

Pyrrolizidine Alkaloid Profiles of Some *Senecio* Species from Egypt

Assem El-Shazly

Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt

Z. Naturforsch. **57c**, 429–433 (2002); received January 4/February 1, 2002

Pyrrolizidine Alkaloids, *Senecio aegyptius*, Senecionine

Alkaloid profiles of two Egyptian *Senecio* species (*Senecio aegyptius* var. *discoideus* and *S. desfontainei*) in addition to a cultivated species (*S. cineraria*) were studied using capillary GLC and GLC-mass spectrometry with respect to pyrrolizidine alkaloids (PAs). Four alkaloids were identified in *S. aegyptius* var. *discoideus*, 8 in *S. desfontainei* and 13 in *S. cineraria*. Some of these alkaloids have not been reported from these plants. The alkaloidal pattern of different plant organs (flowers, leaves, stem, root) were also investigated. Senecionine has been found to be a one of the major alkaloid in all studied species, it was isolated and its structure was elucidated by ¹H- and ¹³C-NMR.

Introduction

Numerous taxa of the genus *Senecio* (Compositae) are rich in PAs exhibiting hepatotoxic, mutagenic, carcinogenic and antitumor activities (reviews in Mattocks 1986; Rizk 1990; Roeder 1995, 1999; Hartmann and Witte 1995; Roberts and Wink 1998).

Reviewing the current literature 3 PAs were previously identified in *S. aegyptius* (Gharbo and Habib 1969; Habib 1981), 4 in *S. desfontainei* (Gharbo and Habib 1969; Habib 1981) and 5 in *S. cineraria* (Habib 1974; Rizk 1990); no information could be obtained concerning the alkaloid content of *S. aegyptius* var. *discoideus*. In the present work and in the course of our study on the Egyptian plants containing PAs (El-Shazly *et al.* 1996a,b; 1998; 1999) we have investigated the alkaloidal pattern of flowers, leaves, stem and root of wild *S. aegyptius* var. *discoideus*, *S. desfontainei* and the cultivated *S. cineraria* species by sensitive phytochemical methods (GLC and GLC-MS). Since capillary GLC in combination with mass spectrometry is a rapid and powerful technique for the analysis of complex PA mixtures (Bicchi *et al.*, 1991; Witte *et al.*, 1993; Stelljes *et al.*, 1991; El-Shazly *et al.*, 1996a–d; 1998; 1999), we were able to unambiguously identify most PAs in these plants and have found a number of alkaloids which were reported for the first time in the studied species.

Materials and Methods

Plant material

Flowering plant of *S. aegyptius* var. *discoideus* Boiss. was collected from the Nile banks at the vicinity of Benha in April 2000. *S. desfontainei* Druce (= *S. coronopifolius* Desf.) was collected from sandy area near Bilbeis in March 2000. *S. cineraria* Dc. (syn. *Cineraria maritima* L.) was collected from plants cultivated in the experimental station of the Faculty of Pharmacy, Zagazig University in May 2000. Identification of these plants was confirmed by Dr. H. Abdel Baset, Faculty of Science, Zagazig University. Voucher specimens are deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University. The different plant organs were separated, air dried and powdered.

Alkaloid extraction

Air dried plant materials (10 g each) were separately extracted twice with 0.5 N HCl (500 ml) through homogenization by Ultra-turrax and left to stand for one hour. The resulting extracts were combined, then defatted with CH₂Cl₂. Half of the aqueous phase was made alkaline with 25% NH₄OH solution (pH 10) and extracted with CH₂Cl₂. The resulting extract was dried over anhydrous Na₂SO₄ and evaporated to dryness to afford the free tertiary bases. To investigate the presence of PA N-oxides, the second aliquot of the aqueous phase was brought to 2 N HCl and reduced with Zn dust under continuous stirring

overnight, filtered and treated as above to obtain the total alkaloids (tertiary bases and *N*-oxides) in the form of tertiary PA bases. The dried residues were weighed and kept at 4 °C for GLC and GLC-MS analysis. PLC [silica gel F₂₅₄ CHCl₃- MeOH-NH₄OH (25%), 85: 15: 1)] of the crude PA tertiary bases of *S. aegyptius* var. *discoideus* resulted in the isolation of a major alkaloid (**4**) with *R_f* 0.68.

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. program, initial temp. 150 °C 5 min isothermal, 150–300 °C 10 °C/ min. 300 °C, 15 min isothermal. Retention index (RI): Kovats indices (Kovats, 1958) were calculated with respect to a

set of co-injected even number hydrocarbons (C10 – C28). Each RI was subjected to library search by comparison with references RI and MS.

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 PA analysis. EI-mass spectra were recorded at 45 eV. Condition: injector 250 °C; temp. program 120 °C 3 min isothermal, 120–300 °C 6 °C / min., 300 °C 10 min isothermal; split ratio 1: 20; carrier gas He 50 kPa.

NMR measurements

¹H and ¹³C NMR spectra were recorded on Varian instrument in CDCl₃, at 300 and 75 MHz, respectively.

Table I. Pyrrolizidine alkaloids of *Senecio aegyptius* var. *discoideus*, *S. desfontainei* and *S. cineraria* separated by capillary GLC and identified by GLC-MS.

Alkaloid*	RI	M ⁺	B. P.	Reference
1 9-Methyl-didehydroretronecine	1387	167	136	1
2 5,6-Dihydro-7,9-dimethoxy-7H-pyrrolizine	1413	181	150	1
3 Senecivernine	2299	335	120	2, 3
4 Senecionine	2310	335	136	4
5 Seneciphylline	2327	333	120	5
6 Spartioidine	2359	333	120	6
7 Integerrimine	2367	335	119	7
8 Jacobine	2432	351	120	3
9 Riddelliine	2471	349	119	2,
10 Acetylseneciphylline	2483	357	136	1
11 Jacoline	2508	369	120	1, 3
12 Retrorsine	2539	351	120	7
13 Jaconine	2548	387	120	1, 3
14 Usaramine	2607	351	119	6
15 Otosenine	2631	381	151	8
16 Florosenine	2776	423	43	1, 9
17 Doronine	2818	459	43	1, 9
18 PA1*	2543	351	80	
19 PA2*	2563	351	138	
20 PA3*	2595	351	138	

1= Tei, 2000; 2= Borstel *et al.*, 1989; 3= Witte *et al.*, 1992; Witte *et al.*, 1993; 5= Roeder and Abdel Ghani 1990; 6= Pieters and Vlietink 1988; 7= Ray *et al.*, 1987; 8 = Resch *et al.*, 1982; 9= Krebs *et al.*, 1996.

RI = retention index, M⁺ = molecular weight, B. P. = base peak.

Identified compounds are listed in order of elution from OV-1 column under condition listed in experimental section. * Mass spectra of unknown compounds; **PA1 (18)**, GLC-EIMS, *m/z* (rel. int.): [M⁺] 351 (10), 333 (20), 262 (8), 137 (27), 136 (27), 125 (60), 120 (70), 119 (67), 111 (60), 106 (56), 94 (30), 80 (100), 43 (85).

PA2 (19), GLC-EIMS, *m/z* (rel. int.): [M⁺] 351 (58), 282 (10), 227 (38), 138 (100), 125 (30), 94 (60), 93 (53), 80 (20), 43 (43).

PA3 (20), GLC-EIMS, *m/z* (rel. int.): [M⁺] 351 (8), 333 (4), 227 (10), 139 (30), 138 (100), 137 (40), 125 (40), 120 (13), 106 (10), 94 (65), 93 (60), 80 (30).

Results and Discussion

Capillary GLC and GLC-mass spectrometry is a valuable method for resolving and identification of complex PA mixtures even in trace amounts or diastereomeric forms. Combination of M^+ , RI and mass group fragmentation pattern provided unequivocal identification of most of these alkaloids. The identification of the compounds was established by direct comparison (MS, RI) with authentic alkaloids or by comparison with literature data. As PAs often occur both as tertiary bases and the corresponding *N*-oxides, the aqueous acidic extract of the plant material was worked up to give separate estimation of the tertiary bases and of the total alkaloid content (tertiary bases and reduced *N*-oxides) as well. The alkaloid contents were estimated gravimetrically and given as w/w% of dried plant materials. 17 PAs were unambiguously identified in the crude tertiary bases of *S. aegyptius* var. *discoideus*, *S. desfontainei* and *S. cineraria* species (Table I). In all studied species most of PAs were present as *N*-oxide rather than the corresponding tertiary bases (see tertiary bases and *N*-oxides) (Table II).

Senecio cineraria is grown in Egypt as an ornamental plant. Previous studies (Habib 1974, Rizk 1990) on this species resulted in isolation and identification of 5 PAs namely; senecionine, seneciophylline, jacobine, otosenine and retrorsine. In the present study only 4 of the previously identified components and additional 9 other alkaloids namely 9-methyl-didehydroretronecine, 5,6-dihydro-7,9-dimethoxy-7H-pyrrolizine, senecivernine, integerrimine, jacoline, jaconine, usaramine, florosenine and doronine are now reported and identified for the first time as alkaloids in *S. cineraria*. On the other hand we have not found any trace of retrorsine. Highest concentration occurs in the flower heads, while other organs contain relatively smaller amounts (Table II).

Concerning *S. desfontainei*, eleven peaks were observed in the GLC and GLC-MS of which 8 alkaloids could be identified three of them were previously reported in this plant viz. senecionine, seneciophylline and riddelliine (Gharbo and Habib 1969; Habib 1981). New alkaloids for this plant are: senecivernine, spartioidine, integerrimine, acetylseneciophylline and retrorsine. The presence

Table II. Pyrrolizidine alkaloid profiles and percentage in *Senecio aegyptius* var. *discoideus*, *S. desfontainei* and *S. cineraria* using capillary gas chromatography.

Alkaloid*	<i>Senecio aegyptius</i> var. <i>discoideus</i>								<i>S. desfontainei</i>								<i>S. cineraria</i>							
	F		L		S		R		F		L		S		R		F		L		S		R	
	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.	Ter.	Tot.
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9.2	10.2	6.9	21.4	18.1	26.8	tr	18.9	1.9	tr	4.1	tr	3.9	tr	1.8	tr	-	-	-	-	-	-	-	-
4	52.3	68.1	12.5	49.1	24.6	41.5	tr	53.7	42.1	55.4	21.3	44.4	37.4	28.3	52.3	70.6	8.6	1.5	-	1.8	3.6	16.8	29.5	33.6
5	23.9	21.7	28.9	28.8	32.9	30.6	55.4	27.5	20.3	34.6	3.5	27.7	4.5	17.9	3.6	8.4	-	0.4	-	tr	tr	tr	9.2	11.4
6	14.6	tr	51.8	0.7	24.4	1.1	44.6	tr	tr	tr	8.5	tr	5.1	tr	9.5	1.1	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	9.1	8.0	13.8	11.0	14.6	12.3	5.8	11.1	0.8	0.8	7.3	tr	7.4	5.9	1.3	6.9
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.6	10.9	28.1	13.8	16.4	14.7	9.3	31.1
9	-	-	-	-	-	-	-	-	tr	tr	tr	tr	tr	tr	tr	tr	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	tr	tr	-	tr	-	tr	-	tr	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4	3.0	10.3	7.8	9.8	5.8	4.1	6.2
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.8	9.7	10.5	63.9	13.2	39.7	1.7	3.7
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.9	25.5	tr	10.7	tr	0.7	3.4
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.1	33.3	tr	2.9	23.9	7.4	4.2	Tr
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42.1	37.5	tr	2.9	13.6	tr	1.3	tr
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.4	10.9	tr	3.9	30.8	tr	tr	tr
Total alkaloid**	0.18	0.47	0.13	0.26	0.14	0.18	0.1	0.1	0.21	0.61	0.06	0.13	0.06	0.11	0.12	0.10	0.26	0.23	0.05	0.12	0.05	0.21	0.04	0.12

* = Numbers of compounds as in Table I, ** = mg/100 mg w/w dried plant material.

F = flowers, L = leaves, S = stem, R = root; tr = trace amounts (< 0.1); - = not detected.

Ter. = tertiary bases (before Zn reduction), Tot. = total alkaloids (tertiary bases and N-oxides after Zn reduction).

of otosenine previously reported from this plant was not confirmed. Three unidentified PAs: PA1 (RI 2543), PA2 (RI 2563) and PA3 (RI 2595) were detected in delayed elution components in GLC and GLC-MS (Table I) and their individual percentages were only in small or trace amounts. The mass spectrum of these alkaloids exhibited a molecular ion $[M^+]$ at m/z 351. This $[M^+]$ indicates a senecivernine, senecionine or integerrimine containing additional oxygen.

Three PAs namely senecionine, otosenine and riddelliine have been previously isolated from *S. aegyptius* L. (Gharbo and Habib 1969; Habib 1981). Our present investigation of *S. aegyptius* var. *discoideus* resulted in identification of 4 alkaloids viz. senecionine, senecivernine, integerrimine and retrorsine and no solid evidence was obtained for the presence of otosenine and riddelliine. The highest total alkaloid contents were found in flower heads (Table II). Before Zn reduction, the dichloromethane extracts had yielded only relatively small amount of PAs indicating that most of these alkaloids were found in the intact plants in *N*-oxide forms. Thus, comparative evaluation of PA patterns of *S. aegyptius* L. and *S. aegyptius* var. *discoideus* Boiss. are clearly distinguished by their PAs contents.

As the ^{13}C NMR spectrum of **4** contained 18 resonances, apparently corresponding to 18 carbons (Table III) and its ^1H NMR spectrum integrated for 25 hydrogens, it was inferred that the molecular composition was $\text{C}_{18}\text{H}_{25}\text{NO}_5$. MS, ^1H - and ^{13}C NMR spectra of compound **4** were identical

Table III. ^1H and ^{13}C NMR spectral data of senecionine (**4**).

Position	^1H NMR	^{13}C NMR
1	–	131.94
2	6.15 1H, d, $J = 5.1$ Hz	136.27
3	H-3 α 3.38, 1H, m	62.92
	H-3 β 3.92, 1H, br d, $J = 15.9$ Hz	
5	H-5 α 2.50 1H, m	53.21
	H-5 β 3.33 1H, m	
6	H-6 α 3.25 1H, m	34.95
	H-6 β 2.35 1H, m	
7	4.99 1H, d, $J = 3$ Hz	75.04
8	4.27 1H, br s	77.73
9	H-9 α 4.01 br d, $J = 11.1$ Hz	60.69
	H-9 β 5.46, 1H, d, $J = 7.2$ Hz	
11	–	178.37
12	–	76.85
13	1.65, 1H, m	38.46
14	H-14 α 1.72, 1H, m	38.58
	H-14 β 2.14, 1H, m	
15	–	133.22
16	–	167.68
18	1.28 3H, s	25.39
19	0.87 3H, d, $J = 6.3$ Hz	11.27
20	5.69 1H, q, $J = 6.6$ Hz, 7.2 Hz	134.11
21	1.80 3H, d, $J = 6.9$ Hz	15.20

with those reported for senecionine (Witte *et al.*, 1993; Logie *et al.*, 1994; Jones *et al.*, 1982; Roeder 1990).

Acknowledgement

Thanks are due to Prof. Dr. M. Wink (University of Heidelberg, Germany) for providing facilities for GLC and GLC-MS and Dr. H. Abdel Baset for identification of plants.

- Bicchi C., Rubiolo P., Frattini C., Sandra P. and David F. (1991), Off-line supercritical fluid extraction and capillary gas chromatography of pyrrolizidine alkaloids in *Senecio* species. *J. Nat. Prod.* **54**, 941–945.
- Borstel K., Witte L. and Hartmann T. (1989), Pyrrolizidine alkaloid patterns in populations of *Senecio vulgaris*, *S. vernalis* and their hybrids. *Phytochemistry* **28**, 1635–1638.
- El-Shazly A., Sarg T., Ateya A., Abdel Aziz E., El-Dahmy S., Witte L. and Wink M. (1996a), Pyrrolizidine alkaloids from *Echium setosum* and *Echium vulgare*. *J. Nat. Prod.* **59**, 310–313.
- El-Shazly A., Sarg T., Ateya A., Abdel Aziz E., El-Dahmy S., Witte L. and Wink M. (1996b), Pyrrolizidine and tetrahydroisoquinoline alkaloids from *Echium humile*. *Phytochemistry* **42**, 225–230.
- El-Shazly A., Sarg T., Ateya A., Abdel Aziz E., Witte L. and Wink M. (1996c), Pyrrolizidine alkaloids of *Cynoglossum officinale* and *Cynoglossum amabile* (Family Boraginaceae). *Biochem. Syst. Ecol.* **24**, 415–421.
- El-Shazly A., Sarg T., Witte L. and Wink M. (1996d), Pyrrolizidine alkaloids from *Cynoglossum creticum*. *Phytochemistry* **42**, 1217–1221.
- El-Shazly A., El-Domiaty M., Witte L. and Wink M. (1998), Pyrrolizidine alkaloids in members of the Boraginaceae from Sinia (Egypt). *Biochem. Syst. Ecol.* **26**, 619–639.
- El-Shazly A., Abdel-All M., Tei A. and Wink M. (1999), Pyrrolizidine alkaloids from *Echium rauwolfii* and *Echium horridum* (Boraginaceae). *Z. Naturforsch.* **54 C**, 295–300.
- Gharbo A. S. and Habib A. M. (1969), Phytochemical investigation of Egyptian *Senecio* species. Part II. Alkaloids of *Senecio aegyptius*, *S. desfontainei*, *S. vulgaris*, *S. petasitis* and *S. mikanioides*. *Lloydia* **32**, 503–508.
- Habib A. M. (1974), Senecionine, seneciphylline, jacobine and otenine from *Senecio cineraria*. *Planta Med.* **26**, 279–282.
- Habib A. M. (1981), Alkaloids from *Senecio aegyptius* and *S. desfontainei*. *Planta Med.* **43**, 290–292.
- Hartmann T. and Witte L. (1995), Chemistry, biology and chemecology of pyrrolizidine alkaloids. In: *Alkaloids: Chemical and Biological Perspective* (Pelletier S. W., ed.). Pergamon, Oxford, Vol. **9**, pp. 155–233.
- Jones A. J., Culvenor C. C. J. and Smith W. (1982), Pyrrolizidine alkaloids- A carbon-13 N.M. R. study. *Aust. J. Chem.* **35**, 1173–84.
- Kovats E. (1958), Gas-Chromatographische Charakterisierung organischer Verbindungen. Teil I: Retentions-Indices aliphatischer Halogenide, Alkaloide, Aldehyde und Ketone. *Helv. Chim. Acta* **41**, 1915–1932.
- Krebs H. C., Carl T. and Habermehl G. (1996), Pyrrolizidine alkaloid composition in six Brazilian *Senecio* species. *Phytochemistry* **43**, 1227–1229.
- Logie C. G., Grue M. R. and Liddell J. R. (1994), Proton NMR spectroscopy of pyrrolizidine alkaloids. *Phytochemistry* **37**, 43–109.
- Mattocks A. R. (1986), *Chemistry and Toxicology of Pyrrolizidine Alkaloids*. Academic Press, London.
- Pieters L. A. and Vlietinck A. J. (1988), Spartioidine and usaramine, two pyrrolizidine alkaloids from *Senecio vulgaris*. *Planta Med.* **54**, 178–179.
- Ray A. C., Williams H. J. and Reagor J. C. (1987), Pyrrolizidine alkaloids from *Senecio longilobus* and *Senecio glabellus*. *Phytochemistry* **26**, 2431–2433.
- Resch J. F., Goldstein S. A. and Meinwald J. (1983), Otenine from *Senecio aureus*. *Planta Med.* **47**, 255.
- Rizk A. M. (1990), *Naturally Occurring Pyrrolizidine Alkaloids*. CRC Press, Boca Raton, FL, USA
- Roberts M. F. and Wink M. (1998), *Alkaloids: Biochemistry, Ecology, and Medicinal Applications*. Plenum, New York and London.
- Roeder E. (1990), Carbon-13 NMR spectroscopy of pyrrolizidine alkaloids. *Phytochemistry* **29**, 11–29.
- Roeder E. (1995), Medicinal plants in Europe containing pyrrolizidine alkaloids. *Pharmazie* **50**, 83–98.
- Roeder E. (1999), Analysis of pyrrolizidine alkaloids. *Curr. Org. Chem.* **3**, 557–576.
- Roeder E. and Abdel Ghani A. (1990), Pyrrolizidine alkaloids from *Petasites paradoxus* (Retz.) Baumg. *Sci. Pharm.* **58**, 403–407.
- Stelljes M. E., Kelley R. B., Molyneux R. J. and Seiber J. N. (1991), GC-MS determination of pyrrolizidine alkaloids in four *Senecio* species. *J. Nat. Prod.* **54**, 759–773.
- Tei A. (2000), Identifizierung und Strukturaufklärung von Alkaloiden und anderen Sekundärstoffen mit Hilfe von GC-MS und Kernresonanzspektroskopie. Dissertation, Ruprecht-Karls-Universität Heidelberg, Germany.
- Witte L., Ernst L., Adam H. and Hartmann T. (1992), Chemotypes of two pyrrolizidine alkaloid-containing *Senecio* species. *Phytochemistry* **31**, 559–565.
- Witte L., Rubiolo P., Bicchi C. and Hartmann T. (1993), Comparative analysis of pyrrolizidine alkaloids from natural sources by gas chromatography-mass spectrometry. *Phytochemistry* **32**, 187–196.