TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. LXVII. STRUCTURE OF CYCLOEXOSIDE B

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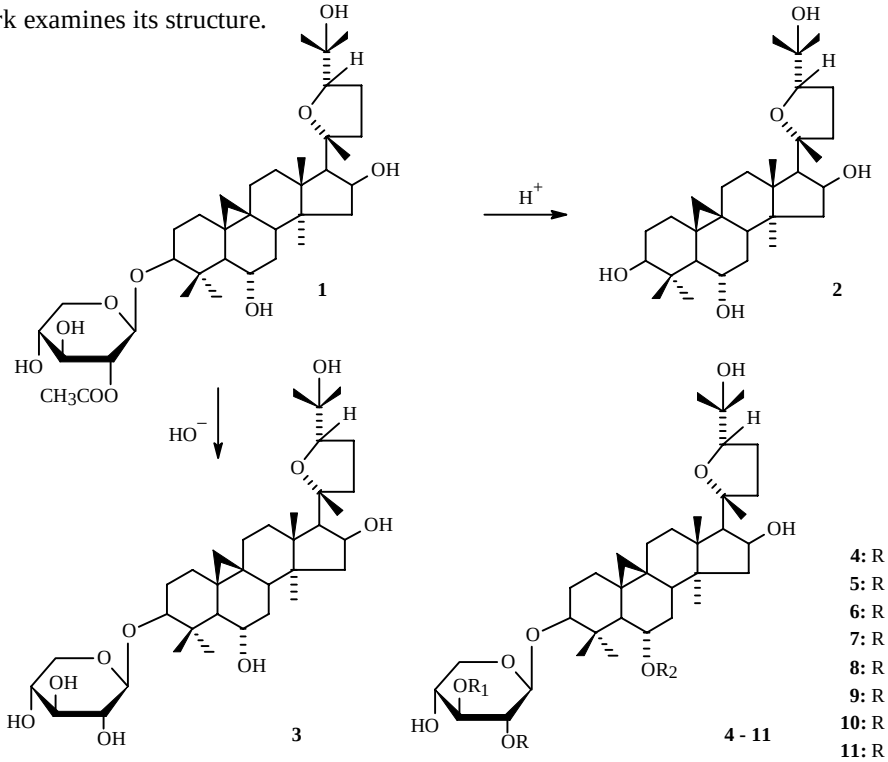
UDC 547.918:547.926

Another seven components from the roots of Astragalus exilis *A.Kor. (Leguminosae) were identified using spectral data and chemical transformations. A triterpenoid of genin nature was identical to cyclosiversigenin. One compound of glycosidic nature turned out to be a new cycloartane glycoside called by us cycloexoside B of structure 20R,24S-epoxycycloartan-3β,6α,16β,25-tetraol 3-O-β-D-(2-O-acetyl)xylopyranoside. Five glycosides were identified as cyclosiversigenin 3-O-β-D-xylopyranoside and cyclosiversiosides B, C, D, and G.*

Key words: triterpenoids, cycloartanes, cyclosiversigenin, cycloexoside B, cyclosiversiosides, *Astragalus*, Leguminosae, PMR, ^{13}C NMR, DEPT, J-modulation.

We continued chemical investigations of isoprenoids from plants of the *Astragalus* genus (Leguminosae) [1] by isolating and identifying from the roots of *A. exilis* A.Kor. four cycloartane glycosides: cycloexoside (**4**) and cyclosiversiosides A (**5**), E (**9**), and F (**10**) [2]. We also isolated another seven substances in continuing the study of this plant. Six of the compounds turned out to be known and were identified as cyclosiversigenin (**2**), cyclosiversigenin 3-O-*β*-D-xylopyranoside (**3**), and cyclosiversiosides B (**6**), C (**7**), D (**8**), and G (**11**) [3]. One compound was a new glycoside called by us cycloexoside B (**1**). This work examines its structure.



4: R = R_1 = Ac, R_2 = H;
5: R = R_1 = Ac, R_2 = *β*-D-Xyl*p*;
6: R = R_1 = Ac, R_2 = *β* - D-Glc*p*;
7: R = Ac, R_1 = H, R_2 = *β*-D-Xyl*p*;
8: R = Ac, R_1= H, R_2 = *β*-D-Glc*p*;
9: R = R_1 = H, R_2 = *β*-D-Xyl*p*;
10: R = R_1 = H, R_2 = *β*-D-Glc*p*;
11: R = *α*-L-Rha*p*, R_1 = H, R_2 = *β*-D-Xyl*p*

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0009-3130/02/3806-0579$27.00 ©2002 Plenum Publishing Corporation

TABLE 1. Chemical Shifts of C Atoms of compounds **1-3** (δ, ppm, C_5D_5N, 0 = TMS)

C atom	Compound		
	1	**2**	**3**
1	32.25	32.81	32.50
2	30.08	31.47	30.35
3	89.01	78.32	88.72
4	42.24	42.46	42.72
5	53.80	54.00	54.12
6	68.09	68.38	68.02
7	38.66	38.85	38.67
8	47.14	47.30	47.07
9	20.95	20.99	21.05
10	29.43	29.92	29.55
11	26.10	26.32	26.25
12	33.30	33.47	33.43
13	44.96	45.09	45.07
14	46.05	46.21	46.16
15	46.61	46.81	46.73
16	73.40	73.48	73.44
17	58.32	58.44	58.40
18	21.54	21.66	21.53
19	30.70	31.02	30.63
20	87.18	87.27	87.25
21	28.63	28.59	28.94
22	34.86	34.97	34.95
23	26.35	26.17	26.44
24	81.67	81.75	81.73
25	71.22	71.27	71.26*
26	27.03	27.17	27.15
27	28.08	28.21	28.19
28	20.12	20.27	20.20
29	28.47	29.44	28.55
30	16.39	16.14	16.68
	β-D-Xyl*p*		
1	104.79 (-2.84)		107.63
2	76.15 (+0.52)		75.63
3	75.63 (-2.87)		78.50
4	71.27		71.26*
5	67.02		67.05
$\underline{CH_3}COO$	21.21		
$CH_3\underline{COO}$	170.09		

*Signals overlap.

The PMR spectrum of **1** contains 1H doublets of an AX system at 0.16 and 0.42 ppm with spin—spin coupling constants (SSCC) ^2J = 4.5 Hz in addition to signals of seven methyls in the range 0.88-1.68 ppm. This indicates that the observed compound is a cycloartane triterpenoid [3, 4]. Therefore, cyclosiversigenin was isolated from the acid hydrolysate of **1** as the genin. Paper chromatography (PC) of the carbohydrate part of the acid hydrolysate detected D-xylose. PMR and ^{13}C NMR spectra of **1** (Table 1), which contain signals of one pentose, indicate that the studied glycoside is a monoxylopyranoside.

The PMR spectrum of glycoside **1** has a 3H singlet at 2.05 ppm, which indicates that this glycoside contains one acetic acid unit. As expected, signals of one acetyl are found in the ^{13}C NMR spectrum of a new glycoside **1** at 21.21 and 170.09 ppm.

Alkaline hydrolysis of **1** produces glycoside **3**, identified as cyclosiversigenin 3-O-β-D-xylopyranoside [3]. Therefore, cycloexoside B is cyclosiversigenin 3-O-β-D-xylopyranoside monoacetate. The site of attachment of the acetyl group was revealed by comparing the PMR and ^{13}C NMR spectra of compounds **1**-**3**.

Atoms C-6, C-16, and C-25 of **1** resonate in the ^{13}C NMR spectrum at 68.09, 73.40, and 71.22 ppm, respectively. These values are practically identical to those in the ^{13}C NMR spectra of cyclosiversigenin and glycoside **3**. Therefore, the acetyl unit is not located in the genin part of **1**. In fact, the PMR spectrum of **1** has at 5.46 ppm a 1H doublet of doublets with SSCCs $^3J_1$ = 9 and $^3J_2$ = 8 Hz that belongs to a proton geminal to the acetoxy unit. These parameters are similar to those for H-2 and H-3 of a β-D-xylopyranoside unit. The magnitude of the chemical shift does not allow unambiguous assignment of this signal. Sometimes H-2 and H-3 of a β-D-xylopyranoside unit resonate as triplets of identical SSCC, which complicates the assignment [2]. In this instance, the signals can be assigned by analyzing the fine multiplicities and the SSCCs of H-1, H-2, and H-3 of the β-D-xylopyranoside unit, which forms a system of vicinally coupled protons. The anomeric proton resonates as a doublet with $J_{1,2}$ = 8 Hz. As already mentioned, the doublet of doublets from the proton geminal to the acetoxy group has the same SSCC ($J_{1,2}$ = 8 Hz) and therefore belongs to H-2 of the pentose. The third proton resonates as a triplet with $J_{2,3} = J_{3,4}$ = 9 Hz at 4.07 ppm. Therefore, it is H-3 of the monosaccharide unit.

This means that the acetyl group is located on C-2 of β-D-xylose. The positive reaction to periodate oxidation of **1**, which indicates the presence of an α-diol in the molecule, confirms the location of the acetyl group.

Additional confirmation of the acetyl position comes from a comparison of the ^{13}C NMR spectra of **1** and **3**. Table 1 shows that the chemical shifts of C-1 (-2.84 ppm), C-2 (+0.52), and C-3 (-2.87) of the monosaccharide unit change considerably on going from **3** to **1**. The signs and magnitudes of these changes agree well with the α- and β-effects of an acetyl located on C-2 of the β-D-xylopyranoside ring [5] and unambigously determine the location of the acyl group.

Thus, **1** has the structure 20*R*,24*S*-epoxycycloartan-3β,6α,16β,25-tetraol 3-O-β-D-(2-O-acetyl)-xylopyranoside.

EXPERIMENTAL

For general comments, see the literature [6]. The following solvent systems were used: $CHCl_3$—CH_3OH (25:1, 1; 15:1, 2), $CHCl_3$—CH_3OH—H_2O (140:14:1, 3; 70:12:1, 4; 70:23:4, 5), *n*-BuOH—C_5H_5N—H_2O (6:4:3, 6).

PMR and ^{13}C NMR spectra were recorded on Bruker DRX-500, Bruker AM-400, and UNITYplus 400 spectrometers in C_5D_5N (δ, ppm, 0 = TMS). ^{13}C NMR spectra were obtained with complete C–H decoupling and under J-modulation and DEPT conditions.

Isolation and Separation of Triterpene Glycosides of *Astragalus exilis.* Another batch of air-dried roots (750 g) of *A. exilis* collected during flowering (July) from a gorge near the Gornaya Khanaki river (southern slopes of the Gissarsk crest, Tadzhikistan) was extracted with CH_3OH (2.5 L ×3). The methanol extracts were evaporated to dryness to afford dry extract (120 g). Chromatography over a silica-gel column with successive elution by $CHCl_3$ and systems 1-5 and rechromatography of the individual fractions isolated 11 pure compounds that are presented in order of increasing polarity.

Known compounds were identified by comparison with authentic samples. PMR and ^{13}C NMR spectral data are given for the new compound **1** and for other compounds to prove their structures.

Cyclosiversigenin (2), 15 mg (0.002%, yields here and hereafter are based on air-dried raw material), $C_{30}H_{50}O_5$, mp 239-241°C (MeOH) [3, 7, 8].

PMR spectrum (400 MHz, C_5D_5N, 0 = TMS, δ, ppm, J/Hz): 0.36 and 0.63 (2H-19, d, ^2J = 4), 1.03, 1.31, 1.34, 1.38, 1.46, 1.59, 1.90 (7×CH_3, s), 2.55 (H-17, d, ^3J = 7.8), 3.12 (H-22, td, $^2J = {}^3J_1$ = 11.3, $^3J_2$ = 9), 3.66 (H-3, dd, $^3J_1$ = 11.5, $^3J_2$ = 4.6), 3.80 (H-6, td, $^3J_1 = {}^3J_2$ = 9.6, $^3J_3$ = 3.6), 3.90 (H-24, dd, $^3J_1$ = 8.8, $^3J_2$ = 5.7), 5.03 (H-16, q, $^3J_1 = {}^3J_2 = {}^3J_3$ = 7.6).

Table 1 lists the ^{13}C NMR spectrum.

Cycloexoside (4), 200 mg (0.27%), $C_{39}H_{62}O_{11}$, mp 193-196°C (MeOH) [2].

Cycloexoside B (1), 70 mg (0.009%), $C_{37}H_{60}O_{10}$. PMR spectrum (500 MHz, C_5D_5N, 0 = TMS, δ, ppm, J/Hz): 0.16 and 0.42 (2H-19, d, ^2J = 4.5), 0.88, 1.16, 1.21, 1.23, 1.32, 1.48, 1.68 (7×CH_3, s), 2.05 (CH_3COO, s), 2.43 (H-17, d, ^3J = 8), 2.97 (H-22, q, $^2J = {}^3J_1 = {}^3J_2$ = 10), 3.38 (H-3, dd, $^3J_1$ = 12, $^3J_2$ = 4), 3.59 (H-5a of D-xylose, dd, ^2J = 11, ^3J = 10), 3.63 (H-6, td, $^3J_1 = {}^3J_2$ = 9, $^3J_3$ = 3), 3.79 (H-24, dd, $^3J_1$ = 9, $^3J_2$ = 5), 4.07 (H-3 of D-xylose, t, $^3J_1 = {}^3J_2$ = 9), 4.11 (H-4 of D-xylose, m),

4.22 (H-5e of D-xylose, dd, $^2J = 11$, $^3J = 5$), 4.74 (H-1 of D-xylose, d, $^3J = 8$), 4.90 (H-16, m), 5.46 (H-2 of D-xylose, dd, $^3J_1 = 9$, $^3J_2 = 8$).

Table 1 lists the ^{13}C NMR spectrum.

Cyclosiversioside A (5), 180 mg (0.024%), $C_{44}H_{70}O_{15}$, mp 229-230°C (MeOH) [3, 9].

Cyclosiversigenin 3-O-β-D-xylopyranoside (3), 35 mg (0.0047%), $C_{35}H_{58}O_9$, mp 263-264°C (MeOH) [3, 10]. PMR spectrum (400 MHz, C_5D_5N, 0 = TMS, δ, ppm, J/Hz): 0.27 and 0.56 (2H-19, d, $^2J = 4$), 0.99, 1.28, 1.30, 1.31, 1.41, 1.56, 1.96 (7×CH_3, s), 2.52 (H-17, d, $^3J = 8$), 3.08 (H-22, q, $^2J = {}^3J_1 = {}^3J_2 = 10$), 3.61 (H-3, dd, $^3J_1 = 12$, $^3J_2 = 4$), 3.71 (H-5a, dd, $^2J = 11$, $^3J = 9$), 3.73 (H-6, td, $^3J_1 = {}^3J_2 = 9$, $^3J_3 = 3$), 3.87 (H-24, dd, $^3J_1 = 9$, $^3J_2 = 5.5$), 4.03 (H-2 of D-xylose, dd, $^3J_1 = 9$, $^3J_2 = 7.4$), 4.12 (H-3 of D-xylose, t, $^3J_1 = {}^3J_2 = 9$), 4.21 (H-4 of D-xylose, td, $^3J_1 = {}^3J_2 = 9$, $^3J_3 = 5.5$), 4.34 (H-5e of D-xylose, dd, $^2J = 11$, $^3J = 5.5$), 4.89 (H-1 of D-xylose, d, $^3J = 7.4$), 5.00 (H-16, q, $^3J_1 = {}^3J_2 = {}^3J_3 = 8$).

Table 1 lists the ^{13}C NMR spectrum.

Cyclosiversioside B (6), 55 mg (0.007%), $C_{45}H_{72}O_{16}$, mp 185-188°C (MeOH) [3, 11].

Cyclosiversioside C (7), 40 mg (0.005%), $C_{42}H_{68}O_{14}$, mp 253-255°C (MeOH) [3, 9].

Cyclosiversioside D (8), 60 mg (0.008%), $C_{43}H_{70}O_{15}$, mp 254-257°C (MeOH) [3,11].

Cyclosiversioside E (9), 890 mg (0.12%), $C_{40}H_{66}O_{13}$, mp 216-218°C (MeOH) [3, 12].

Cyclosiversioside F (10), 3.5 g (0.46%), $C_{41}H_{68}O_{14}$, mp 284-286°C (MeOH) [3, 13].

Cyclosiversioside G (11), 27 mg (0.0036%), $C_{46}H_{76}O_{17}$, mp 237-240°C (MeOH) [3, 14].

Cyclosiversigenin (2) from 1. Cycloexoside B (40 mg) was hydrolyzed by methanolic H_2SO_4 (10 mL, 0.25%) at 60°C for 2.5 h. The reaction mixture was diluted with water. The methanol was evaporated. The resulting solid was filtered off and dried. The solid was chromatographed over a column with elution by system 2. Yield 16 mg of cyclosiversigenin, mp 239-241°C (MeOH).

The aqueous filtrate was condensed to 3 mL, boiled for 1 h, and neutralized by anion exchanger ARA-8p. The solid was removed. The solution was evaporated to dryness. PC in system 6 of the resulting solid detected D-xylose. The PMR and ^{13}C NMR spectra of **1** indicate that this glycoside includes one D-xylose.

Cyclosiversigenin 3-O-β-D-xylopyranoside (3) from 1. Glycoside **1** (20 mg) was hydrolyzed by methanolic NaOH (8 mL, 0.1%) at room temperature for 3 h. Workup of the products and chromatography over a column using system 4 afforded glycoside **3** (11 mg), mp 263-264°C (MeOH), identified as cyclosiversigenin 3-O-β-D-xylopyranoside.

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