclosieversioside F (**2**), mp 260-261°C (from methanol).

Acid Hydrolysis of the Product of the Saponification of Compound (4). The acid hydrolysis of the saponification product (70 mg) led to the genin (5) (43 mg). In the hydrolysate, D-xylose and D-glucose were detected by PC in comparison with authentic specimens.

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