and terpinene-derivatives show analgetic and antirheumatic activities. Insecticidal effects are attributed to some mono- and sesquiterpenes, like terpinenes and cadinenes.

Experimental

1. Material and essential oil extraction

The roots were collected in Yaounde in March 1997. The species was identified by Dr. S. Yonkeu, Institute of Agricultural Research for Development Wakwa, Cameroon. A voucher specimen (no. W.149.) is deposited at this institute.

The essential oil was obtained by steam-distillation of 200 g of powdered roots for 6 h with a yield of 0.02% (v/w) oil.

2. GC/FID and GC/MS

For GC/FID analyses a Shimadzu GC-14A (FID) with the integrator C-R6A-Chromatopac (injector-temp.: 250 °C; detector-temp.: 320 °C; temp.-progr.: 40 °C/5 min. to 280 °C/5 min. with a rate of 6 °C/min.; carrier gas: hydrogen) and the columns: 30 m × 0.32 mm unpolar FSOT-RSL-200 (film thickness: 0.25 μm) and a 30 m × 0.32 mm polar Stabilwax (0.50 μm) was used. The retention times were partly correlated by co-injection of reference compounds and comparison with own data.

GC/MS analyses were done with a Shimadzu GC-17A and the QP5000 mass spectrometer (EI mode; 70 eV; range: 41–450 amu; interface-heating: 230 °C; ion source: 200 °C, carrier gas: helium). Other parameters see GC/FID part. The MS were correlated with NIST-, NBS- and Wiley or with own MS librariers (on-line).

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Steroid saponins from the petals of *Lilium candidum* L.

M. Haladová[1], E. Eisenreichová[1], P. Mučaji[1], M. Buděšínský[2] and K. Ubik[2]

A new steroid saponin (25*R*,26*R*)-3β-{β-D-glucopyranosyl-(1 → 4-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyloxy}spirost-5-en-26-ol (**1**) was isolated from the ethanolic extract of petals of *Lilium candidum* L., together with a known steroidal saponin (**2**) identified as (25*R*,26*R*)-3β-{β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyloxy}-26-methoxy-spirost-5-ene [1].

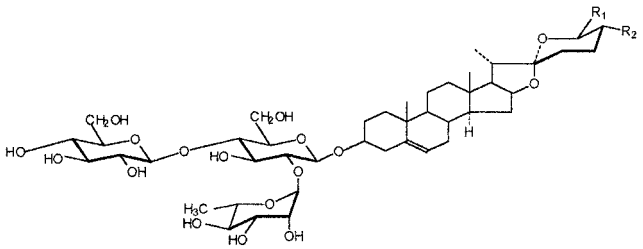
A standard FAB MS of compound **1** provided limited information only. A peak of protonated molecules at m/z 901 is only half in intensity of the peak at m/z 883 arising by the elimination of water. The peak of the protonated aglycon at m/z 431 is also low while ions arising by an elimination of water form the base peak at m/z 413 which is followed by a further elimination of water to give the peak at m/z 395. Peaks characterizing a saccharide chain are low and it is not possible to distinguish them from background peaks.

The daughter ion spectrum of the protonated molecule gives a clear information on a structure of the saccharide

Table: ^{13}C and ^{1}H NMR data of steroid saponin 1 from *Lilium candidum* L.

Position	^{13}C chemical shifts[a]		^{1}H chemical shifts (coupling constants)[b]	
	1 (C_5D_5N)	**1** (CD_3OD)	**1** (C_5D_5N)	**1** (CD_3OD)
Aglycone				
1	37.55	41.45	1.87;[c]	1.88; 1.08
2	30.20	33.20	2.11; 1.87	1.90; 1.61
3	78.33	78.65	3.88 m	3.59 tt (11.5; 4.5)
4	38.98	38.56	2.78 ddd; 2.73 vbt	2.45 ddd; 2.30 vbt
5	140.84	141.91	–	–
6	121.84	122.63	5.30	5.39 m
7	32.35	30.77	1.87; 1.42	2.01; 1.57
8	31.76	32.82	1.54	1.65
9	50.34	51.73	0.96	0.97
10	37.19	38.05	–	–
11	21.16	21.99	[c]	∼1.54 (2 H)
12	39.97	39.57	[c]	1.77; 1.20
13	40.52	40.93	–	–
14	56.73	57.86	1.02	1.15
15	32.24	32.75	1.97; 1.44	1.99; 1.29
16	81.27	81.02	4.63	4.53
17	62.99	63.76	1.87 dd (8.5; 6.5)	1.81
18	16.42	16.76	0.84 s	0.81 s
19	19.47	19.83	1.05 s	1.05 s
20	42.22	42.96	2.01 p	1.92 p
21	15.21	14.92	1.23 d (6.8)	1.01 d (7.0)
22	112.18	113.36	–	–
23	31.71	32.10	[c]	1.70; d
24	28.73	24.03	[c]	1.64; 1.50
25	37.80	38.01	1.74	1.35
26	96.63	97.08	4.40	4.59 b
27	17.58	17.15	1.16 d (6.0)	0.93 d (6.5)
Saccharide part				
Glc: 1′	100.05	100.43	4.97 d (7.4)	4.40 d (7.9)
2′	77.76	77.94	∼4.24	3.20 dd (7.9; 9.2)
3′	76.27	76.26	∼4.25	3.28
4′	81.96	82.40	∼4.25	3.39
5′	77.47	77.82	3.88	3.55
6′	62.17	62.50	4.55 dd (∼12; ∼3.5)	[c]
			4.48 dd (∼12; ∼2.5)	[c]
Rha: 1″	101.91	102.06	6.26 d (1.5)	5.24 d (1.7)
2″	72.49	72.25	4.77 dd (1.5; 3.5)	3.89 dd (1.7; 3.3)
3″	72.82	72.40	4.61 dd (3.5; ∼10)	3.66 dd (3.3; 9.6)
4″	74.14	73.95	4.37	3.39 t (9.6; 9.6)
5″	69.57	69.74	4.96	4.13 dq (9.6; 6.3)
6″	18.72	17.94	1.78 d (6.2)	1.24 d (6.3)
Glc: 1‴	105.20	104.66	5.16 d (8.0)	4.51 d (7.9)
2‴	75.06	75.08	4.08 t (8.0; 8.6)	3.42 dd (7.9; 9.1)
3‴	78.55	79.41	∼4.25	3.65 t (9.1; 8.9)
4‴	71.31	71.42	∼4.29	3.36 t (8.9; 9.0)
5‴	78.22	78.14	4.00 ddd(9.0;2.4;5.2)	3.34 m
6‴	61.90	61.94	4.48 dd (11.4; 2.4)	3.88 dd (12.0; 2.7)
			4.34 dd (11.4; 5.2)	3.83 dd (12.0; 4.0)

[a] The assignment of carbon signals was done using our data for diosgenine and the literature data [3]; [b] proton signals were assigned using 2D-COSY spectra and the literature data [3]; [c] the value of parameter could not be determined



Compound	R_1	R_2
1	OH	CH_3
2	OCH_3	CH_3

part of the molecule. The saccharide chain is branched, formed by two Glc and one Rha with Glc and Rha as terminal saccarides. The parent ion spectrum of the base peak at m/z 413 shows the protonated aglycon as a main precursor having a very low stability and eliminating water.

The ^{1}H NMR spectrum in CD_3OD exhibited signals of three anomeric protons (doublets at δ 4.40 (J = 7.9 Hz), 4.51 (J = 7.9 Hz) and 5.24 (J = 1.7 Hz)), an olefinic proton (δ 5.39 m), two angular methyl groups (singlets at δ 0.81 and 1.05) and three secondary methyl groups (doublets at δ 0.93 (J = 6.5 Hz), 1.01 (J = 7.0 Hz) and 1.24 (J = 6.3 Hz)). The ^{13}C NMR spectrum in CD_3OD showed 18 carbons of three hexoses and a total of 27 carbons arising from the aglycone moiety. A quaternary carbon signal at δ 113.36 and a trisubstituted double bond at δ 141.91 and 122.63 indicated its Δ^5-spirostanol skeleton. Repeated NMR measurements in d_5-pyridine allowed a direct comparison with literature data [2]. Comparison with NMR data of compound **2** [1] showed a very close similarity except of the absence of a methoxy group which is accompanied with significant chemical shift changes in positions 25, 26 and 27. The observed upfield shift of C-26 (−6.69 ppm) and downfield shifts of C-25 and C-27 (2.17 and 0.75 ppm, resp.) are compatible with the expected substitution of the methoxy group with hydroxyl in compund **1**. Compound **1** could then be described as the new steroidal saponin (25*R*,26*R*)-3β-{β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyloxy}spirost-5-en-26-ol.

Experimental

1. Apparatus

The m.p. was measured on a Kofler micro hot-stage. IR spectra were recorded on an Impact 400D (Nicolet) spectrophotometer in KBr discs. MS were measured on a ZAB-EQ instrument (Micromass, Manchester, U.K.) using fast atom bombardment (FAB) with a glycerol matrix and Xe at 8 kV as a bombarding gas. Daughter ion linked scans at B/E = const. and parent ion linked scans at B^2/E = const. were used to determine the sequence of saccharides and a molelular weight of the aglycon.

NMR spectra were recorded on a FT-NMR spectrometer Varian UNITY-500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz) in CD_3OD and d_5-pyridine.

For CC silica gel (Silpearl Kavalier Votice) was used. TLC was carried out on UV 254 or 366 plates and silica gel 60 F_{254} glass plates (Merck).

2. Plant material

Flowers of *Lilium candidum* L. were collected from the Podunajské Biskupice, Slovakia in 1994. A voucher specimen was deposited at the Pharmaceutical Faculty, Comenius University, Bratislava, Slovak Republic.

3. Extraction and isolation

Fresh petals of *Lilium candidum* L. (1.5 kg) were extracted with EtOH. The ethanolic extract was concentrated in vacuo and divided between n-BuOH and H_2O (1:1). The butanolic layer was concentrated in vacuo to give 56.4 g of residue. The butanolic fraction was chromatographed over silica gel (Silpearl Kavalier Votice) with a mixture of $CHCl_3$ and MeOH with increasing MeOH contents. A total of 250 fractions (150 ml) were collected.

Fractions 40–42 were combined and evaporated in vacuo and the residue was chromatographed over silica gel (20 g) with the same solvent system as for the previous fraction. A total of 140 fractions were collected. Fractions 62–75 yield compound **1** (12.5 mg), m.p. 279–281 °C. IR spectrum: ν_{max}^{KBr} cm^{-1}: 3410, 2940, 2855, 1455, 1378, 1248, 1170, 1060, 1043, 980, 920. Standard FAB MS: m/z (% rel. int.): 901 (27%) $[M+H]^+$, 883 (67) $[M+H-H_2O]^+$, 431 (22) $[Aglycon+H]^+$, 413 (100) $[Aglycon+H-H_2O]^+$, 395 (77) $[Aglycon+H-2H_2O]^+$. Daughter ion linked scan for precursor $[M+H]^+$ at m/z 901: 883 $[M+H-H_2O]$, 755 $[M+H-Rha]^+$, 739 $[M+H-Glc]^+$, 721 $[M+H-H_2O-Glc]^+$, 593 $[M+H-Glc-Rha]^+$, 575 $[M+H-H_2O-Glc-Rha]^+$, 431 $[M+H-Glc-Rha-Glc]^+$, 431 $[Aglycon+H]^+$, 413 $[Aglycon+H-H_2O]^+$. Parent ion scan for $[Aglycon+H-H_2O]^+$ at m/z 413: 431 $[Aglycon+H]^+$: 901 $[M+H]^+$ = 8:1. ^{1}H and ^{13}C NMR data: see Table.

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