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Seed flavonoids of the *Podalyrieae* and *Liparieae* (*Fabaceae*)

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Abstract: A study of the phenolic compounds of the closely related papilionoid tribes, *Podalyrieae* and *Liparieae*, proved that the flavonoid patterns of hydrolysed seed extracts are remarkably conservative. Butin (7, 3′, 4′-trihydroxyflavanone), 3′-hydroxydaidzein (7, 3′, 4′-trihydroxyisoflavone), vicenin-2 (6, 8-di-β-D-glucopyranosyl-5, 7, 4′-trihydroxyflavone) and orobol (5, 7, 3′, 4′-tetrahydroxyisoflavone) were isolated and identified as the major flavonoids. The seeds of *Amphithalea*, *Coelidium*, *Liparia*, *Xiphotheca*, *Calpurnia*, *Stirtonanthus* and *Podalyria* accumulated three isoflavone O-glycosides that yielded 3′-hydroxydaidzein on hydrolysis. In contrast, *Virgilia* contained a unique combination of vicenin-2 and orobol. Vicenin-2 was also present in *Calpurnia* as a major compound, but *Stirtonanthus insignis* was the only other species studied that contained orobol (in trace amounts only). Butein, a chalcone, was reported by HARBORNE from the seed of *Cyclopia subternata*. This compound's flavanone analog, butin, was the principal component in *Cyclopia*. A cladistic analysis, using flavonoid, alkaloid and morphological data, showed that the seed flavonoids of the *Podalyrieae* and *Liparieae* behave rather poorly as cladistic characters. They are, however, of considerable taxonomic value at the tribal level favouring the opinion that the two tribes should be combined. The apparent absence of flavonoids in the seed of *Hypocalyptus* supports the suggestion that it should be excluded from the *Liparieae*. Flavonoids also show that the *Argyrolobium*-group is very different from the tribe *Crotalarieae* and support the recent transfer of this group to the tribe *Genisteae*.

Until recently the *Podalyrieae* and *Liparieae* have been distinguished by the degree of fusion of the stamens (POLHILL 1981a). However, the taxonomic value of this character at the tribal level has been questioned (POLHILL 1981a, VAN WYK & SCHUTTE 1995a). In a recent taxonomic revision of the two tribes, SCHUTTE (1995) based the amalgamation of these endemic southern African legume tribes upon three synapomorphies: the general absence of bracteoles, the presence of carboxylic acid esters of alkaloids and persistent antipodal cells. Eight genera were included in the *Podalyrieae* and *Liparieae* by SCHUTTE (1995): *Priestleya* sect. *Aneisothea* was given generic status as the reinstated genus *Xiphotheca* (SCHUTTE & VAN WYK 1993); *Priestleya* sect. *Priestleya* was included in *Liparia* (SCHUTTE & VAN WYK 1994); *Amphithalea* and *Coelidium* were considered to be congeneric;

the yellow flowered species of *Podalyria* were incorporated in the new genus *Stirtonanthus* (VAN WYK & SCHUTTE 1994, 1995b); *Cyclopia*; *Podalyria* and *Calpurnia* (VAN WYK & SCHUTTE 1995b). The expulsion of the genus *Hypocalyptus* from the *Liparieae* was justified by several anomalous characters, such as the chromosome number of 2n = 20, the morphology of the seed micropyle, and the accumulation of canavanine in the seeds (SCHUTTE 1995). The flower anthocyanins are also different from those of the *Podalyrieae* and *Liparieae*. *Hypocalyptus* accumulates the 3-glucoside of malvidin, while all the other genera have cyanidin or peonidin glucosides, which are esterified with acetic acid or coumaric acid (VAN WYK & al. 1995). In a chemotaxonomic survey of phenolic compounds (DE NYSSCHEN, unpubl.), seed flavonoids provided further evidence for the unification of the two tribes and the exclusion of *Hypocalyptus*. The only seed flavonoids reported thus far from the two tribes are 7, 4′-dihydroxyflavone; 7, 3′, 4′-trihydroxyflavone and butein, all from *Cyclopia subternata* (HARBORNE 1971).

Materials and methods

Plant material was collected by A. L. SCHUTTE and B.-E. VAN WYK and herbarium voucher specimens are housed in the Rand Afrikaans University Herbarium (JRAU) unless otherwise stated. Voucher specimen numbers and locality details are set out in Tables 1 and 2.

Seed samples were ground and extracted with methanol for 4 h. The extracts were filtered through celite and prepared for HPLC analysis using solid phase extraction (Macherey-Nagel, Chromabond® column C_{18}/6ml/1000 mg). Samples were taken to dryness on a rotary evaporator, redissolved in methanol, mixed with water (1:1) and subjected to HPLC analysis. Direct seed extracts were then hydrolysed in 4 N HCl at 95 °C for 60 min and filtered. The flavonoids in the hydrolysed extract were concentrated on a Chromabond® column (conditioned with water), washed with water, eluted with methanol, taken to dryness and redissolved in methanol before HPLC analysis. The HPLC system comprised an IB-Sil column (C_{18}, 5 µm, 250× 4.6 mm, flow rate 1 ml min^{-1}, 20µl sample loop) with an Ultrasphere guardcolumn (45 × 4.6 mm) and a 30 to 100% linear gradient of methanol in 1% acetic acid-water over 20 min. Detection was by diode array detector, using two channels (A: 280 ± 40 nm; B: 330 ± 70 nm). Material used for the isolation of the flavonoid aglycones and the C-glycoside was prepared the same way as the hydrolysed analytical samples.

Butin was isolated from *Cyclopia subternata* (ex hort NBI) seed. The compound was isolated by column chromatography using silica gel (silica gel 60, 0.063 – 0.2 mm) and 30% acetone in chloroform as eluant. A final purification was performed using preparative TLC (silica gel 60) and the same solvent system ($R_f = 0.45$; yield : 1.5 $mg.g^{-1}$. Butin was identified by ^1H NMR and El-MS [$d\delta_H$(DMSO – d_6) 7.61 (1H, d, *J* 8.7, 5-H), 6.86 (1H, br.s, 2′-H), 6.72 (1H, br.s, 5′-6′-H), 6.47 (1H, dd, *J* 8.5 and 2.1, 6-H), 6.31 (1H, d, *J* 2.0 Hz, 8-H); m/e (%) 272 (65, M^+), 163 (22), 150 (19), 137 (100), 84 (52), 66 (70)].

Preparative TLC was used to isolate 3'-hydroxydaidzein from seed of *Podalyria cuneifolia* (VAN WYK 2934) using 10% methanol in chloroform as eluant ($R_f = 0.2$; yield: 1.2 $mg.g^{-1}$. 3′-Hydroxydaidzein was identified by ^1H NMR and El-MS [δ_H (DMSO-d_6) 8.18 (1H, S, 2-H), 7.92 (1H, d, *J* 10.0, 5-H), 6.98 (1H, *J* 1.6, 2′-H), 6.88 (1H, d, *J* 8.7, 5′-H), 6.77 (3H, m, 6, 6′, 8-H); m/e (%) 270 (100, M^+), 213 (12), 137 (71), 134 (38)].

Vicenin-2 and orobol were obtained from *Bolusanthus speciosus* (BOLUS) HARMS (*Sophoreae*) seed (ex hort. NBI). Orobol was isolated from the hydrolysed extract using

Table 1. Distribution of major seed flavonoids in the genera of the *Liparieae*. *1* vicenin-2, *2* butin, *3* 3′-hydroxydaidzein, *4* orobol. *tr* compound constitutes less than 10% of total absorbance; + between 10 and 20%; ++ between 20 and 50%; +++ above 50%

Species	Voucher no.	Locality	1	2	3	4
Amphithalea ericifolia (L.) ECKL. & ZEYH. subsp. *ericifolia*	VLOK, VAN WYK & SCHUTTE 83	Kleinmond Kogelberg Forest Reserve			+++	
A. micrantha (E. MEY.) WALP.	SCHUTTE 755	Uniondale, Mannetjiesberg	tr	++	+	
A. violacea (E. MEY.) BENTH.	SCHUTTE 649	Oudtshoorn, Klein Moerasrivier		tr	+++	
Coelidium muralteoides BENTH.	VLOK & SCHUTTE 275	Oudtshoorn, Swartberg Pass			+++	
C. vlokii A. L. SCHUTTE & B.-E. VAN WYK	SCHUTTE 743	Uniondale, Fortkoppie		tr	+++	
Hypocalyptus coluteoides (LAM.) DAHLG.	VAN WYK 3406	Blueliliesbush, Tsitsikama				
H. oxalidifolius (SIMS) BAILL.	VLOK & SCHUTTE 217	Swellendam, Langeberg Mountains				
H. sophoroides (P. J. BERGIUS) BAILL.	VAN WYK & SCHUTTE 3314	Oudtshoorn, Swartberg Pass				
	VAN WYK & SCHUTTE 3330	Ladismith				
	SCHUTTE 822	Oudtshoorn, De Rust				
	VAN WYK 3012	Worcester, Du Toit's Kloof Pass				
Liparia spec. nova, ined.	VAN WYK 2970	Oudtshoorn, Swartberg Pass		tr	+++	
L. calycina (L. BOLUS) A. L. SCHUTTE	SCHUTTE 764	Stanford, Salmonsdam Nature Reserve			+++	
L. laevigata (L.) THUNB.	SCHUTTE 792	Cape Town, Table Mountain		tr	+++	
L. myrtifolia THUNB.	VAN WYK 2780	Somerset West, Boegoekloof		tr	+++	
L. vestita THUNB.	VLOK, VAN WYK & SCHUTTE 87	Kleinmond, Paardeberg		tr	+++	
Xiphotheca fruticosa (L.) A. L. SCHUTTE & B.-E. VAN WYK	SCHUTTE 720	Montagu, Waboomsberg	tr	tr	++	
	VAN WYK 2749	Constantiaberg	tr		++	

Table 1 (continued)

Species	Voucher no.	Locality	1	2	3	4
X. reflexa (THUNB.) A. L. SCHUTTE & B.-E. VAN WYK	SCHUTTE 760	Heidelberg, Verkykerskop		tr	+++	
X. tecta (THUNB.) A. L. SCHUTTE & B.-E. VAN WYK	SCHUTTE & VAN WYK 778	Paarl, Du Toit's Kloof Pass		tr	++	
	SCHUTTE & VAN WYK 779	Paarl, Du Toit's Kloof Pass		tr	++	

Table 2. Distribution of major seed flavonoids in the genera of the *Podalyrieae*. *1* vicenin-2, *2* butin, *3* 3′-hydroxydaidzein, *4* orobol. *tr* compound constitutes less than 10% of total absorbance; + between 10 and 20%; ++ between 20 and 50%; +++ above 50%

Species	Voucher no.	Locality	1	2	3	4
Calpurnia aurea (AIT.) BENTH.	VAN WYK s.n.	Golden Gate	++	tr	++	
C. aurea subsp. *aurea*	VAN WYK 3583	Butgura Rd, Ethiopia	++		++	
	VAN WYK s.n.	Ex WWBG	tr		+++	
C. intrusa (R. BR. ex AIT. f.) E. MEY.	VAN WYK 3249	Oudtshoorn, Meiring spoort	++	tr	++	
Cyclopia aurescens KIES	SCHUTTE & VAN WYK 775	Ladismith, Seven Weeks Poort Peak	tr	++	tr	
C. bolusii HOFMEYER & E. PHILLIPS	SCHUTTE & VLOK 749	Oudtshoorn, Swartberg Pass	+	+++		
C. bowieana HARV.	SCHUTTE 526	Mossel Bay, Ruitersberg Peak	tr	+++	tr	
	VLOK 2568		tr	+++	tr	
C. buxifolia (BURM. f.) KIES	SCHUTTE 766	Paarl, Du Toits Kloof Pass	tr	+++		
C. intermedia E. MEY	SCHUTTE 510b	Joubertina, Misgund East	tr	+++	tr	
	SCHUTTE 518	Uniondale, Prince Alfred's Pass	tr	+++	tr	
	DE LANGE s.n.	Langkloof	tr	+++	tr	
	DE LANGE s.n.	Dennehoek	tr	+++	tr	
C. maculata (ANDREWS) KIES	SCHUTTE 528	Riversdale, Garcia State Forest	tr	+++		
C. meyeriana WALP.	VAN WYK 2779	Somerset West, Dwarsberg	tr	+++		
C. montana HOFMEYR & E. PHILLIPS var. *glabra* HOFMEYR & E. PHILLIPS	SCHUTTE 557	Ceres, Matroosberg	tr	+++		
	SCHUTTE 558b	Ceres, Matroosberg	tr	+++		
C. plicata KIES	SCHUTTE 517	Uniondale, Hoopsberg	++	++		

Table 2 (continued)

Species	Voucher no.	Locality	1	2	3	4
C. sessiliflora ECKL. & ZEYH.	SUTCLIFF s.n.	Ex hort NBG	tr	+++	++	
C. subternata VOGEL	VAN WYK s.n.	Ex hort NBG	tr	+++	tr	
Podalyria calyptrata (RETZ.) WILLD.	SCHUTTE 709	Caledon, Betty's Bay		tr	+++	
P. cuneifolia VENT.	VAN WYK 2934	Port Elizabeth	tr	tr	+++	
P. glauca (THUNB.) DC.	VAN WYK 2945	Joubertina, Misgund East		tr	++	
P. sericea (ANDREWS) R. BR.	VAN WYK 2461	Du Toit's Kloof Pass	tr	tr	+++	
Stirtonanthus chrysanthus (ADAMSON) B.-E. VAN WYK & A. L. SCHUTTE	VAN WYK & SCHUTTE 3297	Ladismith, Waterkloof			+++	
S. insignis (COMPTON) B.-E. VAN WYK & A. L. SCHUTTE	VAN WYK & SCHUTTE 3332f	Montagu, Waboomsberg		tr	++	tr
	SCHUTTE & VAN WYK 72	Montagu, Waboomsberg		tr	++	tr
S. taylorianus (L. BOLUS) B.-E. VAN WYK & A. L. SCHUTTE	VAN WYK 3298	Oudtshoorn, Swartberg Pass,	tr	tr	++	
Virgilia divaricata ADAMSON	VAN WYK 593	Seweweekspoort, Ladismith	++		tr	++
	VAN WYK 656	Van Stadens River Bridge	+++		tr	++
	VAN WYK 3317	Seweweekspoort, Ladismith	+++		tr	++
	VAN WYK s.n.	Fernkloof	+++		tr	++
V. oroboides (P. J. BERGIUS) T. M. SALTER subsp. *oroboides*	VAN WYK 503	Betty's Bay	+++		tr	++
	VAN WYK 506	Platteklip, Table Mountain	++		tr	+++
	SCHUTTE 534	Barrydale, Tradouws Pass	+++		tr	++
	VAN WYK 698	Red Hill, Simonstown	++		tr	++
V. oroboides subsp. *ferruginea* B.-E. VAN WYK	VAN WYK 960	Montagu Pass	++		+	++

preparative HPLC ($R_t = 13.8$ min; yield : 0.95 mg.g^{-1}). The HPLC system consisted of an Ultrasphere column (C_{18}, 5 µm, 250 mm × 10 mm, flow rate 4.5 ml min^{-1}, 1 ml sample loop) and a 30 to 65% linear gradient over 14 min, followed by a gradient of 65 to 100% methanol in 1% acetic acid-water for 2 min. Vicenin-2 was successfully isolated using column chromatography (4 : 3 : 1, $CHCl_3$: MeOH : H_2O; yield: 1.03 mg.g^{-1}). The compounds were identified by ^{1}H and ^{13}C NMR and El-MS [Vicenin-2: δ_H(DMSO – d_6) 13.70

(1H, s, 5-OH), 8.00 (2H, d, *J* 8.5, 2′, 6′-H), 6.89 (2H, d, *J* 8.5, 3′, 5′-H), 6.80 (1H, s, 3-H), 6.00–3.00 (m, carbohydrate CH and OH); δ_c(DMSO – d_6) 182.3, 164.1, 161.2, 160.7, 158.5, 155.1, 129.0 (2c), 121.4, 115.8 (2C), 107.5, 105.3, 103.8, 102.6, 81.9, 80.8, 78.8, 77.8, 74.0, 73.3, 71.9, 70.9, 70.5, 69.0, 61.3, 59.8; m/e (%) 468 (3), 339 (9), 265 (6), 226 (7), 215 (8), 184 (19), 155 (14), 100 (25), 85 (45), 69 (28), 57 (100); Orobol; δ_H(acetone – d_6) 13.04 (1H, s, 5-OH), 8.13 (1H, s, 2-H), 7.12 (1H, d, *J* 1.8, 2′-H), 6.92 (1H, dd, *J* 8.30 and 1.8, 6′-H), 6.84 (1H, d, *J* 8.0, 5′-H), 6.39 (1H, d, *J* 2.2, 8-H), 6.26 (1H, d, *J* 2.2, 6-H); m/e (%) 286 (100, M^+), 153 (64), 134 (36), 84 (25), 66 (44), 50 (28)].

Table 3. Major seed flavonoids of all the genera of the *Podalyrieae* and *Liparieae*. The distribution of these compounds in selected species of the *Sophoreae*, and some of the genera of the *Crotalarieae* and *Genisteae* is shown. (Number of species investigated in brackets). *1* vicenin-2, *2* butin, *3* 3′-hydroxydaidzein, *4* orobol. *S* compound present in some taxa; *M* compound present in most taxa

Genus	1	2	3	4
Liparieae				
Amphithalea (3)	S	M	M	
Coelidium (2)		S	M	
Hypocalyptus (3)				
Liparia (5)		S	M	
Xiphotheca (3)	S	S	M	
Podalyrieae				
Calpurnia (3)	M	S	M	
Cyclopia (11)	S	M	S	
Podalyria (4)	S	S	M	
Stirtonanthus (3)	S	S	M	S
Virgilia (2)	M		S	M
Sophoreae				
Bolusanthus speciosus	M			M
Cadia purpurea	M		M	
Sophora inhambanensis	M		M	
Crotalarieae				
Aspalathus (2)	M			
Crotalaria (8)		M		
Lebeckia (6)	M			
Lotononis (16)	M			M
Pearsonia (2)	M			M
Rafnia (2)	M			
Wiborgia (2)	M			
Genisteae				
(*Argyrolobium* group)				
Argyrolobium (3)				
Dichilus (3)				
Melolobium (3)				
Polhillia (1)				

Seed flavonoid, morphological and alkaloid data were cladistically analysed at both the generic and tribal levels using "Hennig 86" (FARRIS 1986). This was done to demonstrate the pattern of character state changes in flavonoids compared to other character information. For the generic analysis of the *Podalyrieae* and *Liparieae*, flavonoid data were added to the data set of SCHUTTE (1995). At the tribal level the data set from VAN WYK & SCHUTTE (1995a) was used to determine phylogenetic relationships between the *Podalyrieae/Liparieae*, *Crotalarieae* and *Genisteae*, after data from four seed flavonoids were added. A single cladogram resulted from the data matrix in Table 4, with a length of 19 steps and a consistency index of 68. *Bolusanthus speciosus* was chosen as the outgroup due to the shared presence of vicenin-2 and orobol. *Hypocalyptus* was excluded due to the absence of observable flavonoids and *Cadia purpurea* was subsequently included since it produced both vicenin-2 and 3′-hydroxydaidzein (Table 3).

Results and discussion

The results of a survey of 58 seed samples from 39 species representing all the genera of the *Podalyrieae* and *Liparieae* are summarised in Tables 1 and 2. Three major flavonoid aglycones: butin (7, 3′, 4′-trihydroxyflavanone), 3′-hydroxydaidzein (7, 3′, 4′-trihydroxyisoflavone) and orobol (5, 7, 3′, 4′-tetrahydroxyisoflavone), and one C-glycoside, vicenin-2 (6, 8-di-β-glucopyranosyl-5, 7, 4′-trihydroxyflavone), were identified in the seeds of the two tribes (Fig. 1). These compounds have been reported sporadically in various unrelated groups of the *Fabaceae* (BISBY & al. 1994, BUCKINGHAM 1994, INGHAM 1981), and it is not possible to interpret or compare the published information from a taxonomic point of view due to differences between plant organs and incomplete data for many genera. The family is unique in the high insidence of isoflavonoids and the frequent abscence of the 5-

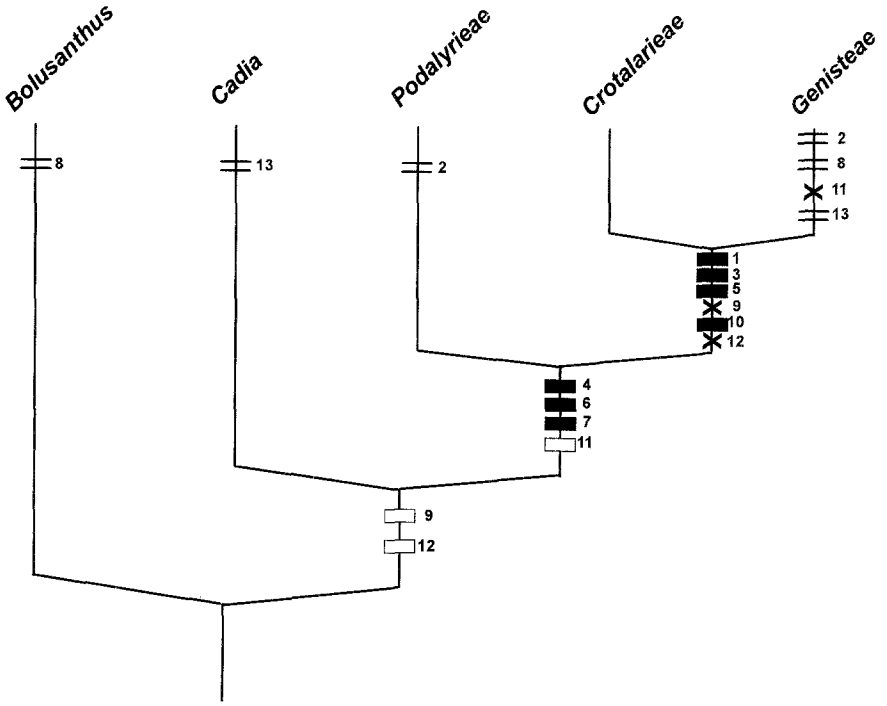

Fig. 1. Major seed flavonoids of the *Podalyrieae* and *Liparieae* (*Fabaceae*)

hydroxyl group (HAGNAUER & GRAYER-BARKMEIJER 1993). Our data agree with these generalizations, as two of the four compounds are isoflavonoids and two of them lack the hydroxyl group at the 5 position.

The accumulation of three O-glycosides, still to be identified, of the aglycone 3′-hydroxydaidzein in the seed of *Amphithalea*, *Coelidium*, *Xiphotheca* and *Liparia* of the *Liparieae* and in *Podalyria*, *Calpurnia* and *Stirtonanthus* of the *Podalyrieae* supports the suggestion that the two tribes are monophyletic and should be combined (VAN WYK & SCHUTTE 1995a, SCHUTTE 1995). The absence of 3′-hydroxydaidzein in hydrolysed seed extracts of the closely related *Crotalarieae* and its presence in *Cadia purpurea* and *Sophora inhambanensis* of the *Sophoreae* (Table 3), emphasizes the importance of determining the identity and distribution of major seed flavonoids of the other tribes of the genistoid alliance sensu POLHILL (1981a, b) such as the *Mirbelieae* and *Bossiaeeae*.

The seeds of *Cyclopia* accumulated O-glycosides of butin. In the other genera butin was sporadically present in trace amounts in hydrolysed extracts, except in *Amphithalea micrantha*. In contrast, none of the species of *Virgilia* contained butin. Genera from other tribes of the genistoid alliance studied so far, did not produce butin except *Crotalaria* (Table 3). In *Virgilia*, vicenin-2 and orobol are the major flavonoids, with trace amounts of 3′-hydroxydaidzein. These compounds were isolated from seed of *Bolusanthus speciosus* (*Sophoreae*). ASRES & al. (1985) isolated a range of isoflavonoids from the seed of *Bolusanthus speciosus*, but did not report the presence of vicenin-2. VAN WYK & SCHUTTE (1995a) considered *Calpurnia* to be closely related to *Virgilia* and included it in the *Podalyrieae*. *Calpurnia* and *Virgilia* are the only genera of the *Podalyrieae* and *Liparieae* that accumulate vicenin-2 as a major compound. Orobol was present in the seed of only one other species of the two tribes, *Stirtonanthus insignis*. Vicenin-2 was present in several genera of the *Crotalarieae*, while orobol primarily accumulated in *Lotononis* and *Pearsonia* (Table 3). VAN WYK & SCHUTTE (1995a) transferred the *Argyrolobium* group from the *Crotalarieae* to the *Genisteae*, with which it shares the trifid lower lip of the calyx and the presence of α-pyridone type quinolizidine alkaloids. Of the ten species from four genera of this group studied none produced any of the seed flavonoids of the *Podalyrieae* and *Liparieae* (Table 3).

The tribal affinity of *Hypocalyptus* is uncertain, and it has been unconvincingly placed in the *Liparieae* (POLHILL 1981b, VAN WYK & SCHUTTE 1995a). Both direct extracts and hydrolysed material of *Hypocalyptus* seed contained negligably low levels of flavonoids. SCHUTTE (1995) assigned the genus tribal status as the *Hypocalypteae* and suggested it might be related to the *Milletieae*. The flavonoid results support this exclusion of *Hypocalyptus* from the *Liparieae*, since it did not accumulate any of the major seed flavonoids of the two tribes (Table 1).

Figure 2 shows the single, fully resolved cladogram of hypothetical relationships at the tribal level. This result is based on a rigorous analysis, using morphological and chemical data listed in Table 4. The topology of the cladogram is similar to the consensus tree obtained by VAN WYK & SCHUTTE (1995a) at tribal level. From the generic analysis of the *Podalyrieae* and *Liparieae* it was concluded that seed flavonoid characters do not contribute much as cladistic characters at the generic level due to repeated reversals or secondary losses. However, the combination of seed flavonoids is often of diagnostic value, usually corresponding

Table 4. Polarization of morphological and chemical character states in the *Crotalarieae, Podalyrieae/Liparieae* and *Genisteae*

Taxa	Characters and character states												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Sophoreae													
Bolusanthus speciosus	0	0	0	0	0	0	0	1	0	0	0	0	0
Cadia purpurea	0	0	0	0	0	0	0	0	1	0	0	1	1
Crotalarieae	1	0	1	1	1	1	1	0	0	1	1	0	0
Podalyrieae/Liparieae	0	1	0	1	0	1	1	0	1	0	1	1	0
Genisteae (*Argyrolobium* group)	1	1	1	1	1	1	1	1	0	1	0	0	1

1. Leaf type: pinnate (at least in some taxa) = 0; digitate or simple = 1
2. Calyx upper lobes: not fused higher up = 0; fused higher up to form an upper lip = 1
3. Stamens: free = 0; fused = 1
4. Anther dimorphism: not dimorphic or slightly dimorphic = 0; strongly dimorphic = 1
5. Carinal anther (size): similar to basifixed anthers = 0; intermediate or similar to dorsifixed anthers = 1
6. Seed micropyle type: ypsaloid = 0; punctate = 1
7. Seed micropyle position: outside the hilum = 0; inside the hilum or on the rim = 1
8. Quinolizidine alkaloids (α-pyridone type): absent = 0; present = 1
9. Carboxylic acid esters of alkaloids: absent = 0; present = 1
10. Vicenin-2: present = 0; absent = 1
11. Butin: absent = 0; present = 1
12. 3′-Hydroxydaidzein: absent = 0; present = 1
13. Orobol: present = 0; absent = 1

with morphologic patterns. Thus *Virgilia* and *Cyclopia* are morphologically unique and also have unique combinations of seed flavonoids.

The cladogram in Fig. 2 gives a visual summary of relationships, not only between the tribes, but also the relation between morphological and flavonoid character state changes. There are three convincing morphological synapomorphies for the three terminal tribes (dimorphic anthers, punctate seed micropyles, positioned within the hilum), and also three synapomorphies for the *Crotalarieae* and *Genisteae* (digitate or simple leaves, fused stamens, and short carinal anthers). The flavonoid data, in contrast, provide only a single synapomorphy, namely the loss of flavone (character 10). All other seed flavonoids behave rather poorly as cladistic characters, since all of them show either reversals or convergences. The small number of seed flavonoids in the two tribes is consistent with the findings of HEGNAUER & GRAYER-BARKMEIJER (1993) and VAN WYK & SCHUTTE (1995a) that seed characters are conservative and consequently indicative of suprageneric relationships. The hypothesis that seed characters are of value at the tribal level is further supported by different seed alkaloid profiles characterising two monophyletic groups (α-pyridones in one group and hydroxylated lupanines in the other) within the tribe *Crotalarieae* (VAN WYK & VERDOORN, unpubl.).

It is apparent that an extensive study of the seed flavonoids of closely related tribes, such as the paraphyletic assemblage currently known as the *Sophoreae*, will

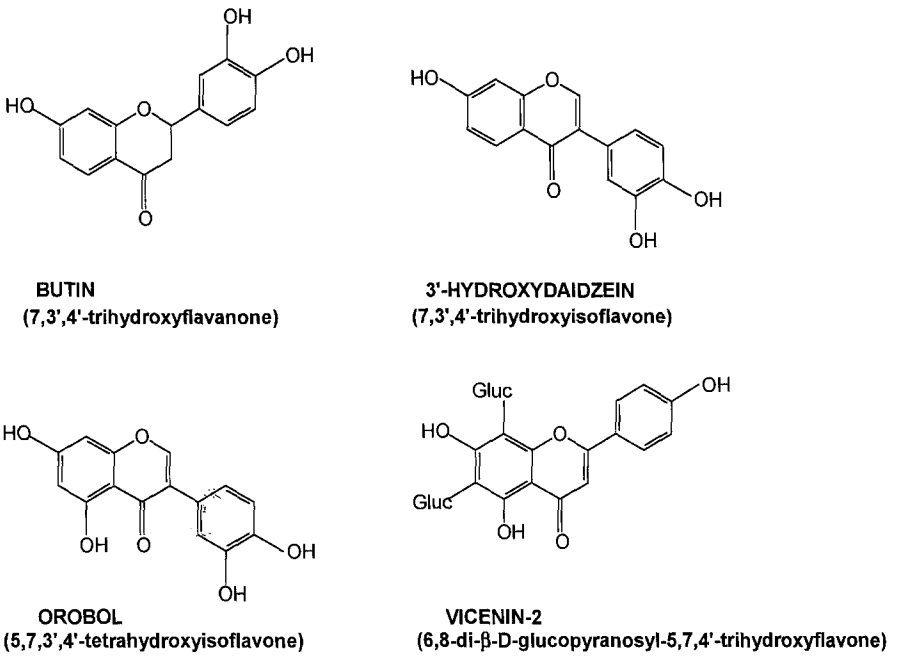

Fig. 2. Cladogram of relationships at the tribal level based on the data set in Table 4. ■ Apomorphy without homoplasy; □ apomorphy with homoplasy higher up; = convergence; × reversal. Numbers of flavonoid characters encircled

lend further insight into phylogenetic relationships. In the *Papilionoideae* conservative characters are generally rare, and seed flavonoids may therefore be valuable to determine relationships amongst the tribes of the genistoid alliance and eventually between all the tribes of the subfamily.

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