The major alkaloids of the genus *Polhillia*

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The major alkaloids of three species of *Polhillia* Stirton and three species of *Argyrolobium* Eckl. & Zeyh. have been identified. The presence of sparteine, lupanine, anagyrine and *N*-methylcytisine as major alkaloids in *Polhillia* and in the morphologically similar *Argyrolobium brevicalyx* Stirton indicates a direct phylogenetic link between *Polhillia* (Crotalarieae) and *Argyrolobium* (Genisteae). The data also supports the transfer of *Melolobium involucratum* (Thunb.) Stirton to *Polhillia*.

Die hoof alkaloïede van drie spesies van *Polhillia* Stirton en drie spesies van *Argyrolobium* Eckl. & Zeyh. is geïdentifiseer. Die voorkoms van sparteïen, lupanien, anagirien en *N*-metielcitisien as hoof alkaloïede van *Polhillia* en van die morfologies-eenderse *Argyrolobium brevicalyx* Stirton dui op 'n direkte filogenetiese verband tussen *Polhillia* (Crotalarieae) en *Argyrolobium* (Genisteae). Die data ondersteun ook die oorplasing van *Melolobium involucratum* (Thunb.) Stirton na *Polhillia*.

Keywords: *Argyrolobium*, chemotaxonomy, generic relationships, *Polhillia*, quinolizidine alkaloids

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Introduction

The genus *Polhillia* was recently described by Stirton (1986a) and comprises 5 rare species of woody shrubs endemic to the south-western Cape Province of South Africa. Based on presumed affinities with *Melolobium* Eckl. & Zeyh. and *Dichilus* DC., the new genus was placed in the tribe Crotalarieae.

In view of obvious morphological similarities with *Argyrolobium* Eckl. & Zeyh., and especially the anomalous *A. brevicalyx* Stirton (1984), we investigated the major alkaloids of three species of *Polhillia* and some species of *Argyrolobium*. *Melolobium involucratum* (Thunb.) Stirton was also included in the study because its major alkaloids were previously shown to be different from those of other *Melolobium* species (van Wyk *et al.* 1988a).

Material and Methods

The species studied and voucher specimens of the material used for alkaloid extraction are listed in the appendix. Authorities for names are not repeated elsewhere.

Methods of extraction and identification are as previously described (van Wyk *et al.* 1988a, b; van Wyk & Verdoorn 1988). All reference samples used in analytical TLC and GC were fully characterized by IR, ^{1}H NMR, ^{13}C NMR and MS spectrometry.

Results and Discussion

The estimated number of alkaloids present in each of the samples and the total yields obtained are shown in Table 1. *Polhillia canescens, P. pallens, P. waltersii, Melolobium involucratum* and *Argyrolobium brevicalyx* (all of which are morphologically similar) seem to differ from *A. crassifolium* and *A. variopile* in both the diversity and total number of alkaloids. The latter two species have only one major alkaloid each, while the others have at least three. The difference is even more distinct if the yields of major alkaloids are taken as percentages of the total (Table 2). The sample size does not allow generalizations (especially for *Argyrolobium*), but it is clear from Table 2 that the species are all rather similar. Anagyrine is by far the most common alkaloid. *N*-methylcytisine, sparteine and lupanine occur at least in trace quantities in all of the species, while cytisine, lusitanine and two unidentified alkaloids

Table 1 Total yields and estimated number of alkaloids extracted from species of *Argyrolobium, Polhilla* and *Melolobium*

Species	Total yield[a] ($\mu g\ g^{-1}$ dry wt)	Estimated number[b] of alkaloids: Major (> 10%)	Minor (> 1%)	Traces (< 1%)	Total
Polhillia canescens	478	3	6	5	14
P. pallens	241	4	1	10	15
P. waltersii	987	4	8	5	17
M. involucratum	387	4	2	13	19
Argyrolobium brevicalyx	731	5	2	11	18
A. crassifolium	590	1	5	6	12
A. variopile	976	1	6	5	12

[a]Yield figures are for purified alkaloidal extracts
[b]Estimated from GC results

Table 2 Distribution of major alkaloids in extracts of *Polhillia* spp., *Argyrolobium* spp. and *Melolobium involucratum* as determined by gas chromatography

Species	Distribution of major alkaloids[a] (% of total alkaloid yield) spar	lupa	anag	cyt	m-cyt	lusi	X1	X2
Polhillia waltersii	1	9	52	13	11	5		tr?
P. pallens	tr	27	25	4	42			tr
P. canescens	tr	4	57	7	24	tr		
Melolobium involucratum	tr	6	23	1	28		3	33
Argyrolobium brevicalyx	1	16	40	tr	18		19	tr
A. crassifolium	tr	tr	90		1			
A. variopile	tr	1	89		2		tr	

Abbreviations: spar = sparteine, lupa = lupanine, anag = anagyrine, cyt = cytisine, m-cyt = *N*-methylcytisine, lusi = lusitanine, X1 & X2 = unknown
[a]Yield figures for sparteine are totally underestimated except in *Argyrolobium crassifolium* and *A. variopile* (see Figure 1). Sparteine was lost from the crude extracts during resin purification — the poor adsorbsion of this alkaloid on cationic exchange resin may be explained by its low basicity compared to the other alkaloids

appear to be less common.

All available information on the major alkaloids of the genera *Lebeckia, Polhillia, Argyrolobium* and *Melolobium* is summarized in Table 3. The presence of minor alkaloids and unidentified major alkaloids is not shown. Sparteine is entered as a major alkaloid despite the low yield figures. The loss of this alkaloid from the crude alkaloidal extracts is explained in the footnotes of Table 2. It is clear that the present generic boundaries do not conform to the alkaloid data. The major alkaloids of *Polhillia canescens, P. pallens, P. waltersii, Melolobium involucratum* and *Argyrolobium brevicalyx* are almost identical. A reappraisal of the circumscription of *Polhillia* seems necessary, as morphological and cytological evidence (van Wyk & Schutte, unpublished data) strongly correlate with the distribution of alkaloids. Our results support the initial idea of Stirton (1986b) to include *Melolobium involucratum* in *Polhillia* rather than the transfer to *Melolobium*. Figure 1 shows the result of thin-layer chromatography of crude alkaloidal extracts of some of the species. It is clear that there are distinct differences between the genera but that the major alkaloids of *Argyrolobium brevicalyx, Polhillia pallens* and *Melolobium involucratum* are very similar.

The position of *Argyrolobium* in the tribe Genisteae is somewhat uncertain (Polhill 1976, 1981). Some of the species treated here are of particular interest in that they may help explain the origins and affinities of *Argyrolobium*. *Argyrolobium brevicalyx* for example, is presently a member of the tribe Genisteae but it is morphologically and cytologically almost identical to the species of *Polhillia* (tribe Crotalarieae). The alkaloids of this species indeed strongly indicate that it should also be transferred to *Polhillia*. Attempts by Salatino & Gottlieb (1980, 1981) to link *Argyrolobium* to *Lupinus* L. and *Sarothamnus* Wimm. have in our opinion rather shown the opposite, as is evidenced by the isolated position of *Argyrolobium* in their affinity diagram (Figure 2 in Salatino & Gottlieb 1981). A connection with *Lebeckia* seems much more likely. The data for *Lebeckia* is incomplete and a more detailed survey of this genus is necessary, especially since it is considered to be one of the basal groups of the tribe Crotalarieae (Polhill 1981). The absence of *N*-methylcytisine, anagyrine and cytisine needs to be confirmed. Biogenetic pathways leading to sparteine and cytisine are now well known and the ability to transform ring A into a pyridone is considered to be an advanced character (Nowacki & Waller 1977; Salatino & Gottlieb 1980). Shrubby and tree species of the Papilionoideae tend to contain sparteine, while derived ones contain more elaborate compounds such as methylcytisine (Nowacki & Waller op. cit.). The common occurrence of α-pyridone alkaloids in southern African genera of the Crotalarieae was not known before, and opens up the possibility of a southern origin for *Argyrolobium*. The species around *Polhillia* thus seem to form a connecting link between *Lebeckia* and *Argyrolobium*, since they contain both phylogenetically primitive sparteine- and lupanine-type alkaloids and advanced cytisine-type alkaloids. In *Melolobium* and the less woody species of *Argyrolobium* the trend appears to be one of increasing specialization in a cytisine-type chemistry. Large amounts of anagyrine and cytisine are characteristic of *Argyrolobium*, while *Melolobium* shows a predominance of thermopsine and the two C_{14} alkaloids leontidine and camoensine.

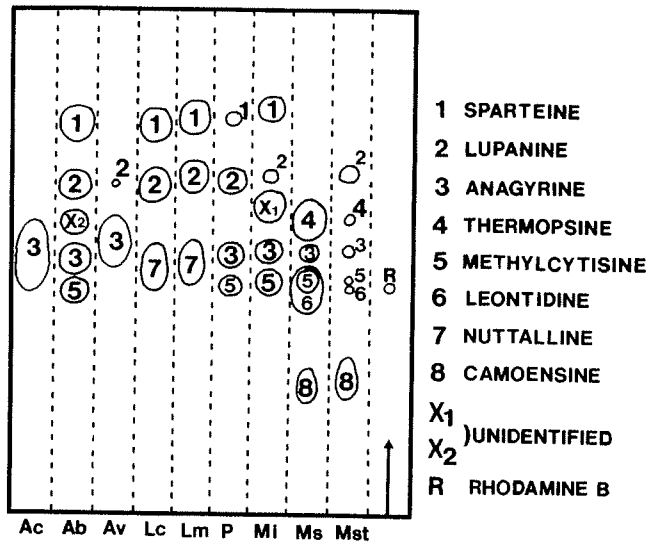

Figure 1 Major alkaloids of some species of *Argyrolobium, Lebeckia, Polhillia* and *Melolobium* as observed by thin-layer chromatography (silica gel: eluent cyclohexane–$CHCl_3$–Et_2 NH, 50:40:10, detecting reagent iodoplatinate). Ac, *Argyrolobium crassifolium*; Ab, *A. brevicalyx*; Av, *A. variopile*; Lc, *Lebeckia cytisoides* Thunb.; Lm, *L. multiflora* E. Mey.; P, *Polhillia pallens*; Mi, *Melolobium involucratum*; Ms, *M. subspicatum* Conrath; Mst, *M. stipulatum* Harv.

Table 3 Distribution of 11 major alkaloids known from the genera *Lebeckia, Polhillia, Argyrolobium* and *Melolobium*. [Data for *Lebeckia* and *Melolobium* from Gerrans *et al.* (1976) and van Wyk *et al.* (1988a, b). Cytisine occurs as a major alkaloid in seeds of *Argyrolobium saharae* Pomel and lusitanine in seeds of *A. zononii* (Turra) P.W. Ball (Greinwald, unpublished data); argyrolobine was reported as the major alkaloid of *A. megarhizum* H. Bol. (Tsuda & Marion 1964)]

	Lebeckia	*Polhillia*	*Melolobium involucratum*	*Argyrolobium brevicalyx*	*Argyrolobium* (other spp.)	*Melolobium* (other spp.)
Nuttalline	+++					+?
Sparteine	+++	+++	+++	+++		
N-methylcytisine		+++	+++	+++		+
Lupanine	+++	++	++	++		++
Anagyrine		+++	+++	+++	++	++
Cytisine		+			+	
Lusitanine					+	
Argyrolobine					+	
Thermopsine						+
Camoensine						++
Leontidine						+

Occurs as a major component in: +++ all species/samples, ++ most species/samples, + at least some species/samples

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

Argyrolobium brevicalyx Stirton: Uitvlug Farm, between Bredasdorp & Swellendam, *Van Wyk 2134*, flowering twigs. *A. crassifolium* Eckl. & Zeyh.: Zuurberg, E. Cape, *B & M Van Wyk 2115*, flowering twigs. *A. variopile* N. E. Br.: Volksrust, Transvaal, *Schutte 364*, flowering twigs. *Melolobium involucratum* (Thunb.) Stirton: Blomfontein Farm, Calvinia district, NW Cape, *Steenkamp sub Schutte 396*, fruiting twigs (fruit not included). *Polhillia canescens* Stirton: Adoonskop, between Bredasdorp & Swellendam, *Van Wyk 2709*, vegetative twigs. *P. pallens* Stirton: Remhoogte, between Bredasdorp & Swellendam, *Van Wyk 2708*, flowering twigs. *P. waltersii* (Stirton) Stirton: Worcester commonage, *Van Wyk 2701*, flowering twigs.