Quinolizidine Alkaloid Profiles of Two Taxa of *Teline maderensis*

Alice Martins[a], Michael Wink[b], Andreas Tei[b], and Amélia P. Rauter[a*]

[a] Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Ed. C8, 5°, Campo Grande, 1749-016 Lisboa, Portugal. Fax: +351217500088. E-mail: aprauter@fc.ul.pt

[b] Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

* Author for correspondence and reprint requests

Z. Naturforsch. **58 c**, 776–778 (2003); received June 10/July 8, 2003

The alkaloid composition of the aerial parts of two taxa of *Teline maderensis* was studied by capillary GLC and GLC-MS. *N*-Methylcytisine was the major alkaloid found in both plants. Contents of cytisine and lupanine were higher in *T. maderensis* var. *paivae* while anagyrine content was more pronounced in *T. maderensis* var. *maderensis*. The alkaloids dehydrocytisine, *N*-acetylcytisine and epibaptifoline appeared only in *T. maderensis* var. *maderensis* and *N*-formylcytisine was identified as a minor constituent in *T. maderensis* var. *paivae*, and detected only in trace amounts in the other variety of the plant.

Key words: Teline maderensis, Quinolizidine Alkaloids, GLC-MS

Introduction

Teline maderensis Webb et Berthel var. *maderensis* [syn: *Genista maderensis* (Webb et Berthel) Lowe] is a rare endemic shrub growing in Madeira, with an approximate height of 5 m, numerous branches, persistent foliage and yellow flowers. It is mainly found in ravines and other localities of Madeira forests (Vieira, 1992).

Teline maderensis Webb et Berthel var. *paivae* (Lowe) Arco (syn: *Genista paivae* Lowe) is an extremely rare shrub endemic to Madeira and Desertas Islands. It can attain a height of up to 1.5 m, with abundance of branches, persistent foliage and also yellow flowers, but with a slight morphological difference of the flowers compared to the previously mentioned variety. It grows on hill-sides near the littoral of Madeira and Desertas (Vieira, 1992).

Both plants belong to the Leguminosae family but sometimes the distinction between the two varieties is quite difficult because they are morphologically too close. The analysis of the alkaloid profiles of these two populations by the sensitive techniques GLC and GLC-MS can offer a chance to separate both plants at an intraspecific level.

Materials and Methods

Plant material

Both plants were collected at the beginning of the flowering period in Madeira Island in June. Their voucher specimens are deposited in Jardim Botânico da Madeira, Funchal, with registration numbers MADJ 8310 for *Teline maderensis* var. *maderensis*, collected in S. Jorge, Ribeiro Bonito, and MADJ 8351 for *Teline maderensis* var. *paivae*, collected in Vereda das Queimadas, Caldeirão Verde.

Alkaloid extraction

Dried and powdered aerial parts of both plants (1047 g, *T. maderensis* var. *maderensis*; 130 g, *T. maderensis* var. *paivae*) were extracted separately with EtOH in a Soxhlet apparatus. The ethanolic extracts were concentrated to dryness under reduced pressure and then acidified with 1 N HCl (pH 2). After filtration (Whatman n° 4), the acid aqueous solutions were basified with concentrated NH_4OH and extracted with CH_2Cl_2. The organic phases were filtered and dried with anhydrous Na_2SO_4, filtered again and concentrated *in vacuo*. The acid base purification was repeated three times to give two dark brown alkaloid residues

0939–5075/2003/1100–0776 $ 06.00

(1.6 g, η = 0.15%, *T. maderensis* var. *maderensis*; 0.19 g, η = 0.15%, *T. maderensis* var. *paivae*).

Alkaloid analysis

Capillary GLC was performed on a Varian gas chromatograph 3300, equipped with a FID detector and a Spectra Physics Integrator SP4290. Conditions: OV-1 fused silica capillary column (15 m × 0.25 mm); carrier gas He; detection temperature 300 °C; injection temperature 250 °C; split 1:20; oven temperature program: initial temperature 120 °C, 2 min isothermal, increased 10 °C min^{-1} to 300 °C.

For GLC-MS an OV-1 fused silica capillary column (30 m × 0.25 mm) was used coupled to a quadropole Finnigan Mat 4515 mass spectrometer. EI-MS were recorded at 40 eV and evaluated with the INCOS DATA SYSTEM. Conditions for alkaloids of *T. maderensis* var. *maderensis*: carrier gas He; splitless; oven temperature program: initial temperature 120 °C, 2 min isothermal, 120 °C–126 °C, 6 °C min^{-1}, 126 °C – 300 °C, 6 °C min^{-1}. Conditions for alkaloids of *T. maderensis* var. *paivae*: carrier gas He; split 1:20; oven temperature program: initial temperature 120 °C, 3 min isothermal, 120 °C–138 °C, 6 °C min^{-1}, 138 °C–300 °C, 6 °C min^{-1}, 300 °C–312 °C, 12 °C min^{-1}.

Chemical ionization mass spectra (CI-MS) were performed using NH_3 as a reactant gas under the same conditions described above.

Kovats retention indices (RI) were calculated by co-injection of standard hydrocarbons.

Results and Discussion

The identification of the alkaloids was accomplished by comparing their mass spectra and Kovats retention indices (Table I) with those reported in the literature (Wink *et al.*, 1983; Wink, 1993; Wink *et al.*, 1995; El-Shazly *et al.*, 1996; Pistelli *et al.*, 2001; Woldemichael and Wink, 2002).

N-methylcytisine and anagyrine are the major alkaloids found in *T. maderensis* var. *maderensis*, while dehydrocytisine, cytisine, rhombifoline, lupanine, *N*-acetylcytisine and epibaptifoline appear in this plant as minor components (Table II). The alkaloids 5,6-dehydrolupanine, aphylline, tinctorine, thermopsine, *N*-formylcytisine and baptifoline were present only in trace amounts.

In *T. maderensis* var. *paivae* we identified *N*-methylcytisine, cytisine and lupanine as major compounds, while *N*-formylcytisine and anagyrine figured as minor constituents (Table II). 5,6-Dehydrolupanine, rhombifoline and tinctorine appeared only in trace amounts.

Quinolizidine alkaloids profiles are of chemotaxonomic value at specific and even subspecific level (Kirch *et al.*, 1995). In this work we reported the alkaloid composition of two populations of *Teline maderensis*. The chemical data obtained may

Table I. GLC-MS data of quinolizidine alkaloids of *Teline maderensis* var. *maderensis* and *Teline maderensis* var. *paivae*.

Alkaloid	RI	M^+	EI-MS	CI-MS
N-Methylcytisine	1975	204	58(100) 117(6) 146(11) 160(9) 204(31)	205 (100)
Dehydrocytisine	1985	188	134(100) 146(61) 148(81) 160(50) 188(98)	n.d.
Cytisine	1995	190	134(23) 146(100) 147(77) 160(22) 190(63)	191 (100)
5,6-Dehydrolupanine	2148	246	84(7) 97(38) 98(100) 134(8) 246(17)	247 (100)
Rhombifoline	2175	244	58(100) 146(8) 160(13) 203(69) 244(2)	245 (100)
Lupanine	2182	248	98(33) 136(100) 149(56) 150(38) 248(39)	249 (100)
Aphylline	2186	248	84(34) 96(43) 136(100) 220(36) 248(25)	249 (100)
Tinctorine	2257	244	58(31) 146(15) 160(12) 203(100) 244(14)	245 (100)
Thermopsine	2300	244	98(100) 136(9) 146(59) 160(13) 244(25)	245 (100)
N-Formylcytisine	2334	218	134(14) 146(100) 160(17) 190(8) 218(46)	219 (100)
N-Acetylcytisine	2344	232	146(100) 147(59) 160(16) 190(8) 232(10)	n.d.
Anagyrine	2410	244	98(100) 136(11) 146(14) 160(10) 244(33)	245 (100)
Baptifoline	2635	260	70(36) 96(26) 114(100) 146(20) 260(24)	n.d.
Epibaptifoline	2656	260	70(38) 96(27) 114(100) 152(12) 260(36)	n.d.

n.d. = not determined.

Table II. Alkaloid retention times (RT) and contents (total alkaloids = 100%) of *Teline maderensis* var. *maderensis* and *Teline maderensis* var. *paivae*.

Alkaloid	*T. maderensis* var. *maderensis*		*T. maderensis* var. *paivae*	
	RT [min]	Content (%)	RT [min]	Content (%)
N-Methylcytisine	9.67	57.83	9.62	38.45
Dehydrocytisine	9.94	0.92	–	–
Cytisine	10.26	4.71	10.00	30.01
Rhombifoline	11.56	2.42	n.d.	tr
Lupanine	11.79	0.64	11.57	18.17
N-Formylcytisine	n.d.	tr	13.08	1.64
N-Acetylcytisine	13.18	4.70	–	–
Anagyrine	13.71	20.80	13.64	2.37
Epibaptifoline	15.93	1.75	–	–

tr = trace amounts.
– = not detected.
n.d. = not determined.

be regarded as a complement of other markers such as chromosome numbers and nucleotide sequences of marker genes (Käss and Wink, 1995) for further unambiguous identification of both taxa.

Acknowledgements

We gratefully acknowledge Susana Fontinha and the staff of the Jardim Botânico da Madeira, Funchal for the collection and identification of plant material. This work was supported by a grant of Fundação para a Ciência e Tecnologia (Portugal), Praxis XXI Program.

Asres K., Tei A., and Wink M. (1997), Quinolizidine alkaloids from the East-African legume *Dicraeopetalum stipulare* Harms. Biochem. Syst. Ecol. **25**, 305–308.

El-Shazly A., Ateya A. M., Witte L., and Wink M. (1996), Quinolizidine alkaloid profiles of *Retama raetam*, *R. sphaerocarpa* and *R. monosperma*. Z. Naturforsch. **51c**, 301–308.

Käss E. and Wink M. (1995), Molecular phylogeny of the Papilionoideae (family Leguminosae): RbcL gene sequences versus chemical taxonomy. Bot. Acta **108**, 149–162.

Kirch J., Veit M., Wätzig H., Greinwald R., and Czygan F. C. (1995), Alkaloid variation in *Genista lobelii* S.I (Fabaceae). Biochem. Syst. Ecol. **23**, 635–643.

Mears A. J. and Mabry T. J. (1971), Alkaloids in the Leguminosae. In: Chemotaxonomy of the Leguminosae (Harborne J. B., Boulter D., and Turnner B. L., eds.). Academic Press, London, p. 88.

Pistelli L., Bertoli A., Giachi I., Morelli I., Rubiolo P., and Bicchi C. (2001), Quinolizidine alkaloids from *Genista ephedroides*. Biochem. Syst. Ecol. **29**, 137–141.

Vieira R. (1992), Flora da Madeira. O interesse das plantas endémicas macaronésicas (Serviço Nacional de Parques, Reservas e Conservação da Natureza., ed.). Lisboa.

Wink M. (1993). Quinolizidine alkaloids. In: Methods in Plant Biochemistry. Alkaloids and Sulphur Compounds (Waterman P. G., ed.). Academic Press, London, vol. 8, pp. 197–239.

Wink M., Meißner C., and Witte L. (1995), Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. Phytochemistry **38**, 139–153.

Wink M., Witte L., Hartmann T., Theuring C., and Volz V. (1983), Accumulation of quinolizidine alkaloids in plants and cell suspension cultures: genera *Lupinus, Cytisus*, *Baptisia*, *Genista*, *Laburnum* and *Sophora*. Planta Med. **48**, 253–257.

Woldemichael G. M. and Wink M. (2002), Concomitant occurrence of pyrrolizidine and quinolizidine alkaloids in the hemiparasite *Osyris alba* L. (Santalaceae). Biochem. Syst. Ecol. **30**, 139–149.