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TRITERPENE GLYCOSIDES OF *Tragacantha* AND THEIR GENINS. CYCLOSTIPULOSIDES A AND B FROM *Tragacantha stipulosa*

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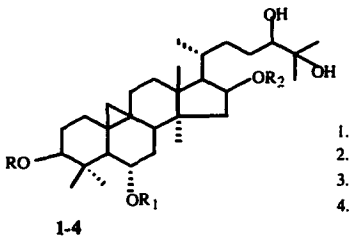
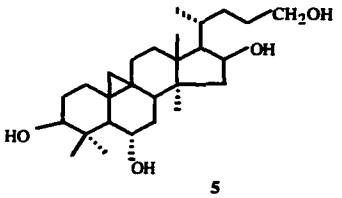
Two new triterpene glycosides of the cycloartane series, which have been called cyclostipulosides A and B, have been isolated in the individual form from the roots of Tragacantha stipulosa *Boviss. Their structures have been established by physicochemical methods. Cyclostipuloside A is 24R-cycloartane-3β,6α,16β,24,25-pentaol 16-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside, and cyclostipuloside B is 24R-cycloartane-3β,6α,16β,24,25-pentaol 6-O-α-L-arabinopyranoside 16-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside. By the acid hydrolysis of cyclostipulosides A and B we have obtained the new glycoside 24R-cycloartane-3β,6α,16β, 24,25-pentaol 16-O-β-D-glucopyranoside.*

Extending the circle of plants of the Leguminosae family containing triterpene glycosides of the cycloartane series that have been studied, we have investigated the roots of *Tragacantha stipulosa* Boviss [1] growing in the Baisunskii region of the Surkhandar'inskaya oblast. From a methanolic extract of the roots of this plant we have isolated two new glycosides of the cycloartane series — cyclostipulosides A (**1**) and B (**4**)[2].



1-4

1. R=β -*D*-Xylp; R_1=H; R_2=β-*D*-Glcp
2. R=R_1=R_2=H
3. R=R_1=H; R_2 =β-*D*-Glcp
4. R=β-*D*-Xylp; R_1= α-*L*-Ara; R_2=β=*D*-Glcp



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In the strong-field region of the PMR spectrum of cyclostipuloside A (**1**) there are the signals of the protons of seven methyl groups, and also one-proton doublets of an *AB* splitting system at 0.29 and 0.50 ppm with ^2J = 4.2 Hz, which are characteristic for the protons of the methylene group of a cyclopropane ring. The presence of a three-membered ring was also confirmed by an absorption band at 2969 cm^{-1} in the IR spectrum of glycoside (**1**).

The acid hydrolysis of cyclostipuloside A led to a genin that was identified by its spectral characteristics and literature information as cycloasgenin C (**2**) [3, 4]. In the hydrolysates *D*-xylose and *D*-glucose were identified by PC. The PMR and ^{13}C NMR spectra also showed that the cyclostipuloside A molecule contained the two above-mentioned residues and was, therefore, a bioside (Table 1).

In the ^{13}C NMR spectrum of cycloasgenin C (**2**) the signals from the C-3, C-6, and C-16 carbon atoms, which bear hydroxy groups, were found at 78.84, 68.25, and 71.77 ppm, respectively. In the spectrum of cyclostipuloside A (**1**) the signals of these carbon atoms were located at 88.81, 67.96, and 83.12 ppm. Consequently, a glycosylation effect was experienced only by the atoms C-3 (+9.97 ppm) and C-16 (+11.35 ppm), while the chemical shift of the C-6 signals remained unchanged.

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TABLE 1. Chemical Shifts of the Carbon Atoms of Cyclostipuloside A (**1**), Cycloasgenin C (**2**), the Monoside (**3**)

C atom	Compound				
	1	**2**	**3**	**4**	**5**
1	32.81	32.81	32.81	32.82	32.81
2	31.93	31.48	31.48	30.23	30.38
3	88.81	78.84	78.84	88.55	78.38
4	42.75	42.43	42.43	42.77	42.47
5	54.07	53.88	53.88	54.02	54.03
6	67.96	68.25	68.25	79.96	68.32
7	38.42	38.56	38.56	38.35	38.65
8	47.88	47.02	47.02	46.82	47.24
9	21.20	21.17	21.17	21.27	21.37
10	29.27	29.55	29.55	29.23	29.41
11	26.26	26.26	26.26	26.26	86.38
12	34.42	34.37	34.37	34.39	33.24
13	45.62	45.55	45.56	45.61	45.75
14	46.88	46.90	46.84	46.84	46.90
15	47.79	47.82	47.82	47.75	48.74
16	83.12	71.77	83.10	83.06	71.77
17	57.54	57.18	57.50	57.52	57.18
18	18.07	18.08	18.08	18.05	18.47
19	30.33	30.30	30.30	30.39	31.47
20	32.58	31.96	31.96	31.92	31.82
21	19.07	19.18	19.18	19.04	19.07
22	30.33	30.63	30.63	30.29	30.03
23	34.42	34.37	34.37	32.58	32.68
24	80.01	79.96	79.96	79.78	61.80
25	72.73	72.70	72.70	72.72	-
26	25.41	25.42	25.36	25.42	-
27	26.33	26.39	26.39	26.20	-
28	20.21	20.25	20.25	20.18	20.26
29	28.95	29.44	29.44	28.69	29.41
30	16.77	16.23	16.23	16.39	16.18
			Xyl		
1	107.82			106.55	
2	75.83			75.77	
3	78.58			77.62	
4	71.82			70.98	
5	67.11			66.59	
			Glc		
1	106.65		106.67	106.60	
2	75.69		75.80	75.77	
3	78.86		78.80	78.78	
4	71.31		71.70	71.83	
5	78.13		78.13	78.02	
6	62.92		62.85	62.91	
			Ara		
1				105.61	
2				73.61	
3				74.31	
4				69.11	
5				66.98	

In addition to the genin, the monoside (**3**) was isolated from the hydrolysis products. Acid hydrolysis of the monoside (**3**) gave cycloasgenin C (**2**). *D*-Glucose was identified in the hydrolysate by PC. The structure of monoside (**3**) was shown completely by an investigation of PMR and ^{13}C NMR spectra. A comparison of the ^{13}C NMR spectra of the monoside (**3**) and of cycloasgenin C (**2**) established that the C-16 signals had undergone a paramagnetic shift of 11.33 ppm in comparison with the genin (Table 1).

Thus, monoside (3) has the structure of 24R-cycloartane-3β,6α,16β,24,25-pentaol 16-O-β-*D*-glucopyranoside and is a new glycoside, obtained by the acid hydrolysis of cyclostipuloside A (1). Consequently, in the cyclostipuloside A molecule of the xylose residue is attached to the C-3 hydroxyl group.

In the region of resonance of anomeric carbon atoms in the ^{13}C NMR spectrum of cyclostipuloside A (1) there were two signals, at 107.82 and 106.55 ppm, showing the presence of two monosaccharide residues. The configurations of the glycosidic centers were determined from the H′ and H″ SSCCs (7.8 and 7.3 Hz), according to which the monosaccharide residues each had the β-configuration, the C1-conformation, and the pyranose form.

Thus, cyclostipuloside A has the structure of 24R-cycloartane-3β,6α,16β, 24,25-pentaol 16-O-β-*D*-glucopyranoside 3-O-β-*D*-xylopyranoside [5].

In the PMR spectrum of cyclostipuloside B, again there were the signals of two protons characteristic for the methylene group of a cycloartane compound, at 0.32 and 0.55 ppm, with $^{2}J = 4.1$ Hz. In the ^{13}C NMR spectrum of cyclostipuloside B (4) there were three signals in the region of resonance of anomeric carbon atoms, and, consequently, it was a trioside (see Table 1).

Partial acid hydrolysis of cyclostipuloside B gave cycloasgenin C (2), the monoside (3), and cyclostipuloside A (1). In the hydrolysate, by PC in comparison with authentic specimens we detected *D*-xylose, *L*-arabinose, and *D*-glucose. Consequently, the *D*-glucose and *D*-xylose residues were attached to the hydroxyl groups at C-3 and C-16, respectively.

In the ^{13}C NMR spectrum of cyclostipuloside B (4), the signal of the C-6 carbon atom was located at 79.96 ppm and, in comparison with the corresponding signal in the spectrum of cycloasgenin C (2) (68.25 ppm), it had shifted downfield by 11.71 ppm. Taken together, all these facts unambiguously show the attachment of the α-*L*-arabinose residue to the hydroxy group at C-6. The Smith oxidation of glycoside (4) [6] led to the tetraol (5), the structure of which agreed with 3β,6α,16β,24-tetrahydroxy-25-norcycloartane [7].

Thus, cyclostipuloside B is 24R-cycloartane-3β,6α,16β,24,25-pentaol 6-O-α-*L*-arabinopyranoside 16-O-β-*D*-glucopyranoside 3-O-β-*D*-xylopyranoside.

EXPERIMENTAL

General Observations. For column and thin-layer chromatography, we used chloroform—methanol—water in ratios of 1) 70:23:3, and 2) 40:7.5:1, and, for paper chromatography, butanol—chloroform—water (6:4:3).

Isolation of the Cycloartanes. Roots (3.5 kg) of *Tragacantha stipulosa* (fam. Leguminosae) were gathered in October, 1995, in the Baisunskii region of the Sukhandar'inskaya oblast. The air-dry comminuted raw material was extracted with 11 liters of methanol (5 times), and the extract was evaporated to dryness, giving a total of 268.48 g of extractive substances. Of this total, 100 g was chromatographed on a column of silica gel. When the column was eluted with system 1, a series of substances of glycosidic nature was obtained.

Cyclostipuloside A (1). We isolated 1.5 g of compound (1) with the composition $C_{41}H_{70}O_{14}$, mp 278—280°C (from methanol). IR spectrum (KBr, ν, cm^{-1}): 3382 (OH), 2969 (cyclopropane group).

PMR spectrum: 0.97, 1.00, 1.02 (d, ^{3}J=6.6Hz), 1.23, 1.37 ,1.47, 1.52, 2.04 (s, each 3H, tertiary methyl groups), 0.29 and 0.50 (each 1H, d, ^{2}J=4.2Hz, 2H-19), 3.67(1H, dd, ^{3}J=11.4 and 4.5Hz, H-3), 3.71(1H, td, ^{3}J=9.5 and 4.0Hz,H-6), 3.75(1H, dd, ^{3}J=10.0 and 2.4Hz, H-24), 4.93 (1H, d, ^{3}J=7.3Hz, H-1′ of xylose), 4.82(1H, d, ^{3}J=7.8Hz, H-1′ of glucose).

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

Acid Hydrolysis. Cyclostipuloside A (1) (200 mg) was hydrolyzed in 30 ml of 0.25% methanolic sulfuric acid with heating on the boiling water bath for 10 h. After cooling, 30 ml of water was added to the reaction mixture and the methanol was distilled off. The precipitate that had deposited was filtered off, washed with water, and chromatographed on a column of silica gel. Using system 2, we obtained 15 mg of the genin (2) with the composition $C_{30}H_{52}O_5$, mp 243—245°C (from methanol), identified by its spectral characteristics and literature information as cycloasgenin C [3, 4] (see Table 1). IR spectrum (KBr, ν, cm^{-1})): 3355 (OH), 2992 (cyclopropane group).

PMR spectrum: 0.99, 1.02, 1.03 (d, ^{3}J = 6.6 Hz), 1.25, 1.40, 1.47, 1.53, 1.94 (s, each 3H, tertiary methyl groups), 0.32 and 0.55 (each 1H, d, ^{2}J = 4.1 Hz). Further elution of the column with the same system led to the isolation of 54 mg of the monoside (3), $C_{36}H_{62}O_{10}$, mp 280—282°C (from methanol). IR spectrum (KBr, ν, cm^{-1}): 3366 (OH), 2996 (cyclopropane group).

PMR spectrum: 0.99, 1.02, 1.03 (d, ^{3}J=6.6Hz), 1.25, 1.40, 1.47, 1.53, 1.94 (s, each 3H, tertiary methyl groups), 0.32 and

0.55 (each 1H, d, ^{2}J=4.1Hz), 3.70(1H, m, H-3), 3.75(1H, m, H-6), 4.41(1H, td, ^{3}J=8.0 and 5.0Hz, H-16), 3.67(1H, m, H-24), 4.87(1H, d, ^{3}J=7.8Hz, H-1' of glucose). For the ^{13}C NMR spectrum, see Table 1.

The aqueous part of the hydrolysate was neutralized with barium carbonate, the filtrate was concentrated in a rotary evaporator, and *D*-xylose and *D*-glucose were detected by PC in system 3.

Hydrolysis of the Monoside (3). The monoside (3) (30 mg) was hydrolyzed under the conditions given above, and 8 mg of the genin (2) was isolated. *D*-Glucose was identified in the aqueous part of the hydrolysate by PC in system 3.

Cyclostipuloside B (4). Compound (4), $C_{46}H_{78}O_{18}$, mp 208—210°C (from methanol) was isolated in an amount of 4.5g.

IR spectrum (KBr, ν,cm^{-1}): 3391 (OH), 2972 (cyclopropane group).

PMR spectrum: 1.01, 1.04, 1.06 (d, ^{3}J=4.1Hz), 1.27, 1.49(2xCH$_3$), 1.54, 2.04(s, each 3H, tertiary methyl groups), 0.32 and 0.55(each 1H, d, ^{2}J=4.1Hz, H-19), 3.65(1H, dd, ^{3}J=12.0 and 4.5Hz, H-3), 3.78(1H, td, ^{3}J=10.3 and 5.6Hz, H-6), 3.77(1H, dd, ^{3}J=11.9 and 2.0Hz, H-24), 4.88(1H, d, ^{3}J=7.8Hz, H-1' of xylose), 4.99(1H, d, ^{3}J=7.0Hz, H-1' of glycose), 5.28(1H, d, J=6.7Hz, H-1' of arabinose). For the ^{13}C spectra, see Table 1.

Acid Hydrolysis. Cyclostipuloside B (4) (700 mg) was hydrolyzed as described above. In the aqueous part of the hydrolysate *D*-xylose, *L*-arabinose, and *D*-glucose were detected by PC in system 3.

The genin part of the fraction was chromatographed on a column with elution by system 2. This gave 35 mg of cycloasgenin C (2). Further elution of the column with the same system led to the isolation of 97 mg of the monoside (3), $C_{36}H_{62}O_{10}$, mp 280—282°C (from methanol).

On continuing the washing of the column with system 2, we obtained 150 mg of a progenin with mp 278—280°C (from methanol), identified from its physicochemical constants and spectral characteristics, and also through direct comparison by TLC in system 1, as cyclostipuloside A (1).

Smith Degradation. A solution of 1 g of cyclostipuloside B (4) in 200 ml of methanol—dioxan (1:1) was treated with 0.4 g of sodium periodate, and the reaction mixture was kept in a dark place at room temperature for 10 h. Monitoring by TLC in system 1.

The unconsumed oxidant was decomposed with ethylene glycol (3 ml) and the reaction mixture was evaporated. The residue was treated with 100 ml of water, and the reaction product was extracted with chloroform. The chloroform was distilled off to dryness, the residue was dissolved in 200 ml of aqueous methanol (1:1), and the solution was treated with 3.5 g of sodium tetrahydroborate in portions, with constant stirring. The reaction mixture was left at room temperature for 20 h (monitoring by TLC in system 2) and was then acidified with sulfuric acid to pH 2.0 and was left for a day. After the addition of 100 ml of water, the methanol was distilled off and the reaction product was extracted with chloroform. The chloroform fraction was washed with water several times and was evaporated. The residue was chromatographed on a column of silica gel. By elution with system 2 we isolated 200 mg of the tetraol (5), composition $C_{27}H_{46}O_4$, mp 191—193°C (from methanol). IR spectrum (KBr, ν, cm^{-1}): 3378 (OH), 2970 (cyclopropane group).

PMR spectrum: 1.06, 1.08, 1.09 (d, ^{3}J=6.6Hz), 1.39, 1.44, 1.92 (s, each 3H,tertiary methyl groups), 0.35 and 0.61(each 1H, d, ^{2}J=4.3 and 3.6Hz, 2H-19), 3.70(1H, dd, J=11.1 and 5.0Hz, H-3), 3.82(1H, m, H-6), 4.70 (1H, m, H-16), 3.94 (1H, m, H-24). For the ^{13}C NMR spectra, see Table 1.

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