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Chemotaxonomic significance of alkaloids in the genus *Spartidium* (Fabaceae — Crotalarieae)

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The presence of alkaloids in the monotypic North African genus *Spartidium* Pomel is reported for the first time. Comparative GC and MS analyses showed that *S. saharae* (Coss.) Pomel contains the bipiperidyl compounds ammodendrine and *N'*-formylammodendrine as major and minor alkaloids respectively. In addition, sparteine (a quinolizidine alkaloid) and two isomers of *N*-cinnamoylhistamine (an imidazole derivative) were present in small quantities. The morphological similarities between *Spartidium* and the genus *Lebeckia* Thunb. are not reflected in the alkaloid patterns. In *Spartidium* only a trace of one quinolizidine alkaloid was detected. Ammodendrine, the major *Spartidium* alkaloid, is absent or occurs only as a trace amount in *Lebeckia*. The results provide supporting evidence for Polhill's (1976) decision to retain *Spartidium* as a distinct genus rather than to consider it a species of *Lebeckia*.

Die teenwoordigheid van alkaloïede in die monotipiese Noord-Afrikaanse genus *Spartidium* word vir die eerste keer gerapporteer. Vergelykende GC- en MS-ontledings het aangetoon dat *S. saharae* (Coss.) Pomel die bipiperidielverbindings ammodendrien en *N'*-formielammodendrien as hoof en ondergeskikte alkaloïede onderskeidelik, bevat. Behalwe hiervoor, was sparteïen ('n kinolisidienalkaloïed) en twee isomere van *N*-sinnamoïelhistamien ('n imidasoolderivaat) teenwoordig in klein hoeveelhede. Die morfologiese ooreenkomste tussen *Spartidium* en die genus *Lebeckia* Thunb. word nie in die alkaloïedpatrone gereflekteer nie. In *Spartidium* is slegs spoorhoeveelhede van een kinolisidienalkaloïed waargeneem. Ammodendrien, die hoof alkaloïed van *Spartidium*, is afwesig of kom slegs as 'n spoorhoeveelheid in *Lebeckia* voor. Die resultate bied ondersteunende getuienis vir Polhill (1976) se besluit om *Spartidium* as 'n afsonderlike genus te behou eerder as om dit as 'n spesie van *Lebeckia* te beskou.

Keywords: Alkaloids, chemotaxonomy, Fabaceae, generic relationships, *Spartidium*

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Introduction

As part of a continuing evaluation of alkaloids as a generic character in the tribe Crotalarieae, we have investigated the North African genus *Spartidium* Pomel. Polhill (1976) was uncertain about the status and correct taxonomic position of this monotypic genus and expressed the hope that cryptic characters might eventually determine its real affinities. He decided to include it in the tribe Crotalarieae, near the genus *Lebeckia* Thunb. Morphologically, *Spartidium* and *Lebeckia* were found to be virtually indistinguishable, the orientation of the seeds and the rather long funicles in *Spartidium* being the only reliable characters to separate it from some species of *Lebeckia*. Polhill also suggested that both genera may be derivatives from a stock near the present-day *Retama* Raf. and *Gonocytisus* Spach of the Genisteae *sensu stricto*. Our aim with the present study was therefore to compare the major alkaloids of *Spartidium* with those of other genera. The distinct pattern observed in *Lebeckia* (van Wyk & Verdoorn 1989) indicated to us that alkaloids may also provide valuable information about the generic status and affinities of *Spartidium*.

Material and Methods

Small samples of the aerial parts of *S. saharae* (Coss.) Pomel were obtained from the following herbarium specimens:

Sample 1 (leaves and twigs; 120 mg dry wt). Morocco: 'Duel el Kherona', April 1913, *Pitard 3276* (K).

Sample 2 (flowers and young pods; 210 mg dry wt). Libya: 'Gebel Nefoussa, Azizia — Rhnem, 200 m', 15 March 1970, *Davis 49544* (K).

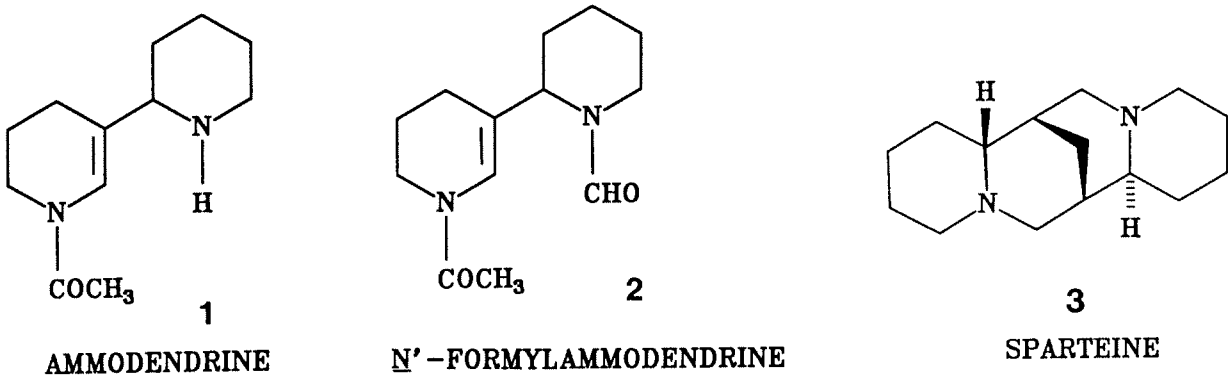
The dry plant material was homogenized in 0.05 M aqueous H_2SO_4 and left at room temperature for 20 min. After filtration, the homogenate was made basic with conc. ammonium hydroxide and the precipitated alkaloids applied to a Chemelut column (0.6 g Chemelut ml^{-1} extract) (ICT, Frankfurt). The mixed alkaloids were eluted with 100 ml chloroform and the extract taken to dryness. The extract was then redissolved in 1 ml MeOH before GC injection. For preliminary identification by comparative gas chromatography, the following conditions were used: DBl 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^{-1} to 250°C, 20°C min^{-1} to 300°C, 10 min isotherm; split ratio 1:30; PND

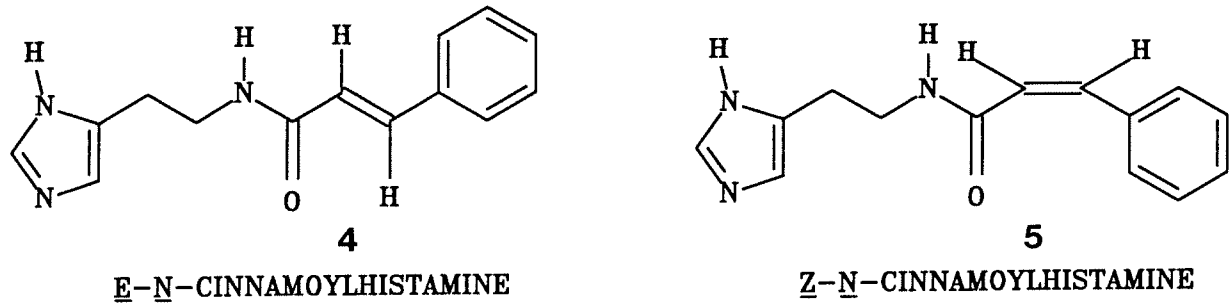
Table 1 Distribution of alkaloids in two samples of *Spartidium saharae*. [Yield figures (given as percentages of the total yield) were estimated from GC results (data obtained from peak area)]

	Retention Index (RI)	Sample 1 (leaves & twigs)	Sample 2 (flowers & young pods)
Total alkaloid yield (mg g^{-1} dry wt):		5.16	7.29
Distribution of alkaloids (% of total yield):			
Ammodendrine	1865	72.4	67.2
N'-formylammodendrine	2210	1.7	1.3
Sparteine	1785	0.2	0.6
N-cinnamoylhistamine (isomer 1)	2440	7.6	15.5
N-cinnamoylhistamine (isomer 2)	2700	5.7	5.5

detection at 300°C). Authentic reference samples of several quinolizidine alkaloids and a number of extracts previously studied by GC–MS were available to us for comparison. Sample 2 was studied by GC–MS under the following conditions: DB1-30W fused silica capillary column (30 m × 0.32 mm i.d.; He as carrier gas; column temperature 150°C to 300°C, 6°C min^{-1}; split ratio 1:20.

The identity of the main alkaloid, ammodendrine (Figure 1) was confirmed by the mass spectrum (Figure 6a). The minor compounds were identified by their retention indices (RI) and mass spectra. Further proof was obtained by the mass spectra of the silylated ammodendrine and cinnamoylhistamines after treating the column-purified extract of sample 2 with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide. Due to the small quantity of material available to us and the very low yields of the minor compounds, the identity of the latter could not be confirmed by other spectroscopic methods. The mass spectra of *N'*-formylammodendrine (Figures 2 & 6b) and sparteine (Figures 3 & 6c) however, were identical to those obtained in several other studies. The mass spectrum of *N'*-formylammodendrine (Figure 6b) [with the characteristic loss of H_2O from the molecular ion (Fitch & Djerassi 1974; Fitch *et al.* 1974)] was identical to those obtained in GC–MS studies of extracts from the genus *Dichilus* (van Wyk *et al.* 1988), where it was listed as 'alkaloid D'. The mass spectra of the compounds (Figures 4, 5 & 6d & e) strongly suggested the *Z* and *E* isomers of *N*-cinnamoylhistamine, an imidazole derivative reported from two species of *Acacia* by Fitzgerald (1964). In the subfamily Papilionoideae, imidazole is known to be widely distributed in the tribe Tephrosieae (Hayman & Gray 1987).





Figures 1–5 Structures of alkaloids. **1.** ammodendrine; **2.** *N'*-formylammodendrine; **3.** sparteine; **4.** *E*-*N*-cinnamoylhistamine; **5.** *Z*-*N*-cinnamoylhistamine.

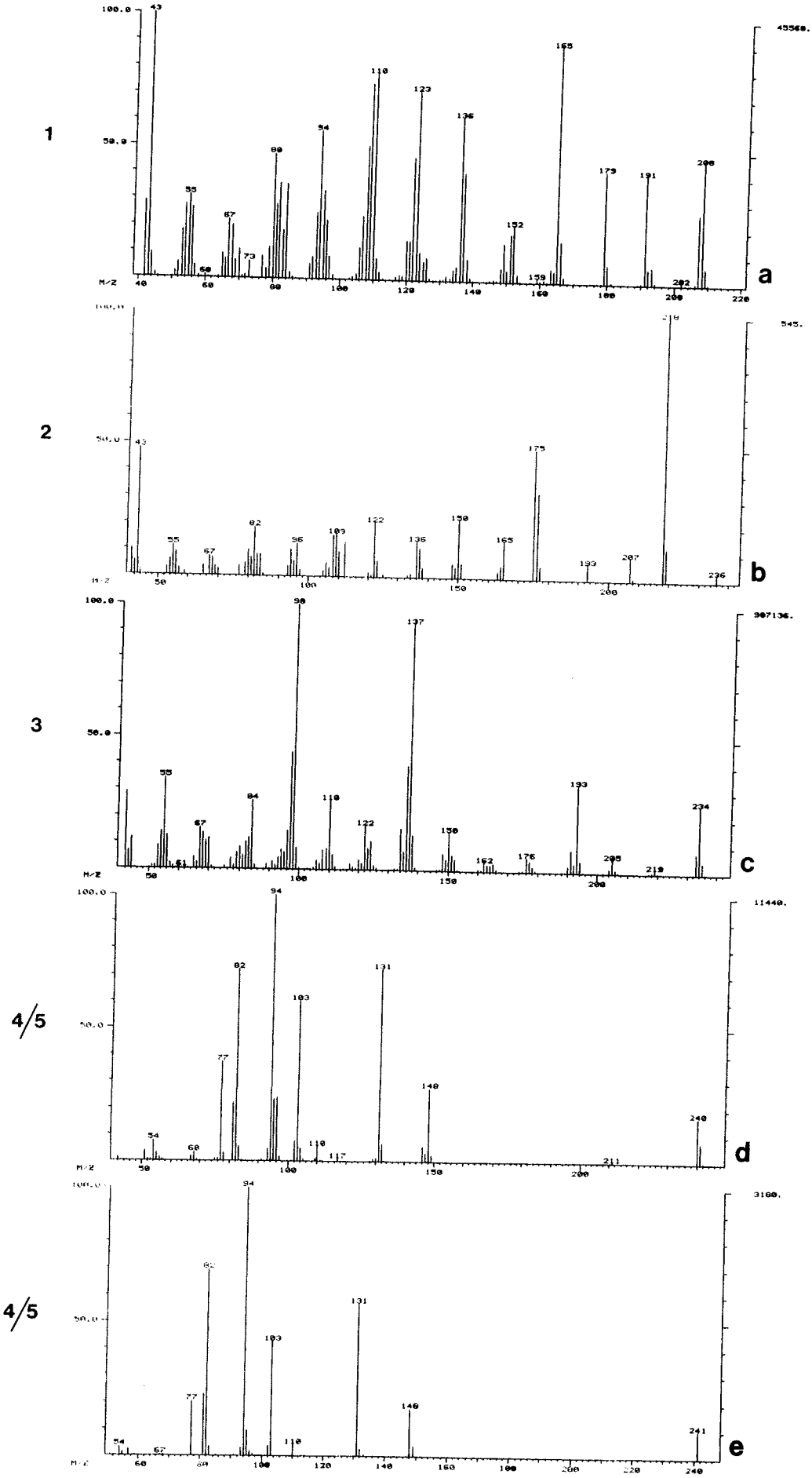


Figure 6 Mass spectra for five alkaloids identified from extracts of *Spartidium saharae* (sample 2; Finnigan-Mat 4515 mass spectrometer; 45 eV electron impact).

Results

Table 1 shows the distribution of alkaloids in the two samples studied. The combination of alkaloids in sample 1 (leaves and twigs) and sample 2 (flowers and young pods) were remarkably similar, despite the difference in age and origin of the material. Ammodendrine (Figure 1) was present as the major compound of both samples and represented 72% and 67% of the total yields respectively. Minor compounds were identified as *N'*-formylammodendrine (Figure 2), sparteine (Figure 3) and two isomers of *N*-cinnamoylhistamine (Figures 4 & 5). The mass spectra obtained for these compounds are shown in Figure 6.

Discussion

A summary of the major alkaloids of some genera of the Crotalarieae is shown in Table 2. With the exception of *Dichilus* DC. (which also produce several piperidyl alkaloids), all genera which contain α-pyridone alkaloids and pyrrolizidine alkaloids were excluded from the comparison. *Retama* and *Gonocytisus* (Tribe Genisteae) were also excluded despite the suggestion by Polhill (1976) that it may be related to *Spartidium*. Both these genera contain α-pyridone alkaloids such as cytisine, thermopsine and anagyrine (summarized in Polhill 1976) and there is no evidence (at least not from the available alkaloid data) of a direct affinity with *Spartidium*.

Table 2 shows that the combination of alkaloids in *Spartidium* is quite different from those of other recently investigated genera of the tribe Crotalarieae. This is true even if the presence of histamine derivatives (not previously reported from any genus of the Genisteae or Crotalarieae) is ignored. Ammodendrine is present in most other genera (at least in trace quantities) but invariably co-occurs with several other major alkaloids. The virtual absence of quinolizidine alkaloids is quite unexpected and represents a significant difference between *Spartidium* and *Lebeckia*. Polhill (1976) found, on the basis of a total characterization, that *Spartidium* is virtually indistinguishable from *Lebeckia*. *Lebeckia* has a characteristic combination of quinolizidine alkaloids and contains large amounts of sparteine, lupanine and nuttalline (van Wyk & Verdoorn 1989). The alkaloids of *Spartidium* therefore strongly support its present generic status.

The available alkaloid data provides no clear evidence of intergeneric relationships, but shows that the combination of alkaloids in each of the genera has diagnostic value at the generic level. The total absence of α-pyridone alkaloids and esters of alkaloids in *Spartidium* and *Lebeckia* agrees with the suggestion (Polhill 1976) of a basal position for these genera in the tribe Crotalarieae.

Table 2 Distribution of alkaloids known from the genera *Spartidium, Lebeckia, Wiborgia, Dichilus* and *Rothia*. [Data for *Lebeckia* from van Wyk & Verdoorn (1989), *Wiborgia* from van Wyk (unpublished data), *Dichilus* from van Wyk *et al.* (1988) and *Rothia* from Hussain *et al.* (1988)]

	Spartidium	*Lebeckia*	*Wiborgia*	*Dichilus*	*Rothia*
No. of species in genus:	1	±35	10	5	2
No. of species examined:	1	14	4	5	2
Tetracyclic quinolizidine alkaloids:					
nuttalline		+++	tr	tr	+
sparteine	tr	+	tr		
lupanine		+++	tr	tr	+
isolupanine		+			tr
thermopsine				++	
Esters of tetracyclic quinolizidine alkaloids:					+++
Piperidyl alkaloids:					
ammodendrine	+++	tr		+++	++
N'-formylammodendrine	tr			tr	
smipine				++	
piperidinone				+	
bipiridyl				+	
1-acetyl-1,2,3,4-tetrahydropyridine				+	
Imidazole derivatives:					
N-cinnamoylhistamine	+++				

Occurs as a major component in: +++ all species/samples, ++ most species/samples, + at least some species/samples. (*N*-cinnamoylhistamine is here entered as a major compound because the combined yield of the two isomers exceeded 10% of the total alkaloid yield in both samples)

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References

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.

FITZGERALD, J.S. 1964. Alkaloids of the Australian Leguminosae. IV. Cinnamoylhistamine, the alkaloid of *Acacia argentea* and *A. polystachya*. *Austr. J. Chem.* 17(3): 375–378.

HAYMAN, A. & GRAY, D. 1987. Imidazole, a new natural product from the Leguminosae. *Phytochem.* 26: 3247–3248.

HUSSAIN, R.A., KINGHORN, A.D. & MOLYNEUX, R.J. 1988. Alkaloids of *Rothia trifoliata* and *Rothia hirsuta*. *J. Nat. Prod.* 51(4): 809–811.

POLHILL, R.M. 1976. Genisteae (Adans.) Benth. and related tribes (Leguminosae). *Bot. Syst.* 1: 143–368.

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. 1989. Chemotaxonomic significance of alkaloids in the genus *Lebeckia*. *Biochem. Syst. Ecol.* 17 (in press).