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TRRITERPENE GLYCOSIDES OF *ASTRAGALUS* AND THEIR GENINS
LIX. STRUCTURE OF CYCLOCANTHOSIDE F

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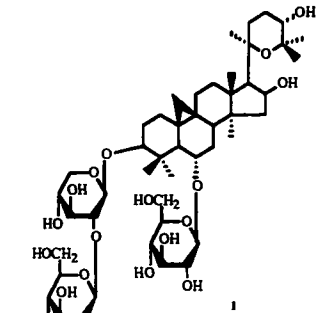
The structure of a new cycloartane glycoside, cyclocanthoside F, has been established on the basis of spectral characteristics as 20R,25-epoxy-24S-cycloartane-3β,6α,16β,24-tetraol 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside] 6-O-β-D-glucopyranoside. The possibility in principle of using the β-effects of glycosylation and ^{13}C NMR spectroscopy for determining a glycosyl substituent at C-6 of a cycloartane has been demonstrated.

Continuing chemical investigations in the cycloartane triterpenoid series [1], we have determined the structure of a new glycoside isolated from *Astragalus tragacantha* Habl. (Leguminosae), which we have called cyclocanthoside F (**1**). The new glycoside corresponds to substance **13** in [2].

Analysis of the ^1H and ^{13}C NMR spectra of the new glycoside (**1**) (Table 1), for the interpretation of which we employed the methods of 2M NMR spectroscopy (^1H—^1H COSY, HMQC, HMBC) and DEPT experiments, showed that this glycoside was a cycloartane derivative [3, 4]. *D*-Glucose and *D*-xylose were detected by paper chromatography in the products of its acid hydrolysis. The ^{13}C NMR spectrum of glycoside (**1**) contained the signals of two *D*-glucose residues and one *D*-xylose residue. In agreement with this, in the ^1H NMR spectrum of the new glycoside (**1**) we observed the signals of the twenty nonhydroxy protons of three monosaccharide residues Consequently, cyclocanthoside F is a trioside containing *D*-glucose and *D*-xylose residues in a ratio of 2:1.



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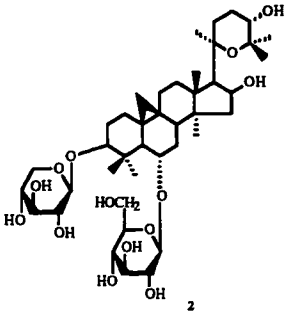

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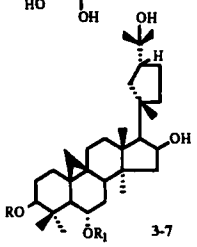
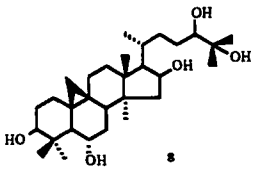


3-7

3. R=R_1=H
4. R=R_1=β-*D*-Xylp
5. R=β-*D*-Xylp, R_1=β-*D*-Glcp
6. R=H, R_1=α-*L*-Rhap
7. R=β-*D*-Xylp, R_1=α-*L*-Rhap



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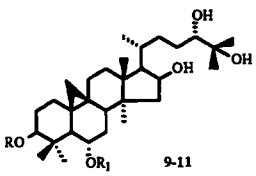

9-11

9. R=R_1=H
10. R=β-*D*-Xylp, R_1=β-*D*-Glcp
11. R=β-*D*-(4-OAc)-Xylp, R_1=β-*D*-Glcp

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TABLE 1. Details of the ^{1}H, ^{13}C, ^{1}H—^{1}H COSY, HMQC, HMBC and DEPT NMR Spectra of Cyclocanthoside F (**1**) and of the ^{13}C NMR Spectrum of Cyclocephaloside I (**2**) (δ, ppm, C_5D_5N, 0 — TMS)

C atom	Compound				
	1				2
	δ_C	DEPT	δ_H	HMBC (C atoms)	δ_C
1	32.26	CH_2	1.54; 1.23		32.3
2	30.12	CH_2	2.29 m; 1.97		30.2
3	88.52	CH	3.44 dd (11.7; 4.4)	X1; 4; 30	88.6
4	42.65	C	-		42.7
5	52.54	CH	1.85 d (8.6)	4; 6; 10; 30	52.7
6	79.33	CH	3.78 td (8.6; 3.9)	$G_1$1	79.5
7	34.56	CH_2	2.26; 1.84		34.8
8	45.72	CH	2.00	6; 9; 14; 28	46.1
9	21.20	C	-		21.1
10	29.01	C	-		29.1
11	26.38	CH_2	1.82; 1.44		26.3
12	34.24	CH_2	1.84; 1.68		34.2
13	45.99	C	-		45.9
14	46.86	C	-		46.8
15	47.32	CH_2	2.37 dd (12.8; 8.2); 1.90	13; 14; 17; 28	47.4
16	74.00	CH	4.92 m	13	74.0
17	60.86	CH	2.13 d (7.5)	12; 13; 16; 18; 20; 21; 22	60.8
18	20.75	CH_3	1.69 s	12; 13; 14; 17	20.9
19	29.01	CH_2	0.19 and 0.58 d (4)	1; 8; 9; 10; 11	29.5
20	78.97	C	-		78.9
21	28.88	CH_3	1.56 s	17; 20; 22	28.8
22	26.74	CH_2	3.15 td (13.9; 5); 1.26	20	26.7
23	24.11	CH_2	2.20; 1.92		24.1
24	68.84	CH	3.70 dd (3.5; 2)		68.7
25	75.25	C	-		75.2
26	28.63	CH_3	1.48 s	24; 25; 27	28.6
27	28.02	CH_3	1.32 s	24; 25; 26	28.0
28	20.07	CH_3	0.95 s	13; 14	20.1
29	28.56	CH_3	1.94 s	3; 4; 5; 30	28.8
30	16.58	CH_3	1.44 s	3; 4; 5; 29	16.7
			β-*D*-Xyl*p* (X)		
1	105.42	CH	4.85 d (7.3)	3	107.7
2	83.87	CH	4.23	X1; X4	75.6
3	77.72	CH	4.20	X2	78.5
4	70.93	CH	4.20		71.8
5	66.55	CH_2	4.29 dd (11.1; 4.6)	X1; X4	67.1
			3.62 dd (11.1; 9.3)	X1; X3; X4	
			6-O-β-*D*-Glc*p* (G_1)		
1	105.02	CH	4.88 d (7.7)	6; $G_1$3; $G_1$5	105.1
2	75.66	CH	4.00 dd (8.9; 7.7)	$G_1$1; $G_1$3	75.6
3	78.97	CH	4.22	$G_1$4; $G_1$5	79.2
4	71.95	CH	4.21	$G_1$3; $G_1$5	71.3
5	77.98	CH	3.90 m		78.1
6	63.19	CH_2	4.49 dd (11.5; 2.7)		63.1
			4.33 dd (11.5; 5.4)	$G_1$5	

TABLE 1. (continued)

C atom	Compound				
	1				2
	δ_C	DEPT	δ_H	HMBC (C atoms)	δ_C
			β-*D*-Glc*p* present at C-2 of β-*D*-Xyl*p* (G_2)		
1	106.24	CH	5.35 d (7.7)	X2	
2	76.94	CH	4.13 dd (9.3; 7.7)	G_21, G_23	
3	77.98	CH	4.26	G_21; G_22 ; G_24	
4	72.00	CH	4.24	G_23	
5	78.14	CH	3.98 m		
6	63.03	CH_2	4.55 dd (11.7; 3.2)		
			4.45 dd (11.7; 4.5)	G_25	

The chemical shifts of protons given without multiplicities and SSCCs were found with the aid of two-dimensional spectra. The details of the ^{13}C NMR spectrum of glycoside (**2**) were taken from the literature [12].

The anomeric protons of the monosaccharide residues of the glycoside under consideration resonated in the PMR spectrum at 4.85 (^3J = 7.3 Hz), 4.88 (^3J = 7.7 Hz) and 5.35 ppm (^3J = 7.7 Hz). As we have shown previously [5], the given SSCCs, together with the β-configuration of the anomeric centers and the $^4C_1$-configuration of the monosaccharide residues, demonstrate the pyranose form of the latter. This conclusion also followed from the chemical shifts of the carbon atoms of the carbohydrate residues. Furthermore, the same parameters indicated that both *D*-glucose residues are terminal. At the same time, the *D*-xylose is substituted by one of the *D*-glucose residues. This was also shown by the HMBC spectrum of glycoside (**1**), where a correlation was traced between the signal of the anomeric hydrogen atom of *D*-glucose and the second carbon atom of *D*-xylose, which were observed at 5.35 and 83.87 ppm. The latter signal correlated in the HMQC spectrum with a proton signal at 4.23 ppm, and this signal, in its turn was linked in the HMBC spectrum, with a geminal interaction constant, with the signal of an anomeric carbon atom observed at 105.42 ppm. Consequently, this signal belonged to *D*-xylose. In the same HBMC spectrum, the signal under consideration correlated with the signal of H-3 of the genin (3.44 ppm). As was to be expected, the signal of C-3 of the genin (88.52 ppm) correlated with the signal of the anomeric proton of the *D*-xylose residue (4.85 ppm).

Thus, cyclocanthoside F is a bisdesmosidic glycoside the carbohydrate components of which are 3-O-[β-D-glucopyranosyl(1→2)-β-D-xylopyranoside] and 6-O-β-D-glucopyranoside.

The conclusion concerning the structure of the above-mentioned disaccharide chain and its position was confirmed in the following way. In the ^{13}C NMR spectra of cycloartane glycosides the signal of the anomeric carbon atom of an unsubstituted *D*-glucopyranosyl residue attached at C-3 of the genin is observed in the range of 107.38—107.60 ppm (Table 2, glycosides **4**, **5**, **7**, and **10**). This situation remains valid even when there is an acetyl group at C-4 of the pentose concerned (cyclocanthoside B (**11**)] [6]. However, on the glycosylation of a *D*-xylopyranose residue at C-2 the signal of the anomeric carbon atom of this *D*-xylose undergoes an upfield shift to 105.16 ppm and the glycosylated C-2 atom resonates in the 83.46—83.86 ppm interval [6—11]. Consequently, the β-influence of glycosylation at C-2 on the chemical shift of the anomeric carbon atom is considerable, and, together with the α-effect may be regarded as a reliable argument showing the position of the glycosylating unit. As can be seen from Table 1, we observed an analogous situation in the ^{13}C NMR spectrum of cyclocanthoside F.

We ascertained the position of the other carbohydrate unit in the following way. In the HMBC spectrum, the signal of the anomeric carbon atom of the β-*D*-glucose residue (105.02 ppm) still remaining unidentified correlated with the proton signal observed at 3.78 ppm in the form of a triplet of doublets with $^3J_1 = {}^3J_2 = 8.6$ Hz and $^3J_3 = 3.9$ Hz. The HMQC spectrum showed that this proton was attached to a carbon atom resonating at 79.33 ppm. The given parameters are characteristic for H-6 and the glycosylated C-6 atom of a cycloartane [4]. This means that the other *D*-glucose reside is attached to the 6α-hydroxy group of the genin.

Establishing the glycosylation of a cycloartane at the 6α-hydroxy group on the basis of the ordinary ^{13}C NMR spectrum of a glycoside containing more than two monosaccharide residues is made difficult by the circumstances given above, and, in our opinion, the elucidation of this question is a matter of interest.

TABLE 2. Details of the ^{13}C NMR Spectra of Compounds (**3**—**10**) (δ, ppm, C_5D_5N, 0 — TMS)

C atom	Compound							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	32.81	31.87	32.12	32.53	32.20	32.81	32.78	32.27
2	31.47	29.93[a]	28.95	31.28	30.06	31.45	31.34	28.77
3	78.32	88.26	88.48	77.60	87.83	78.37	78.34	88.59
4	42.46	42.45	42.58	41.99	42.26	42.44	42.33	42.68
5	54.00	52.03	52.46	52.14	52.03	54.01	53.96	52.52
6	68.38	78.30[b]	79.21	79.69	79.16	68.31	68.29	79.13[a]
7	38.85	33.50	34.55	35.05	34.55	38.62	38.45	34.32
8	47.30	44.22	45.02[a]	46.86	46.16[a]	47.24	47.17	45.61
9	20.99	21.07	21.07	20.67	20.65	21.31	21.35	21.45
10	29.92	27.99[c]	30.12	28.81	28.70	30.37	30.37	30.20
11	26.32	26.33	26.40	26.43	26.38	26.40	26.43	26.30
12	33.47	33.35	33.36	33.35	33.27	33.23	33.33	33.21
13	45.09	45.09	45.02[a]	45.04	45.00	45.74	45.76	45.82
14	46.21	45.72	45.65	46.24	46.16[a]	46.98	46.85	46.94
15	46.81	46.07	46.17	46.71	46.67	48.81	48.41	47.88
16	73.48	73.37	73.37	73.39	73.33	71.78	72.02	72.02
17	58.44	58.02	58.17	58.34	58.23	57.27	57.39	57.20
18	21.66	20.41	19.80	21.69	21.47	18.81	18.24	18.37
19	31.02	29.93[a]	28.52	30.82	30.22	29.36	29.70	28.21
20	87.27	87.20	87.20	87.19	87.15	31.64	28.78	28.57
21	28.59	28.38	28.10	28.56	28.42	19.09	18.97	18.48
22	34.97	34.82	34.86	34.93	34.88	29.62	33.07	33.00
23	26.17	26.17	26.14	26.08	25.95	34.84	27.99	27.90
24	81.75	81.57	81.64	81.73	81.67	80.59	77.22	77.14
25	71.27	71.21	71.16	71.24	71.22	72.69	72.49	72.58
26	27.17	26.95	27.02	27.10	27.05	25.90	25.43	25.77
27	28.21	27.99[c]	28.52	28.17	28.11	26.20	26.46	26.50
28	20.27	19.57	21.07	20.29	20.16	20.30	20.18	19.84
29	29.44	28.45	28.81	29.35	28.51	29.37	29.17	28.65
30	16.14	16.54	16.50	16.58	17.05	16.13	15.98	16.71
				3-O-β-*D*-Xyl*p*				
1		107.38	107.51		107.43			107.60
2		75.38	75.48[b]		75.38			75.61
3		78.30[b]	78.37		78.44			78.48
4		71.09	71.24		71.14			71.25
5		66.77	66.92		66.96			67.02
		β-*D*-Xyl*p*	β-*D*-Glc*p*		α-L-Rha*p*			β-*D*-Glc*p*
1		105.52	105.12	104.12	103.88			105.18
2		75.19	75.48[b]	72.97	72.87			75.56
3		77.80	79.04	72.50	72.56			79.13[a]
4		70.93	71.79	73.76	73.71			71.95
5		66.76	77.98	70.10	70.06			78.06
6			63.02	18.19	18.16			63.20

Signals labeled with the same letter are superposed on one another.

In the ^{13}C NMR spectrum, a nonglycosylated C-6 atom of a 6α-hydroxycycloartane resonates in the range of 68.29—68.38 ppm (Table 2, genins **3**, **8**, and **9**). The C-5 atoms of pentosopyranoses, the C-4 atom of an *L*-arabinopyranose, and the C-5 atom of an *L*-rhamnopyranose resonate fairly close to this region. As we see (Table 1), C-24 of the genins of glycosides (**1**) and (**2**) resonate in the same region (Table 1).

As follows from Table 2, the signal of the glycosylated C-6 atom was observed in the 78.30—79.69 ppm interval. The signals of the C-3 and C-5 atoms of *D*-glucopyranose, of the C-3 atom of *D*-xylopyranose, of the nonglycosylated C-3 atom of

the genin, and of the C-20 atom of the genin of glycosides (**1**) and (**2**) were found in the same interval. Occasionally the ideal superposition of the C-6 signal on one of the above-mentioned signals is observed, as is the case in the spectra of glycosides (**4**) and (**10**). Even DEPT experiments are useless for the analysis of this region of the spectrum in view of the identity of the chemical natures of the carbon atoms under discussion, with the exception of the C-20 atom of the genin of the new glycoside (**1**), In this connection it appeared to us to be possible to make use of the β-effects of glycosylation of the C-6 atom of a cycloartane, i.e., the influence of a glycosyl substituent at C-6 on the spectral behaviors of the C-5 and C-7 atoms.

In the ^{13}C NMR spectra of cycloartanes not glycosylated at C-6, the C-5 atom is revealed in the 53.96—54.01 ppm interval in the form of a fairly isolated individual signal and is identified without difficulty. In the spectra of cycloartanes glycosylated at C-6, the C-5 atoms undergo the β-effect of glycosylation and resonate in a higher field, between 52.03 and 52.52 ppm. The magnitude of the β-effect is approximately 1.5-2 ppm with a minus sign.

The influence of glycosylation on the other β-atom, C-7, is even more pronounced. In the spectra of unsubstituted compounds the signal of the C-7 atom is in the region of 38.45—38.85 ppm, while in the spectra of the glycosides it is at 33.50—35.05 ppm. In this case the magnitude of the β-effect is in the range of 3.4—5.3 ppm, again with a minus sign.

A consideration of the spectra of a large number of glycosides indicates that the β-effects under discussion do not depend on the nature of the glycosylating carbohydrate. These effects can be traced in the spectrum of the new glycoside (**1**).

A comparative analysis of the ^{1}H and ^{13}C NMR spectra of cyclocanthoside F and cyclocephaloside I (**2**) [12], isolated from *Astragalus microcephalus* Willd. in the flora of the Turkish Republic, has shown that the genins of these glycosides have identical structures. The priority for determining the structure of this genin belongs to Bedir et al. [12]. Giving due priority to its possessors, we propose to call the genin of glycosides (**1**) and (**2**) cyclocephalogenin.

These authors [12] determined the relative configurations of C-20 and C-24 of the asymmetric centers of the side-chain that are expressed by formulas (**1**) and (**2**). We shall attempt to show that the given relative configurations of the chiral centers under discussion are absolute; i.e. cyclocephaloside has the 20R,24S-configuration.

Thus, we have shown previously that in the ^{1}H NMR spectra of cycloartanes having the 20R,24S-epoxy-16β,25-diol structural fragment one of protons at C-22 resonates at 3.0 ppm (C_5D_5N, HMDS) in the form of a quartet with broadened lines. Relative to TMS, this signal is observed at 3.12 ppm in the form of a triplet of doublets with the SSCCs $^{2}J = {}^{3}J_1 = 11.3$ Hz and $^{3}J_2 = 9$ Hz (400 MHz). The signal of one of the H-22 protons in the PMR spectrum of glycoside (**1**) was traced at 3.15 ppm in the form of a triplet of doublets with the SSCCs $^{2}J = {}^{3}J_1 = 13.9$ Hz and $^{3}J_2 = 5$ Hz. This fact permits us to conclude that cyclocephaloside has the 20R,24S-configuration.

The 24S- configuration is also logical from biological considerations. Cyclocanthogenin (**9**), which has the 24S-configuration, and its glycosides have been isolated from *A. tragacantha* and *A. microcephalus* . The same compounds are undoubtedly biogenetic precursors of cyclocephalogenin, cyclocephaloside I, and cyclocanthoside F.

Thus, cyclocanthoside F has the structure of 20R,25-epoxy-24S-cycloartane-3β,6α,16β,24-tetraol 3-O-[β-*D*-glucopyranosyl-(1→2)-β-*D*-xylopyranoside] 6-O-β-*D*-glucopyranoside.

EXPERIMENTAL

General Observations. ^{1}H, ^{13}C, $^{1}H—^{1}H$ COSY, HMQC, and HMBC NMR spectra were obtained on Bruker AM 400 and UNITY plus 400 instruments in deuteropyridine with TMS as internal standard. ^{13}C NMR spectra were taken with complete suppression of C—H interactions, and also under DEPT and J-modulation conditions.

For the isolation and separation of the *Astragalus tragacantha* Habl. triterpenoids, see [2, 6].

Cyclocanthoside F (1) — substance **13**: white amorphous powder with the composition $C_{47}H_{78}O_{19}$. For the NMR spectrum, see Table 1.

Acid Hydrolysis of Cyclocanthoside F. Glycoside (**1**) (15 mg) was hydrolyzed with a 0.5% methanolic solution of sulfuric acid. After an appropriate work-up, *D*-glucose and *D*-xylose were detected in the carbohydrate part of the hydrolysate by paper chromatography in the n-butyl alcohol—pyridine—water (6:4:3) system in comparison with authentic specimens.

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