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SPIROSTANOL SAPONINS FROM THE BULBS OF *Lilium candidum*

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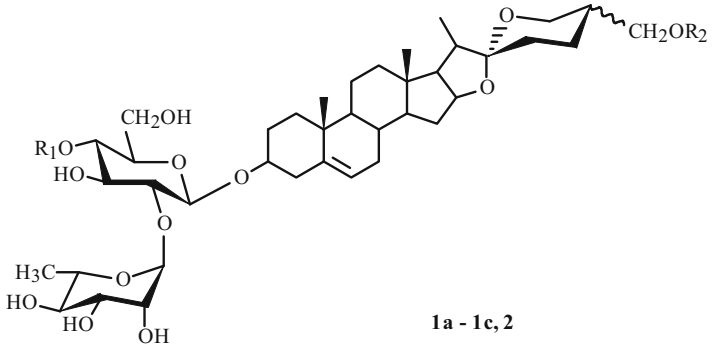
The genus *Lilium* L. contains about a hundred species and, according to Dostal [1], six of them grow in Middle Europe, including *Lilium candidum* L. White Lily belongs to the family of plants used in folk medicine because of its antiinflammatory effect. Several saponins [2, 3] have been isolated from the ethanolic extract of the bulbs and flowers of *Lilium candidum*. Now, we describe the isolation and identification of four steroidal saponins, three of which appear to be new in this plant.

Bulbs of *Lilium candidum* L. were extracted with EtOH and concentrated *in vacuo*. The crude ethanolic extract was dissolved in 1% H_2SO_4 and extracted with diethyl ether. The water portion after extraction was alkalized with NH_3 and extracted with $CHCl_3$. During extraction, a foam-like interphase was created. This interphase was chromatographed over silica gel with a mixture of $CHCl_3$ and MeOH to give compounds **1** and **2**. Additional HPLC separation of compound **1** (S5ODS2 column, mobile system MeOH–H_2O, gradient 30:70% MeOH/60 min, flow 0.6 mL/min, detection 210 nm) resulted in the isolation of compounds **1a**, **1b**, and **1c**.

The structures of the 25*R* (compound **1a**) and 25*S* (compound **1b**) isomers of 3β-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyloxy}spirost-5-en-27-ol were elucidated on the basis of spectroscopic analysis, including two-dimensional NMR spectroscopic techniques. The presence of the 25*R* isomer was described in *Lilium brownii* var. *colchesteri* [4], while the 25*S* isomer was isolated from *Lilium candidum* L. [5]. Their mutual presence and separation have not described in the genus *Lilium* until now. Compound **1c** represents the 3-hydroxy-3-methylglutaryl ester of the isolated 25*R* isomer, and compound **2** is the glucosidic derivative of compound **1c**. These compounds are known in several *Lilium* species [6, 7], but their presence in *Lilium candidum* L. is described for the first time.



1a - 1c, 2

1a: $R_1 = R_2$ = H, 25*R*; **1b:** $R_1 = R_2$ = H, 25*S*
1c: R_1 = H, R_2 = 3-hydroxy-3-methylglutaryl-
2: R_1 = Glc, R_2 = 3-hydroxy-3-methylglutaryl-

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Compound 1a. Positive ion mode: $[M + H]^+$ *m/z* 739.42681, calcd for $C_{39}H_{63}O_{13}$ 739.42632 (0.67 ppm); negative ion mode: $[M – H]^–$ *m/z* 737.41035, calcd for $C_{39}H_{61}O_{13}$ 737.41067 (–0.43 ppm).

^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.195 (1H, H-1α), 1.76 (1H, H-1β), 1.61 (1H, H-2a), 1.92 (1H, H-2b), 3.61 (1H, tt, H-3), 2.295 (1H, H-4a), 2.45 (1H, H-4b), 5.39 (1H, dt, H-6), 1.56 (1H, H-7a), 2.00 (1H, H-7b), 1.66 (1H, H-8), 0.97 (1H, H-9), ~1.56 (2H, m, H-11), 1.08 (1H, H-12α), 1.87 (1H, H-12β), 1.15 (1H, H-14), 1.295 (1H, H-15a), 1.99 (1H, H-15b), 4.42 (1H, ddd, H-16), 1.745 (1H, dd, H-17), 0.808 (3H, s, H-18), 1.050 (3H, s, H-19), 1.86 (1H, H-20), 0.972 (1H, d, J = 7, H-21), 1.38 (1H, H-23a), 1.78 (1H, H-23b), 1.69 (1H, H-24a), 1.96 (1H, H-24b), 1.60 (1H, H-25), 3.58 (1H, H-26a), 3.89 (1H, H-26b), 3.60 (1H, dd, H-27a), 3.75 (1H, dd, H-27b). Glc: 4.48 (1H, d, J = 7.8, H-1), 3.35 (1H, dd, H-2), 3.47 (H-1, t, H-3), 3.27 (1H, t, H-4), 3.24 (1H, H-5), 3.64 (1H, dd, H-6a), 3.845 (1H, dd, H-6b), Rha: 5.19 (1H, d, J = 1.8, H-1), 3.91 (1H, dd, H-2), 3.66 (1H, dd, H-3), 3.39 (1H, t, H-4), 4.14 (1H, dq, H-5), 1.240 (3H, d, H-6).

^{13}C NMR (125 MHz, CD_3OD): 41.40 (C-1), 30.73 (C-2), 79.15 (C-3), 39.48 (C-4), 141.88 (C-5), 122.62 (C-6), 33.17 (C-7), 32.79 (C-8), 51.68 (C-9), 38.03 (C-10), 21.96 (C-11), 38.54 (C-12), 40.89 (C-13), 57.77 (C-14), 32.72 (C-15), 82.42 (C-16), 63.50 (C-17), 16.76 (C-18), 19.84 (C-19), 43.38 (C-20), 15.76 (C-21), 111.05 (C-22), 27.56 (C-23), 21.81 (C-24), 36.50 (C-25), 61.31 (C-26), 62.24 (C-27), Glc: 100.46 (C-1), 79.05 (C-2), 79.35 (C-3), 71.79 (C-4), 77.72 (C-5), 62.78 (C-6), Rha: 102.19 (C-1), 72.20 (C-2), 72.35 (C-3), 73.90 (C-4), 69.76 (C-5), 17.96 (C-6).

Compound 1b. Positive ion mode: $[M + H]^+$ *m/z* 739.42618, calcd for $C_{39}H_{63}O_{13}$ 739.42632 (–0.19 ppm); negative ion mode: $[M – H]^–$ *m/z* 737.41025, calcd for $C_{39}H_{61}O_{13}$ 737.41067 (–0.57 ppm).

Small differences in ^{1}H NMR and ^{13}C NMR between compounds **1a** and **1b** revealed the 25*S* isomer of compound **1b**.

Compound 1c. Positive ion mode: $[M + H]^+$ *m/z* 883.46812, calcd for $C_{45}H_{71}O_{17}$ 883.46858 (–0.52 ppm); negative ion mode: $[M – H]^–$ *m/z* 881.45211, calcd for $C_{45}H_{69}O_{17}$ 881.45293 (–0.93 ppm).

The ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectra of compound **1c** are very similar to those of compound **1a**. The difference in MS between both compounds indicates the presence of the 3-hydroxy-3-methylglutarate moiety for compound **1c**, which was confirmed by NMR.

Compound 2. Mp 228–229°C. Positive ion mode: $[M + H]^+$ *m/z* 1045.52152, calcd for $C_{51}H_{81}O_{22}$ 1045.52140 (0.11 ppm); negative ion mode: $[M – H]^–$ *m/z* 1043.50456, calcd for $C_{51}H_{79}O_{22}$ 1043.50575 (–1.14 ppm). The ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectra of compound **2** are very similar to those of compound **1c**. The difference in MS between both compounds indicates one additional hexose in compound **2**, which was confirmed by NMR.

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