STEROIDAL SAPONINS FROM *Lilium candidum* L.

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(25*S*)-3β-{β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyloxy}spirost-5-en-27-ol and (25*R*,26*R*)-3β-{β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyloxy}-26-methoxyspirost-5-ene were isolated from the ethanolic extract of fresh bulbs and petals of *Lilium candidum* L. Their structures were derived mainly from NMR and mass spectra.

Key words: Steroids; Glycosides; *Lilium candidum* L.; *Liliaceae*; Steroidal saponins; Spirostanol saponins.

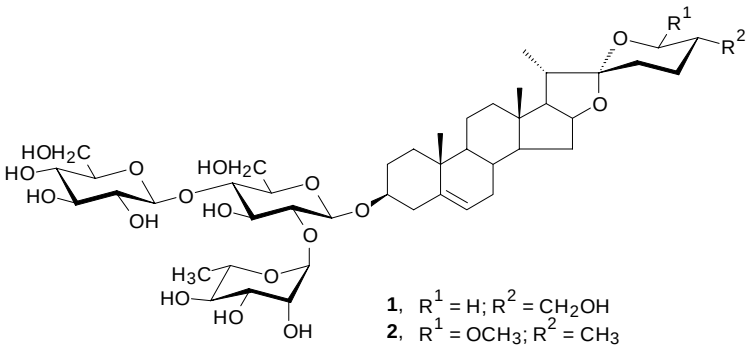
Liliaceae family is well known by occurrence of natural compounds with steroidal character. Steroids and their glycosides are widespread in the genera *Veratrum* L., *Convallaria* L., *Fritillaria* L., and its occurrence was confirmed also in genus *Lilium* L. Steroids of the spirostane and furostane series and their glycosides were isolated from the bulbs of *L. brownii* var. *colchesteri*[1], *L. dauricum*[2], *L. longiflorum*[3], *L. martagon*[4] and others. In our paper we describe the isolation of two steroidal saponins from *Lilium candidum* L. The first one was isolated from the bulbs and the second one from the petals of this plant. Their structures were derived mainly from NMR and mass spectra.

EXPERIMENTAL

The melting points were measured on a Kofler micro hot-stage. IR spectra were recorded on Perkin–Elmer 477 and Impact 400D (Nicolet) spectrophotometers in KBr discs. Optical rotations were measured with a Polamate A (Carl Zeiss, Jena) polarimeter. Mass spectra were measured on Zab-EQ instrument (Micromass, Manchester, U.K.) using fast atom bombardment (FAB) with a glycerol matrix or magic bullet matrix. NMR spectra were recorded on FT NMR spectrometer Varian UNITY-500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz) in CD_3OD and C_5D_5N solutions. For column chromatography silica gel (Silpearl, Kavalier Votice) was used. Thin-layer chromatography (TLC) was carried out on UV 254 or 366 plates and silica gel 60 F_{254} glass plates (Merck).

Extraction and Isolation of Steroidal Saponins **1** and **2**

Compound **1**: Fresh bulbs of *Lilium candidum* L. (20 kg) were extracted with ethanol. Ethanolic extract was evaporated under reduced pressure to yield a crude extract, which was dissolved in 5% HCl and partitioned with light petroleum, ether, chloroform and with chloroform and chloroform–ethanol (2 : 1) (after alkalinization with KOH + $NaHCO_3$, 1 : 1). Chloroform–ethanolic fraction (3.2 g) was subjected to column chromatography over silica gel using chloroform–MeOH mixtures of eluents increasing polarity. A total of 169 fractions (150 ml) were collected and analysed by TLC. Fractions 102–106 afforded 100 mg of **1**, m.p. 290–293 °C ($CHCl_3$–MeOH), $[\alpha]_D$ –88° (*c* 0.25, methanol). Literature[1] gives m.p. 280–285 °C (dec.), $[\alpha]_D$ –82.5° (*c* 0.08). IR spectrum (KBr): 3 410, 2 940, 2 860, 1 635, 1 452, 1 380, 1 250, 1 165, 1 060, 1 040, 978, 960, 920, 875, 840, 820, 805 cm^{-1}. FAB mass spectrum, *m/z* (%): 901 (17) $[M + H]^+$, 755 (5) $[M + H - Rha]^+$, 739 (5) $[M + H - Glc]^+$, 593 (8) $[M + H - Rha - Glc]^+$, 431 (82) $[M + H - Rha - Glc - Glc]^+$ = $[Aglycone + H]^+$, 413 (100) $[Aglycone + H - H_2O]^+$. For ^1H NMR and ^{13}C NMR see Table I.



Compound **2**: Fresh flowers of *Lilium candidum* L. (1.71 kg) were extracted with 70% ethanol. Ethanolic extract was evaporated under reduced pressure and partitioned between butanol and water (1 : 1). The organic layer was evaporated under reduced pressure and the residue (64.82 g) was separated on silica gel column, using $CHCl_3$–MeOH mixtures as eluents of increasing polarity. A total of 202 fractions (150 ml) were collected. Fractions 32–34 gave 20.2 mg of **2**, m.p. 267–272 °C (chloroform–methanol), $[\alpha]_D$ –72° (*c* 0.25, methanol). Literature[2] gives for amorphous powder $[\alpha]_D$ –66.0° (*c* 0.3, methanol). IR spectrum (KBr): 3 446, 2 931, 2 850, 1 456, 1 378, 1 246, 1 170, 1 062, 1 043, 984, 914 cm^{-1}. FAB mass spectrum, *m/z* (%): 937 (11) $[M + Na]^+$, 915 (5) $[M + H]^+$, 897 (6) $[M + H - H_2O]^+$, 883 (37) $[M + H - CH_3OH]^+$, 825 (8), 737 (9) $[M + H - CH_3OH - Rha]^+$, 721 (5) $[M + H - CH_3OH - Glc]^+$, 575 (4) $[M + H - CH_3OH - Rha - Glc]^+$, 567 (21), 459 (11), 445 (16) $[M + H - Rha - Glc - Glc]^+$ = $[Aglycone + H]^+$, 441 (19), 427 (34) $[Aglycone + H - H_2O]^+$, 413 (91) $[M + H - CH_3OH - Rha - Glc - Glc]^+$, 395 (100) $[M + H - H_2O - CH_3OH - Rha - Glc - Glc]^+$. For ^1H NMR and ^{13}C NMR spectrum see Table I.

RESULTS

Mass spectrum (FAB) of compound 1 shows a protonated molecular ion *m/z* 901 $[M + H]^+$ that undergoes fragmentation in two pathways. In the first case it gradually eliminates Glc, Glc + Rha and Glc + Rha + Glc to give a protonated aglycone at *m/z* 431 which

TABLE I
Carbon-13 and proton NMR data of saponins **1** and **2** from *Lilium candidum*

Position	^{13}C chemical shifts[a]			^{1}H chemical shifts (*coupling constants*)[b]		
	1 (CD_3OD)	**1** (C_5D_5N)	**2** (C_5D_5N)	**1** (CD_3OD)	**1** (C_5D_5N)	**2** (C_5D_5N)
			Aglycone			
1	41.43	37.66	37.62	1.88; 1.08	1.83; 1.00	[c]; 1.01
2	33.18	30.29	30.28	1.90; 1.60	2.11; 1.88	2.13; 1.88
3	78.62	78.44[d]	78.44	3.59	3.89	3.90 m
4	38.55	39.10	39.09	2.45; 2.30	2.79; 2.72	2.79; 2.73
5	141.89	141.02	141.05	–	–	–
6	122.65	121.92	121.92	5.38 m	5.33 m	5.35
7	30.76	32.46	32.49	2.00; 1.58	1.90; 1.52	1.93; 1.57
8	32.81	31.85	31.88	1.56	1.58	1.58
9	51.71	50.49	50.55	0.97	0.92 dt (*11; 11; 5.3*)	0.97
10	38.04	37.28	37.29	–	–	–
11	21.98	21.26	21.25	[c]	~1.47; ~1.42	[c]
12	39.55	40.03	39.96	[c]	1.72; 1.12	[c]
13	40.92	40.63	40.66	–	–	–
14	57.80	56.80	56.84	1.15	1.08	[c]
15	32.74	32.36	32.35	1.98; 1.29	2.05; 1.52	2.10; 1.48
16	80.98	81.32	81.56	4.41	4.58 ddd (*8.6; 7.8; 8.6*)	4.70 ddd (*6.4; 7.6; 8.2*)
17	63.75	63.09	63.10	1.76 dd (*6.6; 8.6*)	1.84 dd (*6.4; 8.6*)	1.87 dd (*8.5; 6.5*)
18	16.76	16.44	16.42	0.82 s	0.86 s	0.85 s
19	19.83	19.53	19.53	1.05 s	1.07 s	1.08 s
20	42.96	42.25	42.17	1.92	2.01 p (*6.8*)	2.00 p
21	14.86	15.13	15.11	0.97 d (*7.1*)	1.18 d (*6.8*)	1.16 d (*7.0*)
22	110.87	109.88	112.04	–	–	–
23	31.96	31.72	31.56	[c]	[c]	[c]
24	24.30	24.20	28.50	[c]	[c]	[c]
25	39.30	39.31	35.63	[c]	[c]	1.77
26	65.12[e]	64.57	103.32	3.65; 3.45	3.75 dd (*10.7; 5.4*) 3.68 dd (*10.7; 7.3*)	4.51 d (*8.2*)
27	64.42[e]	64.21	16.83	[c]	4.16; 3.91	0.98; d (*5.8*)
OCH_3	–	–	55.72	–	–	3.54 s

TABLE I
(*Continued*)

Position	^{13}C chemical shifts[a]			^{1}H chemical shifts (*coupling constants*)[b]		
	1 (CD_3OD)	**1** (C_5D_5N)	**2** (C_5D_5N)	**1** (CD_3OD)	**1** (C_5D_5N)	**2** (C_5D_5N)
			Glucose			
1	100.42	100.25	100.22	4.39 d (*7.8*)	4.96 d (*7.5*)	4.97 d (*7.4*)
2	77.92	77.83	77.83	3.20 dd (*7.8; 9.2*)	~4.22	4.22
3	76.25	76.32	76.34	3.36 t (*9.2; 9.2*)	~4.26	4.25
4	82.27	82.16	82.16	[c]	~4.21	4.22
5	77.82	77.59	77.58	[c]	3.87	3.87
6	62.48	62.32	62.31	[c]	4.53 dd (*12.2; 3.7*) 4.47 dd (*12.2; 2.6*)	4.53 dd (*12.2; 4.0*) 4.47 dd (*12.2; 2.5*)
			Rhamnose			
1	102.05	101.94	101.96	5.24 d (*1.7*)	6.24 d (*1.6*)	6.24 d (*1.5*)
2	72.23	72.54	72.54	3.89 dd (*1.7; 3.3*)	4.75 dd (*1.6; 3.6*)	4.75 dd (*1.5; 3.6*)
3	72.39	72.91	72.92	[c]	4.59 dd (*3.6; 9.4*)	4.59 dd (*3.6; 9.4*)
4	73.93	74.28	74.28	3.39	4.34 t (*9.4; 9.4*)	4.35 t (*9.4; 9.4*)
5	69.74	69.58	69.58	4.13 dq (*9.6;6.2*)	4.94 dq (*9.4; 6.2*)	4.94 dq (*9.4; 6.2*)
6	17.94	18.73	18.73	1.24 d (*6.2*)	1.78 d (*6.2*)	1.78 d (*6.2*)
			Glucose			
1	104.64	105.28	105.29	4.52 d (*7.8*)	5.14 d (*7.8*)	5.14 d (*7.8*)
2	75.07	75.12	75.13	3.42 dd (*7.8;9.0*)	4.07 t (*7.8; 7.8*)	4.07 t (*7.8; 7.8*)
3	79.38	78.60[d]	78.61	[c]	4.23	4.23
4	71.41	71.47	71.46	[c]	4.26	4.27
5	78.13	78.40[d]	78.36	[c]	3.99 ddd (*2.5; 5.4; 8.9*)	3.99 ddd (*2.5; 5.3; 8.8*)
6	61.91	62.12	62.12	[c]	4.48 dd (*11.6; 2.5*) 4.33 dd (*11.6; 5.4*)	4.48 dd (*11.8; 2.5*) 4.33 dd (*11.8; 5.3*)

[a] The assignment of carbon signals was done using our data for diosgenine and the literature data (refs[1,2]). [b] Proton signals were assigned using 2D-COSY spectra and the literature data (refs[1,2]). [c] Parameter could not be determined. [d,e] The assignment of signals may be mutually interchanged.

eliminates water. In the second pathway the protonated molecular ion gradually eliminates Rha, Rha + Glc and Rha + Glc + Glc to give again the protonated aglycone. These two fragmentation pathways indicate a branched saccharide chain with Rha and Glc as terminal saccharides.

The ^{1}H NMR spectrum in CD_3OD exhibited signals of three anomeric protons (doublets at δ 4.39, J = 7.8 Hz; 4.52, J = 7.8 Hz; 5.24, J = 1.7 Hz), an olefinic proton (δ 5.38 m), two angular methyl groups (singlets at δ 0.82 and 1.05) and two secondary methyl groups (doublets at δ 0.97, J = 7.1 Hz; 1.24, J = 6.2 Hz). The ^{13}C NMR spectrum showed 18 carbons of three hexoses and a total of 27 carbons arising from aglycone moiety.

Quaternary carbon signal at δ 110.87 and trisubstituted double bond at δ 141.89 and 122.65 indicated its Δ^5-spirostanol skeleton. Replacement of the C-27 methyl with a CH_2OH group was easily recognizable in the spectral data. Repeated NMR measurements in C_5D_5N allowed a direct comparison with literature data[1]. The compound **1** could be then ascribed the (25*S*)-3β-{β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyloxy}spirost-5-en-27-ol described earlier in *L. brownii* var. *colchesteri* and *L. longiflorum*[3].

Mass spectrum (FAB) of compound **2** is rather unusual. Intensities of molecular ions protonated by hydrogen and sodium, respectively, at *m/z* 915 and 937, respectively, such as fragment ions arising from them by elimination of saccharides are relatively very low. The main fragment ions arise by gradual elimination of the saccharides from the precursor at *m/z* 883 that arises by elimination of methanol from protonated molecular ion. The elimination pathways of saccharides are the same as in the case of compound **1** and again indicate the same branched saccharide chain. The protonated aglycone ions at *m/z* 445 form only 1/5 of the intensity of a peak at *m/z* 413 formed by protonated aglycone minus methanol. Both the protonated aglycone and the protonated aglycone minus methanol eliminate water as usual. The ^{1}H NMR and ^{13}C NMR spectra in C_5D_5N are very similar to those of compound **1**. While the saccharide parts are identical, the only significant differences in the aglycone moiety could be interpreted as substitution of CH_2OH group with methyl group at position 25 (signal at δ 16.83 in ^{13}C and doublet at δ 0.98 in ^{1}H NMR spectrum) and introduction of methoxy group into position 26 (OCH_3 signal at δ 55.72 in ^{13}C and singlet at δ 3.54 in ^{1}H NMR spectrum). These substitutions are accompanied with characteristic changes of chemical shifts of carbons 24, 25 and carbon and proton signals in position 26. A detailed comparison with the literature NMR data[2] showed **2** to be identical with (25*R*,26*R*)-3β-{β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyloxy}-26-methoxyspirost-5-ene (**2**) described earlier in *L. dauricum*[2] and *L. longiflorum*[3].

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