Biochemical Systematics and Ecology, Vol. 19, No. 8, pp. 685–695, 1991.
Printed in Great Britain.

0305-1978/91 $3.00+0.00

Alkaloidal Variation in the Genus *Pearsonia*

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Key Word Index—*Pearsonia*; Fabaceae; Crotalarieae; quinolizidine alkaloids; chemotaxonomy; variation study.

Abstract—Several quinolizidine alkaloids, including various angelate esters, are known from the genus *Pearsonia*. In a detailed variation study which included 98 samples from nine of the 11 species, large qualitative and quantitative differences were recorded. The observed variation is ascribed to the following: 1, species (the alkaloids of some species and subspecies are diagnostically different); 2, provenance (various populations of the same species may have unique combinations of alkaloids); 3, developmental stage (in *P. cajanifolia* there is a marked decrease in esterification towards the end of the growing season); 4, plant parts extracted (seeds, for example, have high concentrations of hydroxylated lupanine-type alkaloids and only small amounts of esters). These results highlight some of the problems associated with the use of alkaloids as taxonomic characters.

Introduction

As part of a broad survey of alkaloids as generic characters in the tribe Crotalarieae (Fabaceae), large qualitative and quantitative differences between six species and subspecies of the genus *Pearsonia* Duemmer were reported [1]. Four previously unknown esters of polyhydroxylated lupanines were discovered in extracts of *Pearsonia cajanifolia* and *P. sessilifolia* [2]. In this paper we present the results of a detailed variation study of alkaloids in virtually all the species and subspecies of *Pearsonia*. The sample included different provenances, various plant parts (leaves, stems, root bark and seeds) and also material collected at different times during the growing season.

Materials and Methods

Plant materials. A comprehensive list of voucher specimens of all the taxa studied are given below, with sample numbers as in the tables. *Pearsonia aristata* (Schinz) Duemmer: 1, *Van Wyk 1795* (JRAU); 2, *Van Wyk 2930* (JRAU); 3, *Van Wyk 2728* (JRAU). *P. bracteata* (Benth.) Polhill: 1, *Pont 750* (PRE); 2, *Wells 2353* (PRE); 3, *Van der Schijff 5314* (PRE). *P. cajanifolia* (Harv.) Polhill ssp. *cajanifolia*: 1, *Young 26452* (PRE); 2, *Codd 3183* (PRE); 3, *HBG 26907* (PRE); 4, *Acocks 20894* (PRE); 5, *Grobbelaar 842* (PRE); 6, *Bredenkamp 426* (PRE); 7, *Posthumus 7* (JRAU); 8, *Posthumus 5* (JRAU); 9, *Van Wyk 3048* (JRAU); 10, *Posthumus 6* (JRAU). *P. cajanifolia* (Harv.) Polhill ssp. *cryptantha* (Bak.) Polhill: 1, *Norlindh & Weimarck 4246* (PRE); 2, *Codd 3735* (PRE); 3, *Chase 7927* (PRE); 4, *Wild 6744* (PRE); 5, *Wild 7497* (PRE); 6, *Corby 2063* (PRE); 7, *Vahrmeijer 2405* (PRE); 8, *Moffett 1759* (PRE); 9, *Westfall 1033* (PRE); 10, *Stalmans 1254* (PRE); 11, *Posthumus 1* (JRAU); 12, *Posthumus 3* (JRAU); 13, *Posthumus 4* (JRAU). *P. flava* (Bak f.) Polhill: 1, *Bock 297* (PRE); 2, *Anon 2183* (PRE); 3, *Richards 12047* (PRE). *P. grandifolia* (H. Bol.) Polhill ssp. *grandifolia*: 1, *Galpin 11866* (PRE); 2, *Fischer 954* (PRE); 3, *Acocks 12343* (PRE). *P. grandifolia* (H. Bol.) Polhill ssp. *latibracteolata* (Duemmer) Polhill: 1, *Gilfillan 7182* (PRE); 2, *Codd 3673* (PRE); 3, *Balsinhas 2956* (PRE); 4, *Onderstall 1324* (PRE); 5, *Van Wyk 3047* (JRAU). *P. metallifera* Wild: 1, *Rutherford-Smith 575* (PRE); 2, Drummond 6041 (PRE); 3, *Wild 5610* (PRE); 4, *Bayliss 10441* (PRE). *P. obovata* (Schinz) Polhill: 1, *Marais 104* (PRE); 2, *Mohle 207* (PRE); 3, *Van Wyk 2919* (JRAU). *P. sessilifolia* (Harv.) Duemmer ssp. *filifolia* (H. Bol.) Polhill: 1, *Acocks 13074* (PRE); 2, *Edwards 2994* (PRE); 3, *Germishuizen 3314* (PRE); 4, *Rogers 21051* (PRE); 5, *Germishuizen 2347* (PRE). *P. sessilifolia* (Harv.) Duemmer ssp. *marginata* (Schinz) Polhill: 1, *Van Wyk 2917* (JRAU); 2, *Swanepoel 9a* (JRAU); 3, *Swanepoel 8a* (JRAU). *P. sessilifolia* (Harv.) Duemmer ssp. *sessilifolia*: 1, *Swanepoel 12a* (JRAU); 2, *Van Wyk 3049a* (JRAU); 3, Venter 7747 (PRE); 4, *Pettifer 192* (PRE); 5, *Van Wyk 3192* (JRAU). *P. sessilifolia* (Harv.) Duemmer ssp. *swaziensis* (H. Bol.) Polhill: 1, *Compton 30866* (PRE); 2, *Compton 28302* (PRE); 3, *Compton 28773* (PRE). *P. uniflora* (Kensit) Polhill: 1, *Liebenberg 2728* (PRE); 2, *Krynauw 1130* (PRE); 3, *Ross 2441* (PRE); 4, *Compton 28431* (PRE).

(*Received* 2 *April* 1991)

Procedures. Dry plant material was homogenized in 0.05 M aq. H_2SO_4 and left for 30 min. After filtration, the homogenate (20 ml) was applied to glass columns filled with celite (22 g). The aqueous phase was made basic with ammonia (4 ml) and extracted with 100 ml CH_2Cl_2. Isolation of alkaloids was effected by silica gel 60 column chromatography with $CHCl_3$:cyclohexane:Et_2N (4:5:1) as eluent. Alkaloids were identified by comparative TLC as described below, combined with comparative GC using authentic reference samples obtained in several previous studies [1–4]. Two TLC systems were used for routine work: 1, Merck 60 F_{254} silica gel plates (0.25 mm layer thickness) developed in $CHCl_3$:cyclohexane:Et_2NH (4:5:1) and 2, Merck aluminium oxide F_{254} (type E) plates (0.25 mm layer thickness) with 1.5% MeOH in $CHCl_3$ as eluent. The plates were dried at 100°C for 3 min, studied under UV_{254} and UV_{365} and then sprayed with iodoplatinate reagent. GC spectra were obtained with a DB-1 fused silica capillary column (30 m×0.25 mm i.d.; N_2 as carrier gas at 4 ml min^{-1}; column temperature 150° to 320° at 6° min^{-1}, 15 min isotherm; injector 230°C; PND detection 300°C; split ratio 30:1; injection volume 1 µl). The identity of the major alkaloids was confirmed by GC-MS analyses of several of the extracts. The mass spectra of the various alkaloids were identical to those obtained in other studies. Large-scale extractions of the following species yielded pure samples; *P. cajanifolia* ssp. *cryptantha* (sample 11): 330 mg of pearsonine; *P. obovata* (sample 3): 280 mg of tyramine, 11 mg of 13α-hydroxylupanine; *P. sessilifolia* ssp. *marginata* (sample 2): 350 mg of lebeckianine. Reference samples of the various angelate esters were also available from our previous studies of *Pearsonia* alkaloids [1, 2]. All these pure samples, as well as the alcoholic derivatives of the various esters, were identified by MS, ^1H and ^{13}C NMR. Detailed ^{13}C NMR data of the two new hydroxylupanines and a comparison with structurally related compounds will be published elsewhere [6].

Phenolic amines. Tyramine (**1**): $[M]^+$ 137.

Piperidyl alkaloids. Ammodendrine (**5**): $[M]^+$ 208.

Sparteine- and lupanine-type quinolizidine alkaloids. β-isosparteine (**2**): $[M]^+$ 234. α-Isosparteine (**3**) $[M]^+$ 234. Sparteine (**4**): $[M]^+$ 234. 17-oxo-Sparteine (**6**): $[M]^+$ 248. Δ5,6-lupanine (**7**): $[M]^+$ 246. 11-*epi*-Lupanine (**9**): $[M]^+$ 248. Lupanine (**11**): $[M]^+$ 248. 17-oxo-Lupanine (**12**): $[M]^+$ 262.

Hydroxylated lupanine-type quinolizidine alkaloids. 3β-Hydroxylupanine (**14**): $[M]^+$ 264. Lebeckianine (**17**): $[M]^+$ 280. 13α-Hydroxylupanine (**19**): $[M]^+$ 264. 3β,13α-Dihydroxylupanine (**21**): $[M]^+$ 280. 8α,13α-Dihydroxylupanine (**22**): pale brown oil, $[\alpha]_D^{22}+29°$ (c=2.4 in $CHCl_3$, ν_{max} 3260 br (OH) 1625 (lactam C=O) cm^{-1}; MS *m/z* (rel. int.) 280 (70), 262 (100), 247 (10), 235 (11), 223 (10), 208 (14), 207 (13), 182 (13), 181 (10), 168 (49), 165 (30), 164 (32), 150 (62), 126 (41), 113 (18), 112 (39), 98 (50), 82 (59), 72 (27), 69 (41), 56 (62), 55 (78). 3β,8α,13α-Trihydroxylupanine (**23**): pale brown oil, $[\alpha]_D^{22}+2°$ (c=2.2 in $CHCl_3$), ν_{max} 3300 br (OH) 1620 (lactam C=O) cm^{-1}; MS *m/z* (rel. int.) 296 (100), 279 (23), 278 (23), 277 (16), 261 (7), 251 (6), 239 (5), 225 (4), 181 (9), 168 (19), 166 (16), 164 (15), 150 (30), 126 (15), 114 (14), 96 (16), 82 (17), 69 (23), 57 (28), 55 (31), 46 (28).

Esters of quinolizidine alkaloids. Lupanine-13α-angelate (**24**): $[M]^+$ 346. Cajanifoline (**25**): $[M]^+$ 362. Cryptanthine (**26**): $[M]^+$ 362. Sessilifoline (**27**): $[M]^+$ 362. Pearsonine (**28**): $[M]^+$ 378.

Unknown alkaloids. Unknown 1 (x-hydroxyammodendrine?): MS *m/z* (rel. int.) 224 (32), 207 (74), 181 (31), 179 (32), 165 (37), 152 (34), 137 (23), 123 (28), 110 (43), 109 (53), 108 (44), 94 (45), 82 (39), 80 (41), 67 (18), 56 (30), 43 (100). Unknown 2 (Δx,y-oxo-sparteine?): MS *m/z* (rel. int.) 246 (33), 218 (8), 203 (5), 189 (7), 163 (14), 149 (7), 136 (16), 120 (7), 109 (14), 98 (100), 84 (11), 70 (8), 55 (17), 41 (15). Unknown 3 (Δx,y-lupanine?): MS *m/z* (rel. int.) 246 (100), 218 (21), 205 (5), 191 (15), 175 (5), 164 (14), 147 (13), 136 (100), 122 (24), 109 (22), 98 (69), 96 (58), 82 (43), 67 (19), 55 (52), 41 (47). Unknown 4: MS identical to the MS of 3β-hydroxylupanine. Unknown 5: R_t 22.57, no MS data. Unknown 6: MS *m/z* 308 (15), 207 (100), 191 (9), 178 (7), 165 (15), 163 (16), 134 (14), 120 (13), 107 (9), 96 (8), 82 (13), 55 (14). Unknown 7: R_t 24.86, no MS data.

Results

The distribution of 28 alkaloids in 59 leaf extracts of 14 species and subspecies of *Pearsonia* is shown in Tables 1A, 1B and 1C. The only two species not included in this sample are *P. mesopontica* Polhill and *P. madagascariensis* (R. Viguier) Polhill [5]. To investigate infraspecific variation, leaf samples from three different populations of each of the two subspecies of *P. cajanifolia* were analysed (Table 2). Differences between leaves, stems, root bark and seeds of *P. cajanifolia* are given in Table 3 and a comparison of leaves and seeds of *P. sessilifolia* in Table 4. A seasonal change in the production of hydroxylated lupanines and their angelate esters was observed in two individual plants of *P. cajanifolia* ssp. *cajanifolia.* The result of this study is given in Table 5.

The data show that the genus *Pearsonia* is exceptionally variable and that there are remarkable qualitative and quantitative differences between the various samples and species. The following alkaloids occur as major compounds in at least some of the species: sparteine (**4**), ammodendrine (**5**), Δ5,6-lupanine (**7**), 11-*epi*-lupanine (**9**), lupanine (**11**), 17-oxo-lupanine (**12**), 3β-hydroxylupanine (**14**), lebeckianine (**17**), 13α-hydroxylupanine (**19**), 3β,13α-dihydroxylupanine (**21**), lupanine-13α-angelate (**24**),

TABLE 1A. DISTRIBUTION AND YIELDS OF ALKALOIDS IN LEAF SAMPLES FROM THE *PEARSONIA FLAVA* GROUP

Species:			*P. bracteata*			*P. flava*			*P. uniflora*		
Sample:			1	2	3	1	2	3	1	2	3
Alkaloid no.	Alkaloid name	Retention time* (min)	Distribution of alkaloids (% of total yield; t = trace amounts)								
1	Tyramine	7.28	—	—	—	—	—	—	—	—	—
2	β-Isosparteine	12.08	—	—	—	—	—	—	—	—	—
3	α-Isosparteine	12.62	—	—	—	—	—	—	2	—	—
4	Sparteine	13.77	t	t	t	t	t	2	63	t	t
5	Ammodendrine	15.10	t	t	t	t	8	3	5	t	t
6	17-oxo-Sparteine	18.77	—	—	—	—	—	—	—	—	—
7	Δ5,6-Lupanine	18.87	—	—	—	19	17	14	22	t	t
8	Unknown 1 ([M]$^+$ = 224)	18.91	—	—	—	—	—	—	—	—	—
9	11-*epi*-Lupanine	19.60	t	—	—	—	—	—	t	—	—
10	Unknown 2 ([M]$^+$ = 246)	19.76	—	—	—	—	—	—	—	—	—
11	Lupanine	20.45	t	—	—	28	32	19	3	t	t
12	17-oxo-Lupanine	20.80	t	—	—	—	—	1	t	—	t
13	Unknown 3 ([M]$^+$ = 262)	21.55	—	—	—	—	—	—	—	—	—
14	3-Hydroxylupanine	21.95	—	—	—	—	—	—	—	—	—
15	Unknown 4 ([M]$^+$ = 264)	22.02	—	—	—	—	—	—	—	—	—
16	Unknown 5 ([M]$^+$ unknown)	22.57	—	—	—	—	—	—	—	—	—
17	Lebeckianine	23.43	—	—	—	—	—	t	—	—	—
18	Unknown 6 ([M]$^+$ = 308)	24.20	—	—	—	—	—	—	—	—	—
19	13-hydroxylupanine	24.27	t	—	t	t	t	t	—	—	—
20	Unknown 7 ([M]$^+$ unknown)	24.86	—	—	—	—	—	—	—	—	—
21	3,13-Dihydroxylupanine	25.75	—	—	—	—	—	—	—	—	—
22	8,13-Dihydroxylupanine	26.49	—	—	—	—	—	—	—	—	—
23	3,8,13-Trihydroxylupanine	27.87	—	—	t	—	—	—	—	—	—
24	Lupanine-13-angelate	28.82	—	—	—	—	t	—	—	—	—
25	Cajanifoline	30.30	—	—	—	t	t	t	—	—	—
26	Cryptanthine	30.65	—	—	—	—	—	t	—	—	—
27	Sessilifoline	30.85	—	—	—	—	—	t	—	—	—
28	Pearsonine	32.10	—	—	—	39	43	58	4	—	—
Total yield (mg g^{-1} dry wt):			0.6	0.1	0.2	0.9	1.3	1.0	0.3	0.1	0.1

*For GC parameters see Materials and Methods.

cajanifoline (**25**), cryptanthine (**26**), sessilifoline (**27**) and pearsonine (**28**). Amongst several minor alkaloids, two new hydroxylated lupanines were identified, namely 8α,13α-dihydroxylupanine (**22**) and 3β,8α,13α-trihydroxylupanine (**23**). Spectroscopic details of these new compounds, including a comparison between various hydroxylupanines and their respective angelate esters, will be published elsewhere [6].

Discussion

Despite the complexity of the pattern in Tables 1 to 5, there are some distinct trends that can be ascribed to species, provenance, plant parts and seasonal variation. Each of these sources of variation are discussed below.

Species differences

Some of the species of *Pearsonia* are diagnostically different from one another in their major alkaloids. The main pattern in leaf samples is given in Tables 1A, 1B and 1C. *P. flava* can be distinguished from the morphologically similar *P. bracteata* and *P. uniflora* by the combination of 5,6-dehydrolupanine, lupanine and pearsonine (Table 1A). The yields of alkaloids are very low in these species. In the *P. cajanifolia* group (Table 1B), *P. grandifolia* (morphologically similar to *P. obovata*) can be distinguished from other species by the presence of 17-oxo-lupanine as the major alkaloid. *P. obovata* seems rather similar to *P. cajanifolia*, notably in the presence of pearsonine and other esters.

TABLE 1B. DISTRIBUTION AND YIELDS OF ALKALOIDS IN LEAF SAMPLES FROM THE *PEARSONIA CAJANIFOLIA* GROUP

Species:	*P. grandifolia*								*P. obovata*			*P. cajanifolia*															
Ssp.:	*grandifolia*			*latibracteolata*								*cajanifolia*						*cryptantha*									
Sample:	1	2	3	1	2	3	4	5	1	2	3	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10
Alkaloid no. (see Table 1A)	Distribution of alkaloids (% of total yield; t = trace amounts)																										
1	—	—	—	—	—	—	—	—	t	t	17	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t	—	—	—	—	t	—	—	t	—	—	t	—
3	—	—	—	t	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	t
4	2	2	1	3	2	1	t	t	t	t	t	3	t	2	t	t	t	t	t	t	—	t	—	t	—	t	t
5	t	8	7	t	t	—	t	t	t	4	16	19	8	5	3	32	t	8	t	9	4	t	22	t	t	30	t
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—	—	6	5	2	t	55	t	7	t	8	t	t	18	t	54	17	t
8	—	—	—	—	—	—	—	—	t	4	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	3	1	t	1	t	t	t	2	2	t	t	t	t	16	t	t	t	—	t	t	—	t	t	t	t	16	t
10	27	7	9	6	3	21	12	1	—	t	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—
11	2	45	53	44	3	10	t	59	29	31	6	t	2	t	1	t	1	15	t	t	3	15	t	25	t	t	t
12	35	32	31	32	45	59	70	36	1	—	t	—	t	t	t	—	—	—	—	t	—	—	—	—	—	t	t
13	t	t	t	3	10	1	2	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14	1	t	t	t	32	t	t	—	3	t	1	—	—	t	—	t	—	t	—	—	—	—	—	—	—	—	—
15	3	2	t	t	—	t	14	t	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	—	t	t	—	—	—	—	—	—	t	t	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—
17	—	1	1	3	t	t	t	t	t	—	t	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19	6	4	4	6	t	9	—	1	20	55	2	28	12	16	33	3	22	14	17	33	53	8	60	15	46	27	t
20	—	—	—	—	—	—	—	—	—	t	20	—	t	t	—	6	t	—	—	—	—	—	—	—	—	—	—
21	t	—	—	—	t	—	t	—	t	t	1	6	t	6	50	t	t	t	17	t	t	—	—	—	—	10	—
22	13	t	t	t	5	1	1	t	3	t	—	—	—	—	—	t	—	t	48	—	—	21	t	50	t	—	71
23	—	—	—	—	—	—	—	—	t	t	t	—	t	—	—	t	t	—	—	t	—	—	—	—	—	—	t
24	—	—	—	—	—	—	—	t	3	4	2	18	53	18	t	t	77	15	t	50	40	t	t	—	t	t	t
25	—	—	—	—	—	—	—	—	t	t	3	30	19	25	13	t	t	5	18	t	—	—	—	—	—	—	—
26	7	1	—	—	—	t	—	1	19	—	—	—	—	—	—	5	—	14	—	—	—	54	—	t	t	—	30
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	t	—	17	—	1	—	—	—	—	—	—	20	—	—	—	—	—	—	—	—	t
Total yield (mg g^{-1} dry wt):	1.5	8.4	3.3	3.1	7.8	4.9	9.0	5.9	3.7	5.5	0.9	2.6	4.7	2.9	0.4	2.5	0.5	0.7	0.7	0.9	0.6	1.4	0.5	0.6	1.5	0.4	1.8

TABLE 1C. DISTRIBUTION AND YIELDS OF ALKALOIDS IN LEAF SAMPLES FROM THE *PEARSONIA SESSILIFOLIA* GROUP

Species:	*P. sessilifolia*																*P. aristata*			*P. metallifera*			
Ssp.:	*marginata*			*filifolia*					*sessilifolia*					*swaziensis*									
Sample:	1	2	3	1	2	3	4	5	1	2	3	4	5	1	2	3	1	2	3	1	2	3	4
Alkaloid no. (see Table 1A)	Distribution of alkaloids (% of total yield; t = trace amounts)																						
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	1	t	t	1	1	t	t	t	t	—	—	—	t	t	t	—	—	—	—	—	—	—
4	t	70	5	85	82	87	40	30	22	1	2	5	33	91	86	87	—	t	t	—	—	—	—
5	t	1	—	t	t	3	4	5	5	47	t	t	27	6	t	3	—	t	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	—	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	t	5	1	t	—	—	—	—	—	t	t	3	t	—	—	—	—	—	—	—
9	t	t	—	—	t	t	t	—	2	4	1	—	t	t	t	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	t	1	2	3	t	t	33	60	6	4	t	2	5	t	2	2	t	t	t	t	t	t	t
12	t	3	—	t	2	t	12	—	—	10	—	—	8	t	t	t	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—
14	3	t	39	t	t	t	t	1	t	t	—	—	t	3	1	1	t	—	t	t	t	—	t
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	t	t	t	t	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17	45	4	43	t	1	t	t	t	—	t	—	—	6	t	4	4	—	—	—	—	—	—	—
18	—	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19	—	—	—	4	2	—	11	t	—	t	17	t	7	t	—	—	t	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	17	—	t	t	t	—	t	—	—	—	—	—	2	t	—	—	—	—	—	—	—	—	—
22	t	t	—	—	t	1	—	—	—	—	t	44	—	—	t	t	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	—	—	69	7	—	—	—	—	t	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—	—	—	t	—	t	—	—	—	—	—	—	—	—	—	—
26	10	t	t	t	t	—	—	—	—	—	—	—	—	t	t	t	—	—	—	—	—	—	—
27	18	t	t	—	t	t	—	—	—	—	9	41	—	t	t	t	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total yield (mg g^{-1} dry wt):	7.3	5.7	0.1	4.6	7.3	6.6	8.8	1.2	0.2	0.7	5.4	3.6	2.1	10.3	11.2	8.3	0.5	0.2	0.3	1.0	1.6	1.0	0.9

TABLE 2. DISTRIBUTION AND YIELDS OF MAJOR ALKALOIDS IN LEAVES FROM SIX PROVENANCES OF *PEARSONIA CAJANIFOLIA* (a, b AND c ARE THREE DIFFERENT PLANTS FROM THE SAME POPULATION; ALL PLANTS WERE SAMPLED IN THE FRUITING STAGE)

	ssp. *cajanifolia*									ssp. *cryptantha*								
Provenance:	Kensington			Northcliff			Magaliesberg			Blydepoort			Pilgrims Rest			Lydenburg		
Sample no.:	7a	7b	7c	8a	8b	8c	9a	9b	9c	11a	11b	11c	12a	12b	12c	13a	13b	13c
Alkaloid no. (see Table 1A)	Distribution of alkaloids (% of total yield; t = trace amounts)																	
3	—	—	—	—	—	—	—	—	—	t	—	—	—	—	—	—	—	t
4	9	7	1	t	t	3	—	t	15	t	t	t	—	—	t	1	—	—
5	22	11	2	3	30	27	t	t	t	t	13	t	29	17	17	27	9	35
7	14	32	3	t	17	16	t	t	14	t	t	t	22	32	49	45	42	50
9	3	3	1	t	1	4	t	t	t	t	t	t	t	2	t	3	4	2
11	4	2	15	20	t	2	t	t	t	t	t	t	23	3	t	t	—	—
14	—	—	t	2	—	—	t	—	t	t	—	—	t	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17	—	—	—	—	t	t	—	—	—	—	—	—	—	—	—	—	—	3
19	25	22	54	38	16	13	35	53	22	t	1	t	8	40	23	14	t	6
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	8	7	8	16	10	8	t	t	12	t	t	t	7	5	11	—	—	—
22	—	—	—	—	—	—	65	47	27	—	t	t	—	—	—	—	13	—
23	—	—	—	—	6	—	t	—	—	t	—	t	—	—	—	—	—	—
24	6	4	6	4	—	7	t	—	—	—	t	—	4	t	—	—	—	3
25	8	12	8	16	20	19	t	—	t	—	—	—	5	t	—	—	7	1
26	—	—	—	—	—	—	t	t	9	—	t	—	—	—	—	—	25	t
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	—	99	85	99	t	—	—	—	—	—
Total yield (mg g^{-1} dry wt):	4.2	4.6	9.9	6.3	3.5	3.5	2.2	2.0	2.5	1.8	1.2	0.9	0.7	0.5	0.3	0.8	0.8	0.6

TABLE 3. DISTRIBUTION AND YIELDS OF MAJOR ALKALOIDS IN ROOT BARK, TWIGS, LEAVES AND SEEDS FROM THE TWO SUBSPECIES OF *PEARSONIA CAJANIFOLIA* (a, b AND c ARE THREE DIFFERENT PLANTS FROM THE SAME POPULATION)

	ssp. *cajanifolia*										ssp. *cryptantha*									
Plant parts:	Root bark			Twigs			Leaves			Seeds	Root bark			Twigs			Leaves			Seeds
Sample no.:	7a	7b	7c	7a	7b	7c	7a	7b	7c	7	11a	11b	11c	11a	11b	11c	11a	11b	11c	11
Alkaloid no. (see Table 1A)	Distribution of alkaloids (% of total yield; t = trace amounts)																			
3	—	—	—	—	—	—	—	—	—	—	—	—	—	t	—	—	t	—	—	—
4	—	—	t	2	1	1	9	7	1	t	t	—	t	10	t	t	t	t	t	t
5	4	2	1	21	6	2	22	11	2	t	t	23	t	5	50	t	t	13	t	t
7	9	23	2	19	52	7	14	32	3	—	10	15	5	15	9	12	t	t	t	—
9	—	—	—	t	t	t	3	3	1	—	t	—	—	2	t	t	t	t	t	—
11	t	1	4	8	1	13	4	2	15	t	t	—	t	10	t	10	t	t	t	1
14	—	—	t	t	—	3	—	—	t	—	29	t	19	t	t	t	t	—	—	t
16	—	—	—	—	—	—	—	—	—	—	—	—	t	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19	69	63	77	44	36	65	25	22	54	96	7	10	15	38	30	57	t	1	t	83
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	18	10	16	7	3	9	8	7	8	2	53	52	58	6	6	15	t	t	t	14
22	—	—	—	—	—	—	—	—	—	—	t	t	t	t	t	t	—	t	t	t
23	—	—	—	—	—	—	—	—	—	t	2	t	t	2	t	t	t	—	t	t
24	—	—	t	t	t	t	6	4	6	1	—	—	—	t	—	t	—	t	—	t
25	—	—	—	2	t	t	8	12	8	t	—	—	—	t	t	t	—	—	—	t
26	—	—	—	—	—	—	—	—	—	—	—	—	—	t	—	—	—	t	—	t
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	—	—	t	—	t	11	5	6	99	85	99	t
Total yield (mg g^{-1} dry wt):	5.3	5.3	7.0	4.0	3.6	9.3	4.2	4.6	9.9	9.6	1.4	0.6	1.7	1.0	0.5.	0.5	1.8	1.2	0.9	8.6

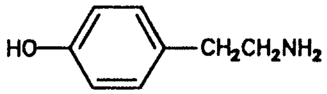
TABLE 4. DISTRIBUTION AND YIELDS OF MAJOR ALKALOIDS IN LEAVES AND SEEDS FROM TWO SUBSPECIES OF *PEARSONIA SESSILIFOLIA* (LEAVES AND SEEDS ARE FROM THE SAME PLANTS; NOTE THE HIGH PERCENTAGE OF TOTAL HYDROXYLUPANINES IN SEEDS)

	ssp. *marginata*		ssp. *sessilifolia*	
Plant parts:	Leaves	Seeds	Leaves	Seeds
Sample no:	2a	2b	5a	5b
Alkaloid no. (see Table 1A)	Distribution of alkaloids (% of total yield; t = trace amounts)			
3	1	—	—	—
4	70	t	33	t
5	t	t	27	t
7	9	t	t	—
9	t	—	t	t
11	1	1	5	t
12	3	t	8	t
14	t	3	t	1
17	4	93	6	33
19	—	—	7	—
21	—	t	2	63
22	t	t	—	—
25	—	—	t	t
26	t	1	—	t
27	t	1	—	1
28	—	t	—	—
Total hydroxylupanines (14 to 22):	4	96	15	97
Total yield (mg g^{-1} dry wt)	5.7	6.6	1.2	4.9

TABLE 5. DISTRIBUTION AND YIELDS OF MAJOR ALKALOIDS IN LEAVES FROM TWO INDIVIDUAL PLANTS OF *PEARSONIA CAJANIFOLIA* SUBSPECIES *CAJANIFOLIA* SAMPLED AT DIFFERENT TIMES DURING THE GROWING SEASON (NOTE THE INCREASE IN TOTAL HYDROXYLUPANINES, THE DECREASE IN TOTAL ESTERS AND THE DECREASE IN TOTAL YIELD)

Provenance:	Darrenwood, Johannesburg			Scheerpoort, Magaliesberg		
Date collected:	12.11.1989	13.03.1990	07.04.1990	12.11.1989	03.12.1989	23.03.1990
Growth phase:	Vegetative	Flowering	Fruiting	Vegetative	Flowering	Fruiting
Sample no.:	10a	10b	10c	9b	9c	9a
Alkaloid no. (see Table 1A)	Distribution of alkaloids (% of total yields; t = trace amounts)					
3	t	t	—	—	—	—
4	1	t	t	t	t	—
5	1	4	5	4	7	t
7	1	16	15	13	7	t
9	t	13	24	t	t	t
11	t	t	t	1	2	1
14	—	—	—	—	—	t
17	—	—	—	—	—	—
19	13	36	49	10	27	34
21	t	t	—	—	—	t
22	1	t	—	4	8	64
23	3	t	—	1	t	t
24	73	21	6	15	24	t
25	t	t	t	—	—	t
26	—	—	—	46	19	t
27	—	—	—	—	—	—
28	t	—	—	—	—	—
Total hydroxylupanines (14 to 23):	17	36	49	15	35	98
Total esters (24 to 28)	73	21	6	61	43	—
Total yield (mg g^{-1} dry wt)	15.8	2.0	1.2	4.3	2.9	2.3

A



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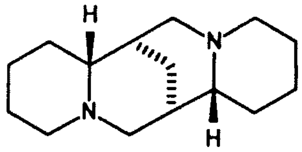

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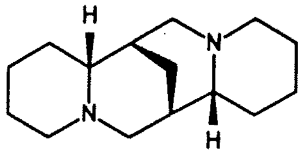

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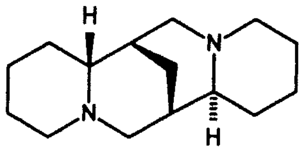

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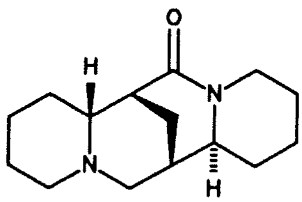

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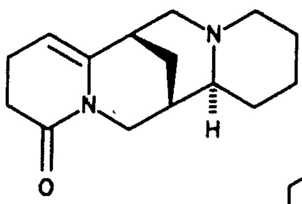

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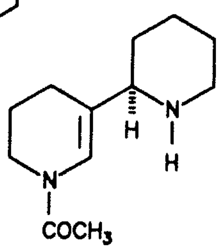

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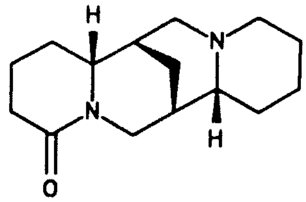

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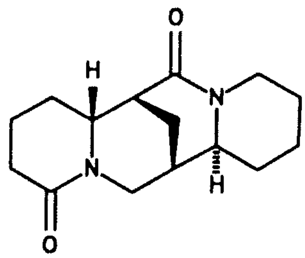

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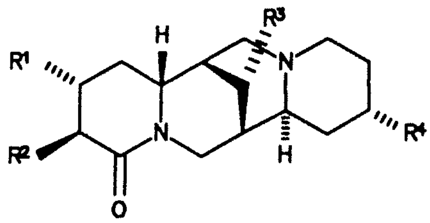

	R[1]	R[2]	R[3]	R[4]
11	H	H	H	H
14	H	OH	H	H
17	OH	OH	H	H
19	H	H	H	OH
21	H	OH	H	OH
22	H	H	OH	OH
23	H	OH	OH	OH
24	H	H	H	OAng
25	H	OH	H	OAng
26	H	H	OH	OAng
27	OAng	OH	H	H
28	H	OH	OH	OAng

The *P. sessilifolia* group is exceptionally variable, both in the total yields that were obtained and in the distribution of major alkaloids (Table 1C). Sparteine is a major alkaloid in most samples of *P. sessilifolia* (particularly in subspecies *filifolia* and *swaziensis*) and occurs only in small quantities elsewhere in the genus. Very low yields were obtained from *P. aristata* and *P. metallifera*, so that the results for these two species are not conclusive. The combination of lebeckianine and sessilifoline appears to be a unique character for *P. sessilifolia* even though it is not present in all the subspecies and provenances. Interestingly, alkaloids seem to have limited value above the species level. The major discontinuities occur between species rather than between the three groups that are listed separately in Tables 1A, 1B and 1C.

Provenance differences

A significant part of the variation within some of the species seems to be determined by geographical origin. The need to sample several populations in order to characterize a species is evident in Table 2. Each of the six populations of *P. cajanifolia* that were sampled had a unique quantitative composition of alkaloids. The high concentration of pearsonine in the Blydepoort provenance is particularly noteworthy. Differences between plants from the same provenance are clearly much smaller than the differences between provenances. Considerable infraspecific variability also occurs in *P. sessilifolia* (see Table 1C) but the samples of *P. flava* and *P. grandifolia*, however, appear to be rather similar.

Plant parts

In *P. cajanifolia*, large quantitative differences were found between various parts of the same plant. Table 3 shows the variable combinations of major alkaloids that occur in root bark, twigs, leaves and seeds of this species. In both populations, leaves contain significantly higher concentrations of esters than the root bark, twigs and seeds. The remarkable difference between leaf and seed samples is also found in *P. sessilifolia*, as shown in Table 4. Note that lebeckianine (**17**) occurs only in seeds of the latter species and not in seeds of *P. cajanifolia*, where this alkaloid appears to be replaced by 13α-hydroxylupanine (**19**). Seeds of both species contain hydroxylated lupanines and only small amounts of esters.

Seasonal differences

Evidence for seasonal variation in the alkaloid contents of leaves was found in samples from two individual plants of *P. cajanifolia* ssp. *cajanifolia*, where a distinct change occurred in the relative amounts of hydroxylated lupanines and their angelate esters. This apparent decrease in esterification towards the end of the growing season is shown in Table 5. Note also the decreasing total yields of alkaloids towards the end of the season. The high yields obtained from seeds (see Tables 3 and 4) suggest that alkaloids are concentrated in the seeds, perhaps as part of a chemical defense mechanism. Similar quantitative differences between leaves and seeds were found in the genus *Virgilia* but here the seeds accumulate esters of alkaloids rather than hydroxylupanines [7]. Seasonal changes have been observed in the tribe Genisteae, where there are distinct quantitative changes during the growing season in *Laburnum watereri* [8] or a decrease in total alkaloid content in *Adenocarpus* [9].

The complex patterns in *Pearsonia* show that alkaloids may be taxonomically significant even at the species level but that the data should be interpreted with caution. Several factors may be responsible for quantitative and qualitative differences and these sources of variation should be accounted for in the sampling procedure. The overall impression is that differences are due to shifts in the biogenetic pathways along which the compounds are formed and that a large part of the alkaloidal complexity in *Pearsonia* can be explained by precursor–product relationships.

Acknowledgements—We thank Carol Posthumus, Danie Swanepoel and Anne Lise Schutte for help with sampling of material and extraction work. Funding from the Rand Afrikaans University is acknowledged.

References

1. Van Wyk, B.-E. and Verdoorn, G. H. (1989) *Biochem. Syst. Ecol.* **17**, 391.
2. Verdoorn, G. H. and Van Wyk, B.-E. (1990) *Phytochemistry* **29**, 1297.
3. Van Wyk, B.-E. and Verdoorn, G. H. (1989) *Biochem. Syst. Ecol.* **17**, 225.
4. Van Wyk, B.-E. and Verdoorn, G. H. (1989) *Biochem. Syst. Ecol.* **17**, 385.
5. Polhill, R. M. (1973) *Kew Bull.* **29**, 383.
6. Verdoorn, G. H. and Van Wyk, B.-E. (1991) *Phytochemistry* **30** (in press).
7. Greinwald, R., Veen, G., Van Wyk, B.-E., Witte, L. and Czygan, F.-C. (1989) *Biochem. Syst. Ecol.* **17**, 231.
8. Greinwald, R., Schultze, W. and Czygan, F.-C. (1991) *Biochemie und Physiologie der Pflanzen* (in press).
9. Greinwald, R., Bachmann, P., Witte, L., Acebes-Ginoves, J. R. and Czygan, F.-C. (1991) *Biochem. Syst. Ecol.* **19** (in press).