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TRITERPENE GLYCOSIDES FROM *Astragalus* AND THEIR GENINS. LXXVII. CYCLOMACROGENIN B, A NEW CYCLOARTANE TRITERPENOID

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*Ten pure compounds, three of which were identified as β-sitosterol, β-sitosterol β-D-glucopyranoside, and D-3-*O*-methyl-*chiro*-inositol, were isolated from roots of* Astragalus macropus *Bunge (Leguminosae). The structure of the new cycloartane triterpenoid cyclomacrogenin B was established as 24*R*-cycloartan-1α,3β,7β,24,25-pentaol.*

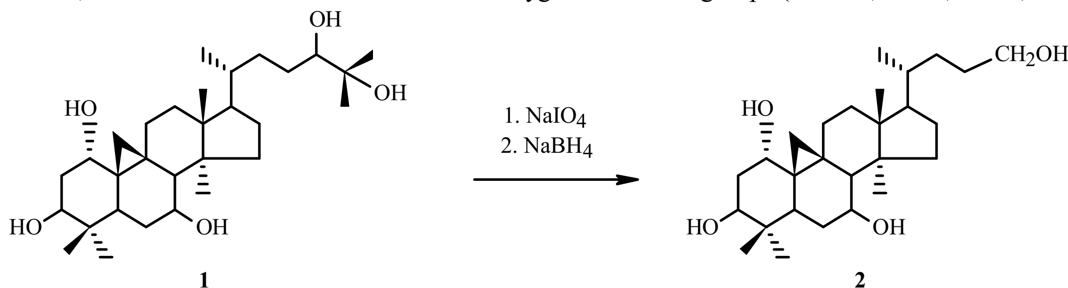
Key words: *Astragalus macropus* Bunge, Leguminosae, cycloartane triterpenoids, cyclomacrogenin B, PMR and ^{13}C NMR spectra, DEPT, ^{1}H—^{1}H COSY, HSQC, HMBC.

In continuation of research on isoprenoids from plants of the genus *Astragalus* (Leguminosae), we studied *A. macropus* Bunge [1]. The methanol extract of its roots contained 10 compounds of triterpenoid and steroid nature that were separated by column chromatography and designated by us in order of increasing polarity as compounds A, B, C, D, E, F, G, H, I, and J. Compounds A, B, and J were identified as *β*-sitosterol, *β*-sitosterol *β*-D-glucopyranoside, and D-3-*O*-methyl-*chiro*-inositol, respectively [2]. Herein the structure of the new compound D, which we called cyclomacrogenin B (**1**), is elucidated.

The observation in the PMR spectrum of **1** of two ^{1}H doublets of an AX system at δ 0.43 and 0.93 with characteristic SSCC ^{2}J = 4.1 and 4.2 Hz (Table 1) and resonances for seven methyls at high field of the same spectrum enabled this compound to be considered a cycloartane triterpenoid [3-6].

The ^{13}C NMR spectrum of **1** exhibited resonances for 30 C atoms, the chemical shifts of which were located in the range δ 13.98-79.06, indicating that **1** did not contain a double bond or carbonyl group.

The DEPT spectrum showed that the C atoms of the new genin **1** consisted of seven methyl, 9 methylene, 8 methine, and 6 C atoms not bonded to H atoms. As expected, five of the six C atoms not bonded to H atoms were quaternary C atoms of the cycloartane skeleton (C-4, C-9, C-10, C-13, C-14) whereas the sixth C atom was a tertiary C atom carrying an oxygen functional group. Because only one methyl was a doublet (CH_3-21), the tertiary C atom with the oxygen functional group was C-25. Furthermore, another four C atoms were bonded to oxygen functional groups (δ 79.06, 72.96, 72.65, 70.14).



Periodate oxidation of **1** with subsequent reduction by sodium borohydride gave nor-product **2**, the structure of which we will discuss below. The preparation of tetraol **2** was consistent with the presence of an *α*-diol at C-24 and C-25 of the new genin **1**. Taking this into account and considering that the PMR spectrum of **1** clearly displayed resonances for four protons geminal to hydroxyls, all oxygen functional groups of the compound were hydroxyls, one of which was located on C-25 and was tertiary. Therefore, cyclomacrogenin B had an acyclic side chain and composition $C_{30}H_{52}O_5$.

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TABLE 1. Chemical Shifts of C and H Atoms and Parameters of DEPT, $^1H—^1H$ COSY, HSQC, and HMBC Spectra of **1** (C_5D_5N, δ, ppm, J/Hz) and **2** (CD_3OD)

C atom	DEPT	1			2
		δ_C	δ_H	HMBC (C atom)	δ_C
1	CH	72.65	3.87 br.s		74.16
2	CH_2	38.95	2.21, 2.40		38.53
3	CH	72.96	4.40 dd (11.9, 4.3)		73.85
4	C	40.98	-		41.31
5	CH	39.36	2.68 dd (13.3, 3.9)		40.05
6	CH_2	32.31	1.42, 2.21		32.20
7	CH	70.14	4.00 td (9.6, 3.9)		71.23
8	CH	55.21	1.95 d (9.2)	7, 9, 14	56.10
9	C	21.00	-		21.95
10	C	31.32	-		31.48
11	CH_2	26.47	1.52, 2.62		26.93
12	CH_2	33.30	1.65, 1.80		33.47
13	C	45.99	-		46.81
14	C	49.13	-		49.60
15	CH_2	37.86	1.52, 1.99		38.04
16	CH_2	28.98[a]	1.83, 1.99		30.45
17	CH	52.38	1.63		52.97
18	CH_3	17.91	1.12 s	12, 13, 14, 17	18.32
19	CH_2	28.98[a]	0.43 d (4.1), 0.93 d (4.2)	1	29.51
20	CH	36.43	1.55		37.25
21	CH_3	18.68	1.00 d (5.8)	17, 20, 22	19.21
22	CH_2	34.22	1.65, 1.80		33.97
23	CH_2	28.20	1.83, 1.83		29.45
24	CH(CH_2)	79.06	3.77* (6.7)		63.60
25	C	72.72	-		-
26	CH_3	25.94	1.50 s	24, 25, 27	-
27	CH_3	26.19	1.53 s	24, 25, 26	-
28	CH_3	19.02	1.25 s	8, 13, 14, 15	18.97
29	CH_3	26.03	1.28 s	4, 30	25.94
30	CH_3	13.98	1.15 s	4, 29	13.72

Chemical shifts given without multiplicities and SSCC were found from 2D spectra; [a]resonances overlapping; *doublet with broad components.

The PMR spectrum of **1** showed the resonance for H-8 at δ 1.95 as a doublet with SSCC $^3J = 9.2$ Hz. Therefore, one of the secondary hydroxyls was located on C-7 and had the *β*-equatorial orientation. This was consistent with the 1H triplet of doublets at δ 4.00 with SSCC $^3J_1 = {}^3J_2 = 9.6$ and $^3J_3 = 3.9$ Hz in the same spectrum that was assigned to H-7 and with the chemical shift of C-7 of δ 70.14 in the ^{13}C NMR spectrum.

The $^1H—^1H$ COSY spectrum of **1** exhibited a doublet of doublets at δ 4.40 with cross peaks for methylene protons resonating at δ 2.21 and 2.40. The protons of this same methylene coupled with another proton geminal to a hydroxyl and resonating at δ 3.87 as a broad singlet. Thus, a spin system of coupled >CH–CH_2–CH< protons that could be located in ring A of the cycloartane skeleton and occupy positions 1, 2, and 3 was observed. This means that the doublet of doublets at δ 4.40 with SSCC $^3J_1 = 11.9$ and $^3J_2 = 4.3$ Hz in the PMR spectrum of **1** belonged to H-3*α* and defined the *β*-orientation of the C-3 hydroxyl. This C atom resonated at δ 72.96 in the ^{13}C NMR spectrum of **1**. Therefore, the other secondary hydroxyl located in ring A was situated on C-1. The proton geminal to the C-1 hydroxyl resonated in the PMR spectrum at δ 3.87 as a broad singlet. This was consistent with the equatorial orientation of this H atom. This fact defines the *α*-orientation of the C-1 hydroxyl. The resonance for H-1 correlated in the HSQC spectrum with that of the C atom resonating at δ 72.65.

Thus, the polycyclic part of cyclomacrogenin B contained $1\alpha,3\beta,7\beta$-hydroxyls.

Atom C-24 resonated at δ 79.06 in the ^{13}C NMR spectrum of **1**. The resonance of C-24 of cycloartanes containing a 16β,24,25-triol group with the 24*R*-configuration was observed at δ 80.5 whereas the chemical shift of a C-24*S* atom was δ 77.2 [4], i.e., the resonance of a C-24*R* atom was located at weak field compared with that of a C-24*S* atom. This principle remained in effect for cycloartanes containing a 24,25-diol. The resonance of the C-24*R* atom was found at δ 79.6 [7]. Therefore, it can be concluded that C-24 of **1** had the *R*-configuration.

Thus, **1** had the structure 24*R*-cycloartan-$1\alpha,3\beta,7\beta$,24,25-pentaol.

As expected, the ^{13}C NMR spectrum of nor-product **2** exhibited resonances for 27 C atoms. The resonance of the primary carbonyl C was found at δ 63.60 and was unambiguously assigned to C-24. This defined **2** as 25-norcycloartan-$1\alpha,3\beta,7\beta$,24-tetraol.

EXPERIMENTAL

General Comments. The following solvent systems were used: $CHCl_3$:CH_3OH (20:1, 1; 10:1, 4), $CHCl_3$:CH_3OH:H_2O (70:12:1, 2; 70:23:4, 3).

NMR spectra were recorded on Bruker AM-300 (**1** in deuteropyridine) and UNITYplus 400 (**2** in deuteromethanol). ^{13}C NMR spectra were obtained with full C–H decoupling under DEPT conditions. 2D spectra of **1** were recorded using standard Bruker programs. Spectra of **1** were obtained without an internal standard; of **2**, with HMDS internal standard. Chemical shifts of protons in **1** were measured relative to the resonance of residual β-protons of deuteropyridine with chemical shift δ 7.19 relative to TMS. Chemical shfits in ^{13}C NMR spectra of **1** were measured relative to the resonance of the β-C atoms of deuteropyridine with chemical shift δ 123.493 relative to TMS.

Isolation and Separation of Isoprenoids from *A. macropus*. Air-dried ground roots (1.2 kg) of *A. macropus* that were collected in June 2004 near Kara-Dygach, Kara-Kuljin region, Osh District, Republic of Kyrgyzstan, were exhaustively extracted with methanol (5 × 7 L). The extract was evaporated to a thick consistency and diluted with methanol to a volume of 1 L. The resulting precipitate, which did not contain the desired compounds, was filtered off. The filtrate was evaporated to dryness to afford total extracted compounds (83.5 g, 6.9% of air-dried raw material). TLC detected about 10 compounds of triterpenoid and steroid nature. These components were designated compounds A-J in order of increasing polarity.

Total extracted compounds (83.5 g) were chromatographed over a column of silica gel (grade L) with elution successively by $CHCl_3$ and systems 1-3. Fractions collected upon elution by $CHCl_3$ and containing compound A were combined and recrystallized from methanol to afford compound A (0.533 g, 0.044%, yield here and henceforth calculated for air-dried raw material).

Further elution of the column with system 1 isolated pure compound B (0.082 g, 0.0067%).

Continued elution of the column by system 2 isolated a mixture of compounds C and D (0.65 g). Further elution with the same system isolated a mixture of compounds E, F, and G (4.58 g) and pure compound H (3.945 g, 0.33%).

Continued elution of the column with system 3 isolated pure compounds I (3.7 g, 0.31%) and J (0.32 g, 0.026%).

Rechromatography of the mixture of C and D (0.65 g) using system 1 produced pure C (0.085 g, 0.007%) and D (0.477 g, 0.039%).

The fraction containing compounds E, F, and G (4.58 g) was rechromatographed using system 2 to isolate pure E (0.196 g, 0.16%), F (0.32 g, 0.027%), and G (0.77 g, 0.064%).

Based on physicochemical constants, direct comparison with authentic samples, and parameters of PMR spectra, compounds A, B, and J were identified as β-sitosterol, β-sitosterol β-D-glucopyranoside, and D-3-*O*-methyl-*chiro*-inositol, respectively [2].

The remaining compounds were cycloartane type triterpenoids [3-6].

***β*-Sitosterol**, compound A, $C_{29}H_{50}O$, mp 131-132°C (methanol) [2].

***β*-Sitosterol *β*-D-glucopyranoside**, compound B, $C_{35}H_{60}O_6$, mp 276-279°C (methanol) [2].

D-3-*O*-Methyl-*chiro*-inositol, compound J, $C_7H_{14}O_6$, mp 189-191°C (methanol) [2].

Cyclomacrogenin B (1), compound D, $C_{30}H_{52}O_5$, mp 209-211°C (methanol).

IR spectrum (KBr, ν_{max}, cm^{-1}): 3426 (OH), 3040 (cyclopropane ring CH_2). Table 1 lists the PMR and ^{13}C NMR, DEPT, ^1H—^1H COSY, HSQC, and HMBC spectra.

25-Norcycloartan-1α,3β,7β,24-tetraol (2) from 1. A solution of **1** (32 mg) in *n*-butanol (2 mL) was diluted with methanol (10 mL), treated with sodium periodate (300 mg) in water (2 mL), and stirred at room temperature for 8 h. The excess of oxidant was destroyed by addition of ethyleneglycol (0.25 mL), after which the solution was poured into water and extracted with ethylacetate. The extract was washed with water and evaporated to dryness. The solid was dissolved in methanol (10 mL), treated in portions with sodium borohydride (200 mg), left at room temperature for 12 h, poured into water, and extracted with ethylacetate. The extract was worked up as usual and evaporated. The solid was chromatographed over a column with elution by system 4 to afford **2** (10 mg), $C_{27}H_{46}O_4$, mp 235-236°C ($CHCl_3$:CH_3OH, 1:1).

PMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz, 0 = HMDS): 0.36 and 0.70 (d, ^2J = 4.7, 2H-19), 0.73 (s, CH_3), 0.86 (d, ^3J = 6.8, CH_3-21), 0.92, 0.96, 0.98 (s, 3 × CH_3), 2.03 (dd, $^3J_1$ = 13.2, $^3J_2$ = 4.4, H-5), 3.43-3.50 (m, H-1, H-7, 2H-24), 3.62 (dd, $^3J_1$ = 10.3, $^3J_2$ = 6, H-3). Table 1 gives the ^{13}C NMR and DEPT spectra.

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