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TRITERPENE GLYCOSIDES FROM *Astragalus* AND THEIR GENINS. LXXXIII. STRUCTURE OF CYCLOMACROSIDE A

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The structure of the novel cycloartane triterpene glycoside cyclomacroside A, which was isolated from Astragalus macropus *Bunge (Leguminosae) roots, was determined as 3-O-α-L-rhamnopyranoside-24,25-isopropylidenedioxy-24*R*-cylcoartan-1α,3β,7β-triol.*

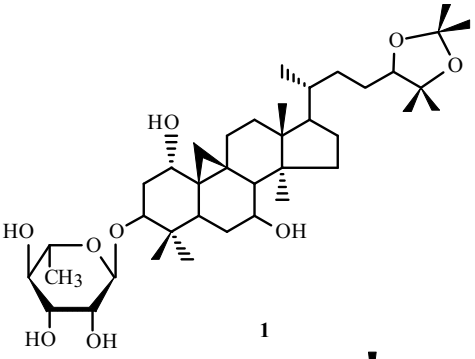
Key words: *Astragalus macropus* Bunge; Leguminosae; cycloartane triterpenoids; cyclomacroside A; cyclomacroside C; cyclomacrogenin B; PMR, ^{13}C NMR, DEPT, COSY, HSQC, and HMBC spectra.

In continuation of research on isoprenoids from *Astragalus macropus* Bunge (Leguminosae) [1], we elucidated the structure of the new cycloartane glycoside **1** that was isolated from the roots of this plant (compound E) [2] and called cyclomacroside A.

The PMR and ^{13}C NMR spectra of **1** (Table 1) indicate that it is a triterpenoid of the cycloartane series [3-6].

The carbohydrate part of the acid hydrolysis products of **1** contained L-rhamnose according to paper chromatography (PC) and comparison with authentic samples and taking into account biogenetic factors.

The PMR and ^{13}C NMR spectra of the glycoside contained resonances for a single L-rhamnose unit. Therefore, cyclomacroside A was a monoside. Chemical shifts and spin—spin coupling constants (SSCC) of C and H atoms in the L-rhamnose were indicative of the pyranose form, the $^1C_4$-conformation, and the α-configuration of the monosaccharide in **1**.



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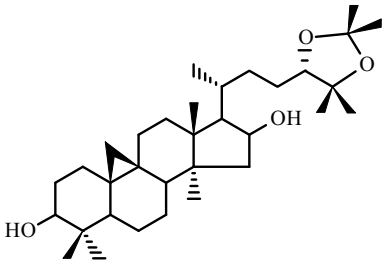

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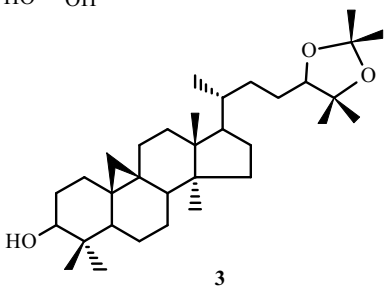

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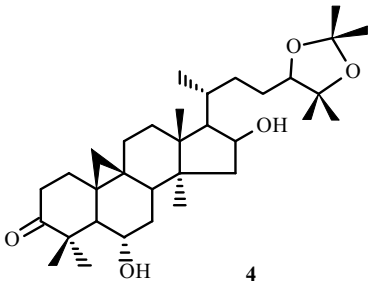

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The PMR spectrum of **1** at strong field contained resonances for 10 methyls, one of which, a doublet at δ 1.57, belonged to L-rhamnose and seven of which were assigned to the genin. The source of the other two methyls was found by analyzing the ^{13}C NMR spectrum of the glycoside, where the resonance of one ketal C atom was clearly visible at δ 106.44.

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TABLE 1. Chemical Shifts of C and H Atoms and DEPT, ^1H–^1H COSY, HSQC, and HMBC Spectra of **1** (C_5D_5N, δ, ppm, J/Hz) and Chemical Shifts of C Atoms of **2** ($CDCl_3$), **3** ($CDCl_3$), and **4** (C_5D_5N)

C atom	DEPT	1			2	3	4
		δ_C	δ_H	HMBC (C atoms)	δ_C [9]	δ_C [10]	δ_C
1	CH	72.41	3.83 br.s		32.0	31.9	31.91
2	CH_2	37.09	2.10, 2.51 m		30.5[a]	30.3	35.86
3	CH	84.23	4.15 dd (11.9, 4)		78.9	78.8	216.75
4	C	40.81	–		40.6	40.5	50.56
5	CH	39.19	2.61 dd (12.8, 3.6)		47.3*	47.1	53.61
6	CH_2	32.09	1.28, 2.10		21.1	21.1	69.22
7	CH	70.01	3.97 td (9, 3)		26.3	28.2	38.32
8	CH	55.30	1.93 d (9.1)	7, 9, 14	48.1	48.0	48.30
9	C	20.99	–		20.0	20.0	21.50
10	C	30.96	–		26.3	26.0	28.28
11	CH_2	26.34*	1.40, 2.55		26.3	26.0[a]	26.23
12	CH_2	33.20	1.60, 1.79		32.9[b]	35.5	33.11
13	C	45.98	–		45.5	45.3	45.75
14	C	49.09	–		46.7	48.8	46.80
15	CH_2	37.90	1.44, 2.00		47.3*	32.9	49.60
16	CH_2	26.71	1.80, 2.05		72.6	26.3[a]	71.20
17	CH	52.06	1.65		57.0	52.2	57.10
18	CH_3	17.95	1.14 s	12, 13, 14, 17	17.7	18.2[b]	18.29
19	CH_2	28.96**	0.47 d (4.2),	1	30.2[a]	29.9	30.71
			0.90 d (4.4)	8			
20	CH	36.62	1.44	17, 20, 22	28.7	36.3	31.03
21	CH_3	18.98	0.96 d (6)		18.9	18.0[b]	19.70
22	CH_2	33.86	1.65, 1.80		32.5[b]	33.1	34.23
23	CH_2	28.25	2.05, 2.05		24.9	26.3[a]	27.24
24	CH	84.01	3.82 br.d (6)		81.4	83.8	84.67
25	C	80.26	–		80.3	80.2	80.31
26	CH_3	23.20	1.16 s	24, 25, 27	26.2	22.9	26.49
27	CH_3	26.34*	1.30 s	24, 25, 26	23.3	26.4[c]	23.17
28	CH_3	18.62	1.25 s	8, 13, 14, 15	20.1	19.3	20.42
29	CH_3	25.68	0.97 s	3, 4, 5, 30	25.5	25.4	28.60
30	CH_3	14.42	0.93 s	3, 4, 5, 29	14.1	14.0	20.55
				α-L-Rha*p* (R)			
1	CH	104.51	5.35 s	3, R2, R3, R5			
2	CH	72.32	4.56 br.d (3)				
3	CH	72.87	4.48 dd (8.5, 3)				
4	CH	74.07	4.29 t (8.7)	R5			
5	CH	69.77	4.30 m				
6	CH_3	18.38	1.57 d (5.7)	R4, R5			
				Isopropylidene (I)			
1	CH_3	28.96**	1.48 s	I2, I3	26.9	26.9[c]	27.19
2	C	106.44	–		106.8	106.3	106.35
3	CH_3	27.16	1.41 s	I1, I2	27.7	28.6	29.00

Resonances marked by asterisks are mutually interchangeable; by letters, assigned arbitrarily.

The resonances of two methyls (δ 1.41 and 1.48) in the HMBC spectrum had correlation peaks with the ketal C atom. This indicated that the glycoside included an isopropylidene substituent. The isopropylidenedioxy group was located in the genin part of the glycoside because the monosaccharide unit was unsubstituted. The chemical shift of the tertiary C atom, which was oxygenated, of δ 80.26 indicated that this tertiary C atom was part of the ketal system. The tertiary carbonyl C atom was C-25

because the resonance for CH_3-21 in the PMR spectrum was a doublet. Therefore, the isopropylidenedioxy group was located on C-24–C-25.

The secondary C atoms of the genin, which remained unidentified, resonated at δ 72.41, 84.23, and 70.01. The chemical shifts, multiplicity, and SSCC of protons geminal to secondary hydroxyl groups and assigned using HSQC spectra indicated that the genin of the new glycoside **1** contained three 1α,3β,7β-hydroxyls. The weak-field shift of the C-3 resonance (δ 84.23) indicated that this C atom was glycosylated. In fact, the HMBC spectrum exhibited a correlation peak between the anomeric proton of L-rhamnose and C-3 of the genin.

Thus, the stereochemistry of C-24 remained in question. Biogenetic considerations such as the isolation from *A. macropus* of cyclomacrogenin B and its glycosides [1, 2, 7, 8] with the 24*R*-configuration suggested that **1** had the same stereochemistry. This means that this glycoside was derived from cyclomacrogenin B and differed from cyclomacroside C by an additional isopropylidene substituent on C-24–C-25.

The conclusion about the stereochemistry of C-24 was confirmed by comparing ^{13}C NMR spectra of **1**, and the 24,25-acetonides **2** [9] and **3** [10]. The C-24 atom in the ^{13}C NMR spectrum of **2** with the 24*S*-configuration resonated at δ 81.4; in the spectrum of **3** with the 24*R*-configuration, at δ 83.8. This C atom in the ^{13}C NMR spectrum of cyclomacroside A was observed at δ 84.01. We recorded the ^{13}C NMR spectrum of the 24,25-acetonide of 3-dehydrocycloasgenin C (**4**) in deuteropyridine because the spectra of **2** and **3** were obtained in $CDCl_3$ whereas that of **1** was obtained in deuteropyridine. The C-24*R* atom was observed in the spectrum of **4** at δ 84.67. These data indicated taht C-24 of **1** had the *R*-configuration.

Thus, the new glycoside **1** was 24,25-isopropylidenedioxy-24*R*-cycloartan-1α,3β,7β-triol 3-*O*-α-L-rhamnopyranoside and was viewed as the 24,25-acetonide of cyclomacroside C.

One natural acetonide, triterpenoid **3** [10], has been described among cycloartane triterpenoids. What considerations argue in favor of **1** being natural? First, cyclomacroside A is the only compound among those isolated from roots of *A. macropus* that is an acetonide whereas cyclomacrogenin B and its other glycosides were isolated under identical conditions. Furthermore, the 2,3-α-diol group of the α-L-rhamnopyranose is more available than the α-diol group on C-24–C-25 for formation of the acetonide because one of the hydroxyls in the latter is tertiary. If **1** is an artifact of the isolation process, then it would seem that we should have isolated other acetonides, even diacetonides. Second, generation of the acetonide requires acid catalysis. Under acid catalysis conditions hydroxyls located in the α-position relative to the cyclopropane ring, i.e., on C-1 and C-11, are readily eliminated, causing migration of the cyclopropane ring. A 1,19-bond arises after cleavage of the 9,19-bond in the first instance; an 11,19-bond after cleavage of the 10,19-bond, in the second [3, 11, 12]. The acetonide of cycloasgenin B with retention of the C-11 hydroxyl and the 9,19-three-membered ring could not be produced because of this [13]. These facts suggest that cyclomacroside A is a native compound although the involvement of the isopropyl group in the biosynthesis and metabolism of plant substances in the organism is unknown.

EXPERIMENTAL

General comments have been published [14]. Descending PC was performed on FN-1 paper using *n*-BuOH:Py:H_2O (6:4:3). Monosaccharides were detected on chromatograms by spraying with anilinium phthalate and heating at 110°C.

NMR spectra were recorded in deuterated Py on Bruker AM-300 (**1**) and UNITYplus 400 (**4**) spectrometers. ^{13}C NMR spectra were obtained with full C–H decoupling under DEPT conditions. Two-dimensional spectra of **1** were recorded using standard Bruker programs. Spectra of **1** were obtained without an internal standard; of **4**, with HMDS internal standard. Chemical shifts of **1** were set relative to the resonance of residual β-protons of deuteropyridine with a chemical shift of δ 7.19 relative to TMS. Chemical shifts of C atoms in **1** and **4** were set relative to the resonance of the β-C atoms of deuteropyridine with a chemical shift of δ 123.493 relative to TMS.

Isolation and separation of isoprenoids from roots of *A. macropus* Bunge has been reported [2].

Cyclomacroside A, $C_{39}H_{66}O_9$, mp 238-241°C (MeOH). IR spectrum (KBr, ν, cm^{-1}): 3450 (OH), 3040 (cyclopropane ring CH_2).

Table 1 lists the PMR, ^{13}C, DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC spectra.

Acid Hydrolysis of Cyclomacroside A. Glycoside **1** (20 mg) was hydrolyzed by methanolic H_2SO_4 (0.5%) under reflux for 2 h. Subsequent work up of the carbohydrate part of the hydrolysate by PC in the presence of authentic samples identified L-rhamnose.

24,25-Isopropylidenedioxy-24*R*-cycloartan-6α,16β-diol-3-one (4) was obtained as before [5].

PMR spectrum (400 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = HMDS): 0.26, 0.56 (2H-19, d, $^2J = 4.2$), 0.89 (CH_3, s), 0.98 (CH_3-21, d, $^3J = 6.5$), 1.05, 1.18, 1.27, 1.30, 1.33, 1.35, 1.63 ($7 \times CH_3$, s), 2.04 (H-17, dd, $^3J_1 = 12.8$, $^3J_2 = 8$), 2.11 (H-5, d, $^3J = 9.7$), 2.36, 2.62 (2H-2, m), 3.58 (H-6, td, $^3J_1 = {}^3J_2 = 10$, $^3J_3 = 3$), 3.71 (H-24, dd, $^3J_1 = 7.7$, $^3J_2 = 5$), 4.53 (H-16, td, $^3J_1 = {}^3J_2 = 7.7$, $^3J_3 = 4.8$).

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

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