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TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS.
LXVI. CYCLOORBICOSIDE C, A NEW BISDESMOSIDE

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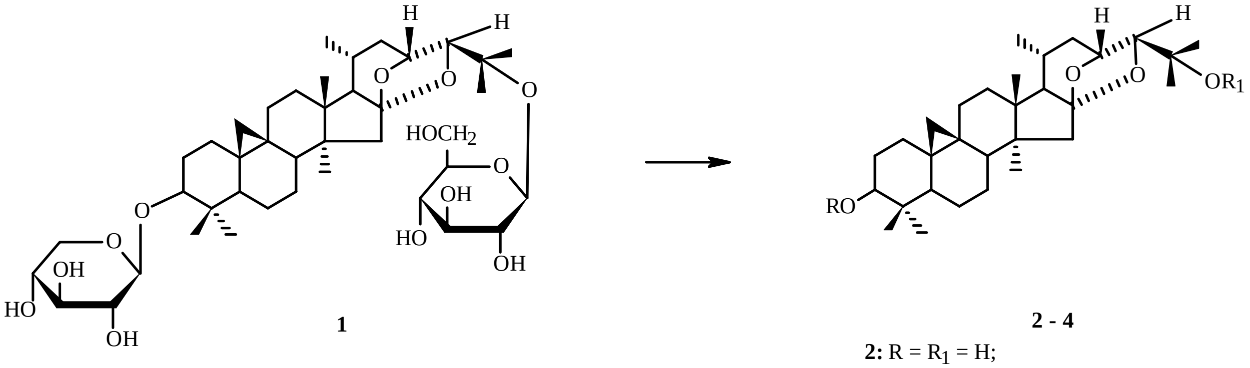
The new triterpene glycoside cycloorbicoside C was isolated from the aerial parts of Astragalus orbiculatus *Ledeb. (Leguminosae) and was identified as (23R,24S)-16β,23;16α,24-diepoxycycloartan-3β,25-diol 3-O-β-D-xylopyranoside 25-O-β-D-glucopyranoside.*

Key words: triterpenes, cycloartanes, glycosides, cycloorbicoside C, hydrocycloorbigenin A, Leguminosae, *Astragalus*, PMR and ^{13}C NMR spectra, DEPT, J-modulation, HSQC, HMBC.

In continuation of studies of triterpenoids of plants of the *Astragalus* genus, we established the structure of cycloorbicoside C (**1**), which was isolated from the aerial part of *Astragalus orbiculatus* Ledeb. (Leguminosae) [1].



1

2 - 4

2: R = R$_1$ = H;
3: R = *β*-D-Xyl*p*, R$_1$ = H;
4: R = H, R$_1$ = *β*-D-Glc*p*

The PMR of glycoside **1** contain two 1H doublets of an AX system at 0.26 and 0.53 ppm with a characteristic spin—spin coupling constant of 4 Hz and signals of seven methyls at high field. This indicates that the examined glycoside is a triterpene cycloartane [2, 3]. In fact, acid hydrolysis of cycloorbicoside C produces genin **2**, identified as dihydrocycloorbigenin A [4].

D-Glucose and D-xylose were detected in the carbohydrate part of the acid hydrolysate by paper chromatography (PC) after neutralization and concentration.

The PMR and ^{13}C NMR (Table 1) of cycloorbicoside C contain one set of signals for each monosaccharide unit. Therefore, glycoside **1** conains D-glucose and D-xylose in a 1:1 ratio and is a bioside.

Stepwise hydrolysis of glycoside **1** produces in addition to dihydrocycloorbigenin A (**2**) two progenins **3** and **4**. Cycloorbicoside C has a bisdesmoside structure.

As expected, both carbinol atoms C-3 and C-25 underwent low-field shifts and resonate at 88.47 and 78.76 ppm, respectively, in the ^{13}C NMR spectrum of compound **1** in comparison with the same atoms of genin **2**.

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TABLE 1. Chemical Shifts of C Atoms of Cycloorbicoside C (**1**) and Its Derivatives **2** and **3** and PMR, HSQC, and HMBC Spectra of glycoside **3** (δ, ppm, J/Hz, C_5D_5N, 0 = TMS)

C atom	Compound					
	1		**2**	**3**		
	d_C	DEPT	d_C	d_C	d_H	HMBC (C atoms)
1	32.16	CH_2	32.41	32.22	1.25; 1.58	
2	30.08	CH_2	31.31	30.19	1.96; 2.37	
3	88.47	CH	77.93	88.50	3.53 dd (11.7; 4.3)	X1, 30
4	41.36	C	41.15	41.42	-	
5	47.54	CH	47.44	47.66	1.35	
6	20.98	CH_2	21.30	21.07	0.76; 1.56	
7	26.59	CH_2	26.75	26.65	1.12; 1.30	
8	47.57	CH	47.79	47.67	1.53	
9	19.60	C	19.51	19.65	-	
10	26.77	C	27.01	26.82	-	
11	26.56	CH_2	26.66	26.65	1.12; 2.04	
12	33.07	CH_2	33.11	33.14	1.55; 1.68	
13	44.60	C	44.59	44.63	-	
14	46.27	C	46.32	46.36	-	
15	46.61	CH_2	46.62	46.64	2.05; 2.05	8, 28
16	114.89	C	114.87	114.84	-	
17	61.30	CH	61.20	61.21	1.59 d (10.9)	
18	19.21	CH_3	19.33	19.32	1.14 s	12, 14, 17
19	30.46	CH_2	30.75	30.55	0.27; 0.54 d (4)	1, 8, 11
20	23.91	CH	23.95	24.00	1.68	
21	19.79	CH_3	19.83	19.90	0.85 d (6.4)	17, 20, 22
22	38.16	CH_2	38.25	38.27	1.01; 2.26	17, 23, 24
23	71.86	CH	71.83	71.86	4.76 d (9.1)	
24	88.28	CH	90.60	90.57	3.70 s	16, 23, 26, 27
25	78.76	C	71.01	71.05	-	
26	22.22	CH_3	27.91	27.93	1.51 s	24, 25, 27
27	24.47	CH_3	24.77	24.81	1.44 s	24, 25, 26
28	19.33	CH_3	19.41	19.40	1.22 s	15
29	25.76	CH_3	26.22	25.84	1.35 s	4, 5, 30
30	15.43	CH_3	14.90	15.53	1.08 s	4, 5, 29
β-D-Xyl*p* (X)						
1	107.52	CH		107.57	4.88 d (7.5)	3
2	75.55	CH		75.62	4.04 t (8)	X1, X3
3	78.56	CH		78.64	4.17 t (8.7)	X2, X4
4	71.24	CH		71.28	4.23 m	X5
5	67.08	CH_2		67.15	3.75 t (10.8) 4.37 dd (11.3; 5.2)	X1, X3, X4 X1, X3, X4
β-D-Glc*p* (G)						
1	98.81	CH				
2	75.23	CH				
3	78.83	CH				
4	71.81	CH				
5	78.19	CH				
6	62.92	CH_2				

Chemical shifts without multiplicities and SSCC were found using 2D spectra.

The PMR and ^{13}C NMR spectra of progenin **3** were interpreted using two-dimensional (2D) HSQC and HMBC spectra in addition to J-modulation. It was noted that this glycoside contains D-xylose. The chemical shifts of the C and H atoms of the D-xylose and the SSCC of the protons indicate that the pentose has the β-D-xylopyranoside structure. The signal of C-3, which is glycosylated, is observed at 88.50 ppm in the ^{13}C NMR spectrum of this same progenin. Therefore, correlations in the HMBC spectrum of glycoside **3** are found between H-3 (3.53 ppm) and the anomeric C atom of D-xylose (107.57 ppm) and between the anomeric proton of the monosaccharide (4.88 ppm) and C-3 (88.50 ppm). These facts unambiguously define the location of the pentose on C-3 of the genin. Therefore, progenin **3** has the structure (23*R*,24*S*)-16β,23;16α,24-diepoxycycloartan-3β,25-diol 3-O-β-D-xylopyranoside.

We previously noted that an anomeric C atom of β-D-xylopyranose located on C-3 of cycloartane genins resonates in the ^{13}C NMR spectrum, as a rule, at 107.38-107.60 ppm. We see that the chemical shifts of the anomeric C atom of D-xylose in the ^{13}C NMR spectra of glycosides **1** and **3** agree completely with this rule and have the values 107.52 and 107.57 ppm.

Therefore, progenin **4** should contain D-glucose on C-25. The anomeric H atom of D-glucose resonates in the PMR of glycoside **4** at 5.14 ppm as a doublet with SSCC $^{3}J = 7.8$ Hz, indicative of the pyranose form, the $^{4}C_1$-conformation, and the β-configuration of this monosaccharide. The chemical shift of the anomeric C atom of β-D-glucopyranoside unit in the ^{13}C NMR spectrum of glycoside **1** (98.81 ppm) indicates that the hexose is actually located on the tertiary C atom. This means that progenin **4** is (23*R*,24*S*)-16β,23;16α,24-diepoxycycloartan-3β,25-diol 25-O-β-D-glucopyranoside.

Thus, the experimental data define cycloorbicoside C as (23*R*,24*S*)-16β,23;16α,24-diepoxycycloartan-3β,25-diol 3-O-β-D-xylopyranoside 25-O-β-D-glucopyranoside.

EXPERIMENTAL

For general comments, see the literature [6]. The following solvent systems were used: $CHCl_3$—CH_3OH—H_2O (70:12:1, 1), $CHCl_3$—CH_3OH (20:1, 2), *n*-BuOH—C_5H_5N—H_2O (6:4:3, 3).

PC was performed on FN-11 paper in descending mode using system 3. Monosaccharides were detected by spraying with anilinium phthalate and subsequent heating for 5-10 min at 100-110°C.

PMR and ^{13}C NMR spectra were recorded on UNITYplus 400 and Bruker DRX-500 spectrometers in C_5D_5N (δ, ppm, 0 = TMS). ^{13}C NMR spectra were obtained with full C–H decoupling and under DEPT and J-modulation conditions. Spectra were interpreted using 2D HSQC and HMBC spectra.

Cycloorbicoside C (1). Fractions containing **5**, which accumulated during isolation of cycloorbicosides A [7] and G [8], were rechromatographed over a column using system 1. In addition to compounds isolated earlier [4], we isolated another 450 mg of **1**, $C_{41}H_{66}O_{13}$, mp 265-266°C (MeOH). IR spectrum (KBr, ν, cm^{-1}): 3520-3290 (OH), 3050 (CH_2 of cyclopropane ring).

PMR spectrum (400 MHz, C_5D_5N, 0 = TMS, δ, ppm, J/Hz): 0.26 and 0.53 (2H-19, d, $^{2}J = 4$), 0.83 (CH_3-21, d, $^{3}J = 6.2$), 1.08 (CH_3-30, s), 1.11 (CH_3-18, s), 1.21 (CH_3-28, s), 1.36 (CH_3-29, s), 1.51 (CH_3-27, s), 1.64 (CH_3-26, s), 3.53 (H-3, dd, $^{3}J_1 = 11.8$, $^{3}J_2 = 4$), 3.76 (H-5α of D-xylose, dd, $^{2}J = 11.2$, $^{3}J = 10.1$), 3.79 (H-24, br.s), 3.98 (H-5 of D-glucose, m), 3.99 (H-2 of D-glucose, dd, $^{3}J_1 = 9$, $^{3}J_2 = 7.7$), 4.06 (H-2 of D-xylose, dd, $^{3}J_1 = 8.7$, $^{3}J_2 = 7.6$), 4.21 (H-3 of D-xylose, t, $^{3}J_1 = {}^{3}J_2 = 8.6$), 4.22-4.29 (H-3, H-4 of D-glucose, H-4 of D-xylose, m), 4.34-4.42 (H-5e of D-xylose and H-6 of D-glucose, m), 4.54 (H-6′ of D-glucose, dd, $^{2}J = 11.8$, $^{3}J = 2.6$), 4.89 (H-1 of D-xylose, d, $^{3}J = 7.5$), 4.92 (H-23, br.d, $^{3}J = 8.6$), 5.15 (H-1 of D-glucose, d, $^{3}J = 7.7$). Table 1 lists the ^{13}C NMR spectrum.

Partial Hydrolysis of Cycloorbicoside C. Glycoside **1** (50 mg) was hydrolyzed by methanolic H_2SO_4 (8 mL, 0.25%) at 40°C for 7 h. The reaction mixture was treated with water (7 mL). The methanol was evaporated. The resulting precipitate was filtered off and dried. The dry solid was chromatographed over a column with elution by system 2 to isolate genin **2** (11 mg), $C_{30}H_{48}O_4$, mp 237-238°C (MeOH), identified as dihydrocycloorbigenin A [4]. Table 1 lists the ^{13}C NMR spectrum.

Continued elution of the column using system 1 isolated progenin **3** (4 mg), $C_{35}H_{56}O_8$, mp 283-285°C (MeOH). Table 1 lists the PMR and ^{13}C NMR spectra.

Elution of the column with the same solvent system isolated progenin **4** (1 mg), $C_{36}H_{58}O_9$.

PMR spectrum (500 MHz, C_5D_5N, 0 = TMS, δ, ppm, J/Hz): 0.31 and 0.58 (2H-19, d, $^{2}J = 4$), 0.84 (CH_3-21, d, $^{3}J = 6.4$), 1.11 (CH-18, s), 1.14 (CH_3-30, s), 1.23, 1.24 (CH_3-28, CH_3-29, s), 1.52 (CH_3-27, s), 1.64 (CH_3-26, s), 3.56 (H-3, dd, $^{3}J_1 = 11.6$, $^{3}J_2 = 4.5$), 3.80 (H-24, br.s), 3.97 (H-5 of D-glucose, m), 3.99 (H-2 of D-glucose, t, $^{3}J_1 = {}^{3}J_2 = 8.3$), 4.20-4.30 (H-3 and H-4 of D-glucose, m), 4.38 (H-6 of D-glucose, dd, $^2J = 11.4$, $^3J = 5.3$), 4.52 (H-6′ of D-glucose, dd, $^2J = 11.7$, $^3J = 2.5$), 4.92 (H-23, br.d, $^3J = 8.6$), 5.14 (H-1 of D-glucose, d, $^3J = 7.8$).

The aqueous filtrate was boiled for 1 h to destroy the methylglycosides, cooled, and neutralized by anion exchanger ARA-8p. PC using system 3 and authentic samples detected in the neutral aqueous solution D-glucose and D-xylose. The PMR and ^{13}C NMR of cycloorbicoside C indicate that glycoside **1** contains one molecule each of these monosaccharides.

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