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TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XL. CYCLOCARPOSIDE B FROM Astragalus coluteocarpus

B. A. Imomnazarov and M. I. Isaev

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In addition to cyclosieversigenin, β-sitosterol β-D-glucopyranoside, and cyclocarposide, we have isolated another three glycosides of triterpene nature from the epigeal part of the plant Astragalus coluteocarpus Boiss. (Leguminosae). On the basis of chemical transformations and spectral characteristics, the structure of one of the new glycosides, which we have called cyclocarposide B, has been established as 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 6-O-α-L-(2-O-acetylrhamnopyranoside) 3-O-β-D-xylopyranoside.

We have previously reported the structure of a cycloartane glycoside, cyclocarposide, isolated from the herb Astragalus coluteocarpus Boiss. (Leguminosae) [1]. Continuing the study of other components of the epigeal part of this plant, we have isolated another five compounds, which have been called substances (1)-(5) in order of increasing polarity. Cyclocarposide corresponds to a 6th substance in this series. The two weakly polar compounds (1) and (2) have been identified as cyclosieversigenin [2] and β-sitosterol β-D-glucopyranoside [3]. The present work was devoted to demonstrating the structure of substance (4), which we have called cyclocarposide B (I).

In the PMR spectrum of cyclocarposide B at 0.23 and 0.42 ppm we observed the one-proton doublets of an AB system that are characteristic for an isolated cyclopropane methylene group. This fact permitted us to assign the glycoside under consideration to the triterpenoids of the cycloartane series [2]. The formation of cyclosieversigenin (II) on the acid hydrolysis of glycoside (I) served as a proof of this conclusion.

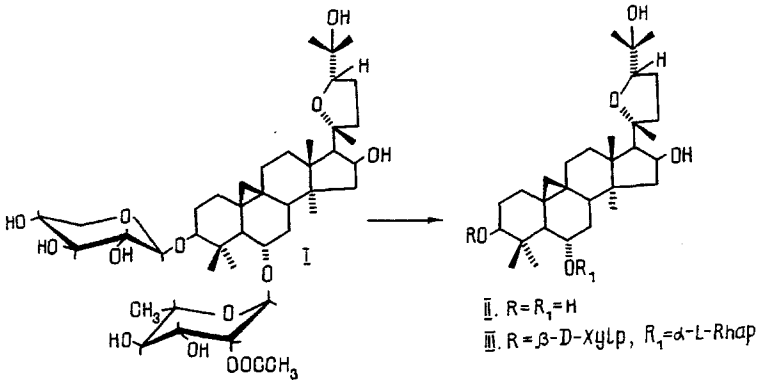

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TABLE 1. Chemical Shifts of the Carbon Atoms of Compounds (I-III) (δ, ppm, 0 – TMS, C_5D_5N)

C atom	Compound			C atom	Compound		
	I	II	III		I	II	III
1	32,16	32,72	32,20	24	81,71	81,57	81,67
2	30,06	31,30	30,06	25	71,22	71,19	71,22
3	87,85	78,21	87,83	26	27 07*	27,04*	27,05*
4	42,27	42,28	42,26	27	28,13*	28,09*	28,11*
5	51,93	53,86	52,03	28	20,18	20,17	20,16
6	79,97	68,27	79,16	29	28,51	29,28	28,51
7	34,13	38,69	34,55	30	16,98	16,16	17,05
8	46,22	47,21	46,16[a]		β-D-Xylp residue		
9	20,04	20,84	20,65	1	107,49		107,43
10	28,77	29,80	28,70	2	75.50		75,38
11	26,39	26,29[a]	26,38	3	78,46		78,44
12	33,28	33,31	33,27	4	71,18		71,14
13	45,02	44,89	45,00	5	66,99		66,96
14	46,19	46,09	46,16[a]		α-L-Rhap residue		
15	46,71	46,69	46,67	1	100,41		103,88
16	73,35	73,35	73,33	2	74,49		72,87
17	58,29	58,26	58,23	3	70,45		72,56
18	21,47	21,51	21,47	4	73,80		73,71
19	30,26	31,00	30,22	5	69,97		70,06
20	87,18	87,17	87,15	6	18.16		18,16
21	28,42	28,46	28,42				
22	34,90	34,81	34,88	CH_3	21.03		
23	25,94	26,29[a]	25,95	COO	170,76		

The signals marked with identical letters are superposed on one another, and the assignments of those with asterisks are doubtful.

It was shown by PC and GLC [4] that cyclocarposide B includes D-xylose and L-rhamnose residues. The GLC results indicated that the glycoside under consideration contained the monosaccharides mentioned in a ratio of 1:1.

The IR spectrum of glycoside (I) contained absorption bands at 1730 and 1250 cm^{-1}, which are characteristic for an ester grouping. The ^1H and ^{13}C NMR spectra, containing a three-proton singlet at 2.04 ppm and signals at 21.03 and 170.76 ppm, respectively, showed the presence of one acetate group in the cyclocarposide B molecule [1].

The position of the acetate group was revealed by a study of the ^{13}C and ^1H NMR spectra of cyclocarposide B (I) and cyclocarposide (III).

It can be seen from a comparison of the ^{13}C NMR spectra (Table 1) of glycosides (I) and (III) that on passing from (III) to (I) substantial changes take place in the chemical shifts of the carbon atoms C-1, C-2, and C-3 of the L-rhamnose residue, the C-1 and C-3 signals undergoing upfield shifts (100.41 – 103.88 = –3.47 ppm; 70.45 – 72.56 = –2.11 ppm) and C-2 a downfield shift (74.49 – 72.87 = +1.62 ppm). This fact unambiguously determines the position of the acetate group at C-2 of the L-rhamnose residue. This conclusion of its position was confirmed by the double-resonance PMR spectrum.

In the PMR spectrum of cyclocarposide B the signal of a proton geminal to an acetoxy group was observed at 5.71 ppm in the form of a doublet of doublets with $^3J_1$ = 3 and $^3J_2$ = 0.9 Hz. The signal of the anomeric proton of the L-rhamnose consisted of a doublet with ^3J = 0.9 Hz resonating at 5.17 ppm. When the latter was irradiated, the doublet of doublets at 5.71 ppm was transformed into a doublet with ^3J = 3 Hz. Consequently, the signal at 5.71 related to the proton at C-2 of the L-rhamnose and the acetate group was present in the same position.

The chemical shifts of the D-xylose and L-rhamnose carbon atoms in glycosides (I) and (III) showed the C1 and 1C configurations, respectively, of these monosaccharide residues.

Thus, cyclocarposide B has the structure of 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 6-O-α-L-(2-O-acetylrhamnopyranoside) 3-O-β-D-xylopyranoside.

EXPERIMENTAL

General Remarks. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) chloroform–methanol–water (70:12:1); and 3) n-butanol–pyridine–water (6:4:3).

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

^1H and ^{13}C NMR spectra were taken on Bruker AM-400, Bruker AC-200, and Tesla BS-567 A instruments in deuteropyridine (δ, ppm, 0 – TMS), ^{13}C NMR spectra were also taken under J-modulation conditions.

Isolation and Separation of the Astragalus coluteocarpus Isoprenoids. The air-dry epigeal part of A. coluteocarpus (11 kg) gathered in the flowering phase on an experimental plot in the Botanical Garden of the Pamir Biological Institute, Tadzhikistan Academy of Sciences, was extracted with methanol (3 × 35 liters). The extract was evaporated to a viscous consistency and was diluted with 2 liters of water. The aqueous solution was treated with n-butanol (6 liters). The butanolic extract was evaporated to dryness, giving a total of 480 g of triterpene compounds. On TLC, this product revealed 6 components, designated as substances (1)-(6) in order of increasing polarity. Part of it (240 g) was chromatographed on a column of silica gel with elution successively by chloroform and systems 1 and 2. Elution of the column with system 1 gave fractions containing mixtures of substances (1) and (2). On continuing elution of the column with system 2, fractions containing substance (3) and (4); (3)-(5); (4) and (5); and (6) were collected.

Rechromatography of the fractions containing substances (1) and (2) on a column in system 1 permitted the isolation of 107 mg of substance (1) (0.0019%; yields here and below are given on the air-dry raw material) and 123 mg of substance (2) (0.0023%). Repeated chromatography of the fractions containing substances (3)-(5) on a column in system 2 led to 205 mg of substance (3) (0.0037%), 315 mg of substance (4) (0.0058%), and 50 mg of substance (5) (0.0009%).

Recrystallization from methanol of the fractions containing substance (6) yielded 13 mg of cyclocarposide (0.2363%).

Cyclosieversigenin (II) – substance (1): mp 239-241°C (from methanol), $[\alpha]_D^{24}$ +52 ± 2° (c 1.0; methanol) [2].

β-Sitosterol β-D-glucopyranoside – substance (2): mp 277-279°C (from methanol), $[\alpha]_D^{24}$ −37 ± 2° (c 1.1; pyridine) [3].

Cyclocarposide B (I) – substance (4): $C_{43}H_{70}O_{14}$, mp 271-273°C (from methanol), $[\alpha]_D^{24}$ 0 ± 3° (c 0.5; pyridine). ν_{max}^{KBr}, cm^{-1}: 3600-3200 (OH); 3045 (CH_2 of a cyclopropane ring); 1730, 1250 (ester group). PMR spectrum: 0.23 and 0.42 (2H-19, d, ^2J = 4 Hz), 0.95; 1.13; 1.28; 1.30; 1.39; 1.56; 1.61 (7 × CH_3, s), 1.62 (CH_3 of L-rhamnose, d, ^3J = 6 Hz), 2.04 (CH_3COO, s), 2.52 (H-17, d, ^3J = 8 Hz), 3.07 (H-22, q, $^2J = {}^3J_1 = {}^3J_2$ = 10 Hz), 3.43 (H-3, dd, $^3J_1$ = 12 Hz, $^3J_2$ = 5 Hz), 3.50 (H-6, td, $^3J_1 = {}^3J_2$ = 9 Hz, $^3J_3$ = 3.5 Hz), 4.76 (anomeric proton of D-xylose, d, ^3J = 7.5 Hz), 4.98 (H-16, q, $^3J_1 = {}^3J_2 = {}^3J_3$ = 8 Hz), 5.17 anomeric proton of L-rhamnose, d, ^3J = 0.9 Hz), 5.71 (H-2 of L-rhamnose, dd, $^3J_1$ = 3 Hz, $^3J_2$ = 0.9 Hz).

Acid Hydrolysis of Cyclocarposide B (I). A solution of 50 mg of glycoside (I) in 15 ml of a 0.5% methanolic solution of sulfuric acid was boiled for 3 h. Then the reaction mixture was diluted with water to a volume of 100 ml, and the methanol was evaporated off. The resulting precipitate was filtered off and was chromatographed on a column, with elution by system 1. This gave 13 mg of cyclosieversigenin (II), mp 239-241°C (from methanol), $[\alpha]_D^{24}$ +52 ± 2° (c 0.7; methanol).

This aqueous solution was concentrated to a volume of 10 ml and was boiled for 1 h. After neutralization with ARA-8p anion-exchange resin and the elimination of the latter, the aqueous solution was evaporated to dryness. D-Xylose and L-rhamnose were detected in the residue with the aid of PC in system 3. GLC [4] showed that cyclocarposide B contained D-xylose and L-rhamnose residues in a ratio of 1.00:0.90.

Alkaline Hydrolysis of Cyclocarposide B (I). To a solution of 40 mg of cyclocarposide B in 10 ml of methanol was added 50 mg of sodium hydroxide in 5 ml of methanol. The reaction mixture was left at room temperature for 5 h. Then the methanolic solution was diluted with a twofold volume of water. The precipitate that deposited was filtered off and was washed with water. Recrystallization from methanol yielded 22 mg of cyclocarposide (III), mp 284-285°C, $[\alpha]_D^{24}$ −28 ± 2° (c 0.9; pyridine), identified by the usual methods.

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PHYTOECDYSTEROIDS OF Rhaponticum carthamoides

III. RHAPISTERONE C

U. A. Baltaev

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A new ecdysteroid, rhapisterone C, has been isolated from seeds of the plant Rhaponticum carthamoides (Willd) Iljin (family Compositae). It has been shown that rhapisterone C is 23ξ-ethylecdysterone.

In studying phytoecdysteroids from Rhaponticum carthamoides seeds [1, 2], we have isolated a new phytoecdysteroid (I) belonging to the ecdysteroids of the C-29 series. The new phytoecdysteroid, which we have called rhapisterone C, was isolated from the moderately polar fraction of a butanolic extract containing the phytoecdysteroids. The amount of rhapisterone C in the plant is very small. For this reason, in separation we used column chromatography and rechromatography on SiO_2 with elution by chloroform–methanol systems [(9:1) and (4:1)].

The IR spectrum of (I) contained bands of hydroxy groups at 3340 and 3500 cm^{-1}, while at 1655 cm^{-1} there was absorption corresponding to a keto group conjugated with a double bond.

The peak of the molecular ion was absent from the mass spectrum of (I). The region of high masses was characterized by the peaks of ions with m/z 472 ($M^+ - 2H_2O$), 454, 439, 436, and 421. The cleavage of the C-20–C-22 bond formed fragments with m/z 363, 345, and 327. An ion with m/z 300 corresponded to breakdown at the C-17–C-20 bond. The above-mentioned fragments of the steroid part of compound (I) were analogous to the mass-spectrometric fragmentation of the steroid part of ecdysterone. In the mass spectra of ecdysteroids with side chains similar to that of ecdysterone, the cleavage of the C-20–C-22 bond after dehydration gives rise to a cyclic fragment with m/z 99. In the mass spectrum of (I), this fragment was displaced by 28 m.u., which means the presence of an additional C_2H_4 group. An ion with m/z 109, formed by the dehydration of the ion with m/z 127, also appeared.

The positions of the substituents in the steroid part and the side chain of (I) were shown with the aid of ^1H and ^{13}C NMR spectra using 2D correlation spectroscopy of ^1H–^1H and ^1H–^{13}C chemical shifts.

The ^{13}C NMR spectrum of compound (I) (Table 1) included at 72.57 ppm the signal of the C-25 carbon atom. When the method of ^1H–^{13}C 2D correlation spectroscopy (2D COSY) was employed, the signal at 72.57 ppm did not appear. This fact is a proof that the C-25 carbon atom did not interact with a proton. Consequently, an OH group and two methyl groups are attached to C-25.

In the PMR spectrum of rhapisterone C, the signal of a proton in the geminal position to a secondary hydroxy group at C-22 appeared in the form of a doublet at 3.87 ppm. This fact is evidence in favor of the assumption that the C_2H_4 residue is attached at C-23, and H-22 interacts with only one proton, in contrast to makisterone C [3, 4], where H-22 inter-

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