

QUINOLIZIDINE ALKALOIDS IN *PETTERIA RAMENTACEA* AND THE INFESTING APHIDS, *APHIS CYTISORUM*

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Key Word Index—*Petteria ramentacea*; Leguminosae; leaves; fruits; stems; quinolizidine alkaloids; cytisine; *Aphis cytisorum*; aphid feeding.

Abstract—Alkaloid extracts of *Petteria ramentacea* (leaves, fruits, stem) contained cytisine, *N*-methylcytisine, anagryrine as major alkaloids, and lupanine, 5,6-dehydrolupanine and rhombifoline as minor constituents. Aphids (*Aphis cytisorum*) feeding on this plant accumulated cytisine in a concentration of 21 mmol/kg fresh weight (0.4%).

INTRODUCTION

Quinolizidine alkaloids are widely distributed in the Leguminosae [1], and isolated occurrences have also been recorded from a number of unrelated families [2, 3]. They are not inert end products of metabolism, but display an active turnover in lupin plants and cell cultures [2, 4]. We have studied their possible function in plants in detail [2, 5–8] and have come to the conclusion that these alkaloids play an important part in the chemical defense of lupins against herbivores and also against bacteria and fungi [2, 3, 8].

It has been shown in a number of cases that the chemical defense of plants is not absolute, but has usually been overcome in nature by a few specialists, which either sequester the secondary compounds or find a way to store and even use these compounds for their own defense [9]. However, compared to the number of potential pathogens which surround a plant, the number of specialists is usually rather small [9]. Whereas lupins of low alkaloidal content, the so-called 'sweet' lupins are challenged by a relatively large number of pests [3, 10, 11], bitter, alkaloid-rich lupins have only a small number of specialized pathogens.

We have recently described that aphids are generally repelled by quinolizidine alkaloids [12], because they are translocated in the phloem sap [4], but that alkaloid-rich broom plants, *Cytisus scoparius*, are infested by two aphid species, *Aphis cytisorum* [12] and *Acyrtosiphon spartii* [13]. We were able to show that *Aphis cytisorum*, which infested *C. scoparius*, contained a series of alkaloids, which were also present in the host plant [12].

In this communication we report on the alkaloid patterns of *Petteria ramentacea* and of *Aphis cytisorum* which also infests this legume species.

RESULTS AND DISCUSSION

Alkaloid extracts of leaves, green fruits, stem bark and stem wood of *Petteria ramentacea* Presl. were analysed by capillary GC and GC-MS, which are suitable for the analysis of complex mixtures [8, 14, 15]. Six alkaloids could be identified in these extracts (Fig. 1, Table 1), with 5,6-dehydrolupanine and rhombifoline as new alkaloids for this species. The others have already been recorded from this species before [16]. Similar to other scrubby

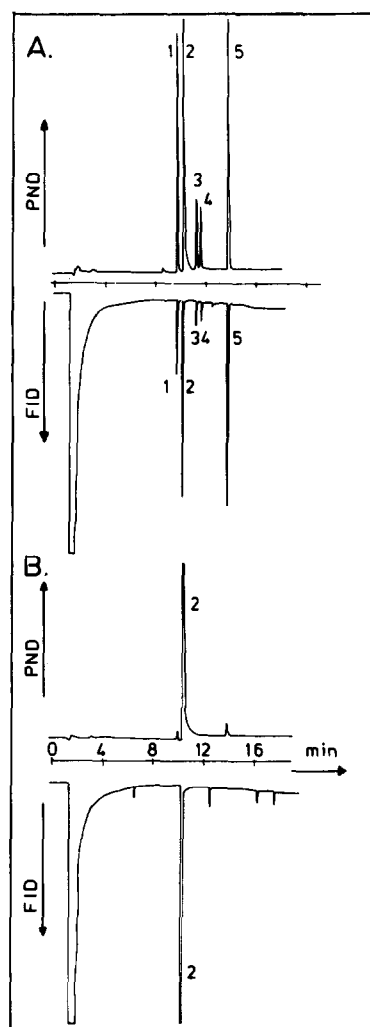


Fig. 1. Separation of alkaloid extracts from *Petteria ramentacea* stem bark (A) and *Aphis cytisorum* (B) by capillary GC. PND = nitrogen detector, FID = flame ionization detector. Numbering as in Table 1.

Table 1. Alkaloid patterns of *Petteria ramentacea* plants and the infesting aphids, *Aphis cytisorum*

	Alkaloid composition (%)						Total alkaloids (mg/kg fr. wt)
	1	2	3	4	5	6	
A. <i>Petteria ramentacea</i>							
Leaves	30	55			15		390
Green fruits	6	94					1800
Stem							
Cortex	17	32	6	2	43	+	1200
Lignum	50	21	+	+	29		160
B. <i>Aphis cytisorum</i>	+	100					4020

Alkaloids were extracted from plants and aphids according to standard procedures (see Experimental). The alkaloid mixtures were analyzed by capillary GC and capillary GC-MS. 1 = *N*-methylcytisine, 2 = cytisine, 3 = 5,6-dehydrolupanine, 4 = lupanine, 5 = anagrine, 6 = rhombifoline; + = traces.

legumes, e.g. *Cytisus scoparius*, *Laburnum anagyroides* [8, 14, 17, 18], alkaloid content of the stem bark and of the fruits was higher than those of leaves and stem wood.

The plants which we investigated were infested by a large number of aphids (*Aphis cytisorum* Hartig). We collected a few grams of immature and mature aphids and have analyzed their alkaloid composition. As can be seen from Fig. 1 and Table 1, the aphids contained cytisine in a high concentration (0.4% or 21 mmol/kg fresh weight). It is confirmed by this finding that alkaloid transport takes place in the phloem [4, 12], since aphids are known to exploit the phloem sap only. The results imply that cytisine is the major alkaloid component which is translocated. It cannot be ruled out at the present stage that the cytisine found in the aphids derived from *N*-methylcytisine by demethylation in the insect gut.

The alkaloid concentration found in the aphids is rather high compared to that of the plant. We have previously shown that pyridone-type alkaloids, e.g. cytisine, have a wide range of biological activities [2, 8, 7]. Cytisine is known as the toxin responsible for the toxicity of *Laburnum* seeds. It repels snails [7] and inhibits growth of seedlings [6]. Half-maximal inhibitory concentrations were in the range of 1–5 mM. It is therefore likely that the aphids, which store cytisine, become toxic themselves. This could be of advantage since aphids are preyed upon by a number of carnivores, e.g. insects and birds.

EXPERIMENTAL

Plant material. *Petteria ramentacea* Presl. plants were grown in the Botanical Garden of Marburg. Plant material was harvested in September 1984.

Alkaloid extraction. Plant material and aphids were homogenized in 0.5 M HCl, made alkaline with 6 N NaOH and applied onto an Extrelut (Merck) column. Alkaloids were eluted with CH₂Cl₂ and concd in a rotavapor.

Alkaloid analysis. Alkaloid extracts were separated by capillary GC on a J & W DB-5 column (0.32 mm × 30 m) employing a Perkin-Elmer gas chromatograph (Sigma 1b). Parallel detection by flame ionization (FID) and nitrogen specific detectors (PND). Carrier gas: Helium; on column injection (J & W injector); oven: 90–170° with 40°/min; 170–300° with 10°/min.

Capillary GC/MS. GC/MS measurements were performed with a Kratos MS 30 instrument as described in refs [14, 18].

Alkaloid identification. These were identified by means of their

specific retention indices [14] and mass spectral fragmentation [8, 14, 15], data which clearly distinguish different stereoisomers: *N*-Methylcytisine (1): RI = 1955; M = 204; ions (relative abundance %): 204 (30), 160 (5), 146 (5), 58 (100). Cytisine (2): RI = 1995; M = 190; 190 (80), 160 (20), 147 (100), 146 (80), 34 (20), 82 (20). 5,6-Dehydrolupanine (3): RI = 2130; M = 246; 246 (60), 148 (10), 135 (10), 98 (100). Lupanine (4): RI = 2170; M = 248; 248 (85), 219 (10), 149 (60), 136 (100), 98 (25). Anagrine (5): RI = 2373; M = 244; 244 (40), 160 (10), 146 (10), 134 (10), 98 (100). Rhombifoline (6): RI = 2155; M = 244; 244 (3), 203 (35), 160 (10), 146 (10), 58 (100).

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