The major alkaloids of the genus *Melolobium*

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The major alkaloids of 12 species of the genus *Melolobium* Eckl. & Zeyh. have been characterized. Variable quantities of anagyrine, camoensine, leontidine, lupanine, *N*-methylcytisine and thermopsine are present in nearly all of the extracts. Sparteine, cytisine and some unidentified alkaloids occur less frequently. If one species [*Melolobium involucratum* (Thunb.) Stirton] is excluded, the genus is morphologically very uniform and also appears to be uniform in its alkaloidal metabolites. Within the tribe Crotalarieae, the combination of thermopsine and the two C_{14} alkaloids leontidine and camoensine may prove to be a unique chemotaxonomic character for *Melolobium*.

Die hoof alkaloïede van 12 spesies van die genus *Melolobium* Eckl. & Zeyh. is gekarakteriseer. Variërende hoeveelhede anagirien, camoensien, leontidien, lupanien, *N*-metielcitisien en thermopsien was teenwoordig in feitlik al die ekstrakte. Sparteien, citisien en enkele ongeïdentifiseerde alkaloïede kom minder algemeen voor. As een spesie [*Melolobium involucratum* (Thunb.) Stirton] buite rekening gelaat word, is die genus morfologies baie eenvormig en skynbaar ook eenvormig in akaloïedmetaboliete. In die tribus Crotalarieae, mag dit blyk dat die kombinasie van thermopsien en die twee C_{14}-alkaloïede leontidien en camoensien, 'n unieke chemotaksonomiese kenmerk vir *Melolobium* is.

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Introduction

In recent review papers on the distribution of alkaloids in the Leguminosae (Mears & Mabry 1971; Salatino & Gottlieb 1980, 1981; Kinghorn & Smolenski 1981), conclusions for the tribe Crotalarieae are based on reports from only two genera (*Lebeckia* Thunb. and *Crotalaria* L.). A preliminary survey (van Wyk *et al.* 1988) has shown the presence of alkaloids in several other genera. The possibility to gain new insights into generic relationships within this predominantly southern African tribe was indicated.

As part of a continuing study of alkaloids in the Crotalarieae, we investigated the genus *Melolobium* Eckl. & Zeyh. (a genus of *ca.* 20 species endemic to southern Africa) and identified the major alkaloids of 12 different species.

Materials and Methods

Voucher specimens of the plant material used in this study are listed in the appendix. The samples consisted of air-dried leaves and twigs, some with flowers and fruit as indicated.

Melolobium subspicatum Conrath was chosen for the isolation and identification of its major alkaloids since extracts of this species contained nearly all of the alkaloids observed in other species. The major alkaloids were isolated, identified and then used as reference samples in the wider survey of several other species.

Extraction methods used are as previously described (van Wyk *et al.* op. cit.; van Wyk & Verdoorn 1988). All crude extracts were purified by elution through columns of Dowex 50 resin prior to gas chromatography. The major alkaloids of *M. subspicatum* were isolated by column chromatography as previously described and the alkaloids identified by m.p., specific rotation, as well as IR, ^1H NMR, ^{13}C NMR and MS spectrometry. The identity of camoensine was confirmed by characterization of the hydrogenated product (camoensidine). All the spectroscopical data obtained was in correlation with literature data (Leonard 1960; Santamaria & Khuong-Huu 1975). GC spectra were obtained with a DB-1 fused silica capillary column (length 15 m, internal diameter 0,25 mm) with PND/FID parallel detection. (Temperature programme: 150°C 2-min isotherm, 10°C min^{-1} to 250°C, 20°C min^{-1} to 300°C, isotherm 5 min; injector 250°C, PND-detector 310°C, split 1:30, flow 2,4 ml min^{-1}, helium as carrier gas at 80 kPa, H_2 as make-up gas for PND at 60 kPa). The GC results confirmed earlier identifications by analytical TLC.

Results and Discussion

Table 1 shows the yields of alkaloidal extract obtained after purification and the estimated total number of alkaloids present in each sample. Major alkaloids, minor alkaloids and traces were taken as those with concentrations of more than 10%, less than 10% and less than 1% of the total extract respectively. The distribution of major alkaloids in 12 species of *Melolobium* as determined by gas chromatography is shown in Table 2. Only those alkaloids that occur as a major component in at least one of the species were

Table 1 Total yields and estimated number of alkaloids extracted from 12 species of *Melolobium*

		Estimated number[b] of alkaloids:			
Species	Total yield[a] ($\mu g\ g^{-1}$ dry wt)	Major (>10%)	Minor (>1%)	Traces (<1%)	Total
M. aethiopicum	1253	2	5	3	10
M. alpinum	445	2	3	10	15
M. cf. *burchellii*	600	1	4	5	10
M. candicans	100	2	4	5	11
M. canescens	149	3	8	3	14
M. exudans	822	2	4	10	16
M. microphyllum	67	3	4	3	10
M. obcordatum	192	2	8	2	12
M. stipulatum	193	2	6	7	15
M. subspicatum	396	3	3	8	14
M. wilmsii	1380	3	2	7	12
M. involucratum[c]	387	4	2	13	19

[a]Yield figures are for purified alkaloidal extracts
[b]Estimated from GC results
[c]Sparteine is taken as a major alkaloid for reasons explained in the text

Table 2 Distribution of alkaloids in extracts of 12 species of *Melolobium* as determined by gas chromatography

Species	Distribution of major alkaloids (% of total alkaloid yield)												
	spar	lupa	anag	ther	leon	camo	cyt	m-cyt	X1	X2	X3	X4	X5
M. aethiopicum		10	7	tr	25	52		tr	3	2		2	
M. alpinum		1	7	tr	29	54	tr?	tr	tr	tr		1	
M. cf. *burchellii*		1	4	1	4	88		tr	tr	tr		2	
M. candicans		20	71	2	tr	1		1	1	1	1		
M. canescens		19	4	1	tr	tr		2	39	2	7	2	
M. exudans	tr?	3	22	tr	6	63	tr	tr		1	tr	2	
M. microphyllum		2	21	tr	tr	10	tr	5			56		
M. obcordatum		9	28	5	1	42		2	5		tr	4	
M. stipulatum		19	4	1	tr	69		tr	tr	2	2	1	
M. subspicatum		tr	11	26	6	8		41	tr	tr		5	
M. wilmsii		1	2	38	2	21		tr	tr	tr		36	
M. involucratum	tr	6	23	tr?	tr	tr	1	28	tr	1			33

Abbreviations: spar = sparteine, lupa = lupanine, anag = anagyrine, ther = *l*-thermopsine, leon = leontidine, camo = camoensine, cyt = cytisine, m-cyt = *n*-methylcytisine, X1 = 4-OH-lupanine?, X2 = 5,6-dehydrolupanine?, X3 = ammodendrine?, X4 = unidentified, X5 = unidentified

entered. These results closely corresponded with earlier identifications made by analytical thin-layer chromatography.

Lupanine, anagyrine, thermopsine, leontidine, camoensine and *N*-methylcytisine are clearly the most common alkaloids in *Melolobium*. Within the Crotalarieae this combination, and especially the common occurrence of thermopsine, camoensine and leontidine may prove to be a unique chemotaxonomic character for *Melolobium*. The two C_{14} alkaloids camoensine and leontidine were previously known only from *Camoensia maxima* Welw. ex Benth. of the tribe Sophoreae (Santamaria & Khuong-Huu op. cit.). Sparteine and cytisine are virtually absent, while other alkaloids provisionally identified as ammodendrine, 4-OH-lupanine (nuttalline) and 5,6-dehydrolupanine seem to occur only in some of the species. Small amounts of isolupanine and 17-oxo-lupanine (not shown in Table 2) appear to be present in *M. alpinum* and *M. canescens* respectively.

Melolobium involucratum, an anomalous species recently transferred from *Argyrolobium* (Stirton 1986) seems to differ from other species of *Melolobium*. Its major alkaloid (X5 in Table 2) does not occur in any of the other species; thermopsine, leontidine and camoensine are absent or virtually absent, while sparteine and cytisine are present. The apparently low concentration of sparteine in *M. involucratum* resulted from a loss of this alkaloid during purification of the extract. This is the only significant discrepancy between the GC results and our earlier TLC results — the latter clearly showed the presence of large quantities of sparteine in both the crude alkaloidal extract and in the methanolic eluent from the resin. The loss of sparteine may be explained by its low basicity compared to other alkaloids (K_1 value of $5{,}7 \times 10^{-3}$ compared to $7{,}7 \times 10^{-7}$ of cytisine, for example). Morphological and cytological evidence strongly suggest that *M. involucratum* is misplaced in *Melolobium* (van Wyk & Schutte, unpublished data). A comparison with the alkaloids of other genera may indicate a more natural position for this species. *Melolobium canescens, M. microphyllum* and *M. wilmsii* also differ from other species in the presence of respectively nuttalline, ammodendrine and an unidentified alkaloid (X4) as the major alkaloids. These alkaloids do however occur as minor compounds elsewhere.

Tetracyclic and tricyclic α-pyridone alkaloids are known to be formed along a biogenetic pathway which starts with lupanine and has methylcytisine as end product (Nowacki & Waller 1977; Salatino & Gottlieb 1980). Each step along the pathway involves a specific enzyme system and leads to an enhancement of alkaloid toxicity, so that cytisine-bearing genera are considered to be phylogenetically advanced (Salatino & Gottlieb op. cit.). The presence of several such specialized quinolizidine alkaloids of the cytisine type in the tribe Crotalarieae is here reported for the first time. Some modifications to previously proposed phylogenetic relationships in the Papilionoideae (Salatino & Gottlieb 1980, 1981) are therefore necessary. An additional route of adaptive radiation is suggested, namely a southern temperate 'cytisine' route. This new route presumably represents an extension of a southern temperate 'pre-cytisine' route proposed by Salatino & Gottlieb (1980).

It is not yet clear how *Melolobium* relates to other genera of the tribe Crotalarieae. The particular combination of alkaloids that occurs in this genus however, may prove to be a unique chemotaxonomic character. The absence of sparteine and the presence of camoensine and leontidine as major alkaloids seem particularly significant. Our results clearly indicate that alkaloids have considerable taxonomic value at the generic level and that it may eventually lead to a better understanding of relationships amongst genera of the Crotalarieae and Genisteae.

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Appendix 1 Plant material of *Melolobium* species used for alkaloid extraction. Voucher specimens are all in the Rand Afrikaans University Herbarium (JRAU)

M. aethiopicum (L.) Druce: Velddrif Rd., 24 km from Cape Town, *Van Wyk 2685*, flowering twigs. *M. alpinum* Eckl. & Zeyh.: Sani Pass, Natal, *Van Wyk 2631*, mostly vegetative twigs. *M.* cf. *burchellii* N.E. Br.: Noupoortnek, 2 km from Clarens, O.F.S., *Schutte 393*, flowering twigs. *M. candicans* (E. Mey.) Eckl. & Zeyh.: ± 20 km from Cathcart to Stutterheim, E. Cape, *Koekemoer s.n.*, flowering and fruiting twigs. *M. canescens* Benth.: Bloemfontein, O.F.S., *Van Wyk 2714*, flowering twigs. *M. exudans* Harv.: Velddrif Rd., ± 20 km from Cape Town, *Van Wyk 2683*, flowering twigs. *M. involucratum* (Thunb.) Stirton: Blomfontein Farm, Calvinia district, NW Cape, *Steenkamp sub Schutte 396*, fruiting twigs. *M. microphyllum* (L.f.) Eckl. & Zeyh.: Naudesberg Pass near Graaff Reinet, Cape, *Van Wyk 2634*, flowering twigs. *M. obcordatum* Harv.: Noupoortnek, 2 km from Clarens, O.F.S., *Schutte 394*, mostly vegetative twigs. *M. stipulatum* Harv.: Verkeerdevlei near Touw's River, Cape, *Van Wyk 2711*, vegetative twigs. *M. subspicatum* Conrath: Irene, Transvaal, *De Beer 38*, flowering twigs. *M. wilmsii* Harms: Hendrina, Transvaal, *Van Wyk 2624*, fruiting twigs.