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The Taxonomic Significance of Alkaloids in the South American genus *Anarthrophyllum*

BEN-ERIK VAN WYK,* ROLAND GREINWALD† and LUDGER WITTE‡

*Department of Botany, Rand Afrikaans University, P.O. Box 524, Auckland Park, Johannesburg, 2006, South Africa;

†Institut für Pharmazeutische Biologie, University of Würzburg, Mittlerer Dallenbergweg 64, D-87000 Würzburg, Germany;

‡Institut für Pharmazeutische Biologie, University of Braunschweig, Mendelssohnstrasse 1, D-3300 Braunschweig, Germany

Key Word Index—*Anarthrophyllum*; Leguminosae; Crotalarieae; Genisteae; quinolizidine alkaloids; chemotaxonomy.

Abstract—The major alkaloids of the genus *Anarthrophyllum* have been identified for the first time. More than 28 alkaloids were detected in nine extracts from six different species. All the extracts showed a typical α-pyridone pattern, with sparteine, β-isosparteine, *N*-methylcytisine, cytisine, 5,6-dehydrolupanine, lupanine, *N*-formylcytisine, *N*-acetylcytisine and anagyrine as major alkaloids. Lupinine, epilupinine, ammodendrine and lamprolobine were also present as major compounds in some of the extracts. Tetrahydrocytisine and structurally related alkaloids occur in most of the species, but rarely in more than trace amounts. The discovery of α-pyridone alkaloids in *Anarthrophyllum* has important taxonomic implications. It provides evidence that the true affinities of the genus are with the *Argyrolobium* group (presently in the tribe Crotalarieae) and *Lupinus* (tribe Genisteae) with which it shares, in addition to the alkaloid pattern, circumcauline stipules, a trifid lower lip of the calyx and a similar chromosome number. The alkaloid data agree with morphological evidence that *Anarthrophyllum* and *Sellocharis* will be better placed near *Lupinus* in the tribe Genisteae.

Introduction

Anarthrophyllum is a South American genus of 15 species, known only from the Andes of Chile and Argentina (Soraru, 1974). In his broad review of relationships in the Genisteae and related tribes, Polhill (1976) expressed uncertainty about the affinities of *Anarthrophyllum* and the closely related *Sellocharis*, a poorly known monotypic genus from southeastern Brazil. Taking similarities with *Argyrolobium* and *Melolobium* at face value, he tentatively included the two genera in the tribe Crotalarieae (Polhill, 1981). As a result of recent cladistic studies (Van Wyk and Verdoorn, 1991; Van Wyk and Schutte, submitted) it became clear that all the genera which have the three lower lobes of the calyx united into a trifid lower lip, form a separate clade within the tribe. The presence of α-pyridone alkaloids in this group of genera (and nowhere else in the tribe) strongly supported a basal dichotomy. *Anarthrophyllum* and *Sellocharis* have the same calyx structure as the genera of the *Argyrolobium* group, but their correct tribal placement has remained undecided. Goldblatt (1981) pointed out that the chromosome number of $2n = 24$ is discordant with the remainder of the tribe Crotalarieae and suggested a possible relationship with *Lupinus* in the tribe Genisteae (several species of *Lupinus* have $2n = 24$). The only available information on the presence of alkaloids in *Anarthrophyllum* is that of Soraru (1974) who briefly mentioned the unpublished results of an analysis in which *A. desideratum* tested positive for alkaloids. Studies of alkaloids in *Dichilus* (Van Wyk *et al.*, 1988a), *Melolobium* (Van Wyk *et al.*, 1988b), *Polhillia* (Van Wyk *et al.*, 1988c) and *Argyrolobium* (Van Wyk and Verdoorn, 1989), have shown that the trifid lower lip of the calyx is correlated with the presence of α-pyridone alkaloids such as cytisine, *N*-methylcytisine and anagyrine. Alkaloidal evidence was therefore an

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obvious next step towards deciding the most likely taxonomic position for *Anarthrophyllum.*

Materials and Methods

Plant materials. Small samples of aerial parts were obtained from the following herbarium specimens. Sample 1, *A. andicolum* (Gill. ex. H. and A.) Phil. (leaves, 484 mg): *Morrison 16736* (K) 06.12.1938. Sample 2, *A. cumingii* (H. and A.) Benth. (leaves, 441 mg): *Sparre 1670* (K) 27.12.1946. Sample 3, *A. desideratum* (D.C.) Benth. (leaves, 489 mg): *O'Donell 3593* (K) 19.11.1945. Sample 4, *A. elegans* (Gill. ex. H. and A.) Phil. (Sample 4a, leaves and twigs, 1784 mg; Sample 4b, flowers, 202 mg): *Comber 50* (K) 24.09.1925. Sample 5, *A. rigidum* (Gill. ex. H. and A.) Hieron. (Sample 5a, leaves, 385 mg; Sample 5b, pods, 200 mg; Sample 5c, seeds, 85 mg); *Elwes s.n.* (K) 09.02.1902. Sample 6, *A. umbellatum* (Clos.) Phil. (leaves, 376 mg): *Worth and Morrison 16592* (K) 18.11.1938.

Procedures. Finely ground material was mixed with 15 ml 0.05 M H_2SO_4 and left standing at room temperature for 20 min. After filtration, the remaining solids were re-extracted with 5 ml 0.05 M H_2SO_4. The aqueous phases were combined, applied to glass columns with celite (24 g), alkalinized with ammonia and extracted (1X) with 100 ml CH_2Cl_2. The CH_2Cl_2 extracts were dried with anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure to leave an alkaloid mixture of pale yellow to pale brown oil. The extracts were dissolved in minimum MeOH and studied by comparative GC and GC–MS. For GC studies we used 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 150–320°C at 6°C min^{-1}, 15 min isotherm; injector 230°C; PND detection 300°C; split ratio 30:1, injection volume 1 µl). To confirm preliminary identifications, two samples (Samples 1 and 5c) were studied by GC–MS under the following conditions: DB-1 fused silica capillary column (30 m×0.32 mm i.d.; He as carrier gas; column temperature 150–300°C at 6°C min^{-1}, split ratio 20:1; injection volume 1 µl). Authentic reference samples from several previous studies were available to us (Greinwald *et al.*, 1990a,b,c, 1991; Van Wyk *et al.*, 1988a,b,c) and comparisons with literature data (Neuner-Jehle *et al.*, 1964; Schumann *et al.*, 1968) allowed the positive identification of all the major and virtually all the minor compounds by their retention indices (*RI*) and mass spectra. Retention indices were calculated according to Kovats, using co-chromatographed standard hydrocarbons. The mass spectrum of lamprolobine from *Anarthrophyllum* was identical to the spectrum of this compound from *Lamprolobium fruticosum* (Hart *et al.*, 1968; Greinwald, unpublished). Due to the small quantities of material, the structures of four partially identified minor alkaloids could not be confirmed by other spectroscopic methods. Mass spectral data of all the alkaloids detected in *Anarthrophyllum* are recorded below (for RI values see Table 1).

Epilupinine: 169 (61), 152 (100); lupinine: 169 (70), 152 (100); α-isosparteine: 234 (39), 137 (62), 98 (100); sparteine: 234 (20), 137 (100), 98 (85); *N*-methyltetrahydrocytisine: 208 (49), 109 (100), 96 (97), 58 (35); β-isosparteine: 234 (18), 193 (15), 137 (100), 98 (63); 11,12-dehydrosparteine: 232 (47), 134 (100), 97 (79); tetrahydrocytisine: 194 (50), 150 (7), 136 (4), 113 (18), 95 (100), 82 (40); ammodendrine: 208 (62), 165 (100); lusitanine: 208 (60), 166 (96), 136 (100); X1 (tetrahydrocytisine isomer?): 194 (63), 151 (15), 136 (30), 112 (22), 110 (19), 96 (38), 97 (100), 95 (67), 84 (38), 83 (50), 55 (33); *N*-methylcytisine: 204 (28), 58 (100); retamine: 250 (8), 232 (35), 207 (10), 148 (22), 134 (38), 98 (100); X2 (dehydrocytisine A): 188 (76), 160 (32), 148 (62, 134 (100); X3 (dehydrocytisine B): 188 (56), 160 (6), 147 (47), 146 (100), 68 (50); cytisine: 190 (76), 146 (100); X4 (retamine isomer?): 250 (8), 232 (25), 207 (20), 150 (22), 134 (38), 98 (100); α-isolupanine: 248 (38), 149 (52), 136 (100), 98 (30); 5,6-dehydrolupanine: 246 (35), 98 (100); *N*-formyltetrahydrocytisine: 222 (78), 193 (18), 163 (20), 150 (100), 113 (74), 95 (27), 55 (38); rhombifoline: 203 (100), 58 (55); lupanine 248 (65), 149 (75), 136 (100); lamprolobine: 264 (24), 235 (4), 222 (17), 152 (38), 138 (100), 136 (19), 124 (17), 110 (43), 97 (50), 83 (48), 55 (21); X5 (tetrahydrocytisine derivative): 266 (10), 207 (100), 167 (5), 154 (10), 112 (15), 58 (35); *N*-formylcytisine: 218 (65), 146 (100); *N*-acetylcytisine: 232 (28), 146 (100); anagyrine: 244 (38), 98 (100); baptifoline: 260 (35), 114 (100); epibaptifoline: 260 (58), 114 (100).

Results

The distribution and yields of alkaloids found in *Anarthrophyllum* are summarised in Table 1. All the extracts had the same basic combination of α-pyridone alkaloids, with anagyrine, cytisine and *N*-methylcytisine as the major alkaloids of most of the samples. Relatively large amounts of sparteine, lupanine, *N*-formylcytisine and *N*-acetylcytisine were present in some extracts. Ammodendrine was invariably present, but rarely represented more than 10% of the total yield. An interesting discovery is that of lamprolobine, which was present as a minor constituent in all the samples studied. The presence of *N*-methyltetrahydrocytisine, tetrahydrocytisine and some minor derivatives could be confirmed by comparisons of their mass spectra with published data (Schumann *et al.*, 1968). Most of the extracts also had a large number of minor alkaloids such as epilupinine, lupinine, β-isosparteine, 11,12-dehydrosparteine, lusitanine, retamine, α-isolupanine, 5,6-dehydrolupanine, rhombifoline, baptifoline

TABLE 1. DISTRIBUTION OF ALKALOIDS IN NINE EXTRACTS FROM SIX SPECIES OF *ANARTHROPHYLLUM*. Alkaloid distributions are given as percentages of total yield, as estimated from GC results using peak area and 4 mg ml^{-1} sparteine as external standard. (– = not detected; + = present in trace amounts only, i.e. less than 0.5% of total yield)

Alkaloids	Retention index (RI)	Species and sample numbers (see Materials and Methods) 1	2	3	4a	4b	5a	5b	5c	6
epilupinine	**1418**	+	**1**	+	–	–	+	+	–	**6**
lupinine	**1420**	+	+	+	+	+	+	+	+	**8**
α-isosparteine	1718	+	+	+	–	–	+	+	+	+
sparteine	**1780**	+	+	**1**	**2**	–	**22**	**1**	+	+
N-methyltetrahydrocytisine	1805	1	1	+	+	–	1	+	+	2
β-isosparteine	**1830**	+	+	**5**	**1**	–	+	+	+	**1**
11,12-dehydrosparteine	1837	+	+	+	+	–	+	+	–	–
tetrahydrocytisine	**1845**	+	+	+	+	**1**	+	+	+	+
ammodendrine	**1863**	**5**	**2**	**6**	**4**	**1**	**5**	**2**	+	**19**
lusitanine	1880	1	1	+	+	+	+	+	+	1
X1	1907	1	1	1	1	+	+	+	+	1
***N*-methylcytisine**	**1953**	**10**	**4**	**40**	**7**	**6**	**5**	**38**	**4**	**22**
X2	1968	+	+	+	+	+	2	+	+	+
retamine	1973	+	–	–	–	–	+	+	–	–
X3	1983	+	+	+	+	+	+	+	+	+
cytisine	1987	46	7	22	50	70	29	35	79	8
X4	2025	+	–	+	–	+	+	–	+	–
α-isolupanine	2100	+	+	+	–	–	+	+	+	–
5,6-dehydrolupanine	**2127**	**1**	**8**	**1**	**1**	–	**1**	**1**	+	+
N-formyltetrahydrocytisine	2148	+	+	+	–	–	+	–	–	–
rhombifoline	2150	+	+	+	–	–	+	–	+	–
lupanine	**2163**	**12**	**12**	**1**	**17**	**2**	**2**	**1**	+	**5**
lamprolobine	**2165**	**2**	**16**	**1**	+	+	**3**	**1**	+	**3**
X5	2302	2	1	+	1	–	+	+	+	**4**
***N*-formylcytisine**	**2315**	**5**	**5**	**3**	**6**	**4**	**8**	**8**	**15**	+
***N*-acetylcytisine**	**2323**	**1**	+	**2**	+	**4**	**1**	+	+	**9**
anagyrine	**2377**	**13**	**37**	**16**	**7**	**11**	**15**	**12**	+	**6**
baptifoline	2630	+	2	+	1	+	2	+	+	1
epibaptifoline	2650	+	1	+	1	+	2	+	+	1
estimated total yield (mg g^{-1})		**3.7**	**1.3**	**2.7**	**0.2**	**0.2**	**4.4**	**4.7**	**2.0**	**0.3**

and epibaptifoline. Only a few alkaloids could not be unambiguously identified by their mass spectral data alone (see Materials and Methods), but these rarely occur in more than trace amounts and are unimportant in terms of the overall pattern.

Discussion

Polhill (1981) concluded that *Anarthrophyllum* and *Sellocharis* are similar to *Argyrolobium, Melolobium* and *Dichilus* but that their true affinities are very uncertain. With the first alkaloidal data now available it is clear that the morphological similarities amongst these genera, such as the trifid lower lip of the calyx and the fusion of stipules (to various degrees) are more than just superficial and that all of them have a similar combination of α-pyridone alkaloids.

The discovery of lamprolobine in *Anarthrophyllum* provides convincing supportive evidence for the suggested connection with *Lupinus*, based on morphological and cytological considerations (Polhill, 1976; Goldblatt, 1981). Lamprolobine is so far known only from *Lamprolobium fruticosum* (Hart *et al.*, 1968), *Lupinus holosericeus* (Keller, 1980, 1981) and *Sophora* species (the latter has epilamprolobine and other structurally related alkaloids—see Murakoshi *et al.*, 1981 and Asres *et al.*, 1986). It may be rewarding to search for this unusual bicyclic quinolizidine in *Argyrolobium, Dichilus, Melolobium* and *Polhillia.*

More distantly, a possible relationship with *Lamprolobium* (tribe Brongniartieae) and *Sophora* (tribe Sophoreae) should be considered. As more and more data on poorly known genera becomes available, the general pattern supports the notion that quinolizidine alkaloids and α-pyridone alkaloids in the Leguminosae may have resulted from single evolutionary events. Most of the anomalies have been due to erroneous reports of alkaloids (based on TLC studies only) or to wrongly placed genera, grouped on the basis of overall similarity or symplesiomorphous character states. An example is the transfer of the *Templetonia* group from the Bossiaeeae (which do not have quinolizidine alkaloids) to the quinolizidine-bearing Brongniartieae by Crisp and Weston (1987). A rigorous comparative study of the Sophoreae, Brongniartieae, Thermopsideae and Genisteae may lead to new interpretations of generic and tribal relationships.

Anarthrophyllum is a highly derived genus with unusual morphological adaptations that cause difficulties in determining taxonomic affinities. The results presented here show the value of alkaloids as independent, conservative characters to evaluate presumed relationships based on morphological similarities. There is indeed considerable congruence between morphological and chemical data and we suggest that *Anarthrophyllum* and *Sellocharis* will be best placed in the tribe Genisteae, close to *Lupinus* and *Argyrolobium*.

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