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A Phytochemical Study of the Quinolizidine Alkaloids from *Genista tenera* by Gas Chromatography–Mass Spectrometry

Alice Martins,[1] Michael Wink,[2] Andreas Tei,[2] Michèle Brum-Bousquet,[3] François Tillequin[3] and Amélia-Pilar Rauter [1]*

[1] Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Ed. C8, 5° piso, Campo Grande, 1749-016 Lisboa, Portugal
[2] Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany
[3] Laboratoire de Pharmacognosie de l'Université René Descartes, UMR/CNRS, no. 8638, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75006 Paris, France

Gas chromatography coupled with mass spectrometry has been used to analyse the alkaloids present in the aerial parts of *Genista tenera*. Anagyrine, cytisine, *N*-formylcytisine, *N*-methylcytisine and lupanine were the major compounds, the last two alkaloids being known for their hypoglycaemic activity. Dehydrocytisine, 5,6-dehydrolupanine, rhombifoline, aphylline and thermopsine were the minor alkaloids. The characterisation of the constituents was based on comparison of their Kovats retention indexes and electron impact–mass spectrometric data recorded on-line with those of reference compounds and literature data. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: GC-MS; quinolizidine alkaloids; *Genista tenera*; Fabaceae.

INTRODUCTION

Genista tenera (Jacq.) Kuntze is included in the Papilionoideae subfamily of the Fabaceae and is a common endemic shrub on the island of Madeira, flowering from March to July. This shrub consists of curved fragile branches and can attain a height of 2.5 m. It grows on slopes of the littoral starting at an altitude of 50 m and reaching medium altitudes of up to 1000–1500 m (Vieira, 1992).

A preliminary phytochemical screening of an infusion of this plant confirmed the presence of flavonoids and alkaloids. A wide range of biological properties is attributed to quinolizidine alkaloids (Kinghorn and Balandrin, 1984; Wink, 1993a, b; Michael, 2002) including hypoglycaemic activity (Perez *et al.*, 1998). The structural characterisation of the flavonoids present in the diethyl ether extract of *G. tenera* has been described (Borges *et al.*, 2001a, b; Martins *et al.*, 2002). In the present work, the alkaloid composition of the aerial parts of *G. tenera* was investigated by GC-MS, a powerful method for the analysis of the quinolizidine alkaloids, since they can be characterised by Kovats retention index together with their characteristic MS (Wink, 1993a, b; Wink *et al.*, 1983, 1995). Both the alkaloids and the flavonoids are important chemotaxonomic markers of the genus *Genista* (Harborne, 1994).

* Correspondence to: A. P. Rauter, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Ed. C8, 5° piso, Campo Grande, 1749-016 Lisboa, Portugal.
Email: aprauter@fc.ul.pt
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EXPERIMENTAL

Plant material. Plants were identified and collected on the island of Madeira at the beginning of the flowering period by Susana Fontinha and Padre Manuel Nóbrega affiliated to the Jardim Botânico da Madeira, Funchal, Madeira. A voucher specimen (MADJ 2508) was deposited in the herbarium of this institution.

Extraction of alkaloids. Dried and powdered aerial parts of *Genista tenera* (1418 g) were extracted with ethanol in a Soxhlet apparatus. The residue (153 g) remaining after concentration to dryness under reduced pressure was acidified with 1 M hydrochloric acid, filtered, and the acid aqueous solution was then basified with concentrated ammonium hydroxide and extracted with dichloromethane. The organic phase was filtered, dried with anhydrous sodium sulphate, filtered again and finally concentrated *in vacuo*. The acid–base purification procedure was repeated three times to give a dark brown semi-solid extract of alkaloids (0.826 g; $\eta = 0.06\%$).

Analysis of alkaloids. The total alkaloid extract was analysed by GC and by GC-MS. GC was performed on a Varian (Palo Alto, CA, USA) gas chromatograph (model 3300), equipped with a flame ionisation detector (FID) and a Spectra Physics model SP4290 integrator. An OV-1 fused silica capillary column (15 m × 0.25 mm) was employed with helium carrier gas, a detection temperature of 300°C, an injection temperature of 250°C, an injection split of 1:20, and an oven temperature initially at 120°C for 2 min and then increased by 10°C/min to 300°C. For GC-MS an OV-1 fused silica capillary column (30 m × 0.25 mm) was employed coupled to a Finnigan

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Table 1. Identification of the alkaloids of *Genista tenera* by GC and GC-MS

Alkaloid	Kovats retention index	M^+ (EI-MS)	CI-MS	%[a]
N-Methylcytisine	1955	204	205 (100)	8.6
Dehydrocytisine	1972	188	n.d.[b]	—
Cytisine	1990	190	191 (100)	16.8
5,6-Dehydrolupanine	2132	246	247 (100)	—
Rhombifoline	2155	244	245 (100)	—
Lupanine	2165	248	249 (100)	30.5
Aphylline	2180	248	249 (100)	—
Thermopsine	2310	244	245 (100)	—
N-Formylcytisine	2315	218	219 (100)	9.6
Anagyrine	2390	244	245 (100)	34.5

[a] Composition of the major alkaloids (total major alkaloids = 100%) as determined by GC.
[b] n.d., not determined.

Mat model 4515 quadrupole mass spectrometer. EI-MS were recorded at 40 eV and evaluated with an INCOS data system. The analytical conditions were: helium carrier gas; splitless injection; injection temperature 250°C, oven temperature initially at 120°C for 3 min and then increased by 8°C/min to 300°C. CI-MS analyses were performed using ammonia as the reactant gas under the following conditions: injection split 1:5; oven temperature initially at 120°C for 3 min and then increased by 6°C/min to 300°C. Kovats retention indices were calculated by co-injection with standard hydrocarbons (C_{12}–C_{28}) and by comparison with reference retention index values stored in the data base of the Institut für Pharmazie und Molekulare Biotechnologie, Heidelberg, Germany.

RESULTS AND DISCUSSION

Ten alkaloids were unambiguously identified in the alkaloid extract of the aerial parts of *Genista tenera* by GC-MS (Table 1). Anagyrine, lupanine, cytisine, *N*-methylcytisine and *N*-formylcytisine were the major alkaloids. Their Kovats retention indexes and EI-MS fragmentation pattern were in agreement with those reported in the literature (Asres *et al.*, 1997; Sagen *et al.*, 2002). Anagyrine and lupanine have already been reported in *Genista ephedroides* (Pistelli *et al.*, 2001). The minor compounds were identified by comparison of their corresponding data with those reported in the literature, namely dehydrocytisine (El-Shazly *et al.*, 1996), 5,6-dehydrolupanine and rhombifoline (Asres *et al.*, 1997; Sagen *et al.*, 2002), aphylline and thermopsine (Wink *et al.*, 1983). Dehydrocytisine, 5,6-dehydrolupanine, rhombifoline, aphylline and thermopsine were detected only in trace amounts by GC-MS. The quantitative pattern of the major alkaloids as determined by GC is given in Table 1.

Lupanine, *N*-methylcytisine and sparteine possess hypoglycaemic activity (Kinghorn and Balandrin, 1984; Wink, 1993a, b). Two of these active principles are reported as the major alkaloids of the aerial parts of *Genista tenera*, namely lupanine and *N*-methylcytisine. Although some quinolizidine alkaloids are known for their toxicity, biomedical applications of plant toxins including anagyrine have recently been reported (James *et al.*, 2004).

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