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Chemotaxonomic Significance of Alkaloids in the Genus *Lebeckia*

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Key Word Index—*Lebeckia*; Leguminosae; Crotalarieae; quinolizidine alkaloids; novel alkaloid; 3β,4α-dihydroxylupanine; chemotaxonomy; generic relationships.

Abstract—The alkaloids of fourteen species of *Lebeckia* have been identified. Sparteine, lupanine and nuttalline were found to be the major alkaloids of all the species studied. α-Isolupanine and a novel alkaloid 3β,4α-dihydroxylupanine (lebeckianine) were identified as minor compounds. *Lebeckia* differs from other quinolizidine-bearing genera of the tribe Crotalarieae by the absence of α-pyridone alkaloids and esters of alkaloids. The combination of major alkaloids seems to be a useful chemotaxonomic marker for the genus and agrees with suggestions that *Lebeckia* is one of the basal groups in the tribe. Despite morphological dissimilarities, species from different sections of the genus are remarkably similar in their alkaloidal constituents.

Introduction

Lebeckia Thunb. comprises an estimated 35 species of woody or suffrutescent papilionoid legumes endemic to the western and southern parts of southern Africa. The genus is considered to be one of the basal groups of the tribe Crotalarieae [1, 2, 3, 4]. A previous investigation of *L. plukenetiana* [5] resulted in the isolation of sparteine, lupanine and 4β-OH-lupanine (nuttalline) as major alkaloids. The same result was obtained for *L. cytisoides* and *L. multiflora* [6].

The aim of the present investigation was to characterize the alkaloidal metabolites of the genus as a whole, so that more definite comparisons could be made with other genera. We studied 14 different species from various sections of the genus in an attempt to show that the combination of sparteine, lupanine and nuttalline may be taken as a chemotaxonomic marker for *Lebeckia*. In terms of a better understanding of generic relationships, the apparent absence of α-pyridone alkaloids such as anagyrine, thermospine, *N*-methylcytisine and cytisine seemed particularly relevant and needed confirmation. The relationship with *Crotalaria* [3] suggested that pyrrolizidine alkaloids may also be present.

Results

Table 1 shows the distribution of seven different quinolizidine alkaloids in 15 extracts from 14 species of *Lebeckia*. Yields were generally very high (more than 10 mg/g in some species) and allowed comparative identification of all the major compounds. Sparteine, lupanine, α-isolupanine and 4β-OH-lupanine (nuttalline) were present in most of the samples. Small quantities of three unknown alkaloids occurred less frequently. One of the latter (isolated from *L. lotonoides* Schltr.) proved to be a new alkaloid. No evidence of α-pyridone alkaloids was found in any of the extracts.

The structure of the novel compound, 3β,4α-dihydroxylupanine (lebeckianine) (1), was established by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. The EI mass spectrum (Fig. 1) showed a fragmentation pattern very similar to that of lupanine type alkaloids in the lower

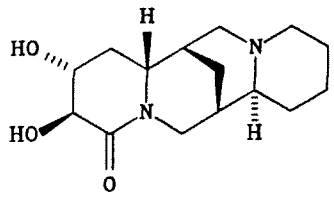

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TABLE. 1. YIELDS AND DISTRIBUTION OF ALKALOIDS IN 15 EXTRACTS FROM 14 SPECIES OF *LEBECKIA*

Species	Yields of major alkaloids (mg/g dry wt)							
	Sparteine	Lupanine	Isolupanine	Nuttalline	Lebeckianine	X1	X2	Total yield
Lebeckia section *Stiza*								
L. macrantha	4.0	0.1	tr	2.6	tr	tr	tr	6.664
L. pungens	2.6	0.2	tr	0.1	tr?	tr	tr?	2.910
Lebeckia section *Phyllodiastrum*								
L. plukenetiana	4.9	1.1	0.3	1.0	tr	0.1	tr	7.454
Lebeckia section *Eu-Lebeckia*								
L. simsiana	2.7	2.1	2.9	7.2	tr	tr	0.5	15.023
Lebeckia section *Calobota*								
L. cystisoides								
Sample 1	4.0	0.2	tr	0.1	—	tr	—	4.274
Sample 2	1.2	0.8	tr	0.3	—	tr	—	2.269
L. lotonoides	1.0	0.1	tr	tr	tr	0.1	—	1.170
L. melilotoides	8.3	0.1	tr	3.7	tr	tr	tr	12.283
L. mucronata	0.6	—	—	0.5	—	—	—	1.129
L. multiflora	3.8	0.2	tr	0.1	tr	tr	—	4.185
L. sericea	1.0	0.7	0.1	2.1	tr	tr	tr	3.832
L. spinescens	2.2	0.2	tr	0.6	0.1	tr	—	3.087
Lebeckia section *Wiborgioides*								
L. bowieana	tr	tr	tr	tr	tr	tr	tr	0.004
L. leipoldtiana	0.1	0.2	tr	0.1	—	tr	—	0.433
L. sessilifolia	tr	tr	tr?	tr	tr	—	tr?	0.002

Yield figures were esimated from GC results. X1 and X2 are unknown alkaloids with mass spectra almost identical to that of lupanine.

mass region. Typical peaks in this part of the mass spectrum are *m/z* 149, 136, 125, 110, 97, 84, 69 and 57. The molecular ion occurs at *m/z* 280, losing a hydroxyl group to produce a [M^+–OH] peak of *m/z* 263 with a 72% intensity. This fragmentation indicated a dihydroxylupanine structure for the compound. The alcoholic character was also revealed in the IR spectrum showing a broad absorption band at 3400 cm^{-1}.

The ^1H NMR spectrum (Fig. 2) of this alkaloid showed a marked similarity with those of lupanine, nuttalline and also calpaurine, a compound extracted from *Calpurnea aurea* (Ait.) Benth. [7]. In spite of overcrowding in the upfield region, the medium and down field regions provided proof for the proposed structure of lebeckianine. Confirmation of the presence of two hydroxyl groups was found in a broad two

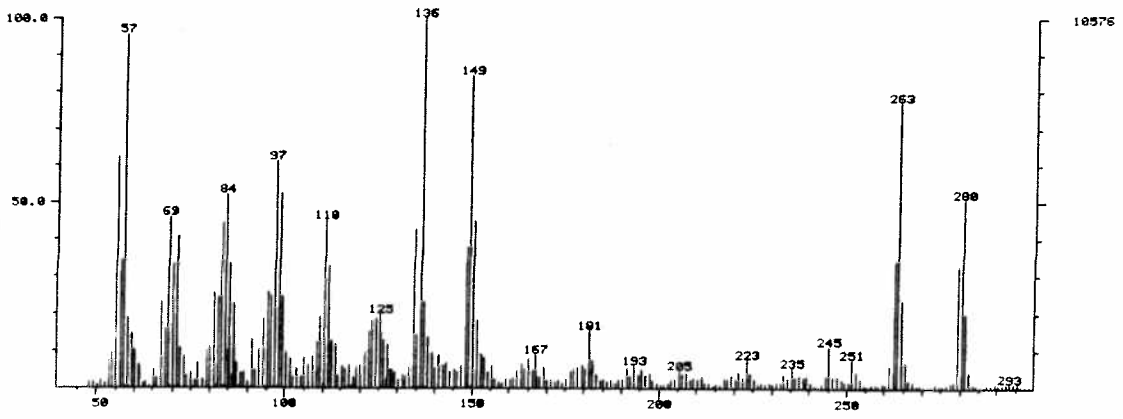

FIG. 1. THE MASS SPECTRUM OF LEBECKIANINE (Finnigan–Matt 8200 spectrometer, 70 eV electron impact).

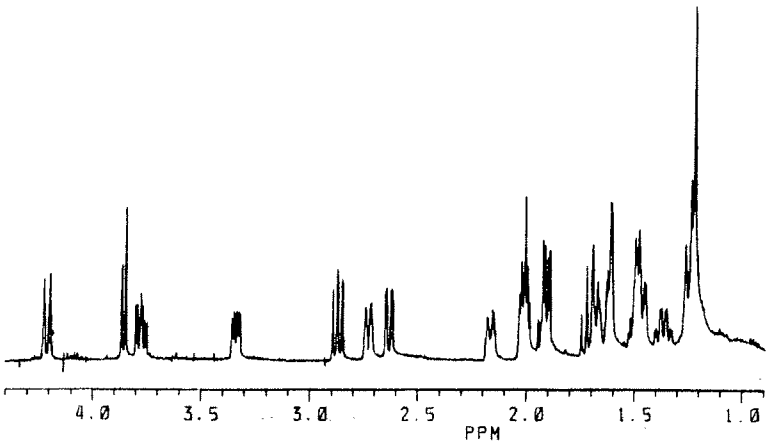

FIG. 2. THE ^{1}H NMR SPECTRUM OF LEBECKIANINE (200 MHz, $CDCl_3$, D_2O added).

proton signal at δ3.22 which was completely exchangeable with D_2O. The splitting pattern of the C-4 proton, which resonates at δ3.78, is practically identical to the C-4 proton of calpaurine. Furthermore, the H-3 is also identical to that of calpaurine. The $J_{3,4}$=9.58 Hz indicates a *trans*-diaxial relationship for these two protons, identical to calpaurine. Other protons in the medium field region such as H-6 (δ3.34), H-10a (δ4.20) and H-10b (δ2.62) compare favourably with the similar protons of nuttaline (δ3.29, 4.25 and 2.50, respectively). Because of overcrowding in the upfield region, the chemical shifts and coupling constants could not be assigned unambiguously for the high field protons. From the ^{13}C NMR spectrum (Fig. 3) the chemical shifts of the respective carbon atoms were assigned by comparison with the equivalent carbon atoms of lupanine and nuttalline, the published data for other structurally related compounds [7, 8] and 2D NMR experiments.

Discussion

It is clear from Table 1 that the various sections and species of *Lebeckia* are remarkably similar in their major alkaloids. Compared to other recently investigated genera of the Crotalarieae (*Melolobium* [9], *Polhillia* [10] and *Dichilus* [11]), the alkaloidal diversity is much less than expected. Qualitative differences between the species seem insignificant and do not reflect morphological dissimilarities. *Lebeckia melilotoides*, for example, is superficially very different from other species, notably in having small indehiscent wind-dispersed pods. Its combination of alkaloids however, is almost identical to that present in the other species.

Unlike the qualitative uniformity, there are distinct quantitative differences. These differences do not appear to be random, but may well be linked to Harvey's [12] sectional classification of *Lebeckia*. Yield figures for the section *Wiborgioides*, for example (*L. bowieana*, *L. leipoldtiana* and *L. sessilifolia*), are extremely low when compared to most other sections. These species are morphologically similar to species of the genus *Wiborgia* Thunb., in which we have found alkaloids to be virtually absent. *Wiborgia* was previously thought not to be distinct from *Lebeckia* at the generic level [13] and the two genera are known to be very closely related [2]. The very low yield figure for *L. mucronata* is also noteworthy. Although traditionally placed in the section *Calobota*, it is morphologically intermediate between the sections *Calobota* and *Wiborgioides* [2].

Generic delimitations in the Crotalarieae have not yet reached stability [14], so that the results seem valuable as supporting evidence for the current generic concept of *Lebeckia* and the close affinity with *Wiborgia*. We have found no evidence of ammodendrine or any other piperidyl alkaloids (characteristic of *Dichilus*) and also no pyrrolizidine alkaloids (common in

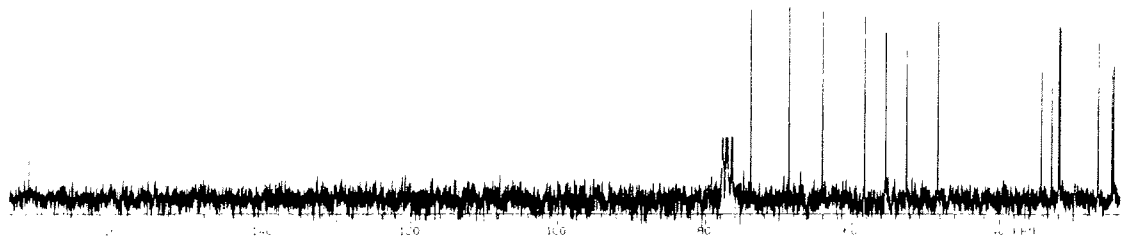

FIG. 3. THE ^{13}C NMR SPECTRUM OF LEBECKIANINE (50 MHz, $CDCl_3$, D_2O added).

Crotalaria and present in *Lotononis* and *Buchenroedera* [15]). Esters of tetracyclic quinolizidine alkaloids, recently found in the genus *Rothia* [16], also appear to be absent in *Lebeckia*. The combination of sparteine, lupanine and nuttalline and the absence of α-pyridone alkaloids and esters of alkaloids is clearly a useful diagnostic character and separates *Lebeckia* (and *Wiborgia*) from all other genera for which alkaloid data is available.

All of the alkaloids found in *Lebeckia* are structurally related to lupanine and the biosynthetic pathway does not seem to proceed beyond this basic type. It has been shown that the elaboration of ring A to α-pyridone requires specialized enzyme systems [17], so that the presence of cytisine-type alkaloids is considered to be a derived character state [18, 19, 20]. The apparant absence of C–C unsaturation in the alkaloids of *Lebeckia* therefore strongly agrees with suggestions [1, 2, 3, 4] that this genus represents part of the original lineage which gave rise to other genera of the tribe.

Experimental

Plant materials. The species studied and voucher specimen details of the material used are listed below. All voucher specimens are housed in the Rand Afrikaans University herbarium (JRAU), some of which have duplicates in various other herbaria.

Lebeckia bowieana Benth.: Uitvlug farm, N of Bredasdorp, *B.–E. van Wyk 2106*; *L. cinerea* E. Mey.: 10 km from Clanwilliam to Van Rhynsdorp, *C. M. van Wyk 2598*; *L. cytisoides* Thunb., sample 1: Goudmyn, between Robertson and Bonnievale, *B.–E. van Wyk 2705*, sample 2: Top of Wildehondkloof Pass, between Barrydale and Montagu, *B.–E. van Wyk 2651b*, sample 3: between Citrusdal and Clanwilliam, *B.–E. van Wyk 2439*; *L. leipoldtiana* Schltr. ex Dahlgr. Between Nieuwoudtville and Grasberg, *A. L. Schutte 295*; *L. lotonoides* Schltr.: hills at Saldanha Bay, *B.–E. van Wyk 2696*; *L. macrantha* Harv.: 5 km from Griquatown to Upington, *B.–E. van Wyk 2534*; *L. melilotoides* Dahlgr.: Verkeerdevlei near Touws River, *B.–E. van Wyk 2562a*; *L. mucronata* Benth.: Elandsberg, N of Patensie, *Stirton 10880*; *L. multiflora* E. Mey.: between Lekkersing and Kuboes, Richtersveld, *B.–E. van Wyk 2836*; *L. plukenetiana* E. Mey.: 40 km from Cape Town on West Coast Road, *B.–E. van Wyk 2694*; *L. pungens* Thunb.: Rooikloof, 23 km SSE of Laingsburg, *B.–E. van Wyk 2147*; *L. sericea* Thunb.: Kamiesberg Pass, *B.–E. van Wyk 2353*; *L. sessilifolia* (Eckl. and Zeyh.) Benth.: De Hoop, Bredasdorp, *B.–E. van Wyk 2120*; *L. simsiana* Eckl. and Zeyh.: Cedarberg, 3 km before Algeria, *C. M. van Wyk 2550*; *L. spinescens* E. Mey.: turn-off to Vlakfontein, 36 km N of Britstown, *C. M. van Wyk 3081*.

Isolation and identification of alkaloids. Ground air-dried leaves and twigs were extracted by refluxing with CH_2Cl_2 for several days. Alkaloidal extracts were obtained from the crude mixtures by water phase separation [6]. Previous experience [9, 10] has shown that differences in basicity may result in a partial loss of some alkaloids during resin purification. We therefore purified the alkaloidal extracts by repeating the water phase separation, followed by filtration through celite. Isolation was effected by silica gel 60 column chromatography with $CHCl_3$–Cyclohexane–Et_2NH (14:4:1) as eluent. Alkaloids were identified by analytical TLC as described previously [6] and GC using authentic reference samples. GC spectra were obtained with an SE-30 capillary glass column (25 m × 0.25 mm; N_2 as carrier gas at 0.5 kg/cm^2; column temperature 250°C isotherm; injector 300°C; FID 275°C). A large-scale extraction of *Lebeckia lotonoides* (2.55 kg) yielded reference samples of sparteine (2712 mg), lupanine (328 mg), 4β-OH-lupanine (107 mg) and 3β,4α-dihydroxylupanine (4.1 mg), all of which were fully authenticated by MS, ^1H and ^{13}C NMR. MS spectra of pure samples of sparteine, lupanine and 4-OH-lupanine extracted from *L. cytisoides* (sample 3) were identical to those from *L. lotonoides*. We obtained further confirmation of our TLC and GC identifications by GC–MS analyses of extracts from *L. plukenetiana* and *L. simsiana*. Sparteine: *Rt* 7.45, M^+ 234; X1 (unknown): *Rt* 10.05, M^+ 248; α-isolupanine: *Rt* 10.64, M^+ 248; lupanine: *Rt* 11.40, M^+ 248; X2 (unknown): *Rt* 12.17, M^+ 248; nuttalline: *Rt* 12.96, M^+ 264; lebeckianine (**1**): *Rt* 15.08, pale brown oil, $[\alpha]_D^{18}$+61° (c = 0.82 in $CHCl_3$), ν_{max} 3400 br (OH) 1640 (lactam C = O) cm^{-1}; ^1H NMR δ4.21 (1H, dt, $J_{10ax,10eq}$ 13.27 and $J_{9,10eq}$ 2.09 Hz, H-10eq), 3.86 (1H, d, $J_{3,4}$ 9.58 Hz, H-3), 3.78 (1H, ddd, $J_{3,4}$ 9.58, $J_{4,5ax}$ 11.83, $J_{4,5eq}$ 4.14 Hz, H-4), 3.34 (1H, ddd, $J_{5ax,6}$ 11.87 $J_{5eq,6}$ 5.48 and $J_{6,7}$ 1.46 Hz, H-6), 3.20 (exchangeable with D_2O) (2H, bs, 2x OH), 2.89 (1H, dd, $J_{7,17a}$ 9.83 and $J_{17a,17b}$ 11.83 Hz, H-17a), 2.74 (1H, m, $J_{15a,15b}$ 12.54 Hz, H-15a), 2.64 (1H, dd, $J_{9,10ax}$ 13.27 and $J_{10ax,10eq}$ 1.96 Hz, H-10ax), 2.18 (1H, m, H-9), 2.04–2.00 (2H, m, H-5eq and H-7), 1.96–1.91 (1H, m, H-14a), 1.92 (1H, dd, $J_{7,17b}$ 3.98 and $J_{17a,17b}$ 11.83 Hz, H-17b), 1.72 (1H, dt, $J_{4,5ax}$ = $J_{5ax,5eq}$

11.83 Hz, $J_{5ax,6}$ 11.87 Hz, H-5ax), 1.70–1.20 (8H, m, 2x H-8, H-11, 2x H-12, 2x H-13 and H-14b); ^{13}C NMR δ171.47 (1C, s, C=O), 73.77 (1C, d, C-3), 68.42 (1C, d, C-4), 63.98 (1C, d, C-11), 58.24 (1C, d, C-6), 55.33 (1C, t, C-15), 52.56 (1C, t, C-17), 48.26 (1C, t, C-10), 34.30 (1C, d, C-7), 32.97 (1C, t, C-8), 31.95 (1C, d, C-9), 31.69 (1C, t, C-12), 26.58 (1C, t, C-5), 24.82 and 24.53 (2C, 2x s, C-13 and C-14); MS *m/z* 280 (51), 263 (72), 251 (7), 245 (11), 235 (5), 223 (7), 205 (4), 193 (6), 181 (15), 167 (9), 149 (84), 136 (100), 125 (20), 110 (45), 97 (61), 84 (52), 71 (40), 69 (46), 57 (96), 55 (62).

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References

1. Dahlgren, R. (1963) *Bot. Notiser* **9**, 254.
2. Dahlgren, R. (1975) *Opera Botanica* **38**, 1.
3. Polhill, R. M. (1976) *Bot. Syst.* **1**, 143.
4. Polhill, R. M. (1981) in *Advances in Legume Systematics* 1 (Polhill, R. M. and Raven, P. H., eds), p. 401. Royal Botanic Gardens, Kew.
5. Gerrans, G. C., Howard, A. S. and Nattrass, M. J. (1976) *Phytochemistry* **15**, 816.
6. Van Wyk, B.-E., Verdoorn, G. H. and Schutte, A. L. (1988) *S. Afr. J. Bot.* **54**, 75.
7. Asres, K., Gibbons, W. A., Phillipson, J. D. and Mascagni, P. (1986) *Phytochemistry* **25**, 1443.
8. Mascagni, P., Gibbons, W. A., Asres, K., Phillipson, J. D. and Niccolai, N. (1987) *Tetrahedron* **43**, 149.
9. Van Wyk, B.-E. Verdoorn, G. H., Burger, L. and Greinwald, R. (1988) *S. Afr. J. Bot.* **54**, 386.
10. Van Wyk, B.-E., Verdoorn, G. H. and Greinwald, R. (1988) *S. Afr. J. Bot.* **54**, 389.
11. Van Wyk, B.-E., Verdoorn, G. H. and Schutte, A. L. (1988) *Biochem. Syst. Ecol.* **16**, 471.
12. Harvey, W. H. (1862) in *Flora Capensis* 2 (Harvey, W. H. and Sonder, O. W., eds), p. 83. Hodges & Smith, Dublin.
13. Dyer, R. A. (1975) *The Genera of South African Flowering Plants* 2, p. 251. Botanical Research Institute, Pretoria.
14. Van Wyk, B.-E. (1988) *S. Afr. J. Bot.* **54**, 175.
15. Van Wyk, B.-E. and Verdoorn, G. H. (1988) *Biochem. Syst. Ecol.* **16**, 287.
16. Hussain, R. A., Kinghorn, A. D. and Molyneux, R. J. (1988) *J. Nat. Prod.* **51**, 809.
17. Nowacki, E. K. and Waller, G. R. (1976) *Rev. Latinoamer. Quim.* **8**, 49.
18. Salatino, A. and Gottlieb, O. R. (1980) *Biochem. Syst. Ecol.* **8**, 133.
19. Salatino, A. and Gottlieb, O. R. (1981) *Biochem. Syst. Ecol.* **9**, 267.
20. Gomes, C. M. R., Gottlieb, O. R., Gottlieb, R. C. and Salatino, A. (1981) in *Advances in Legume Systematics* 2 (Polhill, R. M. and Raven, P. H., eds), p. 465. Royal Botanic Gardens, Kew.