S.Afr.J.Bot., 1991, 57(6): 344 – 347

Taxonomic significance of alkaloids in the genus *Liparia* (Fabaceae – Liparieae)

B.-E. van Wyk*, G.H. Verdoorn† and R. Greinwald‡
*Department of Botany, Rand Afrikaans University, P.O. Box 524, Johannesburg, 2000 Republic of South Africa
†Department of Chemistry and Biochemistry, Rand Afrikaans University, P.O. Box 524, Johannesburg, 2000
Republic of South Africa
‡Institut für Pharmazeutische Biologie, University of Würzburg, Mittlerer Dallenbergweg 64, D-8700 Würzburg, Germany

Accepted 30 August 1991

Comparative GC and GC–MS analyses of the alkaloids of *Liparia splendens* (Burm. f.) Bos & De Wit and *L. parva* Vog. ex Walp. have shown that the two species differ considerably. *L. splendens* contains ammodendrine (a bipiperidyl compound) as major alkaloid, while *L. parva* has sparteine, lupanine and other quinolizidine alkaloids as major alkaloids. Only small quantities of bipiperidyl alkaloids were present in the latter. The two species of *Liparia* are morphologically similar and the different alkaloid patterns are therefore quite unexpected. Our results support the idea that different pollination mechanisms may lead to an enlarged taxonomic distance between closely related species.

Vergelykende GC- en GC–MS-ontledings van die alkaloïede van *Liparia splendens* (Burm. f.) Bos & De Wit en *L. parva* Vog. ex Walp. het aangetoon dat die twee spesies beduidend van mekaar verskil. *L. splendens* bevat ammodendrien ('n bipiperidiel-verbinding) as hoof-alkaloïed, terwyl *L. parva* sparteïen, lupanien en ander kinolisidien-alkaloïede as hoof-alkaloïede het. Slegs klein hoeveelhede bipiperidiel-alkaloïede is in laasgenoemde teenwoordig. Die twee spesies van *Liparia* is morfologies eenders en die verskillende alkaloïed-patrone is derhalwe geheel en al onverwags. Ons resultate ondersteun die idee dat verskillende bestuiwingsmeganismes tot 'n groter taksonomiese afstand tussen naverwante spesies kan lei.

Keywords: Alkaloids, chemotaxonomy, Fabaceae, generic relationships, *Liparia*.

Introduction

Generic delimitations in the Liparieae are considered to be somewhat doubtful (Polhill 1981) and alkaloids may therefore be useful independent criteria for testing presumed relationships based on morphological evidence. In this paper, we report on a study of alkaloids in the genus *Liparia* (L.) DC. The two species of this genus are morphologically similar to some species of the closely related *Priestleya* DC. but can be distinguished from the latter by the dense, many-flowered terminal inflorescences (see Figure 1). *Liparia splendens* (Burm. f.) Bos & De Wit occurs along the Cape coastal region, from the Cape Peninsula to Albertinia, while *L. parva* Vog. ex Walp. is known only from the southern parts of the Cape Peninsula (Bos 1967).

Steinegger and Schlunegger (1970) have isolated (+)-sparteine, (–)-lupanine and (+)-ammodendrine from *Liparia parva*. Based on TLC comparisons, these alkaloids were also reported as the major alkaloids of *Liparia splendens*. To allow a reliable generic characterization and a proper evaluation of the taxonomic significance of alkaloids, a study of the variation within the genus became necessary.

Material and Methods

Small leaf samples of *Liparia splendens* and *Liparia parva* Vog. ex Walp. were obtained from herbarium specimens as listed in Table 1. Collections of fresh material of *L. splendens* (sample 6) and *L. parva* (sample 11) were used for comparison.

The method of extraction was the same as described by Van Wyk and Verdoorn (1991). Dry plant material was homogenized in 0.05M aqueous H_2SO_4 and left for 30 min. After filtration, the homogenate (20 ml) was applied to glass columns with celite (24 g). The aqueous phase was made basic with ammonia (4 ml) and extracted with 100 ml CH_2Cl_2. After evaporation of the solvent under reduced pressure, the residue was dissolved in minimum MeOH. For preliminary identification by comparative gas chromatography, the following two sets of conditions were used. System 1: DB1 fused silica capillary column (30 m × 0.25 mm i.d.; N_2 as carrier gas; column temperature 150 – 320°C at 6°C/min, 15 min isotherm; split ratio 1:30; PND detection at 300°C). System 2: DB1 fused silica capillary column (15 m × 0.25 mm i.d.; He as carrier gas; column temperature 150°C, 2 min isotherm, 10°C/min to 250°C, 20°C/min to 300°C, 10 min isotherm; split ratio 1:30; PND detection at 300°). Authentic reference samples of several quinolizidine alkaloids and a number of extracts previously studied by GC–MS were available to us for comparison. Samples 1, 4, 7 and 9 were studied by GC–MS (Finnigan- Matt 3700 Mass Spectrometer at 70 eV) under the following conditions: DB1 fused silica capillary column (30 m × 0.25 mm i.d.; He as carrier gas; column temperature 150°C, 6 min isotherm, 6°C/min to 320°C; split ratio 1:30; injection volume 1 μl).

The main alkaloids, ammodendrine (**8**), sparteine (**4**) and lupanine (**11**) were identified by comparative TLC, comparative GC and GC–MS. The minor compounds were identified by comparative GC (co-injection of authentic samples) and GC–MS. These minor alkaloids occurred in such low concentrations that it was not possible to confirm their identity by spectroscopic methods. The retention times and mass spectra of α-isosparteine (**3**), *N'*-methylammodendrine (**5**), β-isosparteine (**6**), 17-oxosparteine (**9**), 11-epilupanine (**11**), 17-oxo-lupanine (**12**), 3β-hydroxylupanine

Figure 1 Inflorescences of the species of *Liparia*. A, *L. parva* (Paulsberg, 1990-09-15, *Van Wyk 3149* in JRAU); B, *L. splendens* (Constantiaberg, 1988-01-16, *Van Wyk 2748* in JRAU).

Table 1 Voucher specimens of leaf samples of *Liparia* used for alkaloid studies and total yields of alkaloids obtained[a]

Sample number	Voucher specimen	Locality	Date collected	Quantity extracted (mg dry wt)	Yield (mg/g dry wt)
Liparia splendens (Burm. f.) Bos & De Wit					
subsp. *splendens*					
1.	*Anon. sub PRE 53039*	Between Simonstown and Melbus Point	no date	522	2.1
2.	*Muir sub PRE 5731*	Vryers Berg, Mossel Bay Dist.	May 1915	749	2.3
3.	*Bos 411* (PRE)	Brackenfell	Aug. 1963	170	7.7
4.	*Pillans s.n.* (PRE)	Mountains west of Muizenberg	Dec. 1918	285	5.6
5.	*Acocks 689* (PRE)	Skoorsteen Kop, Hout Bay	Aug. 1936	691	3.2
6.	*Van Wyk 2748* (JRAU)	Constantiaberg	Jan. 1988	820	3.5
subsp. *comantha* (Eckl. & Zeyh.) Bos & De Wit					
7.	*Galpin 3907* (PRE)	Garcias Pass	Sept. 1897	368	6.3
Liparia parva Vog. ex Walp.					
8.	*Pillans 3416* (PRE)	West of Red Hill, Cape Peninsula	June 1918	564	4.6
9.	*Taylor 10341* (PRE)	Froggy Farm, Cape Peninsula	Dec. 1981	440	16.1
10.	*Grobbelaar 2537* (PRE)	Cape of Good Hope Nature Reserve	July 1980	300	8.0
11.	*Van Wyk 3149* (JRAU)	Paulsberg, Cape of Good Hope N.R.	Sept. 1990	986	7.2

[a] Yield figures (in mg/g dry wt) were estimated from GC results, using peak area and 4 mg/ml sparteine as external standard.

(**13**), lebeckianine (**14**) and 13α-hydroxylupanine (**16**), however, were identical to those obtained in several other studies. The mass spectra for piperidyl alkaloids were also identical to results obtained in a detailed study of the mass spectra of piperidyl alkaloids (Fitch & Djerassi 1974; Fitch *et al.* 1974). The mass spectrum of *N*′-methylammodendrine (**5**) from *Liparia* agree exactly with the spectrum of this compound from the genus *Dichilus* (Van Wyk *et al.* 1988). Tyramine (**1**), a phenolic amine, was positively identified by comparison (TLC, GC and MS) with an authentic sample (fully characterized by ^{1}H- and ^{13}C-NMR) extracted from *Pearsonia obovata* (Van Wyk & Verdoorn 1991). The mass spectra of the unknown minor alkaloids suggest than *unknown 1* is a piperidyl alkaloid and that *unknown 2* is an isomer of lebeckianine (14).

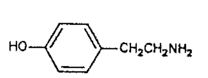
Results

The distribution of alkaloids in 11 leaf samples of *Liparia* is shown in Table 2. There is no obvious difference between the two subspecies of *L. splendens* and the age of the material seems unimportant except for the higher concentrations of tyramine (1) in fresh material. Ammodendrine (8) was present as the major compound in *L. splendens* but occurs only in small amounts in *L. parva*, where sparteine (4) and lupanine (5) are the major alkaloids. Several minor alkaloids were detected, most of which could be positively identified. The major alkaloids and most of the minor alkaloids in *L. parva* are of the quinolizidine type, whereas *L. splendens* contains mostly piperidyl alkaloids. The remarkable quantitative difference between the two species is clear from Table 2.

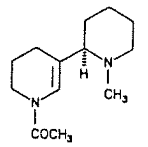
Discussion

Owing to a lack of comparative information for other genera of the tribe, the alkaloids of *Liparia* give no clear indication of affinities at the generic level. Anabasine, reported as a major alkaloid in some species of *Priestleya* by Steinegger *et al.* (1971), is totally absent in *Liparia*. From a morphological point of view, *Liparia* is closely related to the genus *Priestleya*, but available evidence does not allow meaningful comparisons at this stage. The absence of α-pyridone alkaloids in *Liparia* is nevertheless consistent with available evidence for other genera of the Podalyrieae and Liparieae.

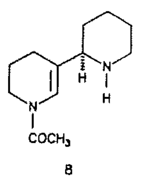
The large quantitative differences between the two species of *Liparia* are quite unexpected and are not reflected in the results of an earlier study by Steinegger and Schlunegger (1970). Alkaloids are rarely diagnostically different in closely related species of the Fabaceae and are generally more useful at the generic level (Kinghorn & Smolenski 1981). Based on variation studies in a large number of samples, Greinwald *et al.* (1989) could show some quantitative differences between the species of *Virgilia* (tribe Podalyrieae). Morphological differences between the two species of *Liparia* are very evident in the structure of the inflorescences and flowers (Figures 1A and 1B). Scott Elliot (1890) assumed that *L. parva* was pollinated by *Xylocopa* species but he did not see the bees actually pollinating. Field observations by one of us (BvW) suggest that *L. parva* may



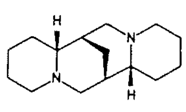
1



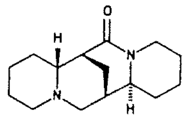
5



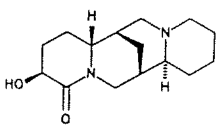
8



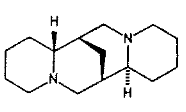
3



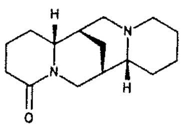
9



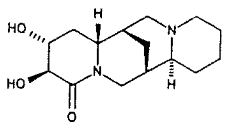
13



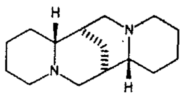
4



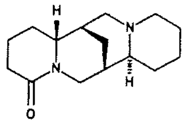
10



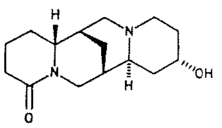
14



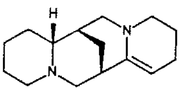
6



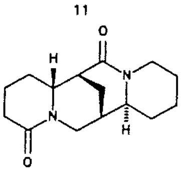
11



16



7



12

Table 2 Distribution of alkaloids in 11 leaf samples of *Liparia*

Alkaloid	Retention	Distribution of alkaloids[a]										
		Liparia splendens							*Liparia parva*			
Sample number	time[b]	1	2	3	4	5	6	7	8	9	10	11
1. Tyramine	7.28	—	tr	tr	10	1	37	tr	4	tr	1	15
2. Unknown 1	12.08	9	38	—	1	tr	2	tr	1	—	—	1
3. α-isosparteine	12.62	—	—	tr	tr	tr	tr	tr	1	1	tr	tr
4. Sparteine	13.77	2	1	1	1	1	2	tr	53	78	58	68
5. *N'*-methylammodendrine	14.52	2	tr	4	tr	1	—	1	2	1	1	1
6. β-isosparteine	14.61	—	—	—	—	—	—	tr	3	3	2	1
7. Δ11,12-sparteine	14.76	—	tr	tr	tr	tr	tr	tr	4	3	6	2
8. Ammodendrine	15.10	72	61	65	85	95	59	96	4	2	6	1
9. 17-oxo-sparteine	18.77	—	tr	tr	tr	tr	—	tr	5	5	11	1
10. 11-epi-lupanine	19.60	—	tr	1	—	—	—	tr	1	tr	1	tr
11. Lupanine	20.45	2	tr	tr	tr	tr	tr	tr	24	7	14	6
12. 17-oxo-lupanine	20.80	2	tr	1	tr	1	tr	2	tr	tr	tr	tr
13. 3β-hydroxylupanine	21.95	4	tr	14	1	tr	tr	tr	tr	tr	tr	tr
14. Lebeckianine	23.43	3	tr	14	1	tr	tr	tr	1	tr	tr	tr
15. Unknown 2 ($M+ = 264$)	24.20	4	tr	tr	tr	—	tr	tr	tr	tr	tr	—
16. 13α-hydroxylupanine	24.27	—	tr	—	—	—	—	tr	—	—	tr	tr

[a] % of total yield; tr = trace amounts.

[b] GC parameters are given under Material and Methods.

be pollinated by small mammals. The inflorescences (Figure 1A) are borne at ground level, usually completely hidden by the surrounding vegetation and strikingly similar in colour, odour and structure to the inflorescences of mammal-pollinated *Protea* species (Wiens *et al.* 1983). It would make an interesting field study to gain empirical evidence for non-flying mammal pollination in *L. parva*. The flowers and inflorescences of *L. splendens* (Figure 1B) show morphological adaptations that are generally associated with bird-pollination but there is also no definite evidence of ornithophily. Orange-breasted sunbirds (*Nectarinia violacea*) and large xylocopid bees visit the flowers (Marloth 1925, Skead 1967) and it seems reasonable to accept that *L. splendens* is at least occasionally or partly bird-pollinated. According to Arroyo (1981), some ornithophilous papilionoid legumes undoubtedly evolved from *Xylocopa*-pollinated species. Judged by peculiar floral characteristics, such an adaptation seems to have occurred in *L. splendens*. A change in pollination syndrome (particularly ornithophily) is known to exaggerate taxonomic gaps (Arroyo 1976) and it is interesting that this exaggerated distance is also reflected in the alkaloid patterns.

Acknowledgements

We thank the curator of PRE for permission to remove small quantities of herbarium material for alkaloid analyses.

References

ARROYO, M.T. KALIN 1976. The systematics of the legume genus *Harpalyce* (Leguminosae: Lotoideae). *Mem. N. Y. bot. Gdn.* 26: 1 – 80.

ARROYO, M.T. KALIN 1981. Breeding systems and pollination biology in Leguminosae. In: Advances in legume systematics 1. eds. Polhill, R.M. & Raven, P.H. pp. 723 – 769. Royal Botanic Gardens, Kew.

BOS, J.J. 1967. The genus *Liparia* L. (Pap.). *J. S. Afr. Bot.* 33: 269 – 292.

FITCH, W.L. & DJERASSI, C. 1974. Mass spectrometry in structural and stereoscopical problems. 263. Functional group interaction. Unusual fragmentations of amides as exemplified by bipiperidyl alkaloids. *J. Am. Chem. Soc.* 96: 4917 – 4927.

FITCH, W.L., DOLINGER, P.M. & DJERASSI, C. 1974. Alkaloid studies 68. Novel piperidyl alkaloids from *Lupinus formosus*. *J. Org. Chem.* 39: 2974 – 2979.

GREINWALD, R., VEEN, G., VAN WYK, B.-E., WITTE, L. & CZYGAN, F.-C. 1989. Distribution and taxonomic significance of alkaloids in the genus *Virgilia*. *Biochem. Syst. Ecol.* 17: 231 – 238.

KINGHORN, A.D. & SMOLENSKI, S.J. 1981. Alkaloids of Papilionoideae. In: Advances in legume systematics 2, eds. Polhill, R.M. & Raven, P.H., pp. 585 – 598. Royal Botanic Gardens, Kew.

MARLOTH, R. 1925. The Flora of South Africa, Vol. 2, p. 72. Darter, Cape Town.

POLHILL, R.M. 1981. Tribe 28. Liparieae (Benth.) Hutch. In: Advances in legume systematics 1, eds. Polhill, R.M. & Raven, P.H., p. 398. Royal Botanic Gardens, Kew.

SCOTT ELLIOT, G.F. 1890. Notes on the fertilisation of South African and Madagascar plants. *Ann. Bot.* 5: 333 – 405.

SKEAD, C.J. 1967. The sunbirds of southern Africa, p. 85. Balkema, Cape Town.

STEINEGGER, E. & SCHLUNEGGER, E. 1970. Alkaloide der südafrikanischen *Liparia parva* Vog. ex Walp. und *Liparia sphaerica* L. *Pharm. Acta Helv.* 45: 369 – 372.

STEINEGGER, E., SCHLUNEGGER, E., SCHNYDER, F. & FREHNER R. 1971. Alkaloidvorkommen in der Gattung *Priestleya* DC. *Pharm. Weekbl. Ned.* 106: 245 – 251.

VAN WYK, B.-E., VERDOORN, G.H. & SCHUTTE, A.L. 1988. The chemotaxonomic value of alkaloids in the genus *Dichilus*. *Biochem. Syst. Ecol.* 16: 471 – 474.

VAN WYK, B.-E. & VERDOORN, G.H. 1991. Alkaloidal variation in the genus *Pearsonia*. *Biochem. Syst. Ecol.* 19 (in press).

WIENS, D., ROURKE, J.P., CASPER, B.B., RICKART, E.A., LAPINE, T.R., PETERSON, C.J. & CHANNING, A. 1983. Nonflying mammal pollination of southern African proteas: a non-coevolved system. *Ann. Missouri Bot. Gdn.* 70: 1 – 31.