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STEROIDAL GLYCOSIDES FROM *Allium cyrillii* BULBS

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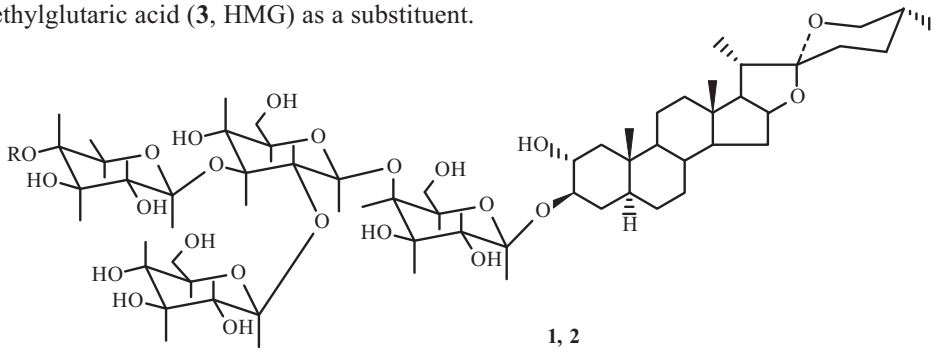
Two spirostanol saponins, one of which was a new compound, were isolated among the steroidal glycosides of Allium cyrillii *Ten. Bulbs. The structures of these glycosides were established using chemical and spectral analytical methods as* β-*D-glycopyranosyl-(1→2)-[* β-*D-xylopyranosyl-(1→3)]-* β-*D-glucopyranosyl-(1→4)-* β-*D-galactopyranosyl-(1→3)-(25*R*)-5*α*-spirostan-2*α*,3*β*-diol and* β-*D-glucopyranosyl-(1→2)-[4-*O*-(3-hydroxy-3-methylglutaryl)-* β-*D-xylopyranosyl-(1→3)]-* β-*D-glucopyranosyl-(1→4)-* β-*D-galactopyranosyl-(1→3)-(25*R*)-5*α*-spirostan-2*α*,3*β*-diol.*

Keywords: *Allium cyrillii*, steroidal glycosides.

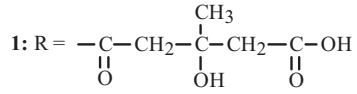
Steroidal glycosides represent a broad class of natural saponins, which are interesting to researchers because of their broad spectrum of biological activity and ecological safety [1].

Plants of the genus *Allium*, which are indigenous to Crimea, are promising in the search for saponin-bearing species. Moreover, data on the steroidal glycosides of the majority of Crimean species have not been reported. Hence the study of the chemical structure of steroidal glycosides from representatives of the Alliaceae family is timely.

Bulbs of *A. cyrillii* yielded chromatographically pure glycosides **1** (0.085 g) and **2** (0.154 g). The aglycon of both compounds was identified after acid hydrolysis as gitogenin [2]. TLC and comparison with authentic sugar samples detected glucose, xylose, and galactose in a 2:1:1 ratio. Alkaline hydrolysis of **1** and TLC using an authentic standard detected 3-hydroxy-3-methylglutaric acid (**3**, HMG) as a substituent.



1, 2

1: R =  **2:** R = H

The empirical formulas of **1** and **2** were determined as $C_{56}H_{90}O_{27}$ and $C_{50}H_{82}O_{23}$. These were confirmed as peaks of negative ions $[M - H]^-$ in mass spectra with *m*/*z* 1193.5602 and 1049.5149 that correlated to molecular weights (MWs) 1194.5680 and 1050.5228 Da (calculated MWs 1194.5670 and 1050.5247 Da, respectively). The ^{13}C NMR spectrum of **1** (Table 1) showed resonances of the carbohydrate part and the HMG residue as 27 C atom resonances including 4 methyl (δ 13.3, 15.0, 16.6, 17.3), 10 methylene, 10 methine, and 3 quaternary (δ 36.8, 40.8, 109.4) atoms. The spectrum of **2** also contained 27 resonances for C atoms of the aglycon. The PMR and ^{13}C NMR spectra indicated that **1** and **2** were spirostane derivatives. Thus, the ^{13}C chemical shift (CS) of C-22 in both compounds was 109.4 ppm. The CSs of C-5 (δ 44.5), C-9 (54.3), and C-19 (13.3) were consistent with *cis*-fusion of rings A and B.

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TABLE 1. Chemical Shifts (Py-d_5, 0 = TMS, δ, ppm) of ^{13}C Atoms of **1** and **2**

C atom	**1**	**2**	C atom	**1**	**2**
1	45.3	45.4	HMG 1*	179.6	
2	70.4	70.4	2*	47.4	
3	84.0	84.0	3*	70.4	
4	33.7	33.8	4*	47.3	
5	44.5	44.5	5*	171.5	
6	28.0	28.1	6*	28.2	
7	32.0	32.1	Gal-1′	102.9	103.0
8	34.5	34.6	2′	72.4	72.5
9	54.3	54.3	3′	75.2	75.3
10	36.8	36.9	4′	79.4	79.4
11	21.4	21.4	5′	75.6	75.7
12	40.0	40.1	6′	60.9	60.8
13	40.8	40.8	Glc-1″	104.2	104.3
14	56.3	56.3	2″	80.8	80.9
15	32.2	32.2	3″	86.7	87.1
16	81.2	81.2	4″	70.0	70.1
17	62.9	62.9	5″	77.2	77.4
18	16.6	16.6	6″	62.5	62.6
19	13.3	13.4	Glc-1‴	104.2	104.4
20	42.0	42.0	2‴	75.6	75.7
21	15.0	15.0	3‴	77.6	77.9
22	109.4	109.4	4‴	71.1	71.2
23	31.7	31.8	5‴	78.2	78.3
24	29.2	29.2	6‴	62.4	62.5
25	30.5	30.6	Xyl-1⁗	104.4	104.8
26	66.9	66.9	2⁗	74.6	74.9
27	17.3	17.3	3⁗	74.3	78.3
			4⁗	72.8	70.6
			5⁗	63.2	67.1

The CSs of the CH_3-27 doublet (δ 0.71–0.72) and the H-26 methylene protons (3.50, 1H, H-26a; 3.61, 1H, H-26b) indicated unambiguously that CH_3-27 had an equatorial orientation (Table 2). Thus, the aglycon of **1** and **2** was a (25*R*)-spirostane. The 25*R*-configuration was confirmed by the CSs of C-20, C-21, C-23, C-24, C-25, and C-27 and a comparison with the literature [3, 4]. The 5α-configuration was determined based on correlations among resonances for protons at δ 0.70 (CH_3-19) and C atoms at δ 36.8 (C-10), 54.3 (C-9), 44.5 (C-5), and 45.3 (C-1) in the 2D HMBC spectrum. Resonances of four methyls at δ 0.81 (3H, s, Me-18), 0.70 (3H, s, Me-19), 0.71 (0.72) (3H, d, J = 7.0 Hz, Me-27), and 1.14 (1.15) (3H, d, J = 7.0 Hz, Me-21) were clearly resolved in the strong-field region of PMR spectra of both glycosides.

The location of the hydroxyl on C-2 of the aglycon was confirmed by the weak-field position of its resonance compared with literature data for the non-hydroxylated aglycons [5] and taking into account the significant positive α-effects of hydroxylation and the small and usually negative β-effects. The 2α,3β-configuration was determined based on an analysis of spin–spin coupling constants of the H-2 and H-3 protons with neighboring protons.

Spectral data were compared with the literature [6] and confirmed the structure of the aglycon as (25*R*)-5α-spirostan-2α,3β-diol. It was also noticed that C-3 was substituted (weak-field shift from 76.5 ppm to 84.0). The ^{13}C NMR spectrum of **1** exhibited six additional resonances for two methylenes (δ 47.3 and 47.4), a methyl (28.2), a carboxylic group (179.6), an ester (171.5), and a tertiary C atom bound to the hydroxyl (70.4 ppm). The CSs of the methylene protons of this substituent were δ 2.75, 2.93, 2.84, and 3.07 ppm; of the methyl protons, 1.57 ppm. A comparison of these values with the literature [7] confirmed that glycoside **1** contained HMG. The acid was bonded at O-4 of a xylose unit as indicated by the weak-field CS of H-4⁗ (δ 5.17) compared with that of **2** (4.12), which did not contain an HMG residue (Table 2). Also, α-effects (for C-4⁗) and β-effects (for C-3⁗ and C-5⁗) of the 4-*O*-acylation of the xylose unit were characteristic in the ^{13}C NMR spectrum (Table 1).

TABLE 2. Observed Correlations in HSQC and HMBC Spectra of the Glycosides

Atom	δ_H HSQC		HMBC	Atom	δ_H HSQC		HMBC
	1	**2**			**1**	**2**	
1a	2.18 dd	2.18 dd	C-2, 10	Gal-1′	4.92 d	4.93 d	C-3
1b	1.19 m	1.18 m	C-2, 10, 19	2′	4.49 m	4.52 m	C-1′, 3′
2	3.96 m	3.98 m	C-3	3′	4.16 m	4.17 m	C-2′, 5′
3	3.89 m	3.91 m	C-2	4′	4.57 m	4.58 m	C-2′, 3′, 1″
4a	1.87 q	1.88 q	C-2, 3, 6, 10	5′	4.08 m	4.10 m	C-1′, 3′, 6′
4b	1.47 m	1.50 m	C-3, 5	6′a	4.55 m	4.56 m	C-5′
5	1.02 m	1.03 m	C-1, 6, 19	6′e	4.27 m	4.27 m	C-4′
6a	1.18 m	1.17 m	C-10	Glc-1″	5.14 d	5.16 d	C-4′, 5″
6b	1.03 m	1.06 m	C-7	2″	4.27 t	4.30 t	C-3″, 1‴
7a	1.51 m	1.53 m	C-5, 6, 8	3″	4.14 t	4.09 t	C-1⁗, 1″, 2″
7b	0.78 m	0.79 m		4″	3.76 t	3.76 t	C-3″, 5″, 6″
8	1.40 m	1.42 m	C-14	5″	3.82 m	3.82 m	C-4″
9	0.58 td	0.58 td	C-19	6″a	4.46 m	4.47 m	
11a	1.44 m	1.47 m	C-8	6″e	4.03 m	4.04 m	C-5″
11b	1.22 m	1.22 m		Glc-1‴	5.58 d	5.59 d	C-2″
12a	1.64 m	1.65 m	C-9, 11, 14	2‴	4.05 m	4.04 m	C-1″, 3‴
12b	1.03 m	1.05 m	C-13	3‴	4.21 m	4.19 m	C-4‴
14	1.05 m	1.03 m	C-13, 18	4‴	4.08 m	4.09 m	C-3‴, 6‴
15a	2.04 m	2.06 m	C-13, 17	5‴	3.94 m	3.96 m	C-2‴
15b	1.41 m	1.42 m	C-14, 16	6‴a	4.50 m	4.53 m	C-4‴
16	4.57 m	4.57 m	C-13	6‴e	4.39 dd	4.41 dd	
17	1.82 t	1.82 t	C-12, 16, 18, 21	Xyl-1⁗	5.23 d	5.21 d	C-3″
18	0.81 s	0.81 s	C-12, 13, 14, 17	2⁗	3.92 t	3.94 t	C-1⁗, 3⁗
19	0.70 s	0.70 s	C-1, 5, 9, 10	3⁗	4.25 m	4.10 m	C-1⁗, 5⁗
20	1.96 m	1.97 m	C-13, 17, 21, 22	4⁗	5.17 m	4.12 m	C-3⁗
21	1.14 d	1.15 d	C-17, 20, 22	5⁗a	3.50 t	3.68 t	C-1⁗, 3⁗
23a	1.69 m	1.69 m	C-25	5⁗e	4.20 m	4.22 m	C-4⁗
23b	1.71 m	1.73 m	C-22	HMG			HMG
24a	1.61 m	1.61 m	C-25	2a*	2.93 d		C-1*, 4*, 6*
24b	1.58 m	1.57 m	C-23	2b*	2.76 d		C-1*, 4*, 6*
25	1.60 m	1.60 m		4a*	3.07 d		C-1*, 3*, 6*
26a	3.61 dd	3.61 dd	C-22, 24	4b*	2.85 d		C-1*, 3*, 6*
26b	3.50 t	3.51 t	C-22, 24, 25	6*	1.57 s		C-2*, 3*, 4*
27	0.71 d	0.72 d	C-24, 25, 26				

Four resonances for anomeric protons of the monosaccharides at δ 5.58 (5.59) (1H, d, J = 7.5 Hz), 5.23 (5.21) (1H, d, J = 7.5 Hz), 5.14 (5.16) (1H, d, J = 7.5 Hz), and 4.92 (4.93) (1H, d, J = 7.5 Hz) appeared at weak field in the PMR spectra of **1** and **2**. Thus, the carbohydrate chain in both glycosides consisted of four sugar units. The spin–spin coupling constants $J_{1,2}$ were indicative of the β-configurations of the glycoside bonds in all monosaccharide residues.

The CSs of all four monosaccharide protons were found using a combination of 2D TOCSY and COSY spectra. The CSs of the resonances of the corresponding ^{13}C atoms were assigned unambiguously using 2D HSQC spectra (Table 2). These data indicated that one β-D-galactopyranose, one β-D-xylopyranose, and two β-D-glucopyranose residues were present.

The correlation between Gal H-1′ and C-3 of the aglycon (Table 2) defined the site of attachment of the carbohydrate chain to the aglycon. A CS of δ 79.4 was observed for Gal C-4′. This characterized the site of attachment of the next monosaccharide unit. The HMBC spectrum showed structurally informative cross-peaks between resonances Xyl H-1⁗ (δ 5.23) and Glc C-3″ (86.7); Glc H-1‴ (5.58) and Glc C-2″ (80.8); and Glc H-1″ (5.14) and Gal C-4′ (79.4) for **1** in addition to Xyl H-1⁗ (5.21) and Glc C-3″ (87.1); Glc H-1‴ (5.59) and Glc C-2″ (80.9); and Glc H-1″ (5.16) and Gal (C-4′) (79.4) for **2**.

Thus, **1** was isolated from *A. cyrillii* and was β-D-glucopyranosyl-(1→2)-[4-*O*-(3-hydroxy-3-methylglutaryl)-β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(1→3)-(25R)-5α-spirostan-2α,3β-diol whereas **2**

had the structure β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(1→3)-(25*R*)-5α-spirostan-2α,3β-diol.

Glycoside **2** was isolated previously from *Digitalis purpurea* L. [8] whereas **1** was an unreported new compound.

EXPERIMENTAL

NMR spectra were taken from Py-d_5 solutions (0.6 mL) with TMS standard on a Varian AS 400 instrument (400 MHz for ^{1}H; 100, for ^{13}C). Mass spectra (negative-ion mode) with electrospray ionization were obtained in a micrOTOF II instrument (Bruker Daltonics). Samples (~10 ng/μL) were dissolved in a H_2O:*i*-PrOH:Et_3N mixture (5:5:0.003) and injected at flow rate 3 μL/min. The potential at the capillary entrance was 3.2 kV; drying-gas temperature, 180°C.

Analytical HPLC was performed on an Agilent 1100 chromatograph equipped with an Agilent light-scattering detector and a Zorbax SB-C18 column (150 × 2.1 mm, 3.5 μm) using gradient elution with aqueous CH_3CN (15%) with added CF_3COOH (TFA, 0.05%) up to MeOH (100%) in 20 min at mobile-phase flow rate 0.25 mL/min and column temperature thermostatted at 35°C. The injected sample volume was 2 μL. The N_2 pressure in the light-scattering detector was 3.5 atm; the vaporizer temperature, 100°C.

Preparative column chromatography was carried out using a column packed with C18 sorbent (100 × 20 mm, 40–63 μm, Aldrich) with elution successively by aqueous MeOH (20%) with added TFA (0.05%) up to MeOH (100%).

Two saponins isolated from *A. cyrillii* bulbs collected on the northeast slope of Paragilmen mountain in 2010 were studied. Freshly collected and ground bulbs (1148 g) were extracted (3×) with EtOH (70%). The extraction was performed with heating of the material on a water bath with a reflux condenser for 3 h. The resulting extract was evaporated in a rotary evaporator at 50°C to a watery residue from which total steroidal glycosides were extracted by *n*-BuOH. The BuOH extract was evaporated to dryness. TLC was performed using $CHCl_3$:MeOH:H_2O (65:35:7) and $CHCl_3$:MeOH (7:3). We used Sorbfil AF-V plates with layer thickness 110 μm for the chromatography. Chromatograms were sprayed with Erlich [9] and Sannie [10] reagents and then heated to 120°C.

Acid Hydrolysis. Pure glycoside (10 mg) was heated at 110°C in H_2SO_4 solution (1%, 5 mL) in EtOH for 2 h, cooled, and diluted with H_2O. The aglycon was extracted by $CHCl_3$. The $CHCl_3$ extracts were washed with H_2O until neutral and concentrated *in vacuo* to a dry solid. The aglycon was identified using TLC and benzene:EtOH (9:1).

The aqueous layers were neutralized with anion exchanger until neutral and evaporated. The sugars were identified using BuOH:Me_2CO:H_2O (4:5:1).

Alkaline Hydrolysis. The glycoside (10 mg) was heated in NaOH solution (10%, 10 mL) for 2 h at 50°C. The hydrolysate was neutralized by cation exchanger, washed with MeOH, and evaporated. TLC analysis was performed using EtOH:NH_4OH:H_2O (1:1:2) with detection by bromocresol solution (0.05%).

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