TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XXXVII. CYCLOARALOSIDE F FROM Astragalus amarus AND Astragalus villosissimus

M. I. Isaev and B. A. Imomnazarov

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The glycosides of the plant Astragalus villosissimus Bunge have been studied. Four glycosides have been isolated from the roots of this plant, and two of them have been identified as β-sitosterol β-D-glucopyranoside and cycloaraloside C. The most polar glycoside, which has also been isolated from the roots of Astragalus amarus Pall., proved to be a new triterpene glycoside of the cycloartane series and has been called cycloaraloside F. It is a trioside of cyclosieversigenin containing two molecules of D-glucose and one molecule of D-apiose. On the basis of chemical transformations and spectral characteristics, the structure of cycloaraloside F has been established as 20R, 24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-[O-D-apio-β-D-furanosyl-(1→2)-β-D-glucopyranoside] 25-O-β-D-glucopyranoside.

Continuing investigations of triterpene glycosides of plants of the genus Astragalus (Leguminosae) [1], we have studied Astragalus villosissimus Bunge. Chromatographic analysis showed that the epigeal part of the plant did not contain the substances sought. By column chromatography, a methanolic extract of the roots yielded four products which were named in order of increasing polarity substances 1—4. Substances 1 and 3 were identified as β-sitosterol β-D-glucopyranoside [2] and cycloaraloside C (V) [3]. The structure of compound 4 was shown to be identical with that of substance 9 [4] isolated from Astragalus amarus Pall., which we have called cycloaraloside F (I).

The presence in the PMR spectrum (C_5D_5N) of glycoside (I) at 0.40 ppm of a one-proton doublet forming a component of an AB system and the signals of seven methyl groups in the high-field region permitted this substance to be assigned to triterpenoids of the cycloartane series [5, 6]. An absorption band at 3050 cm^{-1} in the IR spectrum of compound (I), characteristic for a methylene group in a cyclopropane ring, also showed that the glycoside under consideration belonged to the cycloartane series. (See scheme 1.)

In actual fact, from the products of the acid hydrolysis of cycloaraloside F we isolated the genin (II), which was identified as cyclosieversigenin [6], and the progenin (III), which was identified as cycloaraloside A [7]. In the carbohydrate fraction of the products of acid hydrolysis we detected D-glucose and D-apiose by PC and GLC [8]. It was shown by the GLC method that glycoside (I) contained these monosaccharides in a ratio of 2:1.

The partial hydrolysis of glycoside (I) led to bioside (IV). The latter was identical with cycloaraloside E [4]. Consequently, the second D-glucose residue was present at C-25 of the genin.

Cycloaraloside F was subjected to enzymatic hydrolysis with enzymes obtained from the gastric juice of the Roman snail. From the products cycloaraloside C was isolated and identified [3]. Hence, D-apiose addition on one hydroxyl group at C-2 of D-glucose was present at C-3 of one genin.

The facts given showed that cycloaraloside F 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] 25-O-β-D-glucopyranoside, which is in complete agreement with its ^{13}C NMR spectrum (Table 1).

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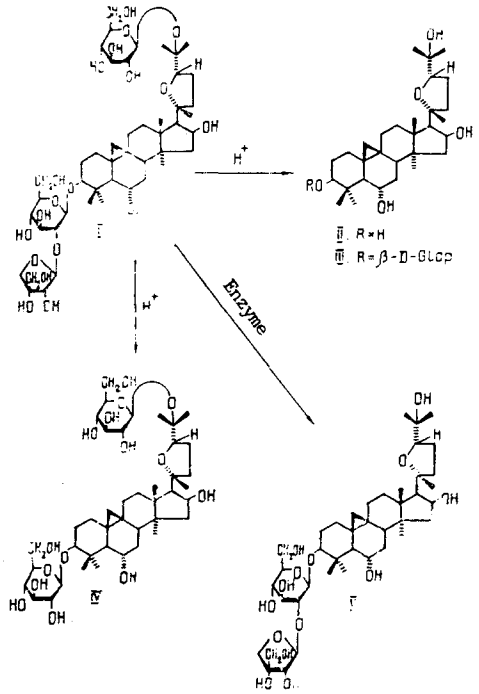
TABLE 1. Chemical Shifts of the Carbon Atoms of Compounds (I-V) (δ, ppm, O-TMS)

C atom	Compound I	II	III	IV	V
1	32,35	32,72	32,41	32,40	32,42
2	30,19	31,30	30,16	30 0	30,15
3	88,84	78,21	88,96	88,95	88,89
4	42,58	42,28	42,58	42,60	42,58
5	53 90	53,86	53,93	53,85	54,01
6	67,81	68,27	67,97	68,10	67,97
7	38,45	38,60	38 54	38,55	38,54
8	46,60	47,21	47,06	46,95	46 98
9	20,73	20,84	20,84	20,70	20 84
10	29,37	29,80	29,51	29,25	29,43
11	26 14	26,29a	26,37a	26,10	26,37a
12	33,41	33,31	33,31	33,45	33,30
13	45,13	44,89	44,97	45,15	45,04
14	45,97	46,09	45,91	46,05a	46,09
15	46,01	46 60	46,09	46 05a	46,61
16	73,47	73,35	73,43	73,80	73 43
17	58,06	58,26	58,26	58,20	58,34
18	21,43	21,51	21,51	21,45	21,51
19	30,34	31,0	30 55	30,45	30,47
20	87,14	87,17	87,17	87,15	87,25
21	27,80	28,46	28,41	27,75	28,16
22	34,95	34,81	34,88	34,95	34,88
23	25,90	26,29a	26,37a	25,80	26,37a
24	82,03	81,57	81 64	82,05	81,72
25	78,52	71,19	71,26	73,45b	71,26

C atom	Compound I	II	III	IV	V
26	22,97*	27,04*	27,04*	22,80*	27,12*
27	25,60*	28,09*	28,53*	25 50*	28,53*
28	19,96	20,17	20,09	19,95	20,17
29	28,75	29,28	28,91	28 65	28,76
30	16,56	16 16	16,66	16,50	16,58
	3-O-β-D-Glcp residue				
1	105,46		106 82	106,95	105,47
2	79,39		75,82	75,75	79,40
3	78,71		78,66	78,45b	78,66
4	71,95		71,78	71,85	72,01
5	78,28		78,06	77,85b	78,21
6	62,85		62,97	62,85	62,89
	D-Apio- β-D-f residue				
1	111,13				111,15
2	77,91				77,83
3	80,56				80,52
4	75,58				75,59
5	66,11				66,11
	25-O-β -D-Glcp residue				
1	98,76		98 70		
2	75,09		75,15		
3	78,44		78,45b		
4	71,28		71,25		
5	77,86		77,85b		
6	62,67		62,70		

The signals marked with identical letters fall upon one another within a column. The assignment of the signal marked with asterisks is uncertain.



Scheme 1

EXPERIMENTAL

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

PC was conducted on type FN-11 paper. For the GLC, TLC, and CC conditions, see [8].

^1H and ^{13}C NMR spectra were recorded on Tesla BS-567 A and Bruker WM-250 instruments in deuteropyridine (δ, ppm; for protons, 0-HMDS; for carbon atoms, 0-TMS).

Isolation and Separation of the Glycosides of Astragalus villosissimus. The air-dry comminuted roots (329 g) of Astragalus villosissimus gathered on the Muinak Peninsula (Muinak region, Karakalpak ASSR) in the middle of July, 1988, were exhaustively extracted with methanol (5 liters). The methanolic extracts were evaporated, giving 41 g of extractive substances. The whole amount of the extractive substances was chromatographed on a column with elution successively by chloroform and systems 1, 2, and 3. Four substances of glycosidic nature were isolated which were designated in order of increasing polarity substances 1—4. In order to free them from pigments, each substance was repeatedly rechromatographed, and the final products were: substance 1 — 48 mg (0.0145%; here and below the yields are calculated on the air-dry raw material); substance 2 — 132 mg (0.040%); substance 3 — 420 mg (0.1277%) and substance 4 — 710 mg (0.2158%).

β-Sitosterol β-D-Glucopyranoside. Substance 1, mp 276-279°C (from methanol), $[\alpha]_D^{24}$ −37 ± 2° (c 1.2; methanol), was identified as β-sitosterol β-D-glucopyranoside from comparison of its PMR spectrum.

Cycloaraloside C (V). Substance 3, mp 242-244°C (from methanol), $[\alpha]_D^{30}$ 4.5 ± 2° (c 1.5; methanol), was identified as cycloaraloside C [3] by direct comparison with an authentic sample in TLC and from its PMR spectrum.

Cycloaraloside F (I). Substance 4 was shown to be identical with substance 1 [4] isolated from Astragalus amarus, which we have called cycloaraloside F. Glycoside (I): $C_{47}H_{78}O_{19}$, amorphous, $[\alpha]_D^{28}$ − 26.1 ± 2°, (c 0.84; methanol) ν_{max}^{KBr}, cm^{-1}: 3600-3160 (OH); 3050 (CH_2 of a cyclopropane ring). PMR spectrum, ppm: 0.40 (H-19, d, ^2J = 4 Hz); 0.82, 1.16, 1.20, 1.26, 1.26, 1.52, 1.83 (7 × CH_3, s); 4.25 and 4.61 (2 H-5 of D-apiose, d, ^2J = 10 Hz), 4.78 (H-2 of D-apiose, br.s.); 4.82 anomeric proton of D-glucose at C-3, d, ^3J = 8 Hz; 4.90 (anomeric proton of D-glucose at C-25, d, ^3J = 8 Hz); the H-16 signal was completely masked by the signals of the anomeric protons of the D-glucose residues; 6.37 (anomeric proton of D-apiose, br.s.).

Cyclosieversigenin (II) and Cycloaraloside A (III) from (I). Glycoside (I) (200 mg) was hydrolyzed with 25 ml of a 0.25% methanolic solution of sulfuric acid at 50°C for 2 h. The reaction mixture was diluted with 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 1. This led to the isolation of 55 mg of cyclosieversigenin (II), mp 239-241°C (from methanol), $[\alpha]_D^{30}$ 50 ± 2° (c 1.0; methanol).

On continuing elution of the column with system 2, 30 mg of glycoside (III) was isolated, with mp 240-242°C (from system 2), $[\alpha]_D^{30}$ + 33.5 ± 2° (c 1.2; methanol). Its PMR spectrum showed the identity of glycoside (III) with cycloaraloside A.

The filtrate was evaporated to a volume of 20 ml and was boiled for 1 h. After neutralization of the solution with type ARA-8p anion-exchange resin and removal of the resin, the water was evaporated off. D-glucose (T_{rel} 0.88, 1.00) and D-apiose (T_{rel} 0.15, 0.16, 0.17, and 0.18) were identified in the residue by PC (system 4) and GLC [8] in comparison with authentic samples. Quantitative analysis of the monosaccharides by GLC [8] showed the presence in glycoside (I) of D-glucose and D-apiose residues in a ratio of 1.00:0.47.

Cycloaraloside E (IV) from (I). Cycloaraloside F (322 mg) was hydrolyzed with 25 ml of 0.05% methanolic sulfuric acid at 50°C for 4 h. Then the reaction mixture was diluted with water and the methanol was evaporated off. The aqueous solution was treated with n-butanol. The butanolic extract was washed with water, and the butanol was distilled off. The dried residue was chromatographed on a column with elution by system 3. This gave 193 mg of glycoside (IV), mp 180-182°C (from chloroform—methanol (1:1)), $[\alpha]_D^{28}$ −5 ± 2° (c 0.8; methanol), which was identified as cycloaraloside E also by its chromatographic behavior in TLC and by its PMR and IR spectra.

Cycloaraloside C (V) from (I). Cycloaraloside F (127 mg) in 20 ml of water was treated with 50 mg of an enzyme preparation obtained from the gastric juice of the Roman snail and two drops of toluene, and the mixture was left at room temperature for 28 days. The dry residue after evaporation of the solvent was chromatographed on a column with elution by system 3. This gave 50 mg of the progenin (V), mp 242-244°C (from methanol), $[\alpha]_D^{25}$ + 4.5 ± 2 (c 1.6; methanol). Progenin (V) was shown to be identical with cycloaraloside C [3] also by its PMR and IR spectra and from its R_f value on TLC in comparison with an authentic sample.

LITERATURE CITED

1. B. A. Imomnazarov and M. I. Isaev, Khim. Prir. Soedin., 653 (1990).
2. L. J. Swift, J. Am. Chem. Soc., 74, 1099 (1952).
3. M. I. Isaev and N. K. Abubakirov, Khim. Prir. Soedin., 656 (1990).
4. M. I. Isaev and N. K. Abubakirov, Khim. Prir. Soedin., 783 (1990).
5. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 431 (1985).
6. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 156 (1989).
7. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 806 (1989).
8. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 719 (1986).

TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS.

XXXVIII. CYCLOALPIGENIN D AND CYCLOALPIOSIDE D FROM Astragalus alopecurus

M. A. Agzamova and M. I. Isaev

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Eight compounds of triterpenoid nature have been isolated from the epigeal parts of the plant Astragalus alopecurus Pall. (Leguminosae) and have been designated in order of increasing polarity as substance 1—8. On the basis of chemical transformations and spectral characteristics, we have established the structures of 4 and 8, which have been called cycloalpigenin D and cycloalpioside D, respectively. Cycloalpigenin D is 20R,24S-epoxycycloartane-3β,7β, 16β,25-tetraol. Cycloalpioside D is cycloalpigenin D 3-O-β-D-xylopyranoside.

Continuing investigations of the cycloartane methylsteroids of plants of the genus Astragalus (Leguminosae) [1], we have begun a study of Astragalus alopecurus Pall. (foxtail milk vetch). In a methanolic extract of the epigeal part of the plant eight products of triterpenoid nature were detected in TLC, and these have been designated in order of increasing polarity as substances 1—8. Chromatography of the total triterpenoids and repeated rechromatography of the individual fractions on a column led to the isolation of the individual compounds 1—8. The first four substances consisted of genins, and the last four were of glycosidic nature. The present work was devoted to proving the structures of the new substances 4 and 8, which we have called cycloalpigenin D (I) and cycloalpioside D (V), respectively.

In the strong-field region of the PMR spectrum of cycloalpigenin D (Table 1), there were the signals of seven methyl groups and of two protons interlinked in the manner of an AB system, at 0.19 and 0.68 ppm with ^2J = 4 Hz, which are characteristic for an isolated cyclopropane methylene group. The presence of the latter was also shown by an absorption band at 3040 cm^{-1} in the IR spectrum of the compound under consideration [2]. The facts given, and also the molecular formula $C_{30}H_{50}O_5$, showed that the new genin (I) belonged to the triterpenoids of the cycloartane series [3, 4]. (see Scheme 1).

The acetylation of the genin (I) with acetic anhydride in pyridine led to the diacetate (II) and the triacetate (III). A comparative analysis of the PMR spectra of compounds (I-III) enabled the signals at 3.42, 3.70, and 4.97 ppm in the spectrum of the genin (I) to be assigned to protons located geminally to secondary hydroxy groups.

In the mass spectra of cycloalpigenin D (I) and its acetates (II) and (III) the maximum peak in each case was that of an ion with m/z 143 arising on the cleavage of the C-17—C-20 bond, while in the PMR spectra of the same compounds, one-proton signals were clearly seen at 3.78, 3.77, and 3.81 ppm, respectively, belonging to H-24. These facts indicated that

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