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TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. LVI. CYCLOPYCNANTHOSIDE* — A NEW CYCLOARTANE GLYCOSIDE†

M. A. Agzamova and M. I. Isaev

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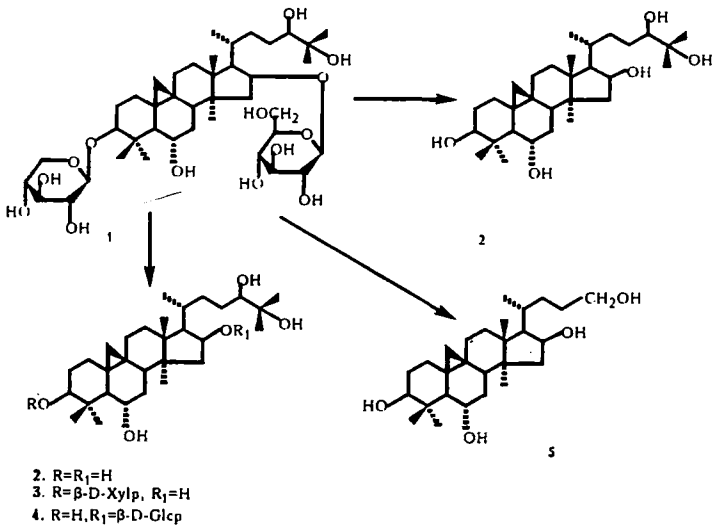
The stems of Astragalus pycnanthus *Boriss. (Leguminosae) contain a number of low-molecular-mass substances, of which β-sitosterol, cyclosieversioside F, and D-3-O-methyl-*chiro*-inositol have been identified, and also a new cycloartane diglycoside — 24R-cycloartane-3β,6α,16β,24,25-pentaol 16-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside.*

Continuing our investigations of cycloartane triterpenoids [1], we have studied the milk vetch *Astragalus pycnanthus* Boriss. (Leguminosae). Compounds of triterpenoid nature have been found in the stems of this plant. In a methanolic extract of the stems we detected by TLC five components, which were designated in order of increasing polarity as substances A, B, C, D, and E. Substances A, C, D, and E were isolated in the individual form.

Components A, C, and E proved to be known compounds and were identified as β-sitosterol [2], cyclosieversioside F [3], and D-3-O-methyl-*chiro*-inositol [4-6], respectively. Substance D was a new glycoside and we have called it cyclopycnanthoside (1). In this paper we present experimental results for establishing the structure of this latter compound.



*Throughout this paper the given Russian name of the compound concerned would correspond to an English "pycanthoside." "Pycnanthoside" agrees better with the name of the source plant — Translator.

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Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 194-200, March-April, 1998. Original article submitted November 10, 1997.

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TABLE 1. Chemical Shifts (δ, ppm), Multiplicities, and SSCCs (J, Hz) of the Protons of Cyclopycnanthoside (1) and Its Derivatives (C_5D_5N, 0 — TMS)

Position of H	Compound				
	1	2	3	4	5
H-3	3.66 dd (11.5; 4.5)	3.66 dd (12; 4)	3.67 dd (11.7; 4.4)	3.68 dd (11.6; 4.6)	3.70 dd (11.4; 4.5)
H-6	3.70 td (9.2; 3.8)	3.80 td (9; 3)	3.80 td (9; 4)	3.74 td (9; 3)	3.84 td (9.5; 2.8)
H-16	4.41 m*	4.70 td (7; 6)	4.73 td (8; 4.7)	4.41 td (7.9; 4.7)	4.70 td (7.3; 4.8)
2H-19	0.28; 0.50 d (4)	0.33; 0.61 d (4)	0.29; 0.58 d (4)	0.34; 0.54 d (4)	0.34; 0.65 m (4)
H-24	3.73 dd (10; 1.7)	3.77 dd (10.3; 2)	3.82 dd (10.3; 2.2)	3.73 dd (10.2; 1.5)	3.95 m (2H)
CH_3 groups	0.98 s, 1.02 d (6.8), 1.23 s, 1.37 s, 1.46 s, 1.51 s, 2.03 s	1.04 s, 1.11 d (6.5) 1.35 s, 1.41 s, 1.47 s, 1.49 s, 1.87 s	1.04 s, 1.13 d (6.5), 1.36 s, 1.42 s, 1.51 s, 1.53 s, 2.02 d	0.99 s, 1.02 d (6.6), 1.23 s, 1.37 s, 1.46 s, 1.51 s, 2.03 s	1.07 s, 1.09 d (6.8), 1.40 s, 1.45 s, 1.93 s
			β-D-Xyl*p* residue		
1	4.93 d (7.3)		4.91 d (7.4)		
2	4.09 dd (9; 7.3)		4.09 dd (8.7; 7.4)		
3	4.18 t (9)		4.18 t (8.7)		
4	4.29 td (9; 5)		4.26 ddd (10; 8.6;5)		
5a	3.74 dd (12; 9)		3.74 dd (11; 10)		
5e	4.38 dd (12; 5)		4.38 dd (11; 5)		
			β-D-Glc*p* residue		
1	4.88 d (7.8)			4.85 d (7.9)	
2	4.00 dd (9; 7.8)			4.01 dd (8.9; 7.9)	
3	4.29 t (9)			4.29 t (9)	
4	4.22 t (9)			4.23 t (9)	
5	3.88 ddd (9; 4.8; 2.7)			3.89 ddd (9; 4.8; 2.7)	
6	4.36 dd (11.6; 4.8)			4.37 dd (11.6; 4.8)	
6"	4.45 dd (11.6; 2.7)			4.46 dd (11.6; 2.7)	

*The signal partially overlaps the H-6′ signal on the one side and the H-5e signal on the other.

TABLE 2. Chemical Shifts of the Carbon Atoms of Compounds (**1-5**) (δ, ppm, C_5D_5N, 0 — TMS)

C atom	Compound				
	1	2	3	4	5
1	32.57	32.81	32.50	32.81	32.81
2	29.26	31.45	29.25	31.50	31.49
3	88.79	78.37	88.73	78.36	78.36
4	42.74	42.44	42.70	42.47	42.48
5	54.05	54.01	54.13	53.92	54.02
6	67.94	68.31	67.93	68.28	68.30
7	38.42	38.62	38.44	38.58	38.66
8	46.86[a]	47.24	47.03	47.06	47.23
9	21.29	21.31	21.37	21.22	21.30
10	30.41	30.37	30.34	30.65	30.37
11	26.25	26.40	26.33	26.35	26.37
12	32.79	33.23	33.19	32.58	33.21
13	45.60	45.74	45.72	45.60	45.73
14	46.86[a]	46.98	46.93	46.88	46.88
15	47.77	48.81	48.73	47.85	48.73
16	83.10	71.78	71.74	83.13	71.74
17	57.52	57.27	57.24	57.54	57.16
18	18.06	18.81	18.77	18.08	18.48
19	30.33[b]	29.36	30.01	29.59	29.37
20	31.97	31.64	31.63	31.98	30.01
21	19.06	19.09	18.97	19.19	19.08
22	30.33[b]	29.62	29.39	30.34	29.60
23	34.40	34.84	34.84	34.41	32.66
24	79.99	80.59	80.58	79.99	61.76
25	72.73	72.69	72.68	72.73	-
26	25.40	25.90	25.89	25.39	-
27	26.32	26.20	26.16	26.30	-
28	20.19	20.30	20.22	20.26	20.26
29	28.95	29.37	28.87	29.44	29.40
30	16.76	16.13	16.69	16.23	16.18
			β-D-Xyl*p* residue		
1	107.67		107.60		
2	75.68		75.62		
3	78.57		78.50		
4	71.29		71.25		
5	67.10		67.04		
			β-D-Glc*p* residue		
1	106.64			106.65	
2	75.81			75.82	
3	78.84			78.85	
4	71.78			71.78	
5	78.12			78.13	
6	62.89			62.91	

*The signals marked with an asterisk [sic] have been assigned arbitrarily, and those marked with the same letters are superposed on one another.

A consideration of the ^1H NMR spectrum of the new glycoside (**1**), containing one-proton doublets of an *AB* system at 0.28 and 0.50 ppm with a characteristic SSCC of ^2J = 4 Hz and also the signals of seven methyl groups in the high-field region (Table 1), permitted us to assign the compound under study to the cycloartane series [3, 7]. In agreement with this, from the products of the acid hydrolysis of cyclopycnanthoside we isolated the genin (**2**), identical with cycloasgenin C [8].

According to the results of PC, the carbohydrate part of the products of the acid hydrolysis of glycoside (**1**) contained *D*-glucose and *D*-xylose. The ^1H and ^{13}C NMR spectra (Tables 1 and 2), containing signals of the protons and carbon atoms of one *D*-glucose and one *D* xylose residue, showed that cyclopycnanthoside was a bioside of cycloasgenin C.

The Smith degradation [9] of glycoside (**1**) led to the tetraol (**5**), which we have described previously [10]. As we have shown [11-14], the formation of the norproduct (**5**) demonstrates glycosylation of the hydroxy group at C-16 of the genin.

In actual fact, a comparative analysis of the ^{13}C NMR spectra of cycloasgenin C and cyclopycnanthoside showed that the two carbinol carbon atoms of the genin moiety at C-3 and C-16 had experienced a glycosylation effect and resonated at 88.79 and 83.10 ppm, respectively.

Partial acid hydrolysis of cyclopycnanthoside led to the formation of the genin (**2**) and two progenins. Progenin (**3**) was identified as cycloasgenin C 3-O-β-xylopyranoside. Consequently, the other progenin, (**4**), contained a *D*-glucose residue

attached to the genin through the hydroxy group at C-16. This result was confirmed by Smith degradation of glycoside (**4**), leading to the formation of tetraol (**5**), and ^{13}C NMR spectra the same as product (**4**), indicating on glycosylation nucleus C-16. The chemical shifts and SSCCs of the protons of the *D*-glucose residue, and also the ^{1}H and ^{13}C NMR chemical shifts of (**1**) and (**4**), showed the pyranose form, the $^{4}C_{1}$ conformation, and the β-configuration of this hexose.

Thus, the experimental facts given enable us to arrive at the unambiguous conclusion that cyclopycnanthoside has the structure 24R-cycloartane-3β,6α,16β,24,25-pentaol 16-O-β-*D*-glucopyroside 3-O-β-*D*-xylopyranoside.

EXPERIMENTAL

For **general observations**, see [15]. The following solvent systems were used: *n*-butyl alcohol – pyridine – water (6:4:3); 2) chloroform – methanol (15:1); 3) chloroform – methanol – water (70:23:4); 4) benzene – chloroform – ethyl acetate (6:1:1); 5) chloroform – methanol – water (70:12:1).

The conditions for TLC and CC are given in [15]. The descending variant of paper chromatography FN-11 paper was conducted in system 1. On PC the monosaccharides were detected by spraying with aniline phthalate and then heating at 100-110°C for 5-10 min. ^{1}H and ^{13}C NMR spectra were taken on Bruker AM 400 and UNITY plus 400 spectrometers in deuteropyridine with protons and also under J-modulation conditions.

Isolation and Separation of the Triterpenoids of *Astraglus pycnanthus* Boriss. The ground air-dry stems (200 g) of *Astragalus pycnanthus*, gathered on November 20, 1992 in the Shakhristan gorge, Zerafshanskii range, at its outlet to the R. Zerafshan, were exhaustively extracted with methanol (5 × 0.5 liter). After evaporation of the extracts and amalgamation of the residues, 33.66 g of dry methanolic extract was obtained. After homogenization with silica gel, the whole of the extract was transferred to a column of silica gel, which was eluted successively with chloroform and systems 2 and 3. When the column was washed with chloroform, a fraction containing substance A was collected.

On continuing elution of the column, with system 2, fractions containing substance B, contaminated with pigments, were obtained. Elution of the column with system 3 permitted the collection of fractions containing the individual substances C, D, and E.

β-Sitosterol. The fraction containing substance A was rechromatographed on a column in system 4. This led to the isolation of 15 mg (0.0075%; here and below the yields are calculated on the air-dry raw material) of substance A with mp 131-132°C (from MeOH), $[\alpha]_D^{22}$ −37.5 ± 2° (*c* 0.9; $CHCl_3$), identified as β-sitosterol [2] by its mass spectrum, as well.

Cyclosieversioside F. The recrystallization of substance C from methanol gave 2.5 g (1.25%) of a product with mp 284-286°C, $[\alpha]_D^{24}$ +40 ± 2° (*c* 0.6; MeOH), identical with cyclosieversioside F [3]. For its ^{13}C spectrum, see [16].

Cyclopycnanthoside (1). By recrystallization from methanol, the fractions containing substance D yielded 700 mg (0.35%) of cyclopycnanthoside (**1**), $C_{41}H_{70}O_{14}$, mp 280-282°C $[\alpha]_D^{19}$ +17.5 ± 2°C (*c* 0.6; MeOH). IR spectrum (KBr, ν, cm^{-1}): 3540-3230 (OH), 3040 (CH_2 of a cyclopropane ring). For the ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

D-3-O-Methyl-*chiro*-inositol. Substance E was recrystallized from methanol, giving 250 mg (0.125%) of a compound with mp 185-186°C, $[\alpha]_D^{20}$ +65 ± 2° (*c* 0.6, H_2O), identical with D-3-O-methyl-*chiro*-inositol [4-6]. For its ^{1}H and ^{13}C NMR spectra, see [4].

Cycloasgenin C (2) from (1). Cyclopycnanthoside (90 mg) was hydrolyzed with 15 ml of a 0.25% methanolic solution of sulfuric acid at the boiling point of the reaction mixture for 12 h. After the usual work-up, by chromatography on a column in system 2 the genin fraction of the hydrolysis products yielded 12 mg of the genin (**2**), mp 244-246°C (from Me_2CO) $[\alpha]_D^{20}$ +34 ± 2° (*c* 0.9, MeOH), identified as cycloasgenin C [8]. For its ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

The aqueous solution of monosaccharides obtained after the removal of the genin was evaporated to a volume of 10 ml and boiled. The reaction mixture was then neutralized with ARA-8p anion-exchange resin and was evaporated. The residue was chromatographed on paper in system 1 in the presence of authentic specimens of monosaccharides. *D*-Glucose and *D*-xylose were identified by PC in the carbohydrate fraction of the eluate. The ^{1}H and ^{13}C NMR spectra of cyclopycnanthoside showed that the molecule of the new glycoside (**1**) contained one residue of each of the monosaccharides mentioned.

Smith Degradation of Cyclopycnanthoside. A solution of 200 mg of sodium periodate in 2 ml of water was added to a solution of 100 mg of glycoside (**1**) in 15 ml of methanol, and the reaction mixture was stirred at room temperature for 4 h. The excess of sodium periodate was destroyed by the addition of a few drops of ethylene glycol and then the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water and evaporated, and the residue was dissolved in 20 ml of methanol. This solution was treated with 200 mg of sodium tetrahydroborate in small

portions and the mixture was left at the same temperature [sic] for 2 h, after which it was acidified by the addition of 0.5 ml of concentrated sulfuric acid.

The acid reaction mixture was left for a day at room temperature and was then diluted with water and treated with chloroform. The residue from evaporation of the chloroform was chromatographed on a column in system 2. This yielded 18 mg of the tetraol (**5**), $C_{27}H_{46}O_4$, mp 192-194°C (from EtOH), $[\alpha]_D^{20}$ +43 ± 2° (*c* 0.7; MeOH), identical with the analogous norproduct described in the literature [10, 13, 14]. For the ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

Partial Hydrolysis of Cyclopycnanthoside. Glycoside (**1**) (300 mg) was hydrolyzed with 40 ml of a 0.25% methanolic solution of sulfuric acid at 60°C for 8 h, after which the reaction mixture was poured into water and extracted with *n*-butyl alcohol. The butanolic extract was washed with water and evaporated. The residue was chromatographed on a column, with elution by system 2. This led to the isolation of 20 mg of cycloasgenin C (**2**), mp 244-246°C (from Me_2CO).

On continuing elution of the column with system 5, 9 mg of progenin (**3**) and 140 mg of progenin (**4**) were isolated.

24R-Cycloartane-3β,6α,16β,24,25-pentaol 3-O-β-*D*-Xylopyranoside (3). Progenin (**3**), mp 252-254°C (from MeOH) was identified as cycloasgenin C 3-O-β-*D*-xylopyranoside. For its ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

24R-Cycloartane-3β,6α,16β,24,25-pentaol 16-O-β-*D*-Glucopyranoside (4). Progenin (**4**), $C_{36}H_{62}O_{10}$, mp 282-284° (from MeOH), $[\alpha]_D^{20}$ +21.5 ± 2° (*c* 0.8; MeOH), is a new glycoside of cycloasgenin C, containing a β-*D*-glucose residue at C-16. IR spectrum (KBr, ν, cm^{-1}): 3530-3180 (OH), 3045 (CH_2 of a cyclopropane ring). For its ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

25-Norcycloartane-3β,6α,16β,24-tetraol (5) from (4). A solution of 150 mg of sodium periodate in 2 ml of water was added to 70 mg of glycoside (**4**) in 10 ml of methanol, and the reaction mixture was stirred at room temperature for 4 h, after which the excess of sodium periodate was destroyed with a few drops of ethylene glycol. Then the mixture was diluted with water and extracted with chloroform. The residue from evaporation of the chloroform was dissolved in 15 ml of methanol and this solution was treated with 150 mg of sodium tetrahydroborate in small portions and was then left at the same temperature for 2 h. After this, it was acidified by the addition of 0.5 ml of concentrated sulfuric acid.

The acid solution was left at room temperature for 12 h. After the usual work-up and chromatography of the reaction products on a column in system 2, 12 mg of the norproduct (**5**) was isolated, with mp 192-194°C (from EtOH), identical with the tetraol obtained from cyclopycnanthoside. For its ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

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