

# Chemical Composition and Biological Activity of the Essential Oils of *Senecio aegyptius* var. *discoideus* Boiss.

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Z. Naturforsch. **57c**, 434–439 (2002); received January 9/February 1, 2002

*Senecio aegyptius* var. *discoideus*, Compositae, GLC-Mass Spectrometry

The essential oil of *Senecio aegyptius* var. *discoideus* flowers, leaves, stems and roots were isolated by hydrodistillation. Analysis of the oils by capillary GLC and GLC-mass spectrometry were performed and 34 out of 37 compounds were identified. The main component was isolated and characterized as 1,10-epoxyfuranoeremophilane using a combination of GLC, GLC-MS, and NMR analyses. The oils of flowers, leaves and stems were rich in monoterpene hydrocarbons while the root oil mainly contains furanoeremophilanes. Flower and leaf volatile oils showed significant level of antifungal activity against *C. albicans*, moderate effect against Gram positive bacteria, however, it has weak activity against Gram negative bacteria. The isolated sesquiterpene (1,10-epoxyfuranoeremophilane) exhibited substantial inhibitory activity against Gram negative bacteria.

## Introduction

*Senecio* is the largest genus in the tribe Seneccioneae (Asteraceae) and more than 1500 species have been reported (Nordenstam, 1977). This genus is rich in pyrrolizidine alkaloids (Rizk, 1990; Hartmann and Witte, 1995), and sesquiterpenes, in particular eremophilanolide derivatives (Bohlmann *et al.*, 1977, 1979). Few reports about essential oil contents of members of this genus have been reported (Mitsuo *et al.*, 1979; Van Dooren *et al.*, 1981; De Pooter *et al.*, 1986; Mangi *et al.*, 1995; Grace and Khattab 1998; El-Shazly 1999).

We already studied the alkaloid composition of *S. aegyptius* var. *discoideus* from Egypt (El-Shazly, 2002). In this communication, we report on the volatile oil contents and composition of different plant organs of *S. aegyptius* var. *discoideus*. As far as we could investigate, such a study has not been undertaken before.

## Material and Methods

### Plant material

The plant material of *S. aegyptius* var. *discoideus* Boiss. was collected during flowering period from wild plants growing on Nile banks at the vicinity of

Benha (province Kalubeya) Egypt, in April 2000. Identification of the plant was confirmed by Dr. H. Abdel Baset, Faculty of Science, Zagazig University. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt.

### Essential oils isolation

Fresh flowers, leaves, stems and roots were hydrodistilled for 4 h and the percentage of the oil for each organ was found to be 0.3, 0.4, 0.1 and 0.05 v / w yield, respectively according to Egyptian Pharmacopoeia 1984. The oils were dried over anhydrous sodium sulphate and kept at 4 °C in sealed brown vials for analysis.

### Isolation of major component

The major component (**35**) was isolated as a colourless oil by PLC [silica gel F<sub>254</sub>, *n*-hexane – methanol, 9 : 1] from the hydrodistilled oil obtained from the whole plant. It has R<sub>f</sub> 0.58 and a violet colour in 50% aqueous H<sub>2</sub>SO<sub>4</sub>.

### Essential oil analysis

#### Capillary GLC analysis

A Carlo Erba ICU 600 gas chromatograph equipped with FID, spectra physics integrator and DB1 fused silica capillary column (15 m × 0.317 mm i.d. 0.25 µm film thickness) was employed. GLC condition: carrier gas He (2 ml / min); detector temp. 300 °C; injector temp. 250 °C; oven temp. programme, initial temp. 50 °C 4 min isothermal, 50–90 °C 4 °C/ min. 90–300 °C 10 °C/ min, the 10 min. isothermal. A 2 mg portion of each oil was dissolved in 1 ml ethylacetate and 1 µl volume was injected. Retention index (RI): Kovats indices (Kovats, 1958) were calculated with respect to a set of co-injected even number hydrocarbons (C9 – C24). Each RI was subjected to library search by comparison with references RI and MS. Percentage of the identified compounds were computed from GLC peak areas. Total oil was set at 100%.

#### GLC-MS analysis

A Carlo Erba HRGC 4160 gas chromatograph equipped with OV1 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled to a quadrupole mass spectrometer Finnigan MAT 4500 was employed for oil analysis. EI-mass spectra were recorded at 45 eV. Condition: injector 250 °C; temp. programme 46 °C 4 min isothermal; 46–100 °C at 4 °C / min.; 100–300 °C at 8 °C/ min. then 10 min isothermal; split ratio 1: 20; carrier gas He 50 kPa. Identification of the constituents was performed by computer library search, retention indices and visual interpretation of mass spectra with those found in the literature (El-Shazly 1999; Adams 1995; Asres *et al.*, 1998; Massada 1967; Ryhage and Sydow 1963; Sydow 1963). The identified compounds are recorded in Table I. The compounds are listed in order of elution from a DB1 and OV1 capillary columns.

#### NMR measurements

<sup>1</sup>H- and <sup>13</sup>C NMR spectra were recorded on Ac Bruker Instrument in CD<sub>3</sub>OD at 300 and 75 MHz, respectively. EIMS was carried out on JEOL (Japan), 70 eV direct inlet.

Compound (35): colourless oil, changed by time to dark brown then black, **RI** 1751, **EIMS**, *m/z*

(rel. int.) [M<sup>+</sup>] 232 (100), 217 (8), 199 (10), 189 (5), 175 (11), 161 (8), 147 (12), 135 (8), 124 (7), 119 (10), 109 (16), 108 (20), 95 (6), 91 (9), 79 (8), 77 (6), 55 (4), 43 (3), 41 (5). <sup>1</sup>H- NMR (CD<sub>3</sub>OD at 300 MHz) δ 3.08 (1H, d, *J* = 4.5 Hz, H-1), 1.86 (1H, m, H-2), 1.95 (1H, m, H-2), 1.33 (1H, m, H-3), 1.73 (1H, m, H-3), 1.48 (1H, m, H-4), 2.12 (1H, d, *J* = 16.2 Hz, H-6), 2.68 (1H, d, *J* = 16.2 Hz, H-6), 2.19 (1H, d, *J* = 16.8 Hz, H-9), 2.98 (1H, d, *J* = 16.8 Hz, H-9), 7.06 (1H, q, *J* = 1 Hz, H-12), 1.88 (3H, δ, *J* = 1Hz, H-13), 1.07 (3H, s, H –14), 1.09 (3H, d, *J* = 5.5 Hz, H-15). <sup>13</sup>C- NMR (CD<sub>3</sub>OD at 75 MHz) δ 64.48 (d, C-1), 22.15 (t, C-2), 25.30 (t, C-3), 39.19 (d, C-4), 36.36 (s, C-5), 33.89 (t, C-6), 120.85 (s, C-7), 147.49 (s, C-8), 31.88 (t, C-9), 64.88 (s, C-10), 117.57 (s, C-11), 139.19 (d, C-12), 8.07 (q, C-13), 21.13 (q, C-14), 16.25 (q, C-15). Assignments were aided by DEPT, <sup>1</sup>H- <sup>13</sup>C- COSY experiments.

#### Screening for antimicrobial activity

Tested microorganisms were *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive bacteria); *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative bacteria); *Candida albicans* (yeast) and *Aspergillus flavus* (fungi). The microorganisms were obtained from the stock cultures of the Department of Microbiology, Faculty of Pharmacy, Zagazig University. Antimicrobial activity was assayed via agar diffusion method. Small cups were taken out of the agar which could take approximately 60 µl of oil solutions. Each cup was filled accurately with 50 µl of oil solutions (20 mg oil was dissolved in 1 ml dimethylformamide, DMF), as well as DMF as a control. The plates were incubated overnight at 37 °C for bacteria and 30 °C for fungi. The observed zones of inhibition were measured (in mm) and compared against standard antibiotics. Results are recorded in Table II.

#### Results and Discussion

A quantitative and qualitative variation between the oils from leaves, flowers, stems and roots was apparent. All oils obtained smell smoke-like, while the odour of intact plant is herbaceous and fruity. The oils have a pale yellow colour which changed by time to dark black.

Table I shows retention indices and relative percentages of the oil constituents. A total of 33 com-

Table I. Chemical profiles of flower, leaf, stem and root essential oils of *Senecio aegyptius* var *discoideus*.

Compound	RI*	Abundance**			
		flowers	leaves	stems	roots
<b>1</b> 1-Nonane	890	17	22	19	tr
<b>2</b> $\alpha$ -Thujene	919	tr	tr	tr	—
<b>3</b> $\alpha$ -Pinene	924	2.1	0.7	4	tr
<b>4</b> Sabinene	959	1.8	0.6	1.5	tr
<b>5</b> $\beta$ -Pinene	961	1.5	0.2	3.9	tr
<b>6</b> Myrcene	981	8.9	3.4	10.6	—
<b>7</b> 1-Decene	988	tr	tr	tr	—
<b>8</b> $\alpha$ -Terpinene	1004	0.3	tr	0.3	—
<b>9</b> <i>p</i> -Cymene	1008	tr	0.2	tr	—
<b>10</b> $\beta$ -Phellandrene	1014	3.7	0.5	3.8	—
<b>11</b> Limonene	1018	0.4	tr	3.6	—
<b>12</b> <i>Z</i> ( $\beta$ )-Ocimene	1029	tr	tr	tr	—
<b>13</b> <i>E</i> ( $\beta$ )-Ocimene	1041	tr	tr	tr	—
<b>14</b> 1-Nonen-3-ol	1072	tr	tr	tr	—
<b>15</b> Terpinolene	1078	tr	tr	tr	—
<b>16</b> $\alpha$ -Pinene oxide	1085	tr	tr	0.4	—
<b>17</b> 1-Undecene	1090	0.3	0.3	0.7	—
<b>18</b> Octen-1-ol acetate	1097	tr	tr	tr	—
<b>19</b> Sabina ketone	1151	tr	tr	tr	—
<b>20</b> Terpin-4-ol	1157	tr	tr	tr	—
<b>21</b> Methyl salicylate	1166	tr	tr	tr	—
<b>22</b> $\alpha$ -Terpineol	1170	tr	tr	tr	—
<b>23</b> Dihydromyrcenol	1195	0.8	0.5	1.1	—
<b>24</b> Nordavanone	1214	tr	tr	tr	—
<b>25</b> Dihydro-linalool acetate	1262	tr	tr	tr	—
<b>26</b> $\beta$ -Elemene	1381	0.8	0.3	0.3	4.2
<b>27</b> <i>E</i> -Caryophyllene	1411	2.3	0.9	1.0	tr
<b>28</b> $\alpha$ -Humelene	1444	0.6	0.4	0.7	0.6
<b>29</b> ( <i>E</i> ) $\beta$ -Farnesene	1448	tr	tr	tr	tr
<b>30</b> Drima-7,9-(11)-diene	1461	tr	tr	tr	19.0
<b>31</b> $\beta$ -Chamigrene	1468	tr	tr	tr	tr
<b>32</b> Valencene	1483	0.2	0.1	0.1	3.4
<b>33</b> Butylatehydroxy toluene	1499	0.2	tr	tr	tr
<b>34</b> Unidentified	1674	2.3	2.5	1.4	1.8
<b>35</b> 1,10-epoxyfuranoceremophilane	1751	55.3	66.3	46.4	69.0
<b>36</b> Unidentified	1842	0.6	0.7	0.4	tr
<b>37</b> Unidentified	1919	0.1	0.1	tr	tr

\* Measured linear retention indices on an OV1 column; \*\* Area%; tr = trace amounts (< 0.1).

Mass spectra of unknown compounds;

(34) GLC-EIMS, *m/z* (rel. int.) [M<sup>+</sup>] 232 (2), 214 (100), 199 (75), 185 (13), 171 (18), 155 (10), 141 (14), 128 (15), 115 (14), 91 (10), 77 (8), 67 (5), 55 (5), 43 (10).

(36) GLC-EIMS, *m/z* (rel. int.) [M<sup>+</sup>] 232 (100), 217 (17), 199 (45), 189 (5), 175 (21), 161 (6), 159 (6), 147 (31), 133 (13), 119 (14), 108 (25), 91 (14), 85 (11), 79 (14), 67 (8), 55 (11), 43 (11), 41 (11).

(37) GLC-EIMS, *m/z* (rel. int.) [M<sup>+</sup>] 230 (75), 215 (100), 197 (8), 187 (13), 173 (30), 159 (95), 145 (45), 131 (14), 115 (16), 103 (5), 91 (15), 83 (3), 77 (8), 65 (4), 55 (13), 43 (10).

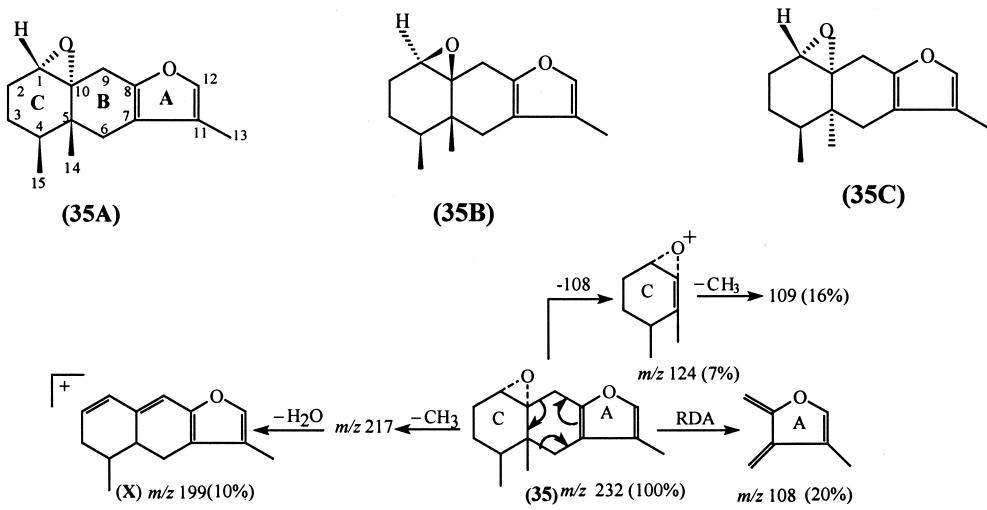
ponents were unambiguously identified by comparing their specific retention indices and mass fragmentations with those reported in literature. In addition three unidentified compounds (34, 36 and 37) were found that occurred only in small or trace amounts. The chemical composition of the flower, leaf and stem oils shows quantitative rather than qualitative variation. The root oil was

devoid of monoterpene hydrocarbons (except traces of  $\alpha$ -pinene,  $\beta$ -pinene and sabinene) and oxygenated monoterpenes whereas sesquiterpene hydrocarbons and furanoeremophilane were abundant.

Compound 35, the major component, was isolated by PLC. The mass spectrum shows a M<sup>+</sup> at *m/z* 232 suggesting a molecular formula C<sub>15</sub>H<sub>20</sub>O<sub>2</sub>.

This formula indicates a sesquiterpene with 6 degrees of unsaturation and/ or double bonds; this was confirmed by the presence of 15 different carbons and 20 protons (3 CH<sub>3</sub>, 4 CH<sub>2</sub>, 3 CH, 5 quaternary carbons) in the <sup>13</sup>C and APT spectra and the <sup>1</sup>H NMR spectrum. A prominent peak at *m/z* 108 (20%) is typical for 3-methylfuranoeremophilane (Bohlmann, *et al.*, 1979; Bohlmann and Zdero, 1974) or 3-methylligularan (Schild, 1971) resulting from Retro Diel's Alder (RDA) fragmentation, or the cleavage of bonds  $\beta$  to the ring system in alkylfurans (Budzikiewicz, 1964). A fragment at *m/z* 217 (8%) for M<sup>+</sup>-CH<sub>3</sub> and the ion peak at *m/z* 199 (10%) is for M<sup>+</sup>-CH<sub>3</sub>-H<sub>2</sub>O. The latter fragment can be attributed to the ion (X) in Scheme 1. This fragmentation implies an oxiran ring at ring C at the junction with ring B in **35**. Fragment at *m/z* 124 (7%) for C<sub>8</sub>H<sub>12</sub>O can be attributed to ions from M<sup>+</sup>-108 from RDA fragmentation; a prominent fragment at *m/z* 109 (16%) can derive from 124-Me. The <sup>1</sup>H NMR spectrum showed the presence of three methyl groups, one of which at  $\delta$  1.88 (d, *J* = 1Hz) is vinylic on a quaternary carbon; was placed on position 11 on biosynthetic reasoning and coupled with the only proton at  $\delta$  7.06 (q, *J* = 1Hz) which confirms that the furan ring is 3, 4, 5 trisubstituted; one at  $\delta$  1.09 (d, *J* = 5.5Hz) is on *sp*<sup>3</sup> methine carbon for H-15 and the other at  $\delta$  1.07 (s) is on *sp*<sup>3</sup> quaternary carbon for H-14. Four *sp*<sup>3</sup> methylenes, two of which are isolated from each other since each is a simple AM system showing

only large geminal coupling at  $\delta$  2.98 (1H, d, *J* = 16.8),  $\delta$  2.19 (1H, d, *J* = 16.8) for H-9 and at  $\delta$  2.68 (1H, d, *J* = 16.2),  $\delta$  2.12 (1H, d, *J* = 16.2) for H-6; each is vinylic to the side of the furan ring and the other side is *sp*<sup>3</sup> quaternary carbons forming the six membered ring B; the other two *sp*<sup>3</sup> methylene at  $\delta$  1.95 (1H, m),  $\delta$  1.86 (1H, m) for H-2 and  $\delta$  1.73 (1H, m), and  $\delta$  1.33 (1H, m) for H-3 are adjacent to each other since each is a complex AB multiplet from geminal and vicinal couplings. Three methines two of which are *sp*<sup>3</sup> at  $\delta$  3.08 (1H, d, *J* = 4.5Hz) for H-1 on oxygenated carbon 1, and at  $\delta$  1.48 (1H, m) for H-4 forming ring C; and one *sp*<sup>2</sup> at  $\delta$  7.06 (1H, q, *J* = 1Hz). The <sup>13</sup>C spectrum showed the presence of five quaternary carbons two of which are *sp*<sup>3</sup> at  $\delta$  64.88 for oxygenated carbon,  $\delta$  36.36 for C-5 and three are *sp*<sup>2</sup> carbons at  $\delta$  147.49, 120.85, and 117.60. A one *sp*<sup>2</sup> methine at  $\delta$  139.19 typical of trisubstituted furan moiety (Atta-ur-Rahmann and Ahmad, 1992). The presence of 2 carbons at  $\delta$  64.88 (s) and at  $\delta$  64.48 (d) confirm the presence of oxirane ring. These data implicate the structural formula **35** as 1,10-epoxyfuranoeremophilane, 8,8a-epoxy-furanoligularan (**35C**) (= 8,8a-Epoxy-3,4a,5-trimethyl-4,4a,5,6,7,8,8a,9-octahydronaphtho-[2,3-b]furan (Schild, 1971) clearly differed from **35** indicating a different stereochemistry for **35**. Also, matching the spectral data with those reported for compounds **35A** and **35B** identified before (Bohlmann, 1979) showed a clear and significant differ-



Scheme 1.

ences with **35A** except C-1 resonance ( $\delta$  63.4 in  $\text{CDCl}_3$  for **35A** as reported by Bohlmann vs.  $\delta$  63.28 in  $\text{CDCl}_3$  and 64.49 in  $\text{CD}_3\text{OD}$  in our measurements) and complete agreement with **35B** except C-1 resonance ( $\delta$  59.0 for **35B** as reported by Bohlmann vs. 63.28 for our's). Assuming that the reported data are valid, our compound would be new albeit with the same structural formula as **35A**, **35B** or **35C**, but with different stereochemistry. But, if the reported data (Bohlmann, 1979) misplaced C-1 resonance for compounds **35A** and **35B**, our compound would be the same as **35B** and it would be the first time to be reported as volatile component in a volatile oil (Adams, 1995).

#### Antimicrobial activity

Flower and leaf volatile oils of *S. aegyptius* var. *discoideus* (Table II) showed significant level of

antifungal activity against *C. albicans*, moderate effect against Gram positive bacteria, however, it has weak activity against Gram-negative bacteria. The isolated sesquiterpene (1,10-epoxyfuranoeremophilane) exhibited marked inhibition activity against Gram negative bacteria, *Bacillus subtilis* and fungi whereas no effect against *Staphylococcus aureus*.

#### Acknowledgements

Thanks are due to Dr. G. Schilling (Universität Heidelberg) for carrying out NMR measurements, Dr. A. Tei for GLC-MS (IPB HD) measurements, Dr. H. Abdel Salam (University of Zagazig) for his help in performing the antimicrobial tests and Dr. H. Abdel Baset for plant identification.

Table II. Results of antimicrobial screening of volatile oils of *Senecio aegyptius* var. *discoideus*.

Oils* and controls	Diameter of inhibition zone in mm.					
	Gram - bacteria		Gram + bacteria		Fungi	
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>A. flavus</i>
Tetracycline 30 $\mu\text{g}$ /disc	—	9	8	16	—	—
Chloramphenicol 30 $\mu\text{g}$ /disc	15	15	20	15	—	—
Penicillin 10 $\mu\text{g}$ /disc	—	—	22	—	—	—
Gentamycin 10 $\mu\text{g}$ /disc	—	18	12	25	—	—
Nystatin 30 $\mu\text{g}$ /disc	—	—	—	—	15	10
Volatile oil of flowers	7	3	10	7	16	8
Volatile oil of leaves	8	3	8	9	20	6
1,10-epoxyfuranoeremophilane	16	17	—	25	29	20

\* All assays consisted of 50  $\mu\text{l}$  of a test solution, containing 20 mg oil in 1 ml DMF; — = no inhibition.

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