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TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS.

XXIV. CYCLOORBICOSIDE G FROM Astragalus orbiculatus

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A bisdesmosidic glycoside – cycloorbicoside G – has been isolated from the epigeal parts of the plant Astragalus orbiculatus Ledeb. (Leguminosae), and on the basis of chemical transformations and spectral characteristics its structure has been established as (23R,24S)-16β,23;16α,24-diepoxycycloartane-3β,7β,25-triol 25-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside.

We have previously reported the determination of the structure of cycloorbingenin [1] and cycloorbicoside A [2] – the main glycoside and its genin that were isolated from the epigeal part of Astragalus orbiculatus Ledeb. (Leguminosae).

Extracts of the epigeal part of this plant [1] contained eight substances of triterpene nature numbered in increasing order of polarity 1-8. Substance 1 corresponded to cycloorbicoside A. Column chromatography on silica gel of a butanolic fraction yielded substance 7. In the present paper we consider the structure of this glycoside, which we have called cycloorbicoside G ((I), Scheme).

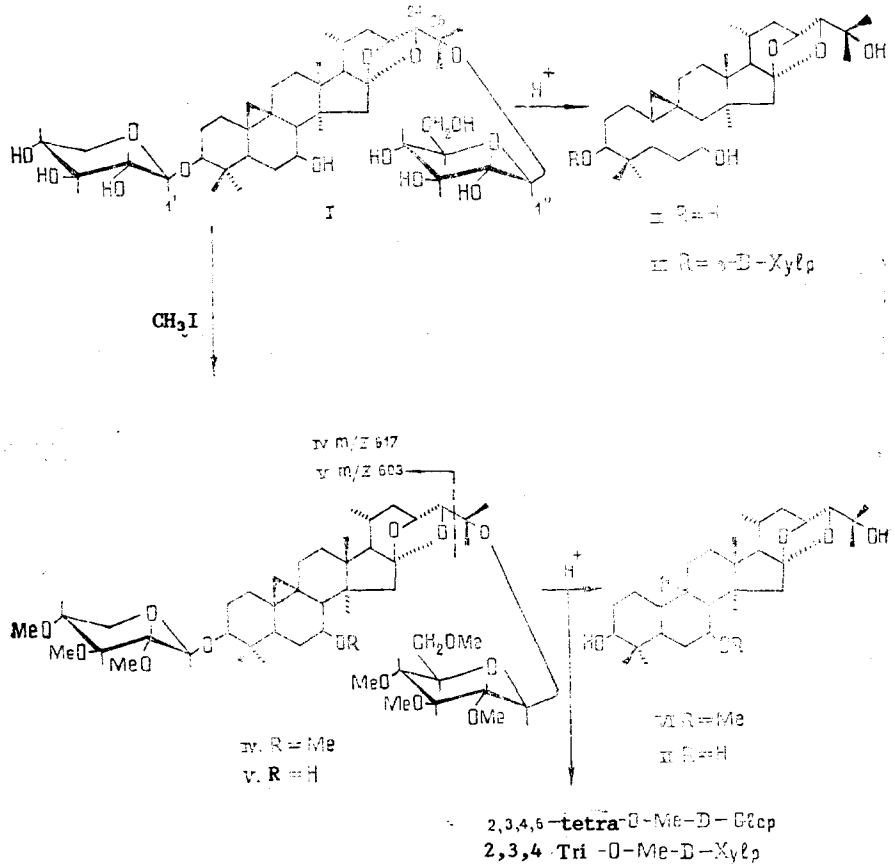

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TABLE 1. Chemical Shifts of the Protons of Cycloorbicoside G (I) and Its Derivatives (δ, ppm, 0 – HMDS)

Compound	Positions of the protons				
	H-3	H-7	2H-15	2H-19	H-23
I	3,38 m		2,40; 2 62 d ^2J=14 Hz	0,16; 0,60 d ^2J=4 Hz	4,73*
II	3,38 q ^3J=12; 5 Hz	3,70 td ^3J=10; 10; 3Hz	2,46; 2,70 d ^2J=15 Hz	0,23; 0,65 d ^2J=4 Hz	4,62 q ^3J=9; 2 Hz
IV				[0,18; 0,70 d ^2J=4 Hz]	[4,37]*
V				[0,30; 0,66d ^2J=4 Hz]	[4,40]*
VI	3,44 q ^3J=12; 5 Hz	2,99m	2,24; 2,36 d ^2J=14 Hz	0,12; 0 62 d ^2J=4 Hz	4,64

Compound	Positions of the protons			
	H-24	H-1′	H-1″	CH_3-groups
I	3,63s	4,71* d	4,95 d ^3J=8 Hz	0,71 d (^3J=6 Hz); 0,93; 1,07; 1,19; 1,28 (2×CH_3); 1,44
II	3,58 s	—	—	0,76d (^3J=6 Hz); 0,99; 1,11; 1,13; 1,26; 1,35 (2×CH_3)
IV		[4,20 d ^3J=7,5 Hz]	[4,35 d ^3J=8 Hz]	[0,79 d (^3J=6 Hz); 0,81; 0,99 (3××CH_3); 1,07; 1,17 (3,22; 3,32; 3,40; 3,45; 3,48; 3,56 — 3××OCH_3)]
V		[4,20 d ^3J=7 Hz	[4,35 d ^3J=8Hz]	[0,81d (^3J=6 Hz); 0,81; 0,96; 1,02; 1,06 (2×CH_3); 1,16 (3,32; 3,40; 3,45; 3,48; 3,56 — 3××OCH_3)]
VI	3,60 s	—	—	0,75d (^3J=6 Hz); 0,98; 1,05; 1,15; 1,16; 1,32; 1,39 (3,09 — — OCH_3)

The spectra were taken in deuteropyridine or deuterochloroform. The signals in the horizontal rows marked with asterisks are superposed upon one another. Where its multiplicity is not shown, the H-23 signal was observed in the form of a doublet (^3J = 9 Hz) with broadened components ($W_{1/2}$ = 2 Hz). The signals of the methyl groups appeared in the form of singlets, with the exception of the CH_3 group at C-20, having a doublet nature; d — doublet; q — quartet; td — triplet of doublets; m — multiplet.

The PMR spectrum of glycoside (I) exhibited characteristic doublets of the AB system of an isolated cyclopropane methylene group at 0.16 and 0.60 ppm (Table 1). This permitted us to assign the compound under discussion to the triterpenoids of the cycloartane series [3]. In actual fact, the acid hydrolysis of cycloorbicoside G gave the genin (II), which was identified as cycloorbigenin [1].

It was shown by GLC [4] that cycloorbicoside G contained D-glucose and D-xylose residues in a ratio of 1:1.

From the products of the partial acid hydrolysis of glucoside (I), in addition to the genin (II), we isolated a progenin (III) which, according to GLC, contained a D-xylose residue. Progenin (III) was identical with cycloorbicoside A [2].

The Hakomori methylation [5] of cycloorbicoside G (I) gave the octa-O-methyl and hepta-O-methyl ethers (IV), M^+ 894, and (V), M^+ 880, respectively. Analysis of the methyl ethers of (IV) and (V) by GLC [6] showed that the carbohydrate components of the two products were the same – 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose residues. This was also shown by the peaks of ions with m/z 219, 187, and 155, and m/z 175, 143, and 115, in the mass spectra of compounds (IV) and (V), characteristic of a tetramethylhexose and a tetramethylpentose, respectively [7, 8]. Consequently, cycloorbicoside G was a bisdesmosidic glycoside and the hydroxy group remaining free in the methyl ether (V) was present in the genin moiety of the molecule.

As was expected, the hydrolysis of the hepta-O-methyl ether (V) led to cycloorbigenin.

Ions with m/z 617 and 603, respectively, were observed in the mass spectra of the methyl ether (IV) and (V). These ions arise on the cleavage of the C-24–C-25 bond and showed the

TABLE 2. Chemical Shifts of the Carbon Atoms of Cycloorbigenin (III) and of Cycloorbicosides A (III) and G (I) (δ, ppm; 0 – TMS)

Carbon atom	Compound			Carbon atom	Compound		
	I	II	III		I	II	III
1	31,89[a]	32,34	32,00*	23	70,08*	70,35*	70,19**
2	29,67	31,22	29,72	24	88,23[b]	90,57	90,56
3	88,23[b]	77,94	88,34	25	78,75[e]	71,16	71,11
4	41,05	40,93	41,10	26	23,82**	23,89**	23,82***
5	46,58	46,57	46,63	27	24,52**	24,83**	24,74***
6	38,29	38,52	38,45	28	18,94[d]	19,10	18,99[a]
7	71,71[c]*	71,87*	71,76**	29	25,72	26,25	25,82
8	55,24	55,46	55,35	30	15,31	14,80	15,37
9	19,65	19,78	19,76	D-Xylose			
10	27,18	27,64	27,23	3a			
11	26,75	26,96	26,80	1′	107,52		107,52
12	33,03	33,24	33,14	2′	75,50		75,50
13	44,19	44,34	44,25	3′	78,54		78,59
14	46,74	46,97	46,85	4′	71,17		71,22
15	48,85	48,93	48,85	5′	67,05		67,19
16	115,21	115,27	115,30	D-Glucose			
17	60,66	60,70	60,61				
18	18,94[d]	19,01	18,99[a]	1″	98,74		
19	29,94	30,00	30,00	2″	75,18		
20	22,09**	27,80**	27,88***	3″	78,75[e]		
21	19,97	20,17	20,08	4″	71,71[c]		
22	31,89[a]	32,18	31,84*	5″	78,10		
				6″	62,82		

The signals denoted by the same letters are superposed on one another. The assignment of the signals marked with asterisks are ambiguous within a column.

locations of the D-glucose residue at C-25. Consequently, in the methyl ether (V) it was the hydroxy group at C-7 that had remained free.

The same conclusions followed from the results of the acid hydrolysis of the permethylate (IV). In the mass spectrum of the monomethyl derivative (VI) (M^+ 502) isolated from the hydrolysis products of the permethylate (IV) the maximum peak of an ion with m/z 443 arising on the cleavage of the C-24–C-25 bond was observed. This showed that the methyl substituent was present in the polycyclic part of the molecule. The signal of the H-7 proton in the PMR spectrum of compound (VI) had undergone a diamagnetic shift by 0.71 ppm as compared with that of cycloorbigenin and was detected at 2.99 ppm. The facts presented determined the monomethyl derivative (VI) as 7-O-methylcycloorbigenin.

In the PMR spectra of cycloorbicoside G (I) and its methyl ethers (IV) and (V), the anomeric proton of the D-glucopyranose residue resonated in the form of a doublet with the SSCC ^3J = 7-8 Hz. Consequently, the D-glucopyranose residue had the C1 conformation, which means the β-configuration [9].

The conclusion of the β-configuration of the glycosidic bond of the D-glucose residue also followed from the difference between the molecular rotations of cycloorbicosides G and A [10].

Thus, cycloorbicoside G has the structure of (23R,24S)-16β,23;16α,24-diepoxycycloartane-3β,7β,25-triol 25-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside.

The structure established for cycloorbicoside G is in full agreement with the features the ^{13}C NMR spectrum given in Table 2.

EXPERIMENTAL

For general observations see [1, 2]. The following solvent systems were used: 1) chloroform–methanol–water (70:23:4); 2) chloroform–methanol (15:1); 3) chloroform–methanol–water (70:12:1); 4) benzene–methanol (15:1); 5) benzene–methanol (15:2); 6) chloroform–methanol (40:1); and 7) benzene–acetone (7:1).

The ^1H NMR spectra were taken in deuteropyridine or deuterochloroform on XL-200 (Varian) and Tesla BS-567 A instruments (δ, ppm: 0 – HMDS), and the ^{13}C NMR spectra in deuteropyridine on a JEOL FX-90Q instrument (δ, ppm; 0 – TMS). The ^{13}C NMR spectra were obtained in the Irkutsk Institute of Organic Chemistry of the Siberian Branch of the USSR Academy of Sciences by M. F. Larin.

For the isolation of the cycloartane triterpenoids from the epigeal part of Astragalus orbiculatus, see [1]. By TLC on Silufol plates impregnated with a 0.3 M solution of sodium dihydrogen phosphate eight compounds were detected in the ethyl acetate and butanol extracts, and also in the fractions obtained in the isolation of cycloorbicoside A [1]. They were designated as substances 1-8 in order of increasing polarity.

Cycloorbicoside G (I). Column chromatography of the butanol fraction (150 g) [1] in system 1 led to the isolation of 9 g (0.16% based on the weight of the air-dry raw material) of substance 7—cycloorbicoside G (I), $C_{41}H_{66}O_{14}$, mp 247-249°C (from methanol), $[\alpha]_D^{24}$ 0 ± 3° (c 0.69; methanol). It was shown with the aid of GLC that glycoside (I) contained D-glucose and D-xylose residues in a ratio of 1.00:1.08.

Cycloorbigenin (II) and Cycloorbicoside (A) III from (I). A solution of 280 mg of glycoside (I) in 20 ml of methanol containing 0.25% of sulfuric acid was boiled for 3 h and was then diluted with an equal volume of water and the methanol was evaporated off. The precipitate that deposited was filtered off, washed with water, and chromatographed on a column with elution by system 2. This gave 84 mg of the genin (II), mp 219-220°C (from methanol), $[\alpha]_D^{24}$ +28 ± 2° (c 1.2; ethanol), which was identified as cycloorbigenin with the aid of TLC, and also from its spectral characteristics [1].

When the column was washed with system 3, 40 mg of progenin (III) containing, according to GLC, D-xylose, was isolated, with mp 264-266°C (from ethanol, $[\alpha]_D^{20}$ +8.3 ± 2° (c 1.2; methanol). The monoside (III) was identified as cycloorbicoside A by the usual means [2].

The Octa-O-methyl (IV) and Hepta-O-methyl (V) Ethers of Cycoorbicoside G from (I). With constant stirring, 800 mg of sodium hydride was added in small portions to a solution of 800 mg of cycloorbicoside G in 100 ml of absolute dimethyl sulfoxide. After 30 min, 10 ml of methyl iodide was added dropwise, and the reaction mixture was stirred for another 4 h. The products were poured into 200 ml of a 2% aqueous solution of sodium hyposulfite and were exhaustively extracted with chloroform. The residue after the usual treatment and evaporation of the chloroform was chromatographed on a column. Elution by system 4 yielded 169 mg of the octa-O-methyl ether (IV), $C_{49}H_{82}O_{14}$, mp 97-99°C (from methanol), $[\alpha]_D^{24}$ +20 ± 2° (c 1.0; methanol). ν_{max}^{KBr}, cm^{-1}: 3040 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M^+ 894 (1.8), 879 (1.6), 862 (0.7), 847 (0.4), 836 (0.6), 821 (0.6), 804 (0.3), 789 (0.3) 733 (0.4), 719 (17.0), 703 (10.6), 688 (2.9), 659 (82.1), 643 (7.7), 627 (8.8), 617 (88.2), 601 (7.0), 585 (22.9), 573 (88.6), 513 (6.4), 484 (17.6), 469 (21.1), 453 (21.1), 443 (18.1), 441 (22.9), 427 (16.5), 401 (29.3), 395 (14.7), 219 (20.5), 187 (79.2), 175 (100), 155 (23.5), 143 (100),115 (47.1), 111 (52.9), 101 (100), 99 (47.1).

Then the elution of the column was continued with system 5, and 200 mg of the hepta-O-methyl ether (V) was isolated: $C_{48}H_{80}O_{14}$, mp 197-198°C (from methanol), $[\alpha]_D^{24}$ +8.8 ± 2° (c 0.9; methanol). ν_{max}^{KBr}, cm^{-1}: 3540-3450 (OH), 3040 (CH_2 of a cycloprpane ring). Mass spectrum, m/z (%): M^+ 880 (0.8), 865 (1.3), 822 (0.8), 720 (0.7), 705 (5.1), 689 (7.1), 688 (7.1), 645 (90.9), 630 (10.1), 627 (5.6), 603 (100), 587 (4.0), 585 (6.1), 574 (3.0), 513 (1.3), 499 (2.1), 485 (4.0), 470 (17.2), 453 (45.5), 429 (15.7), 427 (11.1), 411 (81.8), 385 (10.6), 271 (9.1), 253 (5.1), 219 (10.6), 187 (72.7), 175 (59.1), 155 (18.2), 143 (59.1), 115 (22.7), 111 (36.4), 101 (59.1), 99 (40.9).

Cycloorbigenin (II) from (V). The methyl ether (V) (100 mg) was heated in a 0.25% methanolic solution of sulfuric acid at 48°C for 20 h. After cooling, 10 ml of water was added, the methanol was distilled off, and the precipitate that deposited was filtered off. Chromatography of the residue on a column in system 6 yielded 12 mg of the genin (II), mp 218-219°C (from methanol), $[\alpha]_D^{24}$ +28 ± 2° (c 1.2; ethanol), which was identified as cyclobigenin by the usual means.

(23R,24S)-16β,23;16α,24-Diepoxycycloartane-3β,7β,25-triol 7-Monomethyl Ether (VI) from (IV). A solution of 90 mg of the methyl ether (IV) in 11 ml of methanol containing 0.25% of sulfuric acid was heated at 50°C for 30 h. After the reaction mixture had been worked up in the manner similar to that described in the preceding experiment and the reaction products had been chromatographed on a column in system 7, 24 mg of the 7-monomethyl ether of cycloorbigenin (VI) was isolated: $C_{31}H_{50}O_5$, mp 147-149°C (from methanol), $[\alpha]_D^{24}$ +45 ± 2° (c 0.4; methanol). ν_{max}^{KBr}, cm^{-1}: 3600-3300 (OH), 3040 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M^+ 502 (5.6), 487 (8.5), 470 (17.3) 455 (13.7), 453 (5.2), 443 (100), 441 (8.5), 437 (5.9), 411 (47.1), 393 (10.5), 387 (6.5), 385 (7.8), 363 (7.2), 349 (29.4), 305 (9.8), 303 (17.6), 271 (10.8).

Identification of the Methylated Monosaccharides. The methanolysis of 5 mg of each of the methyl ethers (IV) and (V) in 2 ml of absolute methanol containing 5% of hydrogen chloride was carried out for 4 h.

It was shown with the aid of GLC [6] that the methanolysis products of the two methyl ethers were identical and included two components, identified as 2,3,4,6-tetra-O-methyl-D-glucopyranose (T_{rel}: 1.00, 1.22) and 2,3,4-tri-O-methyl-D-xylopyranose (T_{rel}: 0.40, 0.44).

SUMMARY

A new cycloartane bisdesmoside – cycloorbicoside G has been isolated from the epigeal part of the plant Astragalus orbiculatus Ledeb. (Leguminosae) and has been found to be (23R, 24S)-16β,23;16α,24-diepoxycycloartane-3β,7β,25-triol 25-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside.

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STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES OF PLANTS OF THE Allium GENUS.

XXIII. STRUCTURE OF CEPAGENIN AND OF ALLIOSPIROSIDES C AND D FROM Allium cepa

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Two new steroid glycosides of the spirostan series have been isolated from the fruit of Allium cepa L. (family Liliaceae): alliospirosides C and D. On the basis of chemical transformations and spectral characteristics it has been established that the aglycon of both glycosides is a new steroid sapogenin – cepagenin – having the structure of (24S,25R)-spirost-5-ene-1β,3β,24-triol. Alliospirosides C and D are cepagenin 1-O-[O-α-L-rhamnopyranosyl-(1→2)α-L-arabinopyranoside] and 1-O-[O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside], respectively.

Continuing the fractionation of the total extractive substances from the fruit of the garden onion Allium cepa L. (family Liliaceae) [1], we have isolated two new glycosides which have been called alliospirosides C (II) and D (III). The present publication is devoted to the proof of the structures of these glycosides.

Both compounds (II) and (III) appeared in the form of violet spots when chromatograms (TLC) were treated with vanillin–phosphoric acid. The set of lines in the IR spectra of glycosides (II) and (III) in the "fingerprint" region differed substantially from the pattern characteristic for an unsubstituted spiroketal grouping [2, 3]. Nevertheless, the possibility could not be excluded of assigning alliospirosides C and D to derivatives of

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