TRITERPENE GLYCOSIDES OF ASTRAGALUS AND THEIR GENINS

XLI. CYCLOEXOSIDE FROM ASTRAGALUS EXILIS

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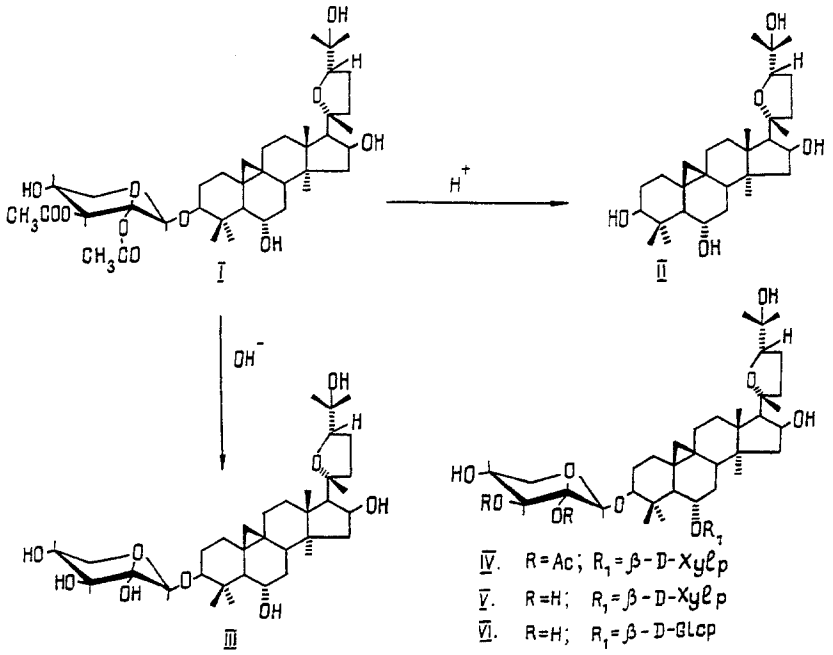
In addition to known glycosides of the cycloartane series – cyclosieversiosides A, E, and F – a new acetylated compound of glycosidic nature – cycloexoside – has been isolated from the roots of Astragalus exilis A. Kor. (Leguminosae). On the basis of chemical transformations and spectral characteristics, the structure of cycloexoside has been established as 20R,24S-epoxycycloartane-3β,6α,16β,25-tetrol 3-O-(2,3-di-O-acetyl-β-D-xylopyranoside).

Continuing investigations of cycloartane methylsteroids from plants of the genus Astragalus (Leguminosae) [1], we have now studied Astragalus exilis A. Kor. No triterpenoids were detected in the epigeal part of the plant. In the methanolic extract of the roots TLC showed the presence of at least ten substances of triterpenoid nature which have been designated in order of their increasing polarity as substances (1-10). By chromatography of the total extractive substances on a column and rechromatography of individual fractions we isolated substances 2, 3, 7, and 8. The remaining substances consisted of minor components of the total material.

Substances 3, 7, and 8 were identified as cyclosieversiosides A (IV), E (V), and F (VI), respectively [2]. Substance 2 proved to be a new glycoside and we have called it cycloexoside (I).



In the PMR spectrum of glycoside (I) at 0.17 and 0.42 ppm we detected the one-proton doublets of an AB system that are characteristic for an isolated cyclopropane methylene.

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

C atom	Compound			C atom	Compound		
	I	II	III		I	II	III
1	32,24	32,72	32,48	21	28,63	28,46	28,54
2	29,98	31,30	29,53	22	34,91	34,81	34,92
3	89,30	78,21	88,70	23	26.40	26,29[a]	26,42
4	42,21	42,28	42,70	24	81,71	81.57	81,71
5	53,81	53,86	54,09	25	71,23	71,19	71,24[a]
6	68,05	68,27	68,01	26	27,11*	27,04*	27,13*
7	38,71	38,69	38,65	27	28,14*	28,09*	28,17*
8	47,17	47,21	47,07	28	20,19	20,17	20.17
9	20,81	20,84	21,02	29	28,52	29,28	28,92
10	29,44	29,80	30,64	30	16,39	16,16	16,67
11	26,18	26,29[a]	26,22		β-D-Xylp residue		
12	33,36	33,31	33,41	1	104,10(—3,51)		107,61
13	45,02	44,89	45,05	2	73,15(—2,45)		75,60
14	46,11	46,09	46,14	3	76,61(—1,87)		78,48
15	46,71	46,69	46,69	4	68,81(—2,43)		71,24[a]
16	73,41	73,35	73,43	5	66,67(—0,36)		67,03
17	58,37	58,26	58,37	CH_3	20,74		
18	21,58	21,51	21,52	|	21,04		
19	30,76	31,0	30,34	COO	169,82		
20	87,20	87,17	87,23		170,47		

Signals marked with identical letters are superposed on one another and those with asterisks have been assigned ambiguously.

The presence of a cyclopropane was also shown by an absorption band in the IR spectrum of glycoside (I) at 3050 cm^{-1} [3]. These facts enabled us to assign glycoside (I) to the triterpenoids of the cycloartane series [2, 4].

The acid hydrolysis of glycoside (I) gave the genin (II), which was identified as cyclosieversigenin [2]. D-Xylose was detected in the carbohydrate fraction of the hydrolysate by the PC method. GLC [5] showed glycoside (I) contained one residue of the monosaccharide.

In the IR spectrum of cycloexoside there were also absorption bands at 1750 and 1260-1240 cm^{-1}, showing the presence of an ester grouping. The ^1H NMR spectrum of glycoside (I) contained two three-proton singlets at 1.89 and 2.03 ppm. Consequently, cycloexoside consisted of a glycoside with two acetate groups. This conclusion was confirmed by the ^{13}C NMR spectrum of cycloexoside, which contains the signals of the carbon atoms of two acetate groups at 20.74, 21.04, 169.82, and 170.47 ppm.

The alkaline hydrolysis of glycoside (I) led to the deacetylated product (III). Glycoside (III) was identified as cyclosieversiogenin 3-O-β-D-xylopyranoside [2].

The positions of the acetate groups were revealed by a comparative study of the ^{13}C NMR spectra of glycosides (I) and (III) (Table 1). The values of the chemical shifts of the carbon atoms of the genin moieties of the two glycosides were practically identical. Consequently, the acetate groups were located in the monosaccharide residue of glycoside (I). As can be seen from Table 1, the signals of the four carbon atoms C-1—C-4 of the β-D-xylopyranoside residue of glycoside (I) had undergone a considerable upfield shift in comparison with those of glycoside (III). This was possible only if the acetate groups were located at C-2 and C-3 of the β-D-xylopyranoside residue.

An analogous conclusion also followed from the ^1H NMR spectrum of glycoside (I), where the signals of protons geminal to acetoxy functions were observed at 5.26 ppm (t, ^3J = 7.7 Hz) and 5.49 ppm (t, ^3J = 7.7 Hz). The multiplicities and parameters of these signals corresponded to the H-2 and H-3 protons, respectively, of a β-D-xylopyranoside residue. This conclusion was confirmed by double homonuclear resonance experiments.

The pre-irradiation of the anomeric proton (4.75 ppm) led to the conversion into a doublet (^3J = 7.7 Hz) of the signal of the gem-acetoxy proton located at 5.26 ppm, while the presaturation of the latter converted the signal of the anomeric proton into a singlet and the signal of the second gem-acetoxy proton, resonating at 5.49 ppm, into a doublet with the SSCC ^3J = 7.7 Hz. Thus, a system of protons interconnected by vicinal spin-spin inter-

actions – H-1, H-2, and H-3 of the β-D-xylopyranoside ring – was observed. Consequently, the acetyl groups were located at C-2 and C-3 of the monosaccharide residue. The experimental facts described permitted us to conclude that cycloexoside is 20R,24S-epoxycycloartane-3β,6α,16β,25-tetrol 3-O-(2,3-di-O-acetyl-β-D-xylopyranoside).

EXPERIMENTAL

General Remarks. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) chloroform–methanol–water (140:14:1); 3) chloroform–methanol–water (70:12:1); 4) n-butanol–pyridine–water (6:4:3).

^1H and ^{13}C NMR spectra were taken on a Bruker AM-400 instrument in deuteropyridine (δ, ppm, p – TMS). ^{13}C NMR spectra were also obtained under the conditions of J-modulation.

Isolation and Separation of the Triterpene Glycosides of Astragalus exilis. The air-dried roots (457 g) of Astragalus exilis collected in the flowering phase (July, 1990) in the gorge of the river Gornaya Khanaka (southern slopes of the Gissar range, Tadzhikistan) were extracted with methanol (3 × 1.5 liters). The methanolic extract was evaporated to dryness. This gave 68 g of extractive substances containing about 10 components of triterpenoid nature which were designated in order of increasing polarity as substances (1)-(10). Substances (1), (4)-(6), (9), and (10) were minor components of the total material. By chromatographing the total material on a column of silica gel with the successive use of chloroform and solvent systems 1-3 and rechromatography we isolated the individual substances (2) (120 mg – 0.026%, the yields here and below being calculated on the air-dry raw material), (3) (105 mp – 0.022%), (7) (510 mg – 0.11%), and (8) (2 g – 0.43%).

Cycloexoside (I) – substance (2), $C_{39}H_{62}O_{11}$, mp 193-196°C (from methanol), $[\alpha]_D^{23}$ –56 ± 2° (c 0.5; methanol. ν_{max}^{KBr}, cm^{-1}: 3600-3240 (OH), 3050 (CH_2 of a cyclopropane ring), 1750, 1260-1240 (ester groups). PMR spectrum: 0.17 and 0.42 (2H-19, d, ^2J = 4 Hz), 0.87; 1.08; 1.20; 1.20; 1.29; 1.44; 1.56 (7 × CH_3, s), 1.89 (CH_3COO at C-3 of D-xylose, s), 2.03 (CH_3COO at C-2 of D-xylose, s), 2.38 (H-17, d, ^3J = 7.7 Hz), 2.90 (H-22, q, $^3J_1 = {}^3J_2 = {}^2J$ = 10 Hz), 3.34 (H-3, dd, $^3J_1$ = 12 Hz, $^3J_2$ = 4 Hz), 3.56 (H-6, m), 3.59 (H-5a of D-xylose, t, $^3J = {}^2J$ = 10 Hz), 3.77 (H-24, dd, $^3J_1$ = 10 Hz, $^3J_2$ = 6 Hz), 4.12 (H-4 of D-xylose, m), 4.20 (H-5e of D-xylose, dd ^2J = 10 Hz, ^3J = 6 Hz), 4.75 (H-1 of D-xylose, d, ^3J = 7.7 Hz), 4.84 (H-16, q, $^3J_1 = {}^3J_2 = {}^3J_3$ = 7.7 Hz), 5.26 (H-2 of D-xylose, t, ^3J = 7.7 Hz), 5.49 (H-3 of D-xylose, t, ^3J = 7.7 Hz).

Cyclosieversioside A (IV) substance (3), mp 229-230°C (from methanol), $[\alpha]_D^{23}$ +21 ± 2° (c 1.07; methanol) [2]. This glycoside was identified on the basis of the PMR spectrum and direct comparison with an authentic sample.

Cyclosieversioside E (V) – substance (7), mp 216-218°C (from methanol), $[\alpha]_D^{23}$ +24 ± 2° (c 0.7; chloroform–methanol (1:1)) [2]. This glycoside was likewise identified by comparison with an authentic sample and from the characteristics of its PMR spectrum.

Cyclosieversioside F (VI) – substance (8), mp 284-286°C (from methanol), $[\alpha]_D^{23}$ +38 ± 2° (c 0.45; methanol) [2]. The PMR spectrum and R_f values in the TLC of the glycoside coincided with those for cyclosieversioside F.

Cyclosieversigenin (II) from (I). Glycoside (I) (30 mg) was hydrolyzed with 10 ml of 0.25% methanolic sulfuric acid at 60°C for 2 h. After the usual working up and chromatography on a column in system 1, the genin fraction of the hydrolysis products yielded 12 mg of cyclosieversigenin (II), mp 239-241°C (from methanol), $[\alpha]_D^{23}$ +52 ± 2° (c 0.8; methanol).

After the separation of the genin part of the hydrolysate, the aqueous filtrate was concentrated to 5 ml and was boiled for 1 h and was neutralized with the anion-exchange resin ARA-8p. The resin was removed and the solution was evaporated to dryness, and then D-xylose was detected in the residue by PC in system 4. It was found by the GLC method [5] in the presence of D-glucose as standard that glycoside (I) contained one D-xylose residue.

Cyclosieversigenin 3-O-β-D-Xylopyranoside (III) from (I). Cycloexoside (25 mg) was hydrolyzed with 10 ml of 0.1% methanolic sodium hydroxide at room temperature for 3 h. After the working up of the products and chromatography on a column in system 3, 15 mg of glycoside (III) was obtained with mp 262-264°C (from methanol), $[\alpha]_D^{23}$ +42 ± 2° (c 0.4; methanol), and this was identified as cyclosieversigenin 3-O-β-D-xylopyranoside likewise from its PMR spectrum and comparison with an authentic sample by TLC.

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TRITERPENE GLYCOSIDES OF ASTRAGALUS AND THEIR GENINS

XLII. CYCLOARTANES OF ASTRAGALUS TRAGACANTHA

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Another six components of Astragalus tragacantha Habl. have been identified on the basis of spectral characteristics and chemical transformations. We have previously described cyclocanthagenin and its 3-O-β-D-xylopyranoside – cyclocanthoside A – as products of the acid hydrolysis of cyclocanthoside D. Cyclocanthosides B, C, E, and G are here described for the first time and are (24S)-cycloartane-3β,6α,16β,24,25-pentol 3-O-(4-O-acetyl-β-D-xylopyranoside) 6-O-β-D-glucopyranoside, (24S)-cycloartane-3β,6α,16β,24,25-pentol 6-O-(6-O-acetyl-β-D-glucopyranoside) 3-O-β-D-xylopyranoside, (24S)-cycloartane-3β,6α, 16β,24,25-pentol 6-O-β-D-glucopyranoside, and (24S)-cycloartane-3β, 6α, 16β, 24, 25-pentol 6-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside], respectively.

Continuing a study of the cycloartane triterpenoids and their glycosides of Astragalus tragacantha Habl. (Leguminosae) [1, 2] we have isolated another two products which have been designated as substances (3) and (12A). The present paper is devoted to a proof of the structures of four new compounds and the identification of two known compounds isolated from Astragalus tragacantha [1, 2]. The substances under consideration were assigned to the cyclartane triterpenoid series on the basis of their ^1H and ^{13}C NMR spectra [3, 4].

Substance (3) was identified as cyclocanthogenin (I), which we had obtained previously from cyclocanthoside D [1]. Substances (6), (9), (10), (12A), and (14) were glycosides, and we have called them cyclocanthosides A, B, C, E, and G, respectively. Cyclocanthoside A is cyclocanthogenin 3-O-β-D-xylopyranoside, identical with the progenin obtained from cyclocanthoside B [2] (see top of following page).

On acid hydrolysis, cyclocanthoside E (III) formed cyclocanthogenin (I). GLC [5] showed that glycoside (III) contained D-glucose and D-xylose residues in a ratio of 1:1. This conclusion also followed from its ^1H and ^{13}C NMR spectra (Tables 1 and 2).

It became clear from a comparison of the ^{13}C NMR spectra of compounds (I) and (III), that the C-3 and C-6 atoms of the genin moiety of glycoside (III) experienced the glycosylation effect, resonating at 88.59 and 79.13 ppm. Consequently, cyclocanthoside E was a bisdesmoside. The SSCCs of the anomeric protons and the chemical shifts of the carbon atoms of the monosaccharide residue showed the β-configuration, the C1-conformation, and the pyranose form of the D-xylose and D-glucoside residues.

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