BSE 121

Biochemical Systematics and Ecology, Vol. 00, No. 0, pp. 000–000,
Printed in Great Britain

0000-1978/89 $3.00+0.00

Distribution and Taxonomic Significance of Major Alkaloids in the genus *Virgilia*

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Key Word Index—*Virgilia*; Leguminosae; Podalyrieae; quinolizidine alkaloids; variation study; chemotaxonomy.

Abstract—As part of a detailed survey of alkaloids in the genus *Virgilia*, the distribution and chemotaxonomic value of major alkaloids have been investigated. Lupinine, sparteine, virgiboidine, virgiline, oroboidine, virgiline–pyrrolyl–carboxyl ester and an unknown alkaloid occur as major compounds in most of the samples studied. Significant quantitative differences were found between the two species and also between various parts of the plant. Only a limited amount of variation can be ascribed to environmental effects and geographical origin. The results strongly support a recent infrageneric classification of the genus. The complete absence of α-pyridone alkaloids is at variance with previous suggestions about generic relationships.

Introduction

The genus *Virgilia* comprises a small group of closely related papilionoid trees endemic to the south-western and southern coastal regions of South Africa. The latest taxonomic treatment [1] was based on an analysis of the considerable morphological variation that exists within the genus. In this paper, we report on the distribution of major alkaloids in 112 samples taken from a wide range of different populations. Our aim was to test the predictivity of the current taxonomic classification, which was based on morphological and ecological evidence alone.

Virgilia is known as a source of several quinolizidine alkaloids with unusual substitution patterns [2, 3, 4]. Our studies have shown the presence of at least 38 different alkaloidal metabolites. Amongst 23 alkaloids not previously reported for the genus, some totally new compounds, such as 4-OH-lupinine and its esters, were found. A detailed account of these is given elsewhere [Veen, G., Greinwald, R., Van Wyk, B.-E., Czygan, F.-C. and Witte, L., in preparation for *Phytochem.*].

(*Received* 16 *August* 1988).

Results

It was assumed that significant results would be obtained only if all possible sources of variation are accounted for in the sampling. We therefore studied material of different populations from the entire natural distributions area of each taxon, including at least one population of each of the different forms within the species and subspecies (Fig. 1). The sample included different individual plants from the same population and different organs (twigs, leaves, fruit pods and seeds) from the same individuals. Material from cultivated plants of known origin allowed us to evaluate possible environmental influences by comparison with material from the original provenance. A complete list of the material used for extraction and the yield of alkaloids obtained are given in Table 1.

Lupinine (**1**), sparteine (**2**), virgiboidine (**3**), virgiline (**4**), oroboidine (**5**), virgiline-pyrrolyl-carboxylic acid ester (**6**) and an unknown alkaloid were present as major compounds in most of the samples. These major alkaloids are not evenly distributed amongst the plant parts studied and also not amongst the species and subspecies. Figure 2 shows that there are distinct differences between twigs, leaves, pods and seeds and that these differences are also clearly

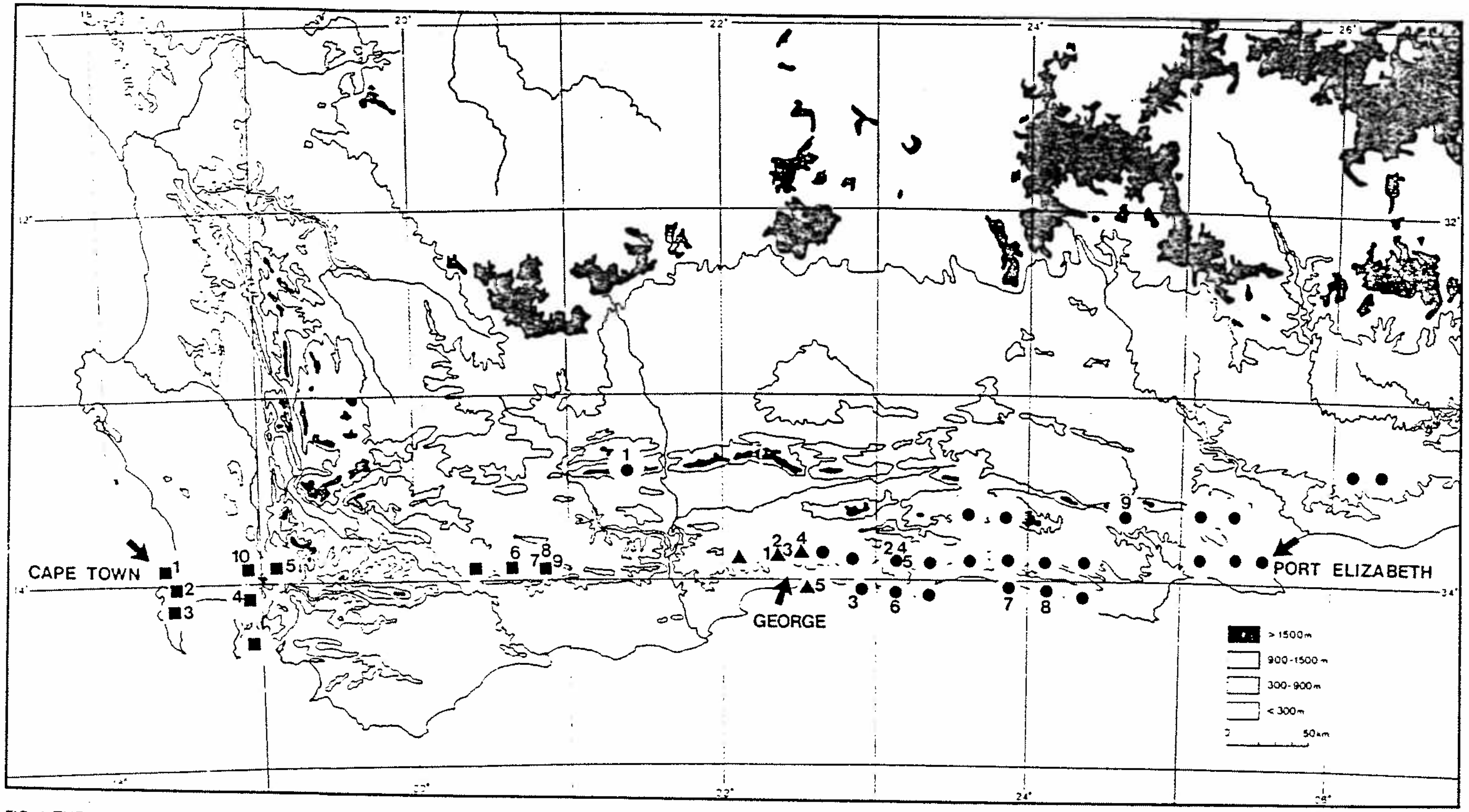


FIG. 1. THE NATURAL GEOGRAPHICAL DISTRIBUTION OF THE GENUS *VIRGILIA*. Symbols indicate all quarter degree square grids from where specimens have been recorded. Localities of the material used for alkaloid studies (see Table 1) are numbered as follows: *V. oroboides* (Berg.) Salter subsp. *oroboides* (■): (1) Platteklip, Table Mountain; (2) Window Stream, Table Mountain; (3) Red Hill, Simonstown; (4) Lourensford, Somerset West; (5) Franschhoek Pass; (6) Tradouws Pass, Barrydale; (7) Grootvadersbosch near Swellendam; (8) Saagkuilkoof, Swellendam district; (9) Duiwenhokskloof, Swellendam district; (10) Kleinplasie, Jonkershoek (and also Stellenbosch, the locality of the trial planting from which some of the materials were collected; see Table 1). *V. oroboides* (Berg.) Salter subsp. *ferruginea* B.-E van Wyk (▲): (1) Geelhoutboom, George; (2) Outeniqua Pass, George; (3) Montagu Pass, George; (4) Swartrivier, on Saasveld Road; (5) Victoria Bay near George. *V. divaricata* Adamson (●) (Swarberg form): (1) Seweweekspoort, Ladismith; (2) Die Vlug, Willowmore district. (Southern Cape and Tsitsikama form): (3) Groenvlei, George district; (4) Keurdraaibos, Plettenberg Bay district; (5) Zeeliesrug, Knysna district; (6) Harkerville, Knysna district; (7) Storms River bridge; (8) Hans Mei River, Humansdorp district, (Eastern Cape form); (9) Baviaanskloof.

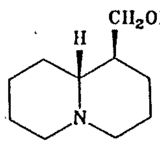
TABLE 1. MATERIAL OF THE GENUS *VIRGILIA* USED FOR ALKALOID STUDIES AND YIELDS OF ALKALOIDAL MATERIAL OBTAINED

Voucher No.	Locality	Yield (mg/g dry weight)				
		Twigs	Leaves	Pods	Seeds	Flowers
Virgilia oroboides (Berg.) Salter subsp. *oroboides*						
2658	Platteklip, Table Mountain	6.32	4.47	3.99	10.6	
2659	Platteklip, Table Mountain	4.53	3.22	2.21	12.6	
506	Platteklip, Table Mountain				3.66	
510	Platteklip, Table Mountain				6.44	
2664	*Platteklip, Table Mountain	4.65	2.34			
2672	*Window Stream, Table Mountain	2.81	2.50			
702	Red Hill, Simonstown				1.95	
2680	Franschhoek Pass	2.59	2.73	6.21	2.54	
2681	Franschhoek Pass	3.27	2.59	1.39	3.73	
2665	*Lourensford, Somerset West	8.70	1.83	1.89	8.57	
2666	*Lourensford, Somerset West	3.04	2.30			
472	Tradouws Pass, Barrydale				9.96	
2715	Grootvadersbosch near Swellendam		7.71			
2716	Saagkuilkloof, Swellendam dist.		3.24			
2717	Duiwenhokskloof, Swellendam dist.		4.55			
V. oroboides (Berg.) Salter subsp. *ferruginea* B.-E. van Wyk						
2661	*Geelhoutboom, George	13.10	9.45	1.33	4.31	10.70
2643	Outeniqua Pass, George	9.80	6.08			
2644a	Outeniqua Pass, George	3.68	4.06			
2644b	Outeniqua Pass, George	7.25	3.37			7.42
956	Outeniqua Pass, George				4.49	
2642	Swartrivier, on Saasveld Road	9.62	10.70			
2663	*Victoria Bay near George	11.10	3.84	1.27	11.30	9.18
2670	*Stellenbosch (cult. tree)	8.96	6.22	0.50	10.40	8.15
V. divaricata Adamson						
(a) Swartberg form						
2645	Seweweekspoort, Ladismith	6.14	10.20	1.82		
2646	Seweweekspoort, Ladismith	6.44	5.98	0.78	9.43	
2647	Seweweekspoort, Ladismith	10.50	8.77	2.09	8.00	
2673	*Die Vlug, Willowmore dist.	1.59	6.60	0.86	1.82	
(b) Southern Cape and Tsitsikama form						
2668	*Groenvlei, George dist.	11.50	6.79	0.76	8.41	
2662	*Keurdraaibos, Plettenberg Bay dist.	2.86	5.32	0.43	5.87	
2641	Zeeliesrug, Knysna dist.	19.00	9.94			
2660	*Harkerville, Knysna dist.	6.60	6.62	2.03	3.87	5.32
2669	*Storms River bridge	2.41	2.65	1.32	5.50	3.33
2667	*Hans Mei River, Humansdorp	4.84	3.97	0.46	8.03	4.35
2674	*Hans Mei River, Humansdorp	9.03	5.68	0.87	3.87	
(c) Eastern Cape form						
2671	*Baviaanskloof	0.87	6.40	0.65	8.57	

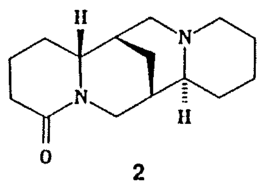
*These materials were collected from a trial planting at Stellenbosch (see [6], where details of the original seed collections are given). Since the trial was planted in a randomized block design, this material is truly comparable—possible environmental effects are excluded.

Voucher specimen numbers refer to the collections of B.-E van Wyk. Numbers below 100 are deposited in the Williams Herbarium, Faculty of Forestry, Stellenbosch (FFS) with duplicates in several other herbaria. Numbers above 2000 are housed in the Rand Afrikaans University Herbarium (JRAU).

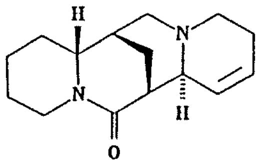
linked to the taxa. Leaves of the two subspecies of *V. oroboides* contain much larger quantities of virgiline-pyrrolyl-carboxylester and much smaller quantities of alkaloid no 8 (a compound of unknown structure, see experimental section) than those of *V. divaricata*. Except for two samples, virgiline-pyrrolyl-carboxylester was found to be almost absent in the leaves of *V. divaricata*. Twigs of *V. divaricata* have more sparteine than those of *V. oroboides* but surprisingly, the leaves do not. Oroboidine is virtually absent from twigs and leaves, present in



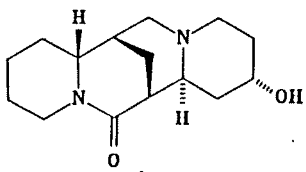
1
LUPININE



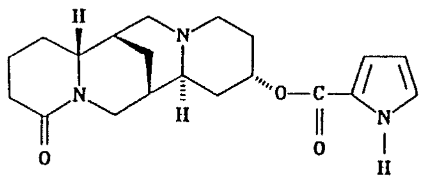
2
SPARTEINE



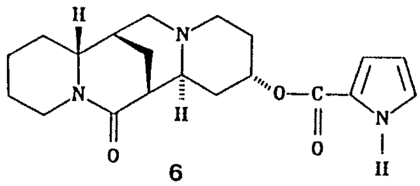
3
VIRGIBOIDINE



4
VIRGILINE



5
OROBOIDINE



6
VIRGILINE-PYRROLYL-
CARBOXYLIC ACID ESTER

small quantities in pods but highly concentrated in all the seed samples. The seeds of the three taxa are remarkably similar even in those alkaloids that occur at almost diagnostically different concentrations in twigs, leaves or pods. The variation in leaf samples amongst different populations of the species and subspecies of *Virgilia* is shown in Fig. 3. There are some significant differences between provenances, but each of the taxa are relatively uniform in its major alkaloids. The low concentrations of alkaloid no. 8 and the very high concentrations of carboxylester in subsp. *ferruginea* confirm its affinity with *V. oroboides* rather than *V., divaricata.* Only two provenances from the eastern parts of the distribution area of *V. divaricata* had significant quantities of the ester compound. The pattern of variation is similar to morphological patterns—individuals from a single locality may often be more variable than individuals from different localities.

Discussion

Unlike twigs, leaves and pods, the seeds of all three taxa are remarkably similar in the concentrations of major alkaloids. The dominance of oroboidine in seeds as opposed to its virtual absence in twigs and leaves seems to be of ecological importance. Seeds of *V. divaricata* are known to retain an almost 100% viability for more than 30 years [5]. This probably represents an adaptation to a forest margin habitat, where fires (a necessity to break seed coat dormancy) occur at low frequencies. Oroboidine may prove to be the most toxic of the major alkaloids and the hard and impermeable seed coat is perhaps not the only protective strategy. Preliminary tests on growth inhibition of some *Agrobacterium* strains have shown that the carboxylester exhibits more toxic properties than sparteine and cytisine. The uniformity of seed alkaloids seems to indicate that its systematic value lies more at the generic than infrageneric level.

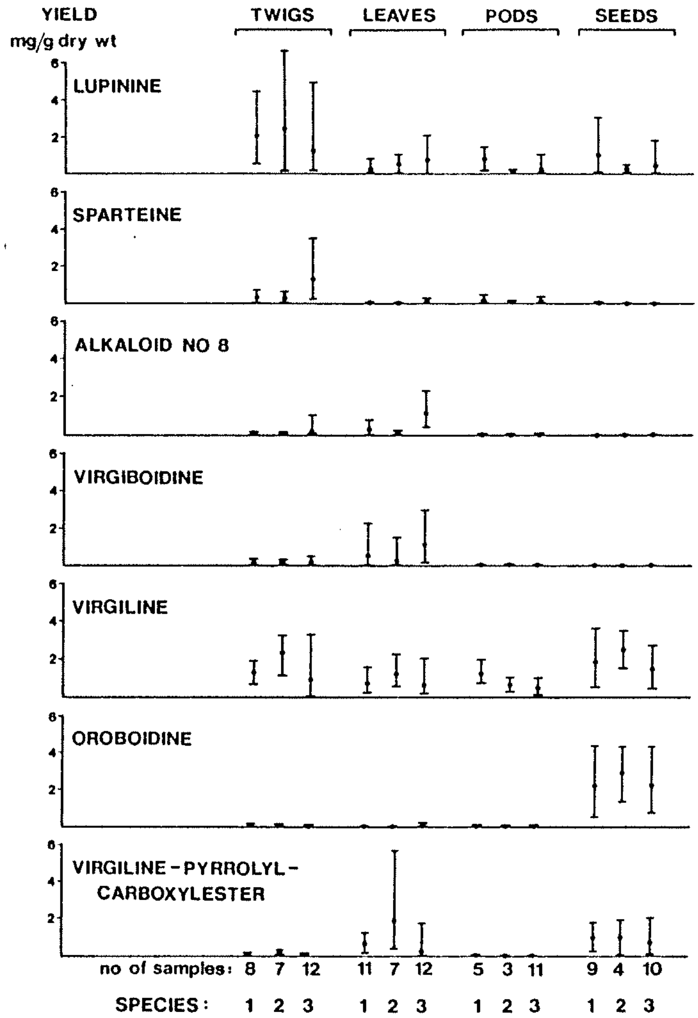


FIG. 2. DISTRIBUTION AND YIELD OF MAJOR ALKALOIDS IN TWIGS, LEAVES, PODS AND SEEDS FROM EACH OF THE SPECIES AND SUBSPECIES OF *VIRGILIA*. The range and mean values for *V. oroboides* subsp. *oroboides*. (1), *V. oroboides* subsp. *ferruginea* (2), and *V. divaricata* (3), are indicated.

Our results indicate that alkaloids may be of diagnostic value even at the specific level. Although we could show no qualitative differences between species and subspecies, there are quantitative differences of such magnitude that sample limitations become unimportant. If a leaf extract contains more than 5% virgiline-pyrrolyl-carboxylester, the sample most likely came from *V. oroboides* and if a twig sample have more than 15% sparteine it is sure to be from *V. divaricata*. Very high concentrations of alkaloid no. 8 and virgiboidine in leaves would indicate *V. divaricata* as the source of the sample. These differences strongly support the taxonomic position of subsp. *ferruginea* as a subspecies of *V. oroboides* rather than V. divaricata. A schematic summary of some of the most conspicuous diagnostic characters of the two species is given in Fig. 4. An overlapping west–east and east–west gradient of character variation is indicated in the figure for *V. oroboides* subsp. *oroboides* and *V. divaricata*, respectively. Subspecies *ferruginea* occupies the region of overlap. Despite the obvious intermediate

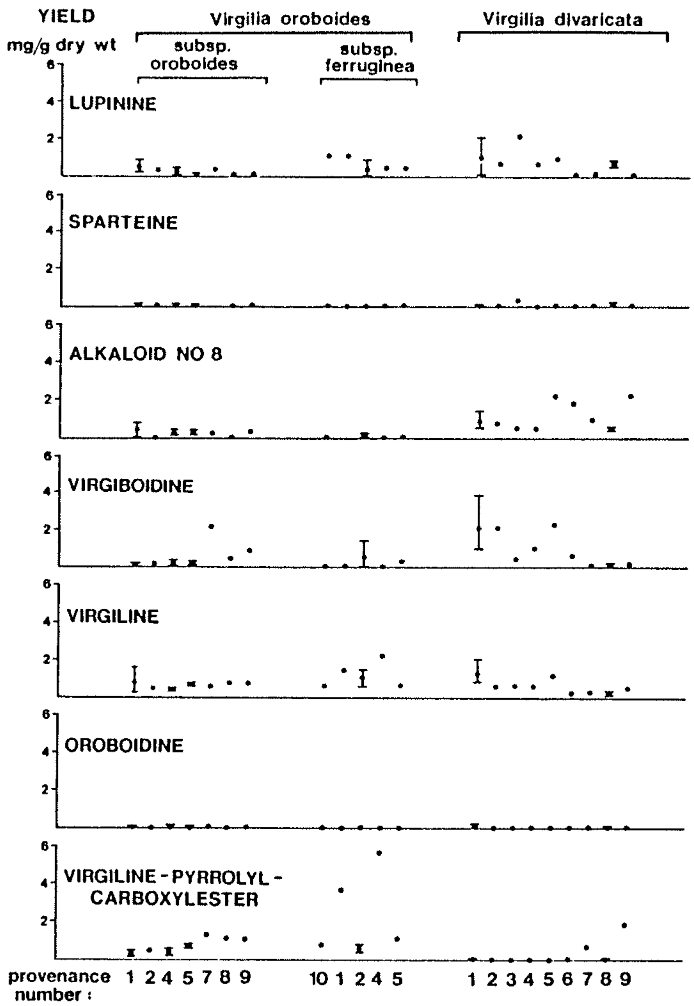


FIG. 3. DISTRIBUTION AND YIELD OF MAJOR ALKALOIDS IN LEAF SAMPLES TAKEN FROM 21 DIFFERENT POPULATIONS OF THE SPECIES AND SUBSPECIES OF *VIRGILIA*. PROVENANCES ARE NUMBERED AS IN FIG. 1.

position of the subspecies between the two typical forms (superficially perhaps more similar to *V. divaricata* than to *V. oroboides* subsp. *proboides*), it was grouped with the latter rather than the former in a recent taxonomic revision [1]. It was argued that the general morphology conforms to the range of variation found in typical *V. oroboides*. Discontinuities in major alkaloids agree with this grouping and the classification therefore seems to have a high predictive value.

We have found cytisine and other α-pyridone alkaloids to be totally absent in *Virgilia*. At higher taxonomic levels, a re-evaluation of affinitites seems necessary. The previously reported presence of cytisine in *Virgilia* has led to suggestions of a connection between this genus and various other cytisine-bearing genera [7, 8]. The assumption that alkaoid toxicity is most efficiently enhanced by the formation of pyridones may not be correct if the same or even a better result is obtained through esterification

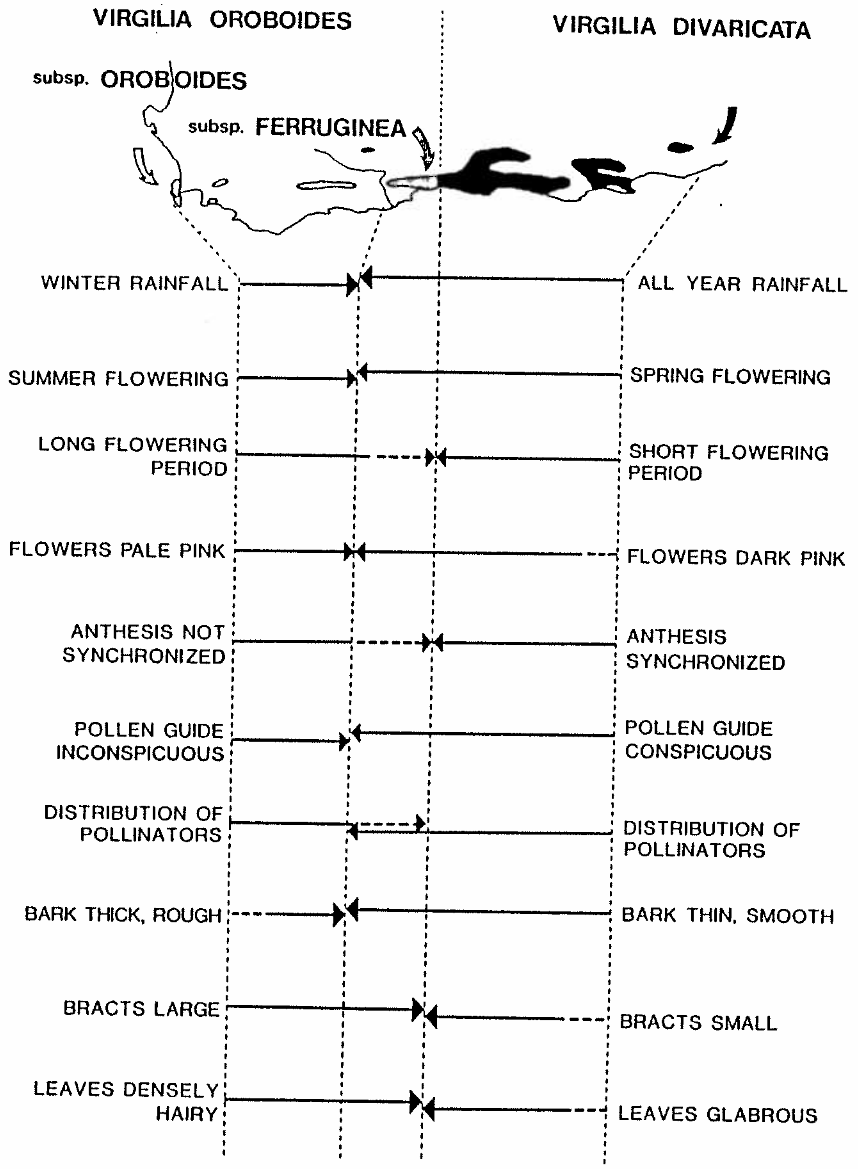


FIG. 4. SCHEMATIC SUMMARY OF THE MOST CONSPICUOUS DIFFERENTIAL CHARACTERS OF *V. OROBOIDES* AND *V. DIVARICATA*. Characters that tend to break down are indicated by broken lines.

by pyrrollyl–carboxylic acid. On the basis of quinolizidine alkaloids, there now seems to remain only evidence for a possible relationship with *Cadia* and *Calpurnia* and not with any other genera.

Experimental

Plant materials. Full details of all the samples used for extraction of alkaloids are given in Table 1.

Procedures. For routine applications, 0.800 g finely ground air-dried material was mixed with 15 ml 1 N H_2SO_4 and left standing at room temperature for 15 min. After centrifugation, the supernatant was filtered and the pellet again extracted with 15 ml 1 N H_2SO_4. After filtration, the supernatants were combined and alkalinized with ammonia. The homogenate was extracted (2×) with 20 ml $CHCL_3$. Chloroform extracts were dried with anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. The alkaloidal mixture was obtained as a pale yellow oil and dissolved in 1.5 ml MeOH. For large scale isolation of specified alkaloids we choose a suitable sample, which was treated under the above-mentioned conditions. The alkaloid mixture was then chromatographed by TLC on Merck Silica 0.5 mm plates with MeOH:$CHCL_3$:-ammonia (85:14:1) and eluted with MeOH. GC separation of alkaloid extracts was performed on a DB1 fused silica capillary colum (15 m × 0.25 mm i.d.; He as carrier gas; column temperature 150° 2 min isotherm, 10° min to 250°, 20° min to 300°, 10 min isotherm; split ratio 1:30; PND detection at 300°). Full details of identification by GC-MS, MS, ^{1}H NMR and ^{13}C NMR are given elsewhere [Greinwald, R., Veen, G., Van Wyk, B.-E., Czygan, F.-C. and Witte, L., in preparation]. Data for alkaloid no. 8: *Rt* 6.35, *m/z* 194 (65), 166 (18), 150 (35), 134 (22), 110 (20), 96 (16), 84 (100), 68 (12), 55 (35).

Acknowledgements—We thank Dave le Maitre, Theo van der Merwe and Jan Vlok for collecting some of the material used in this study and the Fonds der Chemischen Industrie for financial support.

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