Biochemical Systematics and Ecology, Vol. 16, No. 5, pp. 471–474, 1988.
Printed in Great Britain.

0305–1978/88 $3.00+0.00

Chemotaxonomic Value of Alkaloids in the Genus *Dichilus*

BEN-ERIK VAN WYK, GERHARD H. VERDOORN* and ANNE LISE SCHUTTE

Department of Botany and Department of Chemistry*, Rand Afrikaans University, P. O. Box 524, Johannesburg, 2000, South Africa

Key Word Index—*Dichilus*; Leguminosae; Crotalarieae; piperidyl alkaloids; quinolizidine alkaloids; chemotaxonomy; generic relationships.

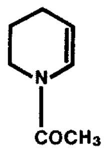
Abstract—Two bicyclic piperidyl alkaloids (ammodendrine and smipine) and a quinolizidine alkaloid (thermopsine) were identified as major alkaloids of the genus *Dichilus*. Other piperidyl alkaloids positively identified were bipyridyl, *N*-methylammodendrine, *N*-acetylhystrine, 1-acetyl-1,2,3,4-tetrahydropyridine and piperidinone. The latter two compounds have not been previously reported from the Leguminosae. In the tribe Crotalarieae, the dominance of piperidyl alkaloids appears to be a unique chemotaxonomic character for *Dichilus*. Our results strongly support the present circumscription and also the isolated position of the genus. An affinity with *Melolobium* rather than *Lebeckia* is suggested.

Introduction

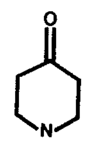
In a general survey of alkaloids in the tribe Crotalarieae [1], we have shown that a distinctive combination of several unidentified alkaloids occur in the genus *Dichilus*. This genus is morphologically remarkably similar to other genera of the tribe and its circumscription on morphological evidence alone is somewhat problematic. Superficial similarities with the genus *Lebeckia* have led to suggestions of affinity with the latter [2], but the chromosome cytology (Van Wyk, B-E. and Schutte, A. L., submitted for publication in *Kew Bull.*) does not support this view. The apparent absence in *Dichilus* of the common quinolizidine alkaloids of other genera has indicated that a full characterization of at least the major compounds would be of considerable chemotaxonomic interest.

Results

The distribution of 18 different alkaloids in 15 extracts of the five species of *Dichilus* is shown in Table 1. Yields of alkaloids were very low and allowed comparative identification of only the major alkaloids, ammodendrine, smipine and thermopsine. Due to their unusual mass spectra [3, 4] however, positive identification of most of the compounds was possible. The presence of 1-acetyl-1,2,3,4-tetrahydropyridine (**1**) and piperidinone (**2**) appears to be a new record for the Leguminosae. We have also found trace amounts of anabasine, 3-(3,4-dihydro-2H-pyrrol-5-yl)-pyridine, 3-(1-pyridinyl)-1,2,3,4-tetrahydropyridine, *N*-acetyl-3-(1-piperidincarboxaldehyde-2-yl)-piperidine and 1-acetyl-5-(1-formyl-2-piperidinyl)-2,3-dihydro-1H-pyrrole. The identity of these minor compounds could not be confirmed however and we considered it best to list them under the unknown alkaloids in Table 1. Trace quantities of alkaloids that were present as single occurrences are also not shown.



I



II

Discussion

Table 1 shows that the species of *Dichilus* are very similar and that the unusual combination of alkaloids leaves little doubt that the group is monophyletic. What differences there are appear to be quantitative only. *Dichilus gracilis*, for example, contained almost exclusively piper-

(*Revised received* 23 *April* 1988)

TABLE 1. DISTRIBUTION OF ALKALOIDS IN 3 EXTRACTS OF EACH OF THE FIVE SPECIES OF *DICHILUS*

Species	*D. strictus*			*D. reflexus*			*D. lebeckioides*			*D. pilosus*			*D. gracilis*		
Sample number	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Piperidyl alkaloids															
Ammodendrine	39	10	25	31	45	16	36	41	59	27	25	45	53	57	38
Smipine	28	18	34	31	24	27	23	3	8	36	9	18	20	13	19
Piperidinone	11	5	14	5	9	4	6	tr	—	7	14	7	6	tr	tr
Bipyridyl	tr	6	11	4	4	3	4	tr	4	4	tr?	3	3	tr	2
1-acetyl-1,2,3,4-tetrahydropyridine	tr	tr	tr	6	—	11	tr	9	2	tr?	tr?	tr?	7	9	19
N'-Methylammodendrine	1	tr	—	5	—	2	—	—	2	tr	5	tr	tr	tr	2
N-Acetylhystrine	5	—	—	3	2	3	2	—	2	—	—	—	—	—	4
Quinolizidine alkaloids															
Thermopsine	2	28	19	tr	5	1	7	21	6	13	7	2	tr	tr	tr
Lupanine	tr	1	tr	1	tr	1	1	tr	tr	tr	1	tr	tr	tr	—
N-Methylcytisine	tr	9	2	2	tr	2	tr	—	1	2	3	2	—	—	—
Anagyrine	—	2	tr	tr	—	2	tr	—	tr	tr	tr	tr	tr	tr	tr
Unknown alkaloids															
A	2	tr	tr	2	2	4	4	tr	1	3	4	3	2	1	2
B	tr	tr	—	2	8	2	8	6	3	3	tr	3	3	3	3
C	1	tr?	1	2	1	2	1	tr	tr	2	2	1	1	4	1
D	2	5	1	tr	1	2	1	1	3	tr	3	tr	tr	2	3
E	—	—	—	—	—	2	1	—	2	—	tr	2	3	—	5
F	—	1	tr?	—	—	1	—	—	tr	tr	tr	—	tr?	—	tr
G	—	—	—	—	—	—	—	—	—	—	2	tr	—	—	1

Figures given are percentages of the total alkaloid yield as estimated from GC results. Authorities for names and voucher specimen details, as well as Rt values and MS data of the unknown alkaloids are given in the experimental section.

idyl alkaloids, but thermopsine and other quinolizidine alkaloids are also present albeit in trace quantities. Qualitative differences between the species do not appear to be very significant and are more likely a result of sample limitations. The diversity and predominance of piperidyl alkaloids are of some interest. It has been postulated [5] that dipiperidine (bipiperidyl) alkaloids are biosynthetic intermediates between a cyclization product of cadaverine and a bicyclic quinolizidine alkaloid. The alkaloidal metabolites in *Dichilus* seem to show connections in a precursor–product relationship on at least two biogenetic pathways — one leading to ammodendrine and related compounds, and a minor one leading to the more conventional α-pyridone alkaloids.

The generic status and isolated position of *Dichilus* in the tribe Crotalarieae is strongly supported by the unusual combination of alkaloids. Other genera investigated so far have as major alkaloids only the more common tetracyclic and tricyclic quinolizidine types, or less frequently, pyrrolizidine alkaloids. A comparison between the genera *Lebeckia, Dichilus, Melolobium, Polhillia* and *Argyrolobium* is given in Table 2. (The position of *Argyrolobium* in the tribe Genisteae is somewhat doubtful and a transfer to the Crotalarieae was recently suggested [Van Wyk and Schutte, *op. cit.*].) *Melolobium* is the only other genus where piperidyl alkaloids have been found (Van Wyk, B-E., Verdoorn, G. H., Burger, L. and Greinwald, R., *S. Afr. J. Botany,* in press). Ammodendrine occurs as a major alkaloid in *M. microphyllum* and at least in trace quantities in several other species. We suggest that *Lebeckia* and *Dichilus* are only superficially similar and that the latter is more closely related to *Melolobium.* Morphological evidence such as the calyx structure and petiole anatomy [Van Wyk and Schutte, *op. cit.*] agrees with this view.

Piperidyl alkaloids have previously been reported from seven genera of the tribes Genisteae, Liparieae, Sophoreae and Bossiaeeae [6]. The presence of piperidyl alkaloids in the Genisteae and Crotalarieae (formerly both part of the Genisteae *sensu lato*), is further evidence of the close relationship between the two tribes. These alkaloids are known to coexist with quinolizidine and pyrrolizidine alkaloids in the genera *Ammodendron*

TABLE 2. DISTRIBUTION OF MAJOR ALKALOIDS IN THE GENERA *LEBECKIA, DICHILUS, MELOLOBIUM, POLHILLIA* AND *ARGYROLOBIUM*

	Lebeckia	*Dichilus*	*Melolobium*	*Polhillia*	*Argyrolobium*
Sparteine	+++			+++	
Lupanine	+++		++	++	
Nuttalline	+++		+?		
Argyrolobine					+
Anagyrine			++	+++	++
Thermopsine		+	+		
Camoensine			++		
Leontidine			+		
N-Methylcytisine			+	+++	
Cytisine				+	+
Lusitanine					+
Ammodendrine		+++	+		
Smipine		++			
Piperidinone		+			
Bipyridyl		+			
1-Acetyl-1,2,3,4-tetrahydropyridine		+			

Data for genera other than *Dichilus* are published elsewhere [Van Wyk *et al., op. cit.*; Van Wyk, B-E., Verdoorn, G. H. and Greinwald, R., *S. Afr. J. Botany*, in press]. +++: occurs as a major component in all species or samples, ++: most species or samples, +: at least some species or samples.

and *Adenocarpus* [6, 7], and are therefore likely to have a wider distribution in the Crotalarieae than is presently known.

Experimental

Plant materials. Collection details and voucher specimens of the species examined are listed below. All samples comprised twigs and leaves in the post-flowering stage. Voucher specimen numbers all refer to our own collections, which are housed in the Rand Afrikaans University herbarium. Yield figures (dry wt) in parentheses.

Dichilus gracilis Eckl. & Zeyh. Sample 1: Fauresmith, Orange Free State, Schutte 352 (130 $\mu g\ g^{-1}$); Sample 2; Jagersfontein, Orange Free State, Schutte 345 (154 $\mu g\ g^{-1}$); Sample 3: Colesberg, Cape, Schutte 337 (112 $\mu g\ g^{-1}$). *Dichilus lebeckioides* DC. Sample 1: Johannesburg, Transvaal, Schutte 380a (45 $\mu g\ g^{-1}$); Sample 2: Pretoria, Transvaal, Schutte 362 (10 $\mu g\ g^{-1}$); Sample 3: Johannesburg, Transvaal, Schutte 380c (47 $\mu g\ g^{-1}$). *Dichilus pilosus* Conrath ex Schinz. Sample 1: Roodepoort, Transvaal, Schutte 370a (84 $\mu g\ g^{-1}$); Sample 2: Roodepoort, Transvaal, Schutte 370b (9 $\mu g\ g^{-1}$); Sample 3: Roodepoort, Transvaal, Schutte 358 (31 $\mu g\ g^{-1}$). *Dichilus reflexus* (N. E. Br.) A. L. Schutte. Sample 1: Sani Pass, Natal, Van Wyk 2630b (100 $\mu g\ g^{-1}$); Sample 2: Pongola, Transvaal, Schutte 369 (126 $\mu g\ g^{-1}$, small sample; 12 $\mu g\ g^{-1}$, 19 kg sample); Sample 3: Pongola, Transvaal, Schutte 365 (53 $\mu g\ g^{-1}$). *Dichilus strictus* E. Mey. Sample 1: Noupoortnek, Orange Free State, Schutte 392 (12 $\mu g\ g^{-1}$); Sample 2: Between Clarens and Golden Gate, Orange Free State, Schutte 146 (73 $\mu g\ g^{-1}$); Sample 3: Reitz, Orange Free State, Schutte 376a (20 $\mu g\ g^{-1}$).

Procedures. Ground air-dried material was extracted by refluxing with CH_2Cl_2 for several days. Alkaloidal material was isolated from the crude extracts by water phase separation [1] and purified by ion exchange resin (Dowex 50 W H^+ form). Alkaloids were identified by analytical TLC and GC by comparison with and coinjection of reference samples that were studied by GC–MS. Pure samples of ammodendrine (120 mg) and smipine (7 mg) were obtained from 19 kg of air-dried leaves and twigs of *D. reflexus* (total alkaloid yield of 230 mg). Isolation was effected by silica gel 60 column chromatography as described previously [8] and Sephadex LH-20 gel filtration in MeOH as eluent. The identity of the two major compounds was confirmed by their MS spectra and characteristic signals of their ^1H NMR and ^{13}C NMR spectra. Both the ^1H and ^{13}C NMR spectra of ammodendrine showed amide isomerism and only ^{13}C signals of the major isomer are given below. Due to impurities, limited sample and amide isomerism, the ^{13}C NMR spectrum of smipine could not be assigned unambiguously. GC spectra were obtained with an OV-17 fused silica capillary column (30 m × 0.3 mm; He as carrier gas at 0.5 kg cm^{-2}, column temperature 50°, 1 min isotherm, 20°/min to 320°, 15 min isotherm; injector 320°; injector mode 1 μl splitless 30:1; FID 350°). Authentic reference samples of anagyrine (Rt 18.40), *N*-methylcytisine (Rt 13.32), thermopsine (Rt 19.32) and lupanine (Rt 17.50) were the same as used in a previous study [Van Wyk *et al., op. cit.*]. MS data were recorded on two samples (*D. reflexus* no. 3 and *D. gracilis* no. 3) and allowed positive identification of seven piperidyl and four quinolizidine alkaloids. The MS data for piperidyl alkaloids were identical to results obtained in a detailed study of the mass spectra of piperidyl alkaloids [3, 4]. Ammodendrine: Rt 14.08, ^{13}C NMR δ20.5, 21.6, 22.6, 24.8, 25.7, 31.5, 40.1, 47.2, 61.3, 119.5, 120.9, 167.7, M^+ 208; smipine: Rt 11.20, M^+ 180; piperidinone: Rt 6.20, M^+ 99; bipyridyl: Rt 10.04, M^+ 156; 1-acetyl-1,2,3,4-tetrahydropyridine: Rt 5.28, M^+ 125; *N*′-methylammodendrine: Rt 13.44, M^+ 222; *N*-acetylhystrine: Rt 15.10, M^+ 206. Data for uncharacterized or partially characterized piperidyl alkaloids — A: Rt 10.48, *m/z* 213 (2), 185 (2), 171 (3), 159 (12), 143 (13), 141 (10), 125 (3), 113 (12), 101 (12), 97 (18), 71 (31), 57 (100). B: Rt 7.24, *m/z* 202 (2), 173 (2), 167 (4), 139 (22), 138 (20), 122 (5), 111 (23), 110 (18), 97 (26), 96 (34), 83 (100), 69 (8), 55 (40), 42 (23). C: Rt 14.66, (no MS data). D: Rt 17.36, *m/z* 236 (9), 218 (100), 207 (20), 193 (15), 175 (63), 165 (25), 150 (31), 136 (19), 122 (34), 108 (26), 82 (31), 57 (22), 43 (47) [*N*-acetyl-3-(1-piperidine carboxaldehyde-2-yl)piperidine?]. E: Rt 15.20, *m/z* 298 (5), 281 (10), 261 (5), 221 (9), 202 (6), 184 (3), 171 (4), 150 (20), 137 (10), 111 (70), 97 (73), 84 (100), 71 (22), 57 (51). F: Rt 18.44, *m/z* 222 (56), 221 (11), 204 (53), 194 (16), 179 (58), 166 (13), 152 (80), 151 (72), 122 (17), 109 (100), 57 (57), 43 (99) [1-acetyl-5-(1-formyl-2-piperidinyl)-2,3-dihydro-1H-pyrrole?]. G: Rt 13.04, *m/z* 253 (4), 225 (17), 209 (30), 166 (21), 152 (19), 150 (28), 149 (28), 138 (100), 136 (70), 110 (59), 98 (89), 83 (72), 55 (51), 43 (40).

Acknowledgements—We thank Mr J. A. Kruger (Department of Chemistry, Rand Afrikaans University), Mr I. Antonowitz (CSIR), Mr P. Mouton (Delta-G) and Dr J. Koekemoer (Delta-G) for recording of spectroscopic data. Funding from the Rand Afrikaans University is acknowledged.

References

1. Van Wyk, B-E., Verdoorn, G. H. and Schutte, A. L. (1988) *S. Afr. J. Botany* **54**, 75.
2. Stirton, C. H. (1986) *S. Afr. J. Botany* **52**, 167.
3. Fitch, W. L., Dolinger, P. M. and Djerassi, C. (1974) *J. Org. Chem.* **39**, 2974.
4. Fitch, W. L. and Djerassi, C. (1974) *J. Am. Chem. Soc.* **96**, 4917.
5. Nowacki, E. K. and Waller, G. R. (1976) *Rev. Latinoam. Quim.* **8**, 49.
6. Kinghorn, A. D. and Smolenski, S. J. (1981) in *Advances in Legume Systematics*, Vol. 2. (Polhill, R. M. and Raven, P. H., eds) p. 585. Royal Botanic Gardens, Kew.
7. Mears, J. A. and Mabry, T. J. (1971) in *Chemotaxonomy of the Leguminosae* (Harborne, J. B., Boulter, D. and Turner, B. L. eds) p. 73. Academic Press, London.
8. Van Wyk, B-E. and Verdoorn, G. H. (1988) *Biochem. Syst. Ecol.* **16**, 287.