

**9g**, which has bulky non-polar groups together with polar amid groups, is inactive to all the tested fungi.

Amino acid derivatives of cinnamic acid, which is biosynthetically related to coumarins, have been synthesized and shown antifungal activity [9]. Their activity has been attributed to a better transport ability into the fungal cell. It has been discussed that an oligopeptide transporter system presents in fungal cell that helps transfer of molecules into the cell. This phenomenon has been described for *Bacillus subtilis* antifungal toxins as well [10]. Therefore, it was decided that the amino acid derivatives of angelicin be synthesized and tested for their antifungal activity. This group of compounds is totally inactive against *Candida*, however, contrary to many other tested compounds in this report, **8d** and **8f** show moderate activity against *Aspergillus*. Although an earlier work [11] suggested that an unprotected Leu-coumarin derivative could be the strongest antifungal in the coumarin series, the furanocoumarins containing amino acids here show a preferred activity while carrying a Phe group. The general MIC values of this group are not considered strong, however they show improvement compared to compound **1b**. This superior activity might be due to simple transformation of carboxyl group of **1b** to a more non-polar moiety. However, the more polar derivatives **8b**, **8d**, and **8f** exhibit improvement in their antifungal activity in many cases, and this may indicate the involvement of other factors.

## Experimental

### 1. Chemistry

The preparation of compounds **1–9** has been reported elsewhere [6].

### 2. Antifungal susceptibility test

The antifungal activity was measured based on the recommendations of NCCLS [12]. The compounds were dissolved in acetone and diluted in a twofold manner in RPMI 1640 (pH = 7.0) in 96 microwell plates. The MIC was the minimum concentration of the agent that shows a full inhibition of the fungal growth in the well, examined by naked eyes.

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Soroush Sardari, Pharm. D., Ph. D.  
Dept. Chemistry  
110 Science Place  
University of Saskatchewan  
Saskatoon, SK  
Canada S7N 5C9  
sardari@sask.usask.ca  
ssardari@hotmail.com

Institute of Pharmaceutical Chemistry<sup>1</sup>, University of Vienna, Austria, and Department of Applied Chemistry<sup>2</sup>, University of Ngaoundere, Cameroon

## Essential oil volatiles of the roots of *Dorstenia psilurus* from Cameroon

L. JIROVETZ<sup>1</sup>, G. BUCHBAUER<sup>1</sup>, W. FLEISCHACKER<sup>1</sup>  
and M. NGASSOUM<sup>2</sup>

*Dorstenia psilurus* (Moraceae) is a small herb reaching 80 cm in height. It grows in the tropical rain forest zone in West, East and Central Africa, whereas it is used especially in traditional medicine: a decoction of leaves and roots is used to treat rheumatism, snake-bites, headache and stomach disorders [1], but no data on the essential oil volatiles of the roots of this *Dorstenia* species were given until now.

The essential oil was olfactorically evaluated by professional perfumers and the odor described as follows: spicy, terpinene- and pinene-like with fresh (limonene) side notes.

Using GC/FID and GC/MS in combination with olfactoric methods 24 components could be identified (see Table; unknown 1.8%).

**Table: Essential oil volatiles of the roots of *Dorstenia psilurus* from Cameroon**

Compound <sup>a</sup>	% <sup>b</sup>	RI <sup>c</sup>
1-Hexen-3-ol	0.3	773
$\alpha$ -Pinene	8.3	941
Camphene	1.1	953
1-Octen-3-ol	0.8	970
Sabinene	15.1	974
$\beta$ -Pinene	7.5	980
para-Cymene	5.5	1012
Limonene	5.2	1023
cis- $\beta$ -Ocimene	1.3	1028
$\beta$ -Phellandrene	5.9	1031
$\gamma$ -Terpinene	11.3	1055
Terpinolene	9.7	1073
Nonanal	0.4	1082
Camphor	0.6	1121
Terpinen-4-ol	1.2	1157
$\alpha$ -Terpineol	0.8	1163
$\beta$ -Cubebene	2.1	1382
trans- $\beta$ -Bergamotene	0.3	1422
$\beta$ -Caryophyllene	4.2	1426
$\beta$ -Gurjunene	6.5	1430
trans- $\beta$ -Farnesene	1.0	1445
$\alpha$ -Humulene	3.8	1452
$\delta$ -Cadinene	5.3	1502

<sup>a</sup> in order of their retention times

<sup>b</sup> calculated as %-peak area of GC/FID analysis

<sup>c</sup> retention indices on unpolar column

As main compounds especially monoterpenes, sesquiterpenes and minor aliphatic derivatives were found. The identified terpinene- and pinene-derivatives as well as limonene are responsible for the characteristic odor of this essential oil.

In correlation to published data [2–8] on the bioactivity of volatiles, monoterpene hydrocarbons (like pinenes and terpinenes) show spasmogenic (on the ileum of pigs) and stimulating (found by special brain wave pattern changes) effects. Antiseptic, bactericidal and fungicidal activity can be attributed to mono- (e.g. pinenes and terpinenes) and sesquiterpenes (e.g. caryophyllene). Camphene-, pinene-

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.-prog.: 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 libraries (on-line).

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Dr. Leopold Jirovetz  
Institute of Pharmaceutical Chemistry  
Althanstraße 14  
1090 Vienna  
Austria  
jirovetz@speedy.pch.univie.ac.at

Department of Pharmacognosy and Botany<sup>1</sup>, Pharmaceutical Faculty, Comenius University, Bratislava, Slovak Republic, and Institute of Organic Chemistry and Biochemistry<sup>2</sup>, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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

M. HALADOVÁ<sup>1</sup>, E. EISENREICHOVÁ<sup>1</sup>, P. MUČAJI<sup>1</sup>,  
M. BUDĚŠÍNSKÝ<sup>2</sup> and K. UBIK<sup>2</sup>

A new steroid saponin (25R,26R)-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 (25R,26R)-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 from 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:** <sup>13</sup>C and <sup>1</sup>H NMR data of steroid saponin **1** from *Lilium candidum* L.

Position	<sup>13</sup> C chemical shifts <sup>a</sup>		<sup>1</sup> H chemical shifts (coupling constants) <sup>b</sup>	
	<b>1</b> (C <sub>5</sub> D <sub>5</sub> N)	<b>1</b> (CD <sub>3</sub> OD)	<b>1</b> (C <sub>5</sub> D <sub>5</sub> N)	<b>1</b> (CD <sub>3</sub> OD)
Aglycone				
1	37.55	41.45	1.87; <sup>c</sup>	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	<sup>c</sup>	~1.54 (2 H)
12	39.97	39.57	<sup>c</sup>	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	<sup>c</sup>	1.70; d
24	28.73	24.03	<sup>c</sup>	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) <sup>c</sup>	
			4.48 dd (~12; ~2.5) <sup>c</sup>	
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)

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