STRUCTURES OF SOME LILIOGLYCOSIDES FROM THE BULBS OF *Lilium regale*

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Six steroid glycosides, which gave been called lilioglycosides B, C, E, F, H, and I have been isolated from fresh bulbs of Lilium regale *Wills. The structure of each compound has been determined by methods of physicochemical analysis. This is the first time that lilioglycosides B, C, H, and I, have been described; they are, respectively: (25S)-spirost-5-ene-3β,27-diol 3-O-β-*D*-glucopyranoside; (25R)-spirost-5-ene-3β,27-diol 3-O-β-*D*-glucopyranoside 27-(3-hydroxy-3-methylglutarate); (25S)-spirost-5-ene-3β,27-diol 3-O-{[O-α-*L*-rhamnopyranosyl-(1→2)],[O-β-*D*-glucopyranosyl-(1→3)]-β-*D*-glucopyranoside}; and (25S)-spirost-5-ene-3β,27-diol 3-O-{[O-α-*L*-rhamnopyranosyl-(1→2)],[O-β-*D*-glucopyranosyl-(1→3)]-O-β-*D*-glucopyranoside} 27-[(S)-3-hydroxy-3-methylglutarate].*

The chemical structures of a number of steroid glycosides isolated from the bulbs of *Lilium regale* Wills have been reported previously [1]. In the present paper we give proofs of the structures of six steroid glycosides obtained from bulbs of *L. regale* growing on the territory of the Republic of Moldova, which we have called lilioglycosides B (**1**), C (**2**), E (**3**), F (**4**), H (**5**), and I (**6**).

An ethanolic extract of the fresh lily bulbs was evaporated to an aqueous residue, from which the total steroid glycosides were extracted with *n*-butanol. By repeated chromatography on a column of silica gel we obtained six steroid glycosides in the individual state.

Lilioglycosides (**3**) and (**4**) were identified as deacylbrownioside and brownioside, which have been isolated from *Lilium brownii* [2, 5]. According to IR spectroscopy (920 < 900 cm^{-1}), lilioglycosides C and I belonged to the spirostanol series with the (25R) configuration, and lilioglycosides B and H to the (25S)-spirostanol series. On complete acid hydrolysis, lilioglycoside B formed an aglycon, identified as narthogenin, and *D*-glucose, as was established chromatographically.

The full structure of lilioglycoside B was determined by ^{13}C NMR spectroscopy (Table 1). When the ^{13}C NMR spectra of lilioglycoside B and diosgenin [3] were compared it became obvious from the downfield shift of the signal that there was a hydroxy group in position 27 of the genin moiety of glycoside B, while the C-3 atom experienced a glycosylation effect and resonated at δ 76.6 ppm. By the NMR-spectroscopic method for detecting NOEs in the two-dimensional variant we also detected a correlation peak of the anomeric proton of β-*D*-glucopyranose with the H-3 proton of the aglycon, which showed the position of the glycosidic bond.

The ^{13}C NMR spectrum of glycoside (**1**), containing the signals of one *D*-glucose residue, determined the glycoside as a monoside. The SSCC (7.2 Hz) of the anomeric proton, H-1, and the chemical shifts of the carbon atoms of the monosaccharide residue confirmed the β-configuration, the C1 conformation, and the pyranose form of the *D*-glucose residue.

Consequently, the chemical structure of the new steroid glycoside lilioglycoside B is described by the formula 3-O-β-D-glucopyranosyl (25S)-spirost-5-ene-3β,27-diol.

The IR spectrum of lilioglycoside C revealed a band at 1725 cm^{-1}, characteristic for a carbonyl group. Analysis of the products of the complete acid hydrolysis of glycoside (**2**) permitted the identification of *D*-glucose and of narthogenin as

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TABLE 1. Details (δ, ppm) of the ^{13}C NMR Spectra of Lilioglycosides B (1), C (2), H (5), and I (6) (Py-d_5, 0 — TMS)

C atom	Chemical shift δ, ppm			
	1	2	5	6
1	37.9	37.9	37.7	37.7
2	31.8	31.8	30.4	30.4
3	76.6	76.6	78.8	78.7
4	40.8	40.8	39.0	39.0
5	142.1	142.1	141.0	140.9
6	121.2	121.2	122.0	122.0
7	37.6	32.6	32.5	32.5
8	32.4	32.2	32.0	32.0
9	50.5	50.4	50.5	50.5
10	37.1	37.1	37.4	37.4
11	21.3	21.3	21.4	21.4
12	40.1	40.6	40.1	40.1
13	40.6	40.6	40.7	40.7
14	56.8	56.8	56.8	56.9
15	32.3	32.3	32.5	32.6
16	81.4	81.3	81.1	81.4
17	63.0	63.0	63.0	63.0
18	16.6	16.5	16.6	16.6
19	19.7	19.7	19.7	19.71
20	42.2	42.2	42.2	42.2
21	15.2	15.2	15.3	15.3
22	109.9	109.6	109.7	109.6
23	31.6	31.1	31.6	31.4
24	24.2	23.9	24.2	24.0
25	39.2	35.8	39.0	35.8
26	64.5	63.1	64.0	63.3
27	64.2	66.4	64.2	66.3
Glc-1				
1	103.1	103.9	100.1	100.1
2	75.4	75.4	79.7	78.9
3	78.3	78.3	89.2	89.5
4	72.0	72.3	70.2	69.8
5	78.4	78.0	77.8	77.9
6	62.9	62.9	62.6	62.7
Rha-1				
1			102.2	102.4
2			72.7	72.2
3			73.0	73.0
4			74.1	74.3
5			69.6	69.8
6			18.7	19.0
Glc-2				
1			104.6	104.7
2			75.0	75.2
3			77.1	77.3
4			71.8	71.7
5			77.8	71.9
6			62.6	62.7
HMG				
1		171.7		171.7
2		46.6		46.7
3		70.7		70.7
4		46.7		46.7
5		171.9		174.9
6		28.7		28.7

the aglycon. The alkaline cleavage of lilioglycoside C led to the formation of glycoside (1), as the deacyl derivative, and 3-hydroxy-3-methylglutaric acid (HMG), these products being identified by TLC in the presence of authentic specimens.

The mass spectrum of glycoside (2) also showed the presence of the acid HMG as a substituent in the aglycon moiety according to the peak of the quasimolecular ion $[M + Na]^+$, m/z 760, and the peak of a protonated ion with a mass of 147.

The ^{13}C NMR spectroscopy of lilioglycoside C revealed a number of signals of carbon atoms not characteristic for monosaccharide residues: δ 28.7 (carbon atom of a methyl group), 46.6, 46.7 (carbon atoms of two methylene groups), 174.9 (carbon atom of a carboxy group), 171.7 (carbon atom of an ester group), and 70.1 ppm (tertiary carbinol carbon atom). In ^1H NMR, the methylene protons of this substituent resonated at δ 3.12 and 3.18 ppm, and the protons of the methyl group at 1.78 ppm. The position of the ester group was determined by comparing the ^{13}C NMR spectra of lilioglycosides C and B. A 2.2-ppm downfield shift of the C-27 carbon atom (δ 66.4 ppm) of lilioglycoside C and a 3.4-ppm diamagnetic shift of C-25 (δ 35.8 ppm) showed the position of the HMG at C-27 of the genin moiety.

According to the facts given above, lilioglycoside C is an acylated monoside and is described by the formula (25R)-spirost-5-ene-3β,27-diol 3-O-β-*D*-glucopyranoside 27-(3-hydroxy-3-methylglutarate).

In the products of the complete acid hydrolysis of lilioglycoside H we identified from their physicochemical constants and ^{13}C and ^{1}H NMR spectra not only narthogenin [2, 5] but also *D*-glucose and *L*-rhamnose.

The sequence of monosaccharide residues in the carbohydrate chain was established after the partial acid hydrolysis of (5), which led to the formation of (1) and a progenin identical with deacylbrownioside (lilioglycoside E) according to TLC and physicochemical constants.

The complete structure of lilioglycoside H was shown by ^{13}C and ^{1}H NMR spectroscopies. A comparison of the ^{13}C NMR spectra of glycoside H and isonarthogenin, obtained after the acid hydrolysis of (5), showed a paramagnetic shift of the C-3 signal of the genin part of the lilioglycoside due to a glycosylation effect. In the region of anomeric atoms in the spectrum there were three signals of glycosidic carbon atoms — δ 100.1, 102.2, and 104.4 ppm — showing the composition of the oligosaccharide. The configurations of the glycosidic centers were determined from the values of their SSCCs (7.4, 7.2, and 1.8 Hz), according to which the glucose residues had the β-configuration, and the rhamnopyranose the α-configuration.

The ^{1}H NMR spectrum was interpreted by the COSY method of two-dimensional correlation spectroscopy and by NOE spectroscopy. The 1Rha*p*/2Glc*p*-1 and 1Glc*p*-2/3Glc*p*-1 correlation peaks in NOESY showed branching in the C-2 and C-3 positions of the substituted glucose residue (Glc*p*-1).

Thus, lilioglycoside H is (25S)-spirost-5-ene-3β,27-diol 3-O-{[O-α-*L*-rhamnopyranosyl-(1→2)]-[O-β-*D*-glucopyranosyl-(1→3)]-β-*D*-glucopyranoside}.

Lilioglycoside I was the most polar spirostanol glycoside, and its IR spectrum contained a band at 1730 cm^{-1}.

The alkaline hydrolysis of (6) took place with the formation of a deacyl derivative identical with glycoside (5) and HMG, according to TLC and physicochemical constants.

Complete acid hydrolysis of (6) led to the formation of the aglycon narthogenin and two monosaccharides — *D*-glucose and *L*-rhamnose, according to paper chromatography in the presence of authentic specimens.

We determined the positions of the ester bond (C-27) and the carbohydrate chain (C-3) in lilioglycoside I by NMR spectroscopy. The SSCC values (7.2, 7.4, and 2 Hz) showed the β-configuration of the glycosidic bonds of the glucopyranoses and the α-configuration of the rhamnopyranose glycosidic center.

Lilioglycoside I was subjected to alkaline methanolysis, which led to the formation of a deacyl derivative identical with glycoside (5), and the monomethyl ester of HMG, for which we recorded the ^{1}H NMR spectrum and compared the results with those in the literature. According to its physicochemical constants and ^{1}H NMR spectrum, the monomethyl ester of HMG was identical with monomethyl (S)-3-hydroxy-3-methylglutarate (7) [4-6].

$$R{-}O{-}\underset{\|}{\overset{}{C}}{-}CH_2{-}C(OH)(CH_3){-}CH_2{-}\underset{\|}{C}{-}OH \xrightarrow[MeOH]{MeONa} R{-}OH + HO{-}\underset{\|}{C}{-}CH_2{-}C(OH)(CH_3){-}CH_2{-}\underset{\|}{C}{-}OCH_3$$

6 7

R- [illegible]*

On the basis of the facts given above, lilioglycoside I was ascribed the formula (25R)-spirost-5-ene-3β,27-diol 3-O-{[O-α-*L*-rhamnopyranosyl-(1→2)]-[O-β-*D*-glucopyranosyl-(1→3)]-β-*D*-glucopyranoside} 27-[(S)-3-hydroxy-3-methylglutarate].

1: R = β-D-Glcp, R' = H
2: R = β-D-Glcp, R' = AI A
5: R = β-D-Glcp ←(2)— α-L-Rhap, R' = H; ↑(3) β-D-Glcp
6: R = β-D-Glcp ←(2)— α-L-Rhap, R' = AI A; ↑(3) β-D-Glcp

*Illegible, as in Russian original — Translator.

EXPERIMENTAL

General Observations. Thin-layer chromatography (TLC) was conducted on Silufol UV-254 plates and plates with silica gel 5/40 μm + 13% of gypsum. For the identification of the aglycon we used silica gel 5/40 μm impregnated with 2% of silver nitrate. Revealing agents: concentrated sulfuric acid and the Sannié and Ehrlich reagents. Chromatographic solvent systems: 1) chloroform–methanol (9:1); 2) chloroform–methanol (4:1); 3) chloroform–methanol (7:3); 4) chloroform–methanol–water (65:35:5) (lower layer); 5) benzene–diethyl ether (7:3); 6) benzene–diethyl ether (4:1).

Column chromatography was conducted on silica gel L 100/250, 100/400, 100/160, 40, and 100 in the above-mentioned solvent systems. For paper chromatography we used solvent system 7) butan-1-ol–benzene–pyridine–water (5:1:3:1) with aniline phthalate as the spot reagent.

Mass spectra were taken on a Sel'my 252-Cf MSBX instrument, IR spectra on an IR-20 instrument (tablets with KBr). ^{13}C and ^{1}H NMR spectra were taken on Bruker WM-250 and Bruker AM-400 instruments (δ, ppm, 0 — TMS). COSY spectra were obtained by the Bruker standard method.

Isolation and Individualization of the Lilioglycosides. Fresh *Lilium regale* W. bulbs (2 kg) were comminuted, defatted with chloroform (3 × 3 liters), and extracted with 70% aqueous ethanol (4 × 3 liters) with heating. The ethanol extracts were combined and evaporated to an aqueous residue. The latter was diluted with water and treated with 200-ml portions of *n*-butanol, and the butanol extracts were evaporated to dryness. For the qualitative evaluation of the extracts the total butanol extract was subjected to TLC in solvent systems 1, 3, and 4.

The butanol extract after evaporation to dryness was dissolved in the minimum amount of methanol and the total glycosides were precipitated with acetone. The residue was filtered off and dried in vacuum. The total steroid glycosides so obtained were chromatographed repeatedly on columns of silica gel with the successive use of systems 1, 2, 3, and 4 as eluents.

As a result, the following lilioglycosides were isolated in the individual state: B (31.2 mg), C (30.6 mg), E (52.4 mg), F (52.4 mg), H (52.1 mg), and I (122.7 mg).

Lilioglycoside B (1) — $C_{33}H_{59}O_9$, mp 258-264°, $[\alpha]_D^{20}$ −110° (*c* 0.25; CH_3OH), IR spectrum (KBr, ν, cm^{-1}): 3500, 3300, 1180, 980, 921 < 900, 860. For the ^{13}C NMR spectrum, see Table 1.

Complete Acid Hydrolysis of Lilioglycoside B. A solution of 10 mg of lilioglycoside B in 4 ml of 1 N HCl in H_2O–dioxane (1:1) was heated on the water bath for 2 h. The course of the reaction was monitored every 30 min by TLC in systems 1 and 2 in the presence of markers. After the end of the reaction, the mixture was neutralized with 2 M NaOH and evaporated to dryness. The residue was dissolved in distilled water, and the solution was treated with chloroform (3 × 4 ml). The chloroform fractions were combined and evaporated, and the residue was chromatographed on Silufol plates and plates of silica gel impregnated with 2% of $AgNO_3$, in solvent systems 1 and 5. Narthogenin was identified in the presence of markers.

The aqueous hydrolysate was evaporated to a syrupy residue, which was chromatographed on paper in system 7, revealing the presence of *D*-glucose.

Lilioglycoside C (2) — $C_{39}H_{60}O_{13}$, mp 201-208°, $[\alpha]_D^{20}$ −87° (*c* 0.25; CH_3OH), IR spectrum (KBr, ν, cm^{-1}): 3420, 2920, 2860, 1720, 1440, 921, 901, 835. Mass spectrum, *m/z*: 760 [M + Na], 147 [ΓΜΓ + 2H]$^+$; for ^{13}C NMR, see Table 1.

The complete acid hydrolysis of glycoside (2) took place with the formation of narthogenin and *D*-glucose.

Alkaline Hydrolysis of Lilioglycoside C. Glycoside (2) (10 mg) was treated with 10 ml of 2 N NaOH at 50°C for 3 h. The reaction mixture was neutralized with Amberlite IR-120B ion-exchange resin, which was then filtered off and washed with methanol. In the combined fractions, after evaporation to dryness, glycoside (1) was identified by TLC in systems 1, 2, and 3 in the presence of markers.

For the chromatography of 3-hydroxy-3-methylglutaric acid we used as the solvent system ethanol–water–ammonia (1:2:1) and as revealing agent a 0.05% solution of Bromocresol [sic] (R_f 0.28).

Lilioglycoside H. — $C_{45}H_{72}O_{19}$, mp 272-276°, $[\alpha]_D^{20}$ −69 (*c* 1.0; Py). IR spectrum (KBr, ν, cm^{-1}): 3410, 2930, 2895, 1440, 990, 975, 955, 905. For ^{13}C NMR, see Table 1.

Complete Acid Hydrolysis of Lilioglycoside H. The hydrolysis of glycoside (5) (10 mg) was conducted by the procedure used for (1). In the chloroform fractions evaporated and purified on plates coated with SiO_2 impregnated with 2% of $AgNO_3$ we identified narthogenin in system 6. *D*-Glucose and *L*-rhamnose were detected in the aqueous hydrolysate.

Partial Acid Hydrolysis of Glycoside (5). A solution of 25 mg of substance (5) in 20 ml of 0.2 M HCl in dioxane–water (1:1) was heated on the water bath for 30 min, the course of the reaction being monitored by TLC in systems

1, 2, and 3. As a result, two progenins were obtained, which were identical with glycoside (**2**) (6.62 mg, mp 266°C, $[\alpha]_D^{20}$ $-108°$ (MeOH)) and lilioglycoside E (12.1 mg, mp 238°C, $[\alpha]_D^{20}$ $-90°$(MeOH).

Lilioglycoside I (6). — $C_{51}H_{80}O_{23}$, mp 230-236°, $[\alpha]_D^{20}$ $-53°$ (*c* 0.5; MeOH). IR spectrum (KBr, ν, cm^{-1}): 3420, 2940, 1725, 1445, 1245, 955 < 835, 805. For the ^{13}C NMR spectrum, see Table 1.

Complete Acid Hydrolysis of Glycoside (6). Glycoside (**6**) (20 mg) was subjected to acid hydrolysis by the procedure given above for lilioglycoside B. The aglycon was purified by column chromatography in systems 5 and 6. This gave 3.6 g of narthogenin 238-242°, $[\alpha]_D^{20}$ $-112°$ (*c* 0.25; $CHCl_3$)), M *m/z* 430. IR spectrum (KBr, ν, cm^{-1}): 3520, 3250, 2850, 915, 895, 860. ^{13}C NMR spectrum (δ, ppm): C-1(37.1), C-2(36.2), C-3(71.3), C-4(43.5), C-5(142.0), C-6(121.0), C-7(32.6), C-8(31.9), C-9(50.3), C-10(37.1), C-11(21.2), C-12(40.0), C-13(40.5), C-14(56.8), C-15(32.2), C-16(81.2), C-17(63.0), C-18(16.4), C-19(19.6), C-20(42.1), C-21(15.1), C-22(109.7), C-23(31.6), C-24(24.1), C-25(39.2), C-26(64.4), C-27(64.1).

Alkaline Hydrolysis of Lilioglycoside I. A solution of 60 mg of substance (**6**) in 20 ml of 3% sodium methanolate in methanol was kept at room temperature for 1 h. It was then passed through a column of cation-exchange resin, which was washed with methanol. The eluates were combined and evaporated to dryness in vacuum. The residue was chromatographed on a column of Sephadex LH-20, which was washed with methanol and then with the chloroform–methanol–25% ammonia (100:25:2) system. TLC in system 3 revealed a deacetyl derivative identical with glycoside (**5**) (180 mg, mp 275°C, $[\alpha]_D^{20}$ $-68°$ (*c* 0.25; MeOH)).

The monomethyl ester of HMG was identified from its physicochemical constants. R_f 0.49 on TLC in the chloroform–methanol–25% ammonia (7:4:1) system; $[\alpha]_D^{20}$ $+3.2°$ (*c* 0.25; $CHCl_3$). IR spectrum (KBr, ν, cm^{-1}): 3452 (–OH); 2954, 2856, 1716 (>C=O), 1430, 1373, 1260, 1204, 1116, 1092, 1013, 979, 895, 802. ^1H NMR ($CDCl_3$): 3.73 (OMe), 2.74 and 2.67 (for 2H-2 or 2H-4, J = 13.9 Hz), 2.72 and 2.65 ($CDCl_3$): for 2H-2 or 2H-4, J = 13.9 Hz, 1.41 ppm (CH_3–).

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