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A Chemotaxonomic Survey of Major Alkaloids in *Lotononis* and *Buchenroedera*

BEN-ERIK VAN WYK* and GERHARD H. VERDOORN†

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

Key Word Index—*Buchenroedera*; *Lotononis*; Leguminosae; Crotalarieae; macrocyclic pyrrolizidine alkaloids; quinolizidine alkaloids; co-occurrence; chemotaxonomy.

Abstract—A survey of alkaloids in five species of *Buchenroedera* and 27 species of *Lotononis* has shown the presence of several pyrrolizidine, quinolizidine and piperidyl alkaloids. Senecionine, integerrimine, platyphylline, neoplatyphylline, sparteine, lupanine, 11-epi-lupanine, nuttalline and ammodendrine have been positively identified. The co-occurrence of quinolizidine and macrocyclic pyrrolizidine alkaloids in the same genus is a new record for the Leguminosae and also appears to be a unique chemotaxonomic character for *Buchenroedera* and *Lotononis*. The distribution of alkaloids in *Lotononis* does not support the traditional infrageneric classification.

Introduction

A general survey of the tribe Crotalarieae [1] has shown the presence of alkaloids in several genera and indicated distinct differences between the genera. Small quantities of alkaloids were also found in *Buchenroedera* Eckl. & Zeyh. and *Lotononis* (DC.) Eckl. & Zeyh. but only the presence of integerrimine has so far been reported from these genera [2]. As part of a taxonomic study of *Buchenroedera* and *Lotononis*, we investigated the value of alkaloids as a chemotaxonomic character at the generic and infrageneric level.

Results

A total of 62 samples from 52 species were extracted but only those extracts in which alkaloids could be detected by TLC were considered for further analyses. Despite the low yields in most of the species, we did, however, positively identify nine different alkaloids that were present as major compounds in at least some of the extracts. The presence of four macrocyclic pyrrolizidine esters, four tetracyclic quinolizidine alkaloids and one piperidyl alkaloid in five species of *Buchenroedera* and 27 species of *Lotononis* is shown in Table 1.

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Senecionine (**1**) and integerrimine (**2**) are the most common pyrrolizidine alkaloids, but relatively large quantities of platyphylline (**3**) and neoplatyphylline (**4**) were also isolated from some of the species. Sparteine (**5**), lupanine (**6**), 11-epi-lupanine (**7**), nuttalline (**8**) and ammodendrine (**9**) occur in several species but rarely in more than trace quantities.

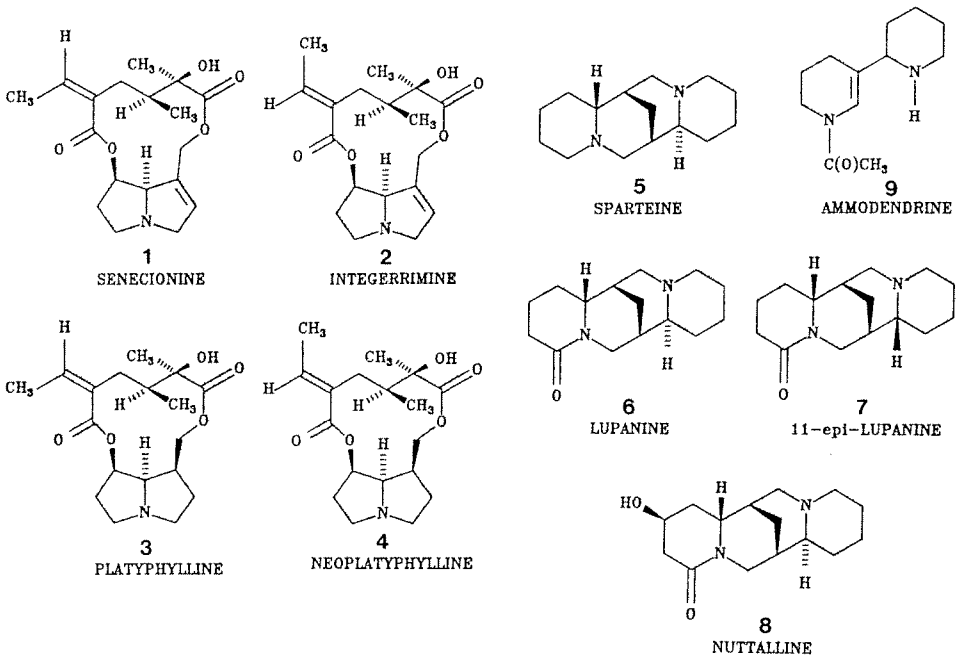
Discussion

The morphological complexity of the genus *Lotononis* is also reflected in the alkaloid data and the variation is much greater than in other recently investigated genera of the tribe [3–9]. At the generic level, the co-occurrence of quinolizidine alkaloids, piperidyl alkaloids and macrocyclic pyrrolizidine esters is here reported for the first time. This appears to be a unique chemotaxonomic character for *Lotononis* and the closely related *Buchenroedera*. *Crotalaria* is the only other genus in the Leguminosae known to contain macrocyclic pyrrolizidine alkaloids. Despite several detailed studies, not a single quinolizidine alkaloid has ever been reported from this genus [10, 11]. *Adenocarpus*, *Cytisus* and *Laburnum* are also known to contain pyrrolizidine bases (which may co-occur with quinolizidine alkaloids) [10–13], but the macrocyclic "*Senecio*-type" is known only from *Crotalaria* and now also *Lotononis*. Recent studies on other

TABLE 1. DISTRIBUTION OF MAJOR ALKALOIDS IN 41 EXTRACTS FROM 32 SPECIES OF *LOTONONIS* AND *BUCHENROEDERA*

	Major alkaloids								
	Pyrrolizidine				Quinolizidine				Piperidyl
	int	sen	plat	neo	spar	lupa	iso	nutt	ammodendrine
Buchenroedera									
B. lotononoides 1, 2 & 3	+	tr							
B. meyeri	tr	tr							tr?
B. multiflora	tr	+							tr?
B. tenuifolia 1 & 2	tr	+							tr?
B. trichodes	tr	tr							
Lotononis section *Krebsia* (Eckl. & Zeyh.) Benth.									
L. caerulescens 1, 2 & 3	tr	tr							
L. divaricata 1, 2 & 3	+	tr							tr
L. trisegmentata 1 & 2	tr	+							
Lotononis section *Aulacinthus* (E. Mey.) Benth. and related species									
L. comptonii		+							tr
L. involucrata aff.		+							
L. purpurescens	+	tr							
L. rigida	tr								
Lotononis section *Telina* (E. Mey.) Benth.									
L. azurea aff.	+								
L. elongata	+	+							
Lotononis section *Lipozygis* (E. Mey.) Benth. and related species									
L. brevicaulis	+								
L. longicephala	tr?								tr
L. polycephala		+							
L. serpens	+								
Lotononis section *Oxydium* Benth. and related species									
L. brachyloba			+	+					
L. fruticoides			+	+					
L. lenticula			+	tr					
Lotononis angolensis group									
L. bainesii					tr	+	tr	+	tr
L. listii						tr	tr	+	tr
Lotononis section *Leptis* (Eckl. & Zeyh.) Benth. and related species									
L. carinata 1 & 2						+	tr		tr
L. adpressa						tr	tr		
L. calycina						+	tr		tr?
L. curvicarpa						+			
L. eriantha						+	tr	tr	tr
L. hirsuta						+			
L. lanceolata					tr	+	tr		
L. mucronata						+	tr		
Lotononis section *Leobordea* (Del.) Benth.									
L. platycarpa					+	tr	tr	tr	

int = Integerrimine, sen = senecionine, plat = platyphylline, neo = neoplatyphylline, spar = sparteine, lupa = lupanine, iso = α-isolupanine (11-epi-lupanine), nutt = nuttalline. Authorities for names, voucher specimens and approximate yield figures are given in the Experimental section.



genera of the Crotalarieae [3–9] have shown the presence of several quinolizidine and piperidyl alkaloids, but none of the pyrrolizidine type have so far been found. Esters of quinolizidine alkaloids are characteristic for *Rothia* [7] and *Pearsonia* [9], but no evidence of these compounds was found in *Lotononis.* In past taxonomic treatments, some species of *Rothia* and *Pearsonia* were included in *Lotononis,* but the alkaloidal evidence now points to a superficial similarity rather than a direct phylogenetic relationship.

The distribution of major alkaloids indicates anomalies in the existing sectional classification of Duemmer [14]. Species with the same major alkaloids are presently placed in different sections despite morphological and cytological similarities. Some of the patterns that emerged from the present study are therefore taxonomically significant and may provide supporting evidence for a more natural infrageneric classification. *Lotononis hirsuta* (presently in section *Krebsia*), *L. eriantha* and *L. lanceolata* (presently in section *Lipozygis*) and *L. adpressa* and *L. calycina* (presently in section *Leptis*), for example, are obviously closely related and have a very similar combination of quinolizidine alkaloids (pyrrolizidine alkaloids at best only in trace quantities) but are distributed amongst different sections. If these species and their allies are excluded from their respective sections and grouped together, a much more predictive classification would result. Another example is the *L. angolensis* group of section *Polylobium,* which is morphologically quite distinct and deserves formal recognition, at least at the sectional level. An isolated position also seems to be indicated by the absence of pyrrolizidine alkaloids and the presence of nuttalline as the major alkaloid of *L. bainesii* (a well-known pasture legume) and *L. listii.* Nuttalline is one of the major alkaloids of *Lebeckia* [8] and an affinity with the latter is worth considering. *Lotononis angolensis* and related species represent the only group in *Lotononis* with well-developed bracteoles, a character which leaves little doubt about a basal position in the genus.

The available evidence in Table 1 also shows that the arrangement of the sections should be reconsidered. In view of the known distribution of pyrrolizidine esters in the plant kingdom [12,

13], it seems reasonable to interpret the accumulation of these compounds as a derived condition. The traditional sequence of species, originally conceived by Bentham [15], starts with the woody groups (superficially similar to *Lebeckia*) and ends with the presumably more derived herbaceous ones (Fig. 1A). A reversal of this sequence would more logically explain the alkaloid pattern and would also agree closely with morphological and cytological evidence. This somewhat paradoxical modification is shown diagramatically in Fig. 1B. *Lebeckia* is considered to be the least specialized genus of the Crotalarieae [16, 17] and it is therefore a logical outgroup for deciding the polarity of character states. Those species of *Lotononis* at the base of the sequence in Fig. 1B have the same chromosome number as *Lebeckia* and have a similar combination of alkaloids. The diagram suggests a gradual replacement of quinolizidine alkaloids by pyrrolizidine alkaloids, which is linked to other general trends such as a more localized geographical distribution, cyanogenesis [18], a change in chromosome base number from 9 to 7 [19], a loss of bracteoles and an increase in the incidence of other presumably apomorphic character states such as blue flowers, biramous hairs and inflated pods.

The alkaloids of *Lotononis,* although often present only in trace quantities, provide some insight into infrageneric relationships and may be useful as supportive evidence for the correct taxonomic position of some of the species. As previously suggested [2], the generic status of *Buchenroedera* is not supported by the alkaloid data. In the Crotalarieae, macrocyclic pyrrolizidine alkaloids appear to be restricted to *Lotononis* and *Crotalaria* and is a useful generic character to distinguish these two derived genera from other genera of the tribe.

Experimental

Plant materials. Voucher specimens of the species examined (all housed in the Rand Afrikaans University Herbarium),

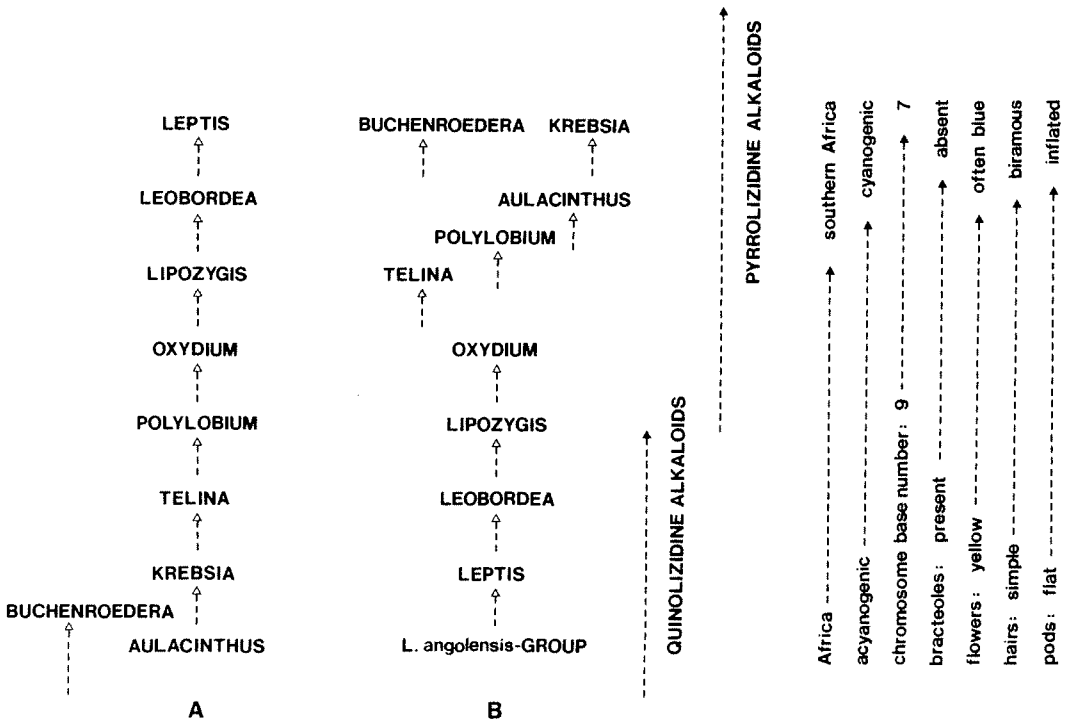


FIG. 1. THE SECTIONS OF THE GENUS *LOTONONIS*: (A), AS ORIGINALLY ARRANGED BY BENTHAM [15], AND (B) SUGGESTED REARRANGEMENT BASED ON THE DISTRIBUTION OF ALKALOIDS AND OTHER DATA.

correct authorities for names (not repeated elsewhere), and approximate yields of alkaloidal material (dry wt) are given below. (Collections of B-E. van Wyk abbreviated as VW.)

Buchenroedera lotononoides Scott Elliot sample 1: VW 2630a (140 μg/g), sample 2: VW 2630b (25 μg/g), sample 3: VW 2630c (104 μg/g); *B. meyeri* Presl: VW 1765 (82 μg/g); *B. multiflora* Eckl. & Zeyh.: B & M van Wyk 1523 (88 μg/g); *B. tenuifolia* Eckl. & Zeyh. var *pulchella* (E. Mey.) Harv. (sample 1): VW 1334 (57 μg/g); *B. tenuifolia* Eckl. & Zeyh. var *tenuifolia* (sample 2): VW 1593 (35 μg/g); *B. trichodes* Presl. VW 1693 (40 μg/g); *Lotononis adpressa* N. E. Br.: VW 1567 (32 μg/g); *L. azurea* Eckl. & Zeyh. aff.: Vlok 2030 (187 μg/g); *L. bainesii* Bak.: Koekemoer 43 (121 μg/g); *L. brachyloba* (E. Mey.) Benth.: VW 2442 (78 μg/g); *L. brevicaulis* B-E. van Wyk: VW 2212 (199 μg/g); *L. caerulescens* (E. Mey.) B-E. van Wyk sample 1: VW 2034 (2 μg/g), sample 2: VW 1614 (13 μg/g), sample 3: VW 1632 (5 μg/g); *L. calycina* (E. Mey.) Benth.: VW 2735 (11 μg/g); *L. carinata* (E. Mey.) Benth. sample 1: VW 2614 (37 μg/g), sample 2: VW 2816 (31 μg/g); *L. comptonii* B-E. van Wyk: VW 2186 (53 μg/g); *L. curvicarpa* B-E. van Wyk ined.: VW 2725 (8 μg/g); *L. divaricata* (Eckl. & Zeyh.) Benth. sample 1: VW 2597 (5 μg/g), sample 2: VW 2729a (11 μg/g), sample 3: VW 2729b (15 μg/g); *L. elongata* (Thunb.) D. Dietr.: VW 2635 (76 μg/g); *L. eriantha* Benth.: Schutte 383 (11 μg/g); *L. fruticoides* B-E. van Wyk ined.: VW 2137 (68 μg/g); *L. hirsuta* Schinz: VW 2734 (19 μg/g); *L. involucrata* (E. Mey.) Benth. aff.: VW 2704 (167 μg/g); *L. lanceolata* (E. Mey), Benth.: VW 1884 (91 μg/g); *L. lenticula* (E. Mey.) Benth.: VW 2018 (8 μg/g); *L. listii* Polhill: VW 2473 (4 μg/g); *L. longicephala* B-E. van Wyk ined.: VW 2201 (91 μg/g); *L. mucronata* Conrath: VW 1804 (23 μg/g); *L. platycarpa* (Viv.) Pich.-Serm.: VW 2822 (203 μg/g); *L. polycephala* (E. Mey.) Benth.: VW 2408 (35 μg/g); *L. purpurescens* B-E. van Wyk: VW 2720 (128 μg/g); *L. rigida* (E. Mey.) Benth.: VW 2876 (7 μg/g); *L. serpens* (E. Mey.) R. Dahlgr.: Schutte 257 (11 μg/g); *L. trisegmentata* Phillips forma *robusta* Phillips (sample 1): VW 1561 (2 μg/g); *L. trisegmentata* Phillips forma *sericea* Phillips (sample 2): VW 1968 (14 μg/g).

Procedures. Ground air-dried aerial parts were 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 H^+ form). The crude alkaloidal extract, dissolved in minimum MeOH, is slowly eluted through a small column of resin (activated with 4 N HCl and rinsed with distilled H_2O until pH 5.5). The resin is then washed with MeOH to remove non-basic impurities. Alkaloids are stripped from the column starting with H_2O–ammonia–MeOH (8:1:1) and the resin continuously rinsed with increasing proportions of ammonia until no more alkaloids are detected in the eluent. Alkaloids were identified by comparative TLC and GC using reference samples that were fully authenticated by m.p., specific rotation, MS-, IR-, ^1H NMR-, and ^{13}C NMR spectroscopy. GC conditions were the same as used in a previous study [8]. Reference samples were extracted from the following species: nuttalline from *Lebeckia lotonoides* Schltr. [8]; lupanine from *Lotononis hirsuta*; integerrimine from *Buchenroedera lotononoides*; senecionine from *Lotononis involucrata* and platyphylline and neoplatyphylline from *Lotononis fruticoides*. These pure alkaloid samples were obtained by column chromatography on silica gel and Sephadex LH-20 (cyclohexane–$CHCl_3$–Et_2NH, 5:4:1 and MeOH, respectively, as eluents). All spectroscopic data closely correlated with that given in the literature [12, 13, 20–23]. Extracts from *Buchenroedera lotononoides* (sample 1), *Lotononis bainesii*, *L. divaricata* (sample 3) and *L. longicephala* were studied by GC–MS and the results confirmed all earlier identifications by TLC and GC.

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