Table 2: Number of colonies (CFU) of the tested *Yersinia* strains after treatment with Oxadin®

Test strain	Time (h)	Dilutions 1 : 2 (50)[1]	1 : 4 (25)	1 : 8 (12.5)	1 : 16 (6.25)	1 : 32 (3.125)	1 : 64 (1.56)	Control
	24	–	–	–	9 ± 2	236 ± 40	SC[2]	CG[3]
YP	48	–	–	–	–	164 ± 25	SC	CG
IP 2969	72	–	–	–	–	39 ± 14	SC	CG
	96	–	–	–	–	–	348 ± 74	CG
	24	–	–	–	2	24 ± 6	SC	CG
YE	48	–	–	–	–	8 ± 2	540 ± 60	CG
IP 8896	72	–	–	–	–	1	110 ± 21	CG
	96	–	–	–	–	–	32 ± 6	CG

Legend: YE – Yersinia enterocolitica
YP – Yersinia pseudotuberculosis;
[1] Quantity of Oxadin® (mg/ml) in 10% concentration
[2] Semi–confluent bacterial growth
[3] Confluent bacterial growth

tuberculosis IP 2969 containing 6.25 mg/ml Oxadin® (dilution 1 : 16), only nine colonies were detected. Later, after 48 h contact at a dilution of 1 : 32, 164 colonies were counted. The total inhibiting effect was proved after 96 h at the same dilution. When *Y. enterocolitica* IP 8896 was used as test strain, similar dynamics of the inhibitory effect was observed. The number of bacterial cells reported periodically at every 24 h at a dilution of 1 : 16 and 96 h at a dilution of 1 : 32 is equal to zero. The MIC determinations for tetracycline (4 μg/ml), chloramphenicol (8 μg/ml), gentamycin (4 μg/ml), and cotrimoxazol (0.2 μg/ml) are in agreement with those reported by other authors [12–14]. Serotype or strain specific patterns of susceptibility were not found, irrespective of the geographic and host origin of the strains used.

The data received allow the conclusion that Oxadin® has a well expressed inhibiting and bactericidal *in vitro* effect on both *Yersinia* pathogens, which are in accordance with the factors concentration and time of contact. Further experiments aiming to establish an antibacterial effect of Oxadin® *in vivo* are in progress in our laboratory.

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A new steroidal saponin from the bulbs of *Lilium candidum* L.

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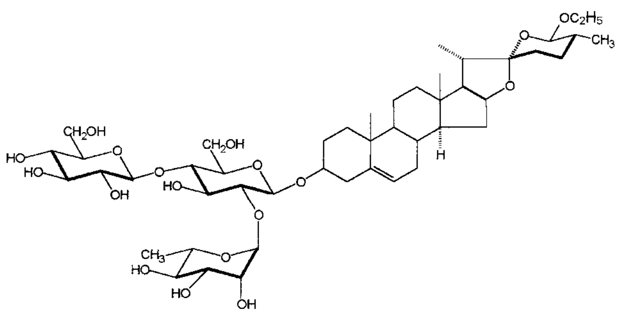
In preceding communications [1, 2], we reported the isolation of three steroidal saponins from *Lilium candidum* L. These glycosylated compounds contain two molecules of

Table: ^{13}C and ^{1}H NMR data of the new steroidal glycoside 1

Position	Carbon-13 chemical shifts (CD_3OD)	Proton chemical shifts (coupling constants) (CD_3OD) Aglycone
1	41.44	1.88; 1.07
2	33.18	1.90; 1.60
3	78.64	3.59 m
4	38.55	2.45 ddd; 2.30 bt
5	141.90	–
6	122.63	5.39 m
7	30.75	2.01; 1.58
8	32.80	1.57
9	51.70	0.97
10	38.04	–
11	21.97	c
12	39.55	c
13	40.90	–
14	57.84	c
15	32.77	2.00; 1.28
16	80.97	4.51 ddd (*6.7; 7.6; 8.4*)
17	63.90	1.80 dd (*6.5; 8.5*)
18	16.74	0.81 s
19	19.82	1.05 s
20	42.94	1.94 p
21	14.99	1.00 d (*6.8*)
22	113.16	–
23	31.94	c
24	28.98	c
25	36.28	1.41 m
26	103.23	4.36 d (*8.6*)
27	16.83	0.90 d (*6.6*)
26-OR	65.35	3.49 dq and 3.82 dq (*9.6; 7.1*)
	15.68	1.20 t (*7.1*)
		Saccharide part
Glc: 1′	100.41	4.40 d (*7.8*)
2′	77.92	3.20 dd (*7.8; 9.3*)
3′	76.25	3.36 t (*9.3*)
4′	82.54	c
5′	77.82	c
6′	62.48	c
Rha: 1″	102.06	5.24 d (*1.6*)
2″	72.24	3.89 dd (*1.6; 3.3*)
3″	72.39	3.66 dd (*3.3; 9.5*)
4″	73.93	3.39 t (*9.5*)
5″	69.74	4.13 dq (*9.5; 6.3*)
6″	17.94	1.24 d (*6.3*)
Glc: 1‴	104.64	4.52 d (*7.8*)
2‴	75.08	3.42 dd (*7.8; 9.0*)
3‴	79.38	3.65 t (*9.0*)
4‴	71.41	c
5‴	78.13	c
6‴	61.91	c

c the value of parameter could not be determined



compound **1**

glucose and one molecule of rhamnose in their saccharide chain. This paper deals with the isolation and structural elucidation of a new steroidal saponin (**1**) from the ethanolic extract of the bulbs of *Lilium candidum* L. The new compound was separated chromatographically and characterized by ^{1}H, ^{13}C NMR, and MS and identified as (25*R*, 26*R*)-3β-{β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyloxy}-26-ethoxy-spirost-5-ene.

Experimental

The m.p. was measured on a Kofler micro hot-stage.

1. Equipment

MS were recorded on 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 the molecular 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. 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

Bulbs of *Lilium candidum* L. were collected near Bratislava, Slovak Republic.

3. Extraction and isolation

Fresh bulbs of *Lilium candidum* L. (1.7 kg) were extracted with EtOH at room temperature. The ethanolic extract was concentrated *in vacuo* (89 g) and partitioned between n-BuOH and H_2O (1 : 1). The butanolic layer was concentrated *in vacuo* and chromatographed over silica gel (Silpearl Kavalier Votice) with a mixture of $CHCl_3$ and MeOH (9 : 1), with increasing MeOH contents. A total of 82 fractions (100 ml) were collected.
Fractions 35–37 were combined and evaporated *in vacuo* and the residue was chromatographed over silica gel with the same solvent system as for the previous fraction to give compound **1** (30 mg), m.p.: 206–208 C. Standard FAB MS: m/z (% rel.int.): 951 (81) $[M + Na]^+$, 929 (5) $[M + H]^+$, 883 (22) $[M + H-C_2H_5OH]^+$, 825 (9), 737 (5) $[M + H-C_2H_5OH-Rha]^+$, 721 (4) $[M + H-C_2H_5OH-Glc]^+$, 441 (10) $[Aglycon + H-H_2O]^+$, 413 (35) $[M + H-C_2H_5OH-Rha-Glc-Glc]^+$, 395 (72) $[M + H-H_2O-C_2H_5OH-Rha-Glc-Glc]^+$, 253 (100). For ^{1}H and ^{13}C NMR data see Table.

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Sesquiterpenoids from *Scorzonera hispanica* L.

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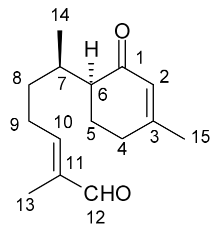
Scorzonera hispanica L. is a perennial herb, which is native to Southern Russia, the Ukraine, Kazakhstan, Eastern Central, South Eastern and South Western Europe [1]. In Central Europe it is widely cultivated as a vegetable and in former times it was also used in folk-medicine as a mucolytic [2]. In our continuing study of the phytochemistry of the Lactuceae tribe of the Asteraceae family we reinvestigated the constituents of *S. hispanica*. Prior studies led to the isolation and identification of 3,4-dimethoxy-cinnamic acid methyl ester, β-sitosterol, the lignan (3a*R*)-1*c*,4*c*-bis-4β-D-glucopyranosyloxy-3,5-dimethoxy-phenyl-(3ar,6ac)-tetrahydro-furo-3,4-*c*-furan as well as the sesquiterpenoid scorzoneroside [3–5].
Repeated CC and subsequent semi-preparative HPLC of methanol extracts of subaerial parts of *S. hispanica* yielded compounds **1–3**. The bisabolane derivative puliglutone (**1**) was identified on the basis of its ^{1}H NMR, ^{13}C NMR and HMBC spectra and in comparison with ^{1}H NMR data given in the literature [6]. This substance has been reported from the Asteraceae genera *Senecio*, *Oldenburgia* and *Pulicaria*, but up to now neither from the genus *Scorzonera* nor from any other genus of the Lactuceae [6–8]. As ^{13}C NMR data for compound **1** have not been published yet, they are given in the experimental section. Compound **2**, could be identified by ^{1}H NMR, ^{13}C NMR and HMBC experiments as ixerisoside D, which represents the 11,13-dehydro-derivative of scorzoneroside [9]. This substance has been isolated from *Ixeris repens*, an Asian species of the Lactuceae tribe, subtribe Crepidinae [9].
The ESIMS of compound **3** showed quasimolecular ion peaks at m/z 526 $[M + NH_4]^+$ and 509 $[M + H]^+$. HRFABMS established the molecular formula of $C_{26}H_{36}O_{10}$ showing a signal at m/z 509.2381 $[M + H]^+$

1



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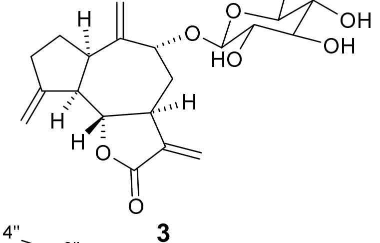

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