TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XXXV. CYCLOARALOSIDE C FROM Astragalus amarus

M. I. Isaev and N. K. Abubakirov

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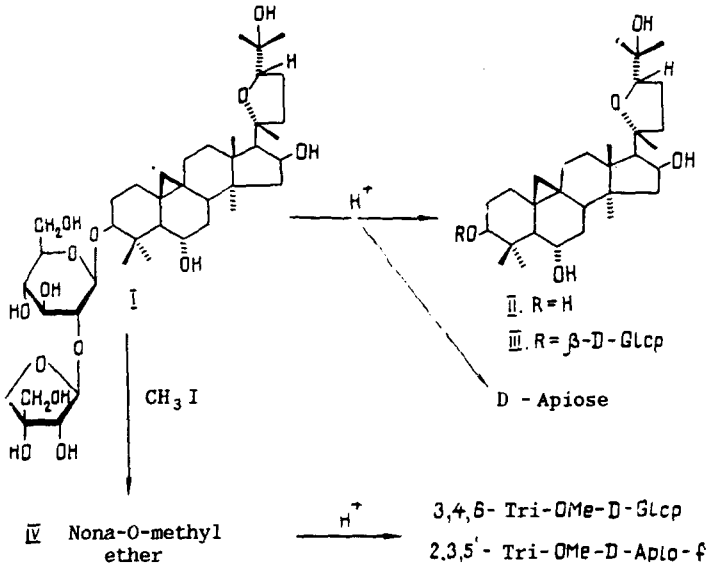
A new triterpene glycoside of the cycloartane series (cycloaraloside C) has been isolated from the roots of the plant Astragalus amarus Pall. (Leguminosae). Cycloaraloside C is a bioside of cyclosieversigenin including one D-glucose residue and one D-apiose residue. The structure of the glycoside has been shown on the basis of the chemical transformations and spectral characteristics as 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-[O-(D-apio-β-D-furanosyl)-(1 → 2)-β-D-glucopyranoside]. This is the first time that D-apiose has been found among cycloartane glycosides.

Continuing a study of the triterpenoids of Astragalus amarus Pall. (Leguminosae), we have established the structure of substance 6 [1], which we have called cycloaraloside C (I).

The observation in the PMR spectrum of glycoside (I) of the one-proton doublets of an AB system at 0.21 and 0.53 ppm, which are characteristic for protons of a 1,1,2,2-tetrasubstituted cyclopropane ring and also of the signals of seven methyl groups, permitted the compound under consideration to be assigned to the triterpenoids of the cycloartane series [2, 3]. This was confirmed by the formation of the cyclosieversigenin (II) on the acid hydrolysis of cycloaraloside C.



D-Glucose and D-apiose were identified in the carbohydrate fraction of an acid hydrolysate of glycoside (I) by PC, TLC, and GLC [4]. GLC [4] showed that glycoside (I) contained the above-mentioned monosaccharides in a ratio of 1:1. This conclusion was in full harmony with the ^{13}C and ^{1}H NMR spectra of cycloaraloside C where the signals of two anomeric carbon atoms could clearly be seen at 105.47 and 111.15 ppm (Table 1), as could the signals of two anomeric protons at 5.17 and 6.58 ppm, respectively.

From the products of the patial hydrolysis of cycloaraloside C was isolated a progenin (III) which was identified as cycloaraloside A [5], and also D-apiose.

Institute of Chemistry of Plant Substances, Academy of Sciences of the Uzbek, Tashkent.
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TABLE 1. Chemical Shifts of the Carbon Atoms of Compounds (I-III) (C_5D_5N, δ, ppm, 0 – TMS)

Atom C	Compound			Atom C	Compound		
	I	II	III		I	II	III
1	32,42	32,72	32,41	23	26,37[a]	26,29[a]	26,37[a]
2	30,15	31,30	30,16	24	81,72	81,57	81,64
3	**88,87**	**78,21**	**88,96**	25	71,26	71,19	71,26
4	42,58	42,28	42,58	26	27,12*	27,04*	27,04*
5	54,01	53,86	53,93	27	28,53*	28,09*	28,53*
6	67,97	68,27	67,97	28	20,17	20,17	20,09
7	38,54	38,69	38,54	29	28,76	29,28	28,91
8	46,98	47,21	47,06	30	16,58	16,16	16,66
9	20,84	20,84	20,84	β-D-Glcp residue			
10	29,43	29,80	29,50				
11	26,37[a]	26,29[a]	26,37[a]	1	105,47		106,82
12	33,39	33,31	33,31	2	**79,40**		**75,82**
13	45,04	44,89	44,97	3	78,66		78,66
14	46,09	46,09	45,91	4	72,01		71,78
15	46,61	46,69	46,09	5	78,21		78,06
16	73,43	73,35	73,43	6	62,89		62,87
17	58,34	58,26	58,26	D-Apio-β-D-f residue			
18	21,51	21,51	21,51				
19	30,47	31,0	30,55	1	111,15		
20	87,25	87,17	87,17	2	77,83		
21	28,16	28,46	28,41	3	80,52		
22	34,88	34,81	34,88	4	75,59		
				5	66,11		

[a]Signals superposed upon one another within a column. The assignment of the signals marked by asterisks is unambiguous within a column [9].

Cycloaraloside C was methylated by Hakomori's method [6]. The carbohydrate moiety of an acid hydrolysate of the nona-O-methyl ether (IV) consisted of two components, which were separated by column chromatography. The polar component was a D-glucose derivative which was found by sodium periodate oxidation and the GLC method [4] to be 3,4,6-tri-O-methyl-D-glycopyranose. Consequently, glycoside (I) was a monodesmoside and the D-apiose residue was terminal. As was to be expected, the mass spectrum of the nona-O-methyl ether (IV) showed the peak of an ion with m/z 379 corresponding to the permethylated disaccharide chain, and peaks characterizing a terminal pentose (m/z 175, 143, 115). This meant that the weakly polar methylated monosaccharide must have been a trimethyl derivative of D-apiose, and its structure was determined in the following way.

A comparative analysis of the ^{13}C NMR spectra of compounds (I-III) showed that in the molecule of (I) the C-3 atom of the genin part and the C-2 part of the D-glucose residue experienced a glycosylation effect. The values of the chemical shifts of the carbon atoms of the D-apiose residue showed the D-apio-β-D-furanoside structure of the branched monosaccharide [7]. Consequently, the trimethyl derivative of D-apiose was 2,3,5-tri-O-methyl-D-apio-D-furanose. A calculation of molecular rotation differences between glycoside (I) and (III) and also the SSCC of the anomeric protons of the D-apiose residue, ^3J = 2.0 Hz in the PMR spectrum of compound (I) and ^3J = 2.5 Hz in the PMR spectrum of the permethylate (IV) confirmed the conclusion of the β-configuration of the glycosidic bond of the pentose [7, 8].

Thus, cycloaraloside C has the structure of 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O[O-(D-apio-β-D-furanosyl)-(1 → 2)-β-D-glucopyranoside].

This is the first time that D-apiose has been found in the cyucloartane glycoside series.

EXPERIMENTAL

General Observations. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) n-butanol–pyridine–water (6:4:3); 3) n-butanol–methanol–water (5:3:1); 4) chloroform–methanol–water (70:12:1); 5) chloroform–methanol–water (70:23:4); 6) benzene–ethyl acetate) (1:1); 7) chloroform–acetone (5:2).

PC was performed on type FN-11 paper. The conditions for GLC, TLC, and CC are given in [4].

The ^{13}C and ^1H NMR spectra were taken in deuteropyridine or deuterochloroform on Tesla BS-567A and Bruker WM-50 instruments (δ, pm, for ^{13}C, 0 – TMS, and for ^1H, where not otherwise stated, HMDS).

For other observations, see [4].

Cycloaraloside C (I) – substance 6 [1], $C_{41}H_{68}O_{14}$, mp 242-244°C (from methanol), $[\alpha]_D^{30}$ + 4.9 ± 2° (c 1.63; methanol). ν_{max}^{KBr}, cm^{-1}, 3580-3200 (OH), 3055 (CH_2 of a cyclopropane ring). PMR spectrum (250 MHz, C_5D_5N, 0 – TMS: 0.21 and 0.53 (2H-19, d, ^2J = 4 Hz), 1.02; 1.33; 1.34; 1.43; 1.45; 1.60; 2.03 (7 × CH_3, s), 2.55 (H-17, d, ^3J = 7.5 Hz), 3.12 (1H-22, g, ^2J = ^3J, = $^3L_2$ = 10 Hz), 3.58 (H-3, dd, $^3J_1$ = 4 Hz, $^3J_2$ = 11 Hz), 4.45 and 4.81 (2H-NB of D-apiose, d, ^2J = 9 Hz), 5.0 (H-2 of D-apiose, d, ^3J = 2.0 Hz), 5.17 (anomeric proton of D-glucose, d, ^3J = 7.5 Hz), 6.58 (anomeric proton of D-apiose, d, ^3J = 2.0 Hz).

Acid Hydrolysis of Cycloaraloside C (I). Glycoside (I) (180 mg) was hydrolyzed with 25 ml of a 0.5% methanolic solution of sulfuric acid at 60°C for 4 h. The reaction mixture was diluted with water, and the methanol was diluted off. The precipiate that deposited was filtered off, washed with water, and chromatographed on a column with elution by system 1. This led to the isolation of 75 mg of the genin (II), mp 239-241°C (from methanol), $[\alpha]_D^{30}$ + 50 ± 2° (c 1.1; methanol), which was identified as cyclosieversigenin also by the indices of its PMR, IR, and mass spectra.

The filtrate after the separation of the precipitate was evaporated to a volume of 15 ml and was boiled for 1 h. After cooling, the solution was neutralized with type ARA-8p anion-exchange resin. The resin was filtered off and the filtrate was evaporated to dryness. D-glucose (T_{rel} 0.88; 1.00) and D-apiose (T_{rel} 0.15; 0.16; 0.17; 0.18) were detected in the residue by PC (system 2), TLC (system 3), and GLC [4]. It was shown by the GLC method [4] that glycoside (I) contained D-glucose and D-apiose residues in a ratio of 1.00:0.84. $[M]_{DI}$ + 38°; $[M]_{DIII}$ + 215°; $\Delta[M]_{D(I-III)}$ −177; $[M]_D$ of the methyl glycosides of D-apio-D-furanose: α, +221°; β, −167° [7, 8].

Cycloaraloside A (III) and D-Apiose from (I). Cycloaraloside C (1.0 g) was hydrolyzed with 100 ml of a 0.05% methanolic solution (sic] at 50°C for 2 h. The methanol was evaporated off to small volume, the residue was diluted with water, and the precipitate that deposited was filtered off, washed with water, and chromatographed on a column with elution by system 4. This yielded 650 mg of cycloaraloside A (III), mp 240-242°C (from system 4), $[\alpha]_D$ +33 ± 2° (c 1.10; methanol), identical with an authentic sample also from the indices of its PMR spectrum.

The filtrate was concentrated to a volume of 40 ml and was boiled for 1 h, and, after cooling, it was neutralized with type ARA-8p anion-exchange resin. The dry residue after the elimination of the resin and evaporation was chromatographed on a column, with elution by system 5. This gave 80 mg of D-apiose in the form of a syrupy mass, $[\alpha]_D^{30}$ + 10.7 ± 2° (c 1.12; water).

Nona-O-methyl Ether (IV) from (I). With constant stirring, 320 mg of sodium hydride was added in small portions to 305 mg of glycoside (I) in 30 ml of dry dimethyl sulfoxide, and the mixture was stirred for another 1 h. After this, 3 ml of methyl iodide was added dropwise and stirring was continued for 4 h. Then the reaction mixture was poured into 150 ml of 2% aqueous sodium hyposulfite and, after the usual working up, the products were chromatographed on a column with elution by system 6. This led to the isolation of 70 mg of the amorphous nona-O-methyl ether (IV), $C_{50}H_{86}O_{14}$, $[\alpha]_D^{30}$ +23.6 ± 2° (c 1.1; methanol. The IR spectrum of product (IV) lacked the absorption band of hydroxy groups.

Mass Spectrum. m/z (%): M^+ 910(0.07), 895(0.8), 585(0.9), 530(10.0), 514(7.1), 499-(12.9), 483(12.9), 467(10.0), 451(17.1), 379(58.6), 347(98.6), 201(37.1), 189(74.3), 175(62.9), 173(40.0), 157(100), 143(98.6), 125(65.7), 115(40.0). PMR spectrum (C_5D_5N): 0.40 (H-19, d, ^2J = 4 Hz); 0.90; 1.08; 1.12; 1.16; 1.24; 1.31; 1.39; (7 × CH_3, c), 3.02; 3.14; 3.16; 3.26; 3.28; 3.37; 3.38; 3.42; 3.52 (9 × OCH_3, s), 3.88 (H-2, D-apiose, d, ^3J = 2.5 Hz), 4.15; 4.35; (2H-5 of D-apiose, d, ^2J = 11 Hz); 4.51 (anomeric proton of D-glucose, d, ^3J = 7 Hz), 5.74 (anomeric proton of D-apiose, d, ^3J = 2.5 Hz). PMR spectrum ($CDCl_3$): 0.16 and 0.43 (2H-19, d, ^2J = 4 Hz), 0.90; 0.90; 1.04; 1.04; 1.10; 1.10, 1.16; 1.20 (7 × CH_3, s); 3.05; 3.16; 3.19; 3.30; 3.32; 3.36; 3.42; 3.44; 3.54 (9 × OCH_3, s), 4.00 (2H-5' of D-apiose, s), 4.24 (anomeric proton of D-glucose, d, ^3J = 7 Hz), 5.40 (anomeric proton of D-apiose, br.s).

Identification of the Methylated Monosaccharides. The nona-O-methyl ether (IV) (220 mg) was hydrolyzed with 25 ml of 0.5% methanolic sulfuric acid at the boiling point of the reaction mixture for 5 h. Then the reaction mixture was diluted with an equal volume of water and the methanol was evaporated off. The precipitate that had deposited was filtered off; it consisted of products difficult to separate and we have not studied it.

The filtrate was boiled for 1 h. The cooled solution was neutralized with type ARA-8p anion-exchange resin. The residue after the elimination of the resin and the evaporation of the water was chromatographed on a column with elution by system 7. This led to the isolation of 10 mg of a syrupy mass which consisted of a tri-O-methyl derivative of D-apiose. On GLC [4], this compound had two peaks with relative retention times (T_{rel}) of 0.42 and 0.53. The chemical shifts of the carbon atoms of the D-apiose residue in the ^{13}C NMR spectrum of glycoside (I) (Table 1) determined the methylated monosaccharide under consideration as 2,3, 5-tri-O-methyl-D-apio-D-furanose.

On continuing the elution of the column with the same system, we obtained 6 mg of a methylated D-glucose. To 2 mg of the latter in 0.2 ml of methanol was added 8 mg of sodium periodate in 0.2 ml of water, and the mixture was left at room temperature for 30 min. TLC in system 7 showed that the partially methylated D-glucose had been oxidized. By GLC [4], the methylated monosaccharide under consideration was identified as 3,4,6-tri-O-methyl-D-glucopyranose (T_{rel} 1.28). After the silylation of the methyl glycoside of this monosaccharide, two peaks appeared on GLC with relative retention times (T_{rel}) of 2.06 and 2.24.

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STRUCTURES OF FOUR NEW TRITERPENE GLYCOSIDES FROM THE HOLOTHURIAN Cucumaria japonica

S. A. Avilov, V. A. Stonik, and A. I. Kalinovskii

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Four new triterpene glycosides of the holostane series have been isolated from an alcoholic extract of the economically important Pacific Ocean holothurian Cucumaria japonica: cucumariosides A_1-2 (I), A_2-3 (II), A_2-4 (III), and A_4-2 (IV). The structures of these substances have been established by the methods of carbohydrate chemistry and ^{13}C NMR spectroscopy.

We have previously [1] reported the structure of a new triterpene glycoside from the holothurian Cucumaria japonica – cucumarioside A_2-2 (I). Continuing our investigations, with the aid of reversed-phase chromatography we have isolated another three new glycosides from this holothurian: cucumariosides A_2-3 (II), A_2-4 (III), and A_4-2 (IV). In the present paper we describe the determination of the structures of these substances (I–IV).

Analysis of the ^{13}C NMR spectra (Table 1) showed that cucumarioside A_2-2 had as the native aglycon 3β-hydroxyholosta-7,25-dien-16-one [2, 3], which had been obtained previously from the total glycosides of this holothurian.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, Academy of Sciences of the USSR, Vladivostok.

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