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Chemotaxonomic Significance of Alkaloids in the Genus *Robynsiophyton*

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Key Word Index—*Robynsiophyton*; Fabaceae; Crotalarieae; quinolizidine alkaloids; esters of alkaloids; chemotaxonomy; generic relationships.

Abstract—The alkaloids of the monotypic genus *Robynsiophyton* have been studied for the first time. Most of the quinolizidine alkaloids and esters of quinolizidine alkaloids found in the genus *Pearsonia* are also the major compounds in *Robynsiophyton*. These include lupanine, 3β-hydroxylupanine, lebeckianine, lupanine-13α-angelate, cajanifoline, cryptanthine, sessilifoline and pearsonine. The results support the idea of a close affinity between *Robynsiophyton*, *Pearsonia* and *Rothia*. Except for the apparent absence of tigliate esters in *Pearsonia*, differences between the genera seem quantitative only.

Introduction

The genera *Pearsonia* Duemmer, *Rothia* Pers. and *Robynsiophyton* Wilczek are morphologically similar and are therefore considered to be closely related [1]. Within the tribe Crotalarieae, these genera are the only ones which have floral characters that are associated with a gullet-type pollination mechanism [2, 3]. In view of recent studies of the alkaloids of *Pearsonia* [4–7] and *Rothia* [8], in which several esters of quinolizidine alkaloids were reported, we were interested to see if the alkaloids of *Robynsiophyton* agree with its accepted taxonomic position [1]. The monotypic *Robynsiophyton* is indeed one of the last remaining genera of the Crotalarieae for which no alkaloid data are available [9].

Materials and Methods

Plant materials. Robynsiophyton vanderystii Wilczek: sample 1 (leaves, 147 mg), Zaire, Madimba territory, Kimbambu, 24.06.1981, *Pauwels 6470* (PRE); sample 2 (leaves, 83 mg), Zambia, near Chilongowelo turning on Mpulungu-Abercorn Road, 08.04.1959, *Richards 11097* (PRE). *Rothia hirsuta* (Gwill. & Perr.) Bak.: sample 1 (aerial parts, 298 mg), South Africa, Kruger National Park, Punda Milia, 19.03.1976, *Van Rooyen 494* (PRE); sample 2 (aerial parts, 377 mg), Transvaal, Dongola Reserve, Farm De Klundert 759, 16.03.1948, *Codd & Dyer 3889* (PRE).

Procedures. The plant material was homogenized in 0.05 M aq. H_2So_4 and left for 30 min. After filtration, the homogenate (20 ml) was applied to glass columns with celite (22 g). The aqueous phase was made basic with ammonia (4 ml) and extracted with 100 ml CH_2Cl_2. Due to the small quantities of plant material that were available, we used analytical GC and GC-MS to identify the alkaloids. Authentic reference samples were available from several other studies [4–7]. MS spectra were obtained for all the major and minor alkaloids of all four extracts and were identical to those obtained in other studies. GC spectra were obtained with 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° to 320° at 6° min^{-1}, 15 min isotherm; injector 230°C; PND detection 300°C; split ratio 30:1; injection volume 1 μl). The following parameters were used for GC-MS analyses: DB-1 fused silica capillary column (30 m×0.25 mm i.d.; H_2 as carrier gas at 4 ml min^{-1}; column temperature 150°, 3.5 min isotherm, then 150° to 320° at 6° min^{-1}, 15 min isotherm; injector 230°C; split ratio 30:1, injection volume 1 μl.

Piperidyl alkaloids. Ammodendrine: R_t 15.10, $[M]^+$ 208.

Quinolizidine alkaloids. Sparteine: R_t 13.77, $[M]^+$ 234. 11-*epi*-Lupanine: R_t 19.60, $[M]^+$ 248. Lupanine: R_t 20.45, $[M]^+$ 248. 3β-Hydroxylupanine: R_t 21.95, $[M]^+$ 264. Lebeckianine: R_t 23.43, $[M]^+$ 280. X1: R_t 24.30, MS identical to that of lebeckianine, probably an isomer of the latter. 3β,13α-Dihydroxylupanine: R_t 25.75, $[M]^+$ 280. 8α,13α-Dihydroxylupanine: R_t 26.49, $[M]^+$ 280. 3β,8α,13α-trihydroxylupanine: R_t 27.87, $[M]^+$ 296.

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Esters of quinolizidine alkaloids. Lupanine-13α-angelate: R_f 28.82, $[M]^+$ 346. Lupanine-13α-tigliate: R_f 29.10, $[M]^+$ 346. Cajanifoline: R_f 30.30, $[M]^+$ 362. Cryptanthine: R_f 30.65, $[M]^+$ 362. Sessilifoline: R_f 30.85, $[M]^+$ 362. Pearsonine: R_f 32.10, $[M]^+$ 378.

Results

The total yields and distribution of alkaloids in two extracts from *Robynsiophyton vanderystii* Wilczek are shown in Table 1. To facilitate the comparison with *Rothia* and to supplement available literature data [8], we also extracted two samples of the aerial parts of *Rothia hirsuta* (Gwill. & Perr.) Bak. Six different esters of quinolizidine alkaloids could be positively identified. These were lupanine-13α-angelate (13α-angeloyloxylupanine), lupanine-13α-tigliate, cajanifoline, cryptanthine, sessilifoline and pearsonine. Lupanine, 3β-hydroxylupanine and lebeckianine occurred as major alkaloids in both extracts. Small amounts of sparteine, 11-*epi*-lupanine (α-isolupanine), ammodendrine, 3β,13α-dihydroxylupanine and 3β,8α,13α-trihydroxylupanine were present in both extracts.

TABLE 1. DISTRIBUTION (AS A PERCENTAGE OF TOTAL YIELD) AND YIELDS (mg g^{-1} DRY WT) OF ALKALOIDS IN AERIAL PARTS OF *ROBYNSIOPHYTON VANDERYSTII, ROTHIA HIRSUTA* AND *ROTHIA TRIFOLIATA*

Species:	*Robynsiophyton vanderystii*		*Rothia hirsuta*		*Rothia trifoliata*
Sample number:	1	2	1	2	data from [8]
Sparteine	t	t	—	t	—
Ammodendrine	t	t	t	t	11.8
11-*epi*-Lupanine	t	t	t	t	1.2
Lupanine	11	40	8	7	29.3
3β-Hydroxylupanine	9	11	72	55	1.2
Lebeckianine	35	20	12	15	—
13α-Hydroxylupanine	—	—	—	—	2.1
X1 (Unknown, $[M]^+ = 280$)	t	16	—	—	—
3β,13α-Dihydroxylupanine	1	2	—	—	—
3β-8α-13α-Trihydroxylupanine	t	t	—	—	—
Lupanine-13α-angelate	8	1	—	—	23.4
Lupanine-13α-tigliate	t	—	—	—	0.1
Cajanifoline	21	t	—	—	—
Cryptanthine	3	2	3	8	—
Sessilifoline	7	4	4	5	—
Pearsonine	5	t	t	4	—
Total yield:	1.3	1.1	5.8	2.7	

t = trace amounts.

Discussion

The general pattern of alkaloids in Table 1 provides strong supporting evidence for the suggested close affinity between *Robynsiophyton* and *Rothia.* Most of the alkaloids have also been found in *Pearsonia* [6], so that the genera now appear to differ only in the relative quantities of alkaloids and not in the types of compounds that are produced. For various reasons given below, the apparent infrageneric variability within *Rothia* should perhaps not be taken too seriously at this stage. A comparison between the three genera in Table 2 shows that the absence of tigliate esters in *Pearsonia* is the only obvious qualitative difference. *Pearsonia* was previously thought to be unique in the accumulation of esters of higher oxidized lupanines [4, 5] but these compounds are indeed also present in both *Rothia* and *Robynsiophyton* (Table 1).

Our results for *Rothia* differ somewhat from those reported in the literature [8] but this may be partly due to the different plant parts that were extracted (we used aerial parts of *R. hirsuta* and not pods) and also to provenance and species differences (see [6]). The reported presence of cinevanine as a major alkaloid of *R. hirsuta* could not be

TABLE 2. SUMMARY OF THE KNOWN DISTRIBUTION OF ALKALOIDS IN THE GENERA *PEARSONIA, ROTHIA* AND *ROBYNSIOPHYTON*

	Pearsonia	*Rothia*	*Robynsiophyton*
Piperidyl alkaloids			
Ammodendrine	++	++	t
Tetracyclic quinolizidine alkaloids			
Sparteine	+	t	t
11-*epi*-Lupanine	+	t	t
Lupanine	++	++	++
3β-Hydroxylupanine	+	++	++
13α-Hydroxylupanine	++	t	—
Lebeckianine	+	++	+++
Esters of quinolizidine alkaloids			
Lupanine-13α-angelate	+	+	+
Other angelate esters	++	++	++
Tigliate and various other esters	—	++	t

Occurs as a major component in: +++ all species/samples; ++ most species/samples; + at least some species/samples. t = trace amounts.

confirmed. It is clear from our results that the alkaloid identified as nuttalline (4β-hydroxylupanine) is in fact 3β-hydroxylupanine [5]. In the ^{1}H NMR data of lupanine-type esters from *Rothia trifoliata* [8], a multiplet at δ*ca.* 4 was assigned to H-4$_{eq}$. We differ from this assignment on the basis that these compounds are not functionalized at C-4, therefore H-4$_{eq}$ cannot be at medium field in the ^{1}H NMR spectrum and should be at much higher field. This chemical shift for H-4$_{eq}$ relates closely to the H-3$_{ax}$ of alkaloids such as cajanifoline and pearsonine. The ^{13}C NMR data, however, seem to be in accordance with the structures reported. The presence of lupanine-type esters in *Robynsiophyton* shows that the morphological similarities with *Pearsonia* and *Rothia* can be taken at face value and that they are not likely to be merely a result of convergence.

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