Observations on the occurrence and distribution of alkaloids in some genera and species of the tribe Crotalarieae (Fabaceae)

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A general survey of alkaloids in the tribe Crotalarieae showed that useful chemotaxonomic data may be obtained. Although the pyrrolizidine alkaloids of *Crotalaria* L. have been studied in considerable detail, virtually nothing is known for the other, predominantly southern African genera. The genera *Dichilus* DC., *Lebeckia* Thunb., *Melolobium* Eckl. & Zeyh. and *Polhillia* Stirton were found to contain several alkaloids in sufficient quantities to warrant further investigation. The major alkaloids (presumably all of the quinolizidine type) seem to be characteristic for each of these genera. *Aspalathus* L., *Buchenroedera* Eckl. & Zeyh., *Lotononis* (DC.) Eckl. & Zeyh., *Pearsonia* Duemmer, *Rafnia* Thunb. and *Wiborgia* Thunb. appear to produce much smaller amounts. The possibility of obtaining useful information from these genera seems limited in view of the large quantities of plant material required to enable proper identification of the compounds. Methods of extraction and detection are described. The R_f values of the major alkaloids are given for the different thin-layer chromatographic systems used. Some preliminary identifications were confirmed by mass spectrometry.

'n Algemene opname van alkaloïede in die tribus Crotalarieae het aangetoon dat nuttige chemotaksonomiese data verkry kan word. Hoewel die pirrolisidien-alkaloïede van *Crotalaria* L. reeds deeglik bestudeer is, is feitlik niks bekend vir die ander, hoofsaaklik suidelike Afrikaanse genera nie. Daar is bevind dat die genera *Dichilus* DC., *Lebeckia* Thunb., *Melolobium* Eckl. & Zeyh. en *Polhillia* Stirton verskeie alkaloïede in genoegsame hoeveelhede bevat om verdere ondersoeke te regverdig. Die hoof alkaloïede (vermoedelik almal van die quinolisidien-tipe) blyk kenmerkend te wees van elkeen van hierdie genera. *Aspalathus* L., *Buchenroedera* Eckl. & Zeyh., *Lotononis* (DC.) Eckl. & Zeyh., *Pearsonia* Duemmer, *Rafnia* Thunb. en *Wiborgia* Thunb. produseer blykbaar veel kleiner hoeveelhede. Die moontlikheid om bruikbare inligting van hierdie genera te verkry is skynbaar beperk, aangesien groot hoeveelhede plantmateriaal nodig sal wees om die verbindings behoorlik te identifiseer. Metodes van ekstraksie en waarneming word beskryf. Die R_f-waardes van die hoof alkaloïede vir die verskillende dunlaagchromatografiese sisteme wat gebruik is, word aangegee. Sommige voorlopige identifikasies is deur massaspektrometrie bevestig.

Keywords: Alkaloids, Crotalarieae, Fabaceae, southern Africa

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Introduction

More than 350 different alkaloids have been identified from about 60 genera of the legume subfamily Papilionoideae (Mears & Mabry 1971; Kinghorn & Smoleski 1981). Quinolizidine (lupine) alkaloids are known to occur in the tribes Sophoreae, Loteae, Bossiaeeae, Podalyrieae, Liparieae, Crotalarieae, Euchresteae, Thermopsideae and Genisteae. The record for the Crotalarieae is based on a single study of *Lebeckia plukenetiana* E. Mey. (Nattrass 1973; Gerrans *et al.* 1976), which was found to contain tetracyclic alkaloids of the sparteine type. The pyrrolizidine alkaloids of the genus *Crotalaria* have been the subject of numerous studies, but surprisingly, there has not been a single report of the occurrence of quinolizidine alkaloids in this genus (Kinghorn & Smolenski *op. cit.*). If *Crotalaria* is excluded, it means that only one out of an estimated total of more than 500 species representing 16 genera has ever been studied analytically. This paper documents a survey of some genera and species to determine the occurrence of alkaloids and to find out where to concentrate future efforts.

Materials and Methods

A total of 38 samples, including 36 species and 11 genera of the Crotalarieae and two species of the tribe Genisteae were extracted in boiling methylene chloride for at least 2 days. Full details of the species and plant material used are given in Table 1. The alkaloids were extracted as salts with 2N hydrochloric acid and then re-extracted with methylene chloride after basifying the aqueous phase with ammonia. Evaporation of the solvent under reduced pressure yielded a brown viscid substance and varying quantities of a white crystalline matter. The latter was found to be very polar and immobile on silica gel thin-layer plates with the systems we used. The presence of this presumably non-alkaloid material and other impurities greatly influenced the yield. As a result, the figures in Table 1 give only an indication of relative yields and do not accurately reflect the actual quantity of alkaloids in each sample.

The crude extracts were diluted in methylene chloride (1:10 mass/vol) and chromatographed on Whatman K6F- and LK6DF silica gel plates (catalogue nos. 4861-820 and 4866-821) in the following systems:

a: cyclohexane – chloroform – diethylamine (50:40:10)
b: cyclohexane – diethylamine (90:10)
c: methanol – chloroform – ammonium (85:20:1)
d: ethyl acetate – methanol (80:20)
e: *n*-butanol – acetic acid – water (12:3:5)

The sheets were oven-dried at 100°C for 3 min, studied for fluorescence in ultraviolet light (254 and 365 nm) and then sprayed with iodoplatinate reagent. To allow for variations in adsorption, the dye Rhodamine B was co-chromatographed on all plates to standardize the R_f values (Waldi 1965).

Preliminary identifications were made by comparison with authentic samples of 6 different quinolizidine alkaloids. For mass spectrometry, pure samples of some of the major alkaloids were obtained by separation on preparative silica gel plates. No attempts were yet made to elucidate the structures of any alkaloids.

Table 1 List of material used for alkaloid extraction and yields of crude extract obtained. All samples consisted of air-dried leaves and branches. The voucher specimens are all in the Rand Afrikaans University Herbarium (JRAU)

Species	Provenances	Voucher specimens	Mass of sample (g)	Mass of extract (g)	Yield (%)
Argyrolobium crassifolium Eckl. & Zeyh.	Zuurberg, E. Cape	*B-E. & M. van Wyk 2115*	59	0,16	0,27
Argyrolobium variopile N.E.Br.	Volksrust, Transvaal	*A.L. Schutte 364*	148	0,76	0,51
Aspalathus hirta E. Mey. subsp. *hirta*	Villiersdorp, SW Cape	*B-E. van Wyk 2070*	44	0,17	0,39
Buchenroedera lotononoides Scott Elliot	Sani Pass, Natal	*B-E. van Wyk 2630*	83	0,07	0,09
Buchenroedera multiflora Eckl. & Zeyh.	Zuurberg, E. Cape	*B-E. & M. van Wyk 1523*	61	0,14	0,23
Crotalaria capensis Jacq.	Ivuna, Natal	*B-E. van Wyk 2525*	98	0,17	0,18
Dichilus gracilis Eckl. & Zeyh.	Fauresmith, Orange Free State	*A.L. Schutte 351*	125	0,22	0,18[a]
Dichilus lebeckioides DC.	Johannesburg, Transvaal	*A.L. Schutte 362*	50	0,16	0,32[b]
Dichilus pilosus Conrath ex Schinz	Roodepoort, Transvaal	*A.L. Schutte 359*	119	0,12	0,10[b]
Dichilus reflexus (N.E.Br.) A.L. Schutte *ined.*	Pongola, Transvaal	*A.L. Schutte 368*	82	0,22	0,27
Dichilus strictus E. Mey.	Harrismith, Orange Free State	*A.L. Schutte 372c*	122	0,13	0,11[b]
Lebeckia cytisoides Thunb.	Clanwilliam, NW Cape	*B-E. van Wyk 2441*	200	0,52	0,25[a]
Lebeckia multiflora E. Mey.	Kamieskroon, NW Cape	*B-E. van Wyk 2353*	295	0,78	0,26[a]
Lebeckia sessilifolia (Eckl. & Zeyh.) Benth.	De Hoop, SW Cape	*B-E. van Wyk 2120*	96	0,11	0,12[a]
Lotononis acuminata Eckl. & Zeyh.	Humansdorp, S. Cape	*B-E. van Wyk 2580*	95	0,06	0,06
Lotononis cf. *adpressa* N.E.Br.	Ermelo, Transvaal	*B-E. van Wyk 2618*	138	0,27	0,20
Lotononis brachyloba (E. Mey.) Benth.	Ceres, SW Cape	*B-E. van Wyk 2244*	65	0,04	0,06[a]
Lotononis caerulescens (E. Mey.) B-E. van Wyk *ined.*	Cradock, E. Cape	*B-E. van Wyk 1614*	61	0,15	0,24
Lotononis divaricata (Eckl. & Zeyh.) Benth.	Burgersdorp, NE Cape	*B-E. van Wyk 2597*	165	0,15	0,09
Lotononis foliosa H. Bol.	Johannesburg, Transvaal	*B-E. van Wyk 2607*	30	0,11	0,36[c]
Lotononis gracilis (E. Mey.) Benth.	Ceres, SW Cape	*B-E. van Wyk 2265*	228	0,21	0,09
Lotononis leucoclada (Schltr.) Duemmer	Clanwilliam, NW Cape	*B-E. van Wyk 2434*	116	0,10	0,08
Lotononis listii Polhill	Bloemfontein, Orange Free State	*B-E. van Wyk 2475*	114	0,06	0,05[b]
Lotononis myriantha Bak. f. *in sched.*	Bethal, Transvaal	*B-E. van Wyk 1825*	145	0,13	0,09[c]
Lotononis oxyptera (E. Mey.) Benth.	Citrusdal, NW Cape	*B-E. van Wyk 2319*	69	0,07	0,09
Lotononis polycephala (E. Mey.) Benth.	Kamieskroon, NW Cape	*B-E. van Wyk 2408*	93	0,07	0,07[a]
Lotononis transvaalensis Duemmer	Pongola, Transvaal	*B-E. van Wyk 2614*	86	0,14	0,16[a]
Lotononis umbellata (L.) Benth.	Swellendam, SW Cape	*B-E. van Wyk 2110*	52	0,22	0,43[a]
Lotononis woodii H. Bol.	Harrismith, Orange Free State	*A.L. Schutte 374*	75	0,14	0,18[b]
Melolobium alpinum Eckl. & Zeyh.	Sani Pass, Natal	*B-E. van Wyk 2631*	58	0,15	0,25
Melolobium microphyllum (L.f.) Eckl. & Zeyh.	Rouxville, Orange Free State	*B-E. van Wyk 2628*	107	0,14	0,13
Melolobium obcordatum Harv.	Harrismith, Orange Free State	*A.L. Schutte 373a*	126	0,05	0,04
Melolobium subspicatum Conrath	Irene, Transvaal	*De Beer 38*	160	0,46	0,29
Melolobium wilmsii Harms	Hendrina, Transvaal	*B-E. van Wyk 2624*	123	0,93	0,76
Pearsonia sessilifolia (Harv.) Duemmer	Roodepoort, Transvaal	*A.L. Schutte 382*	81	0,34	0,43[c]
Polhillia pallens C.H.Stirton	Bredasdorp, SW Cape	*B-E. van Wyk 2095*	100	0,21	0,21
Rafnia perfoliata E. Mey.	Villiersdorp, SW Cape	*B-E. van Wyk 2067*	159	0,18	0,12[b]
Wiborgia sericea Thunb.	Laingsburg, SW Cape	*B-E. van Wyk 2193*	43	0,09	0,21[a]

[a], [b], [c] — small, medium and large quantities respectively of crystalline material present

Results

Figure 1 shows the thin-layer chromatography results for systems a and b of all extracts in which at least some alkaloids were detected. The estimated number of major alkaloids in all the samples totals 34. Their respective R_f values and spot characteristics are given in Table 2. Identities not confirmed by mass spectrometry are tentative only (see Table 3), since thin-layer chromatography is known to lack precision. Closely related compounds may often not be distinguished by this method alone.

Discussion

The results seem to indicate that the genera represented differ considerably both in the quantity and the numbers of alkaloids produced. Within each genus there is much less variation and morphologically similar species also tend to have similar alkaloids (Table 3). What differences there are may be attributed to inadequate sampling. Cranmer & Mabry (1966) have shown that many factors affect the actual quantity of alkaloid within a sample. Alkaloids that are apparently absent may well be present in some individuals of a species, albeit in trace amounts. The only genus of which the species are all included (*Dichilus*), shows remarkably little variation and it seems that at least two of the alkaloids (nos 11 and 14) may turn out to be taxonomic markers for this genus. Our results differ markedly from those of Stirton (1986a), whose findings are difficult to interpret because he only extracted small quantities of seeds and did not include any standards in his chromatograph. He suggested similarities between *Dichilus strictus* and *Lebeckia*, but our data is in contrast to this view. It is tempting to equate alkaloid no. 11 with lupanine and alkaloid no. 14 with no. 10 (which would indeed make the two genera similar) but the UV-fluorescences are totally different.

It is obvious from Table 3 that the genera *Lebeckia, Melolobium, Polhillia* and *Dichilus* warrant further study. In view of the somewhat anomalous position of *Argyrolobium* (Polhill 1976, 1981) and uncertainty regarding its affinities with *Polhillia, Melolobium* (Stirton 1986b) and perhaps *Dichilus*, this genus should be included in comparative studies. The two

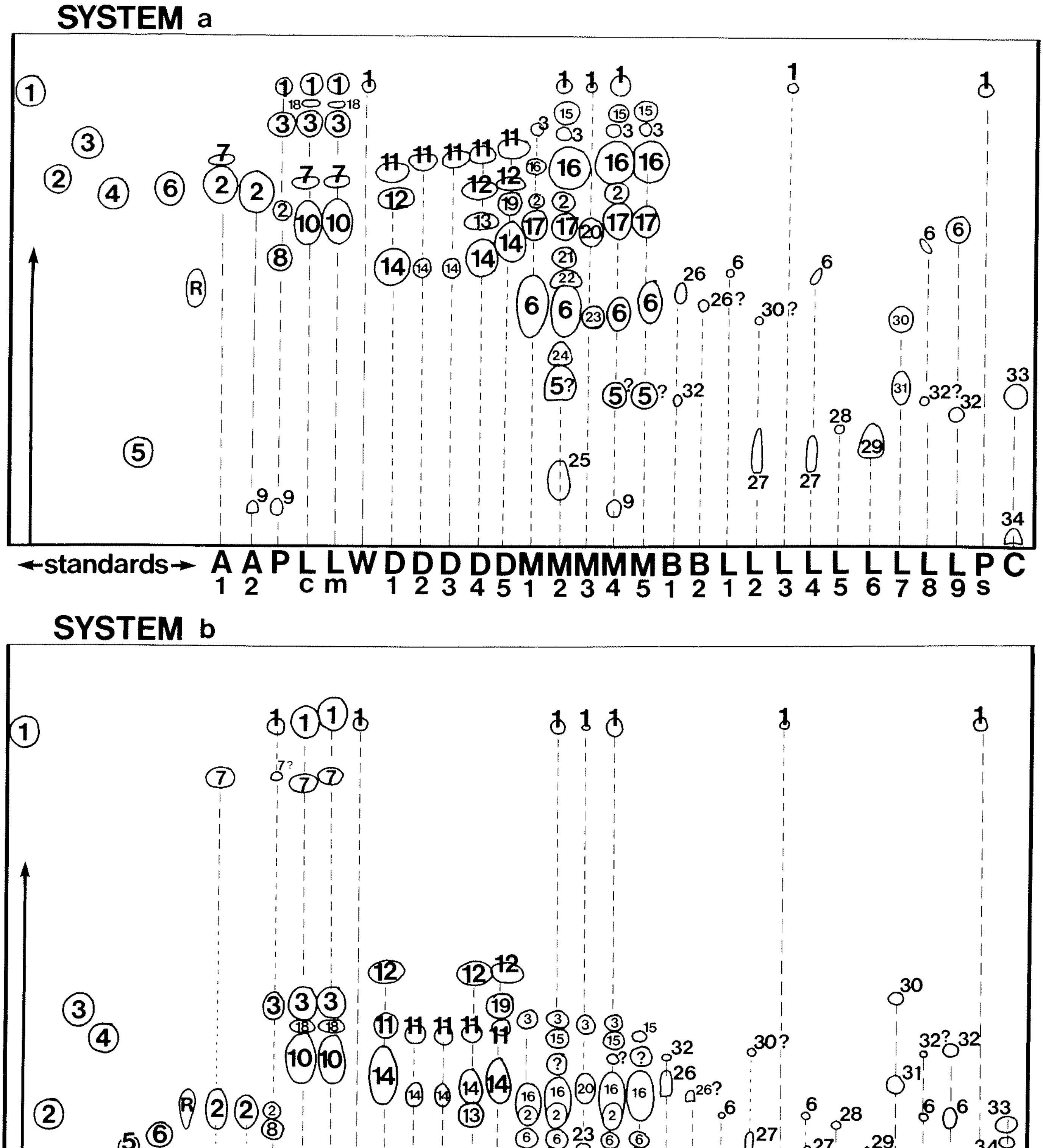

Figure 1 Thin-layer chromatography results (systems a and b) of all crude extracts in which at least some alkaloids were detected. Standards and unknown alkaloids are numbered as in Tables 2 and 3, (R: Rhodamine B). A1, *Argyrolobium variopile*; A2, *Argyrolobium crassifolium*; P, *Polhillia pallens*; Lc, *Lebeckia cytisoides*; Lm, *L. multiflora*; W, *Wiborgia sericea*; D1, *Dichilus gracilis*; D2, *D. lebeckioides*; D3, *D. pilosus*; D4, *D. reflexus*; D5, *D. strictus*; M1, *Melolobium alpinum*; M2, *M. microphyllum*; M3, *M. obcordatum*; M4, *M. subspicatum*; M5, *M. wilmsii*; B1, *Buchenroedera lotononoides*; B2, *B. multiflora*; L1, *Lotononis* cf. *adpressa*; L2, *L. brachyloba*; L3, *L. foliosa*; L4, *L. leucoclada*; L5, *L. listii*; L6, *L. myriantha*; L7, *L. oxyptera*; L8, *L. polycephala*; L9, *L. transvaalensis*; Ps, *Pearsonia sessilifolia*; C, *Crotalaria capensis*.

species represented in our sample contain significant quantities of anagyrine as the dominant alkaloid and this appears to be a new record for *Argyrolobium*. Only one species has been studied before. Tsuda & Marion (1963) isolated cytisine, aphyllidine and argyrolobine from *A. megarhizum* H. Bol.

In the lotononoid genera, the prospects of obtaining useful information seem limited. There is no clear pattern and the amount of plant material required for extraction would be prohibitive for many of the species. An interesting question needs to be answered, however. Is the remarkable similarity

Table 2 Spot characteristics of major alkaloids on silica gel plates (systems a, b, c, d and e) with iodoplatinate as detecting reagent. The dye Rhodamine B was used to allow standardization of the R_f values. Spot shapes: r = round, t = transversely oblong, o = oblong, e = extended, s = streaked. The standards and unknown alkaloids are numbered as in Figure 1

		R_f values and spot shapes					UV – visible			
		a	b	c	d	e	254 nm	365 nm	Colour UV 365 nm	Colour iodoplatinate
1	Sparteine	0,79 r	0,76 r	0,30 s	0,06 e	0,23 s	–	–	–	greyish-blue
2	Anagyrine	0,55 r	0,09 r	0,94 t	0,26 s	0,20 e	+	+	bluish	brown to purple
3	Lupanine	0,65 r	0,28 r	0,77 r	0,04 e	0,23 o	–	–	–	purple
4	Lupinine	0,50 r	0,24 r	0,91 r	0,06 e	0,31 e	–	–	–	blue
5	13-OH-Lupanine	0,17 r	0,02 r	0,44 o	0,04 e	0,20 o	–	–	–	blue
6	*N*-Me-cytisine	0,48 r	0,05 r	0,82 r	0,22 s	0,19 e	+	–	–	blue-grey
7		0,80 t	0,75 t	>0,9 t	? ?	? ?	–	+	pink	pale blue
8		0,61 r	0,06 r	>0,9 r	? ?	? ?	+	–	–	greenish-grey
9		0,08 o	0,08 ?	? ?	? ?	? ?	+	–	–	purplish-brown
10		0,60 r	0,20 o	0,75 r	? ?	? ?	+	+	violet-blue	violet-purple
11		0,73 t	0,25 r	0,94 ?	? ?	? ?	+	+	purple	pale purple
12		0,70 t	0,49 t	0,30 e	0,28 r	? ?	–	+	purple	pale blue
13		0,69 t	0,09 r	>0,9 ?	<0,1 ?	0,12 r	–	+	purple	pale purple
14		0,55 r	0,16 o	0,52 e	<0,1 ?	0,37 r	+	–	–	brown and blue
15		0,81 t	0,26 r	>0,8 ?	0,25 r	? ?	–	–	–	blue
16		0,65 r	0,14 o	0,94 t	0,41 o	0,32 o	+	+	pale blue	blue and green
17		0,50 r	0,11 o	0,87 ?	? ?	? ?	–	–	–	greenish-grey
18		0,81 t	0,27 t	? ?	? ?	? ?	–	+	bluish	pale blue
19		0,70 r	0,30 r	>0,9 ?	0,54 r	0,61 r	+	+	purplish	pale blue
20		0,50 r	0,13 o	0,85 t	0,07 e	0,37 r	+	+	purple	pale purple
21		0,46 r	0,33 r	0,84 ?	? ?	? ?	–	–	–	pale blue
22		0,42 t	0,06 ?	? ?	? ?	? ?	–	+	bluish	purple
23		0,40 r	0,02 r	0,82 ?	0,07 ?	0,28 r	+	–	–	purple
24		0,30 t	<0,1 ?	>0,9 ?	0,65 s	0,67 s	–	+	blue	reddish-violet
25		0,12 o	<0,1 ?	>0,9 ?	0,15 o	? ?	–	–	–	reddish-purple
26		0,45 o	0,10 e	0,78 o	0,15 e	0,24 r	–	+	purple	pink
27		0,22 o	0,02 e	0,04 o	0,03 e	? ?	+	–	–	orange-brown
28		0,25 r	0,05 r	0,26 r	0,04 e	0,62 t	+	–	–	purple
29		0,25 o	0,01 o	0,29 o	0,37 s	0,48 e	+	+	pale yellow	bluish-purple
30		0,43 r	0,23 r	0,23 s	0,07 s	0,41 r	–	–	–	purple
31		0,34 o	0,11 r	0,23 ?	0,07 s	0,31 r	+	–	–	brownish-purple
32		0,27 r	0,15 r	0,38 r	0,01 r	0,19 r	–	–	–	blue
33		0,30 r	0,05 r	0,30 o	0,04 e	0,40 r	+	–	–	brownish-purple
34		0,03 o	0,02 r	0,13 o	0,01 r	0,32 t	+	–	–	brownish-purple
Rhodamine B		0,45 o	0,10 o	0,74 o	0,12 e	0,65 o	+	+	bright red	bright red

between species of *Lotononis* and *Crotalaria* merely the result of convergence? The two genera do appear to be chemically different. Many species of *Lotononis* are cyanogenic, suggesting a nitrogen metabolism directed towards the production of glucosides rather than alkaloids. The occurrence of at least some quinolizidine alkaloids in *Lotononis* (here reported for the first time) does not preclude the possibility that pyrrolizidine alkaloids may also be present. *Lotononis* and *Buchenroedera* appear to be similar, but the alkaloids are in such low concentrations that more detailed studies to prove relationship seem unpractical unless more sophisticated extraction methods are used.

The systematic value of alkaloid data at the generic level is now well established (Cranmer & Turner 1967; Kinghorn & Smolenski *op. cit.*). In the Crotalarieae, it is precisely at this level where more information is needed. Our preliminary results indicate that a more thorough investigation of some genera holds great promise.

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Table 3 Distribution of the major alkaloids extracted from 38 species of the Crotalarieae and Genisteae ([c]identity confirmed by mass spectrometry)

	Distribution of major alkaloids																																	
	Identified						Unidentified																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Argyrolobium crassifolium		2							?																									
Argyrolobium variopile		2[c]					7																											
Aspalathus hirta																																		
Buchenroedera lotononoides																										26						32		
Buchenroedera multiflora																										?								
Crotalaria capensis																																	33	34
Dichilus gracilis											11	12		14																				
Dichilus lebeckioides											11			14																				
Dichilus pilosus											11			14																				
Dichilus reflexus											11	12	13	14																				
Dichilus strictus											11	12		14					19															
Lebeckia cytisoides	1[c]		3				7			10								18																
Lebeckia multiflora	1		3[c]				7			10								18																
Lebeckia sessilifolia																																		
Lotononis acuminata																																		
Lotononis cf. *adpressa*						6																												
Lotononis brachyloba																											27			?				
Lotononis caerulescens																																		
Lotononis divaricata																																		
Lotononis foliosa	1																																	
Lotononis gracilis																																		
Lotononis leucoclada						6																					27							
Lotononis listii																												28						
Lotononis myriantha																													29					
Lotononis oxyptera																														30	31			
Lotononis polycephala						6																										?		
Lotononis transvaalensis						6																										32		
Lotononis umbellata																																		
Lotononis woodii																																		
Melolobium alpinum *		2	3			6										?	17																	
Melolobium microphyllum	1	2	3		?	6									15	16	17				21	22		24	25									
Melolobium obcordatum	?		3																	20			23											
Melolobium subspicatum	1	2[c]	3		?	6			9						15	16	17																	
Melolobium wilmsii					?	6									15	16	17																	
Pearsonia sessilifolia	1																																	
Polhillia pallens	1	2	3				?	8	9																									
Rafnia perfoliata																																		
Wiborgia sericea	1																																	

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