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The major alkaloids of the genus *Argyrolobium*

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The major alkaloids of 12 morphologically dissimilar species of *Argyrolobium* Eckl. & Zeyh. have been identified. All extracts contain large quantities of anagyrine as the dominant alkaloid. Cytisine, *N*-methylcytisine, lupanine, sparteine, 5,6-dehydrolupanine and ammodendrine are present as minor compounds in most of the species and as major compounds only in a few of them. The data do not reflect morphological dissimilarities and the species are remarkably uniform in their major alkaloids. Our conclusions are that *Argyrolobium* is closely related to the genus *Polhillia* Stirton, that large quantities of anagyrine may be taken as a chemotaxonomic marker for *Argyrolobium* and that a more detailed study of alkaloids may provide valuable taxonomic evidence in a genus without any obvious morphological specializations.

Die alkaloïede van 12 morfologies-verskillende spesies van *Argyrolobium* Eckl. & Zeyh. is geïdentifiseer. Alle ekstrakte bevat anagirien as die dominante alkaloïed. Sitisien, *N*-metielsitisien, lupanien, sparteïen, 5,6-dehidrolupanien en ammodendrien is teenwoordig as ondergeskikte verbindings in meeste van die spesies en as hoof verbindings in slegs 'n paar van hulle. Die data weerspieel nie morfologiese verskille nie en die spesies is merkwaardig eenvormig in hul hoof alkaloïede. Ons gevolgtrekkings is dat *Argyrolobium* naverwant is aan die genus *Polhillia* Stirton, dat groot hoeveelhede anagirien as 'n chemotaksonomiese merker vir *Argyrolobium* beskou kan word en dat 'n meer volledige studie van alkaloïede waardevolle taksonomiese getuienis mag bied in 'n genus sonder enige ooglopende morfologiese spesialisasies.

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

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Introduction

The genus *Argyrolobium* Eckl. & Zeyh. comprises an estimated 70 species of papilionoid legumes, 50 or more of which occur in southern Africa. There is no convincing evidence to support its traditional position in the tribe Genisteae and a transfer to the predominantly southern African tribe Crotalarieae has been suggested (Polhill 1976, 1981; van Wyk & Schutte 1989). It has also been shown that the alkaloids of some *Argyrolobium* species are similar to those of other genera of the Crotalarieae, notably *Polhillia* Stirton and *Melolobium* Eckl. & Zeyh. (van Wyk *et al.* 1988a, b). In this study we investigated 12 morphologically dissimilar species of *Argyrolobium* to evaluate the taxonomic significance of alkaloids at the generic and infrageneric level.

Materials and Methods

To obtain a reasonable representation of the variation in the genus, species from different sections (Harvey 1862) were chosen. It was assumed that if all these species turn out to be similar in their major alkaloids, then at least a preliminary characterization of the genus would be possible.

The taxonomy of *Argyrolobium* is in such a state of confusion that some of the material could not be positively identified to species. Care was taken to keep voucher specimens of all the samples for future reference and verification. The species, authorities for names, and voucher specimens of the material used are listed in the appendix.

Methods of extraction and identification were as previously described (van Wyk *et al.* 1988a, b; van Wyk & Verdoorn 1988). The extracts were all purified by ion exchange resin (Dowex 50W H^+ form). All reference samples used in analytical TLC and GC were fully authenticated by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. Identifications by analytical TLC and GC were confirmed by GC-MS studies of three extracts (two samples of *A. tomentosum* and one of *A. frutescens*).

Results and Discussion

Table 1 shows the yields of purified alkaloidal material obtained and the distribution of major alkaloids in 15 different samples. All the major alkaloids (>10% of total yield) and most of the minor ones (<10%) could be positively identified. The presence of unidentified minor compounds is not shown.

The distribution of alkaloids is surprisingly uniform and the variation is much less than expected. Anagyrine is by far the most common alkaloid and rarely represents less than half of the total yield. When the concentration of anagyrine is relatively low, large quantities of cytisine or *N*-methylcytisine are usually present. Lupanine, sparteine, 5,6-dehydrolupanine, ammodendrine and an alkaloid tentatively identified as aphylline are very often present but rarely as major compounds.

Our results differ from those of Tsuda & Marion (1964), who reported argyrolobine and aphyllidine as major alkaloids of *Argyrolobium megarhizum* H. Bol. Argyrolobine has never been reported since and no

Table 1 Yield and distribution of major alkaloids in 15 extracts from 12 species of *Argyrolobium*

Species	Material extracted (V)egetative twigs (F)lowering twigs	Total yield (mg g^{-1} dry wt)[a]	Distribution of major alkaloids (% of total alkaloid yield)[b]							
			anag	cyt	m-cyt	lupa	spar	5,6-deh	ammo	aphy
A. crassifolium										
sample 1	F	1,05	98	1	tr	tr	tr	tr	tr	–
sample 2	F	0,20	84	3	1	tr	–	2	–	–
A. frutescens	V	0,01	50	5	1	8	6	6	3	3
A. lanceolatum										
sample 1	F	1,33	96	tr	tr	2	tr	1	tr	–
sample 2	V	1,46	97	tr	tr	tr	1	tr	–	tr
A. lunare	F	0,24	56	3	5	21	3	3	–	tr
A. molle	F	0,10	41	3	2	4	34	2	11	–
A. rupestre	V	0,05	58	6	2	2	3	2	1	1
A. sankeyi	F	0,01	61	2	tr	6	4	6	1	1
A. speciosum	V	0,03	75	tr	19	2	1	2	tr	tr
A. tomentosum										
sample 1	F	0,60	79	10	7	1	–	tr	tr	1
sample 2	F	0,27	25	36	6	1	7	tr	tr	2
A. sp. cf. *A. tysonii*	F	0,03	53	16	2	2	12	9	tr	–
A. variopile	F	0,38	92	tr	tr	1	2	tr	–	–
A. velutinum	V	0,06	18	35	34	3	2	tr	3	1

Abbreviations: anag = anagyrine, cyt = cytisine, m-cyt = *N*-methylcytisine, lupa = lupanine, spar = sparteine, 5,6-deh = 5,6-dehydrolupanine, ammo = ammodendrine, aphy = aphylline?

[a]Yield figures are for purified extracts

[b]Estimated from GC results

voucher specimens of the plant which yielded this alkaloid could be traced. The reported presence of cytisine and *N*-methylcytisine agrees with our results, but the conspicuous absence of anagyrine and the presence of argyrolobine and aphyllidine as major alkaloids in *A. megarhizum* should be verified.

The uniformity in major alkaloids (much less variable than in *Melolobium*, for example) supports Polhill's (1976) conclusion that *Argyrolobium* is a uniform and natural genus. The relatively high yield figures and at least some indications of specific differences suggest that a more detailed survey may provide useful information about infrageneric relationships. There are no obvious morphological specializations (Polhill 1976) and it may be difficult to devise a natural infrageneric classification on morphological evidence alone. The most conspicuous difference between the species appears to be the proportion of anagyrine relative to sparteine, lupanine, cytisine or *N*-methylcytisine. Some of the northern species of *Argyrolobium* contain cytisine and lusitanine in the seeds (van Wyk *et al.* 1988b) but our results from leaf and twig samples are not directly comparable. Different organs of a plant may produce quite different combinations of major alkaloids (Cranmer & Mabry 1966; Greinwald *et al.* 1989) so that an appropriate sampling procedure is required to show significant differences between species. Seasonal fluctuations in the biosynthesis of alkaloids should also be considered. Such variations in the production of cytisine (known to be highly toxic) have been recorded in European species of the tribe Genisteae (R. Greinwald, unpublished). The observed variation in *Argyrolobium* may therefore be partly a result of seasonal differences, but perhaps also a result of the relative proportions of stems, leaves and flowers in the samples. Due to these sample limitations, it is not yet possible to evaluate the taxonomic significance of alkaloids at the infrageneric level. Qualitative discontinuities seem unlikely, but it may be worthwhile to investigate the relatively large quantitative differences between some of the species in a more detailed study.

At the generic level, however, the results indicate that high concentrations of anagyrine (less frequently also in combination with cytisine and *N*-methylcytisine) may be taken as a chemotaxonomic marker for *Argyrolobium*. The major alkaloids are similar to those in *Melolobium* (van Wyk *et al.* 1988a) and especially very similar to those in *Polhillia* (van Wyk *et al.* 1988b). It differs from the latter in the much higher concentrations of anagyrine and the lower concentrations of sparteine, lupanine and *N*-methylcytisine. The differences between the two genera appear to be quantitative only, so that the combination of alkaloids in some species of *Argyrolobium* is rather similar to that in *Polhillia*. Thermopsine, camoensine and leontidine are highly characteristic of *Melolobium* but these alkaloids have not been found in *Argyrolobium*. Although it also has thermopsine as a

major alkaloid, the genus *Dichilus* differs from both *Argyrolobium* and *Melolobium* in the predominance of piperidyl alkaloids (van Wyk *et al.* 1988c). The combination of alkaloids in *Argyrolobium* clearly suggests a direct relationship of this genus with *Polhillia* and also appears to be a useful generic character.

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

A. crassifolium Eckl. & Zeyh.: Zuurberg, E. Cape, 16/4/87, *B. & M. van Wyk 2115* (sample 1); N. slope of Van Stadensberg, E. Cape, 24/1/87, *van Wyk 2584* (sample 2). *A. frutescens* Burtt Davy: 19 km from Nelspruit on Kaapsehoop road, E. Transvaal, 20/2/88, *van Wyk 2815*. *A. lanceolatum* Eckl. & Zeyh.: Top of Du Toit's Kloof Pass, SW Cape, 4/7/87, *van Wyk 2698* (sample 1); Top of Constantiaberg, Cape Peninsula, 16/1/88, *van Wyk 2758* (sample 2). *A. lunare* (L.) Druce: Rotary road, Hermanus, SW Cape, 7/10/86, *van Wyk 2087*. *A. molle* Eckl. & Zeyh.: Zuurberg, E. Cape, 15/7/87, *B. & M. van Wyk 2131*. *A. rupestre* (Eckl. & Zeyh.) Walp.: 19 km from Nelspruit on Kaapsehoop road, E. Transvaal, 20/2/88, *van Wyk 2819*. *A. sankeyi* Harms: 19 km from Nelspruit on Kaapsehoop road, E. Transvaal, 20/2/88, *van Wyk 2817*. *A. speciosum* Eckl. & Zeyh.: 19 km from Nelspruit on Kaapsehoop road, E. Transvaal, 20/2/88, *van Wyk 2818*. *A. tomentosum* (Andr.) Druce: Mhlahlane, Transkei, 25/1/86, *van Wyk 1756* (sample 1); Magoebaskloof, NE Transvaal, 30/6/88, *Koekemoer 107* (sample 2). Argyrolobium sp. cf. *A. tysonii* Harms: 3 km from Harrismith to Van Reenen's Pass, E. Orange Free State, 13/3/86, *van Wyk 1924*. *A. variopile* N.E. Br.: Volksrust, Transvaal, 21/2/87, *Schutte 364*. *A. velutinum* Eckl. & Zeyh.: Hills at Saldanha Bay, W. Cape, 3/7/87, *van Wyk 2697*.