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Chemotaxonomic Value of Anthocyanins in the Tribe Liparieae (Fabaceae)

BEN-ERIK VAN WYK, PIETER J. D. WINTER and ANNE LISE SCHUTTE
Department of Botany, Rand Afrikaans University, P.O. Box 524, Johannesburg 2006, South Africa

Key Word Index—Fabaceae; *Amphithalea*; *Coelidium*; *Hypocalyptus*; *Liparia*; anthocyanins; chemotaxonomy; generic relationships.

Abstract—The major anthocyanins of the tribe Liparieae have been identified. Pink and purple flowers in *Amphithalea* and *Coelidium* contain the acetic acid and coumaric acid esters of cyanidin, closely similar to the pattern reported for the tribe Podalyrieae. The purple flowers of *Hypocalyptus*, however, differ markedly from all the genera of both tribes; they contain malvidin rather than cyanidin and peonidin; the malvidin pigment is present as the 3-glucoside in fresh flowers, and it is not esterified as in all other purple-flowered genera. The petals of *Liparia* flowers contain the 3-sophorosides of cyanidin and pelargonidin, while the bracts have the 3-glucosides of cyanidin and peonidin. These results support the idea of a close relationship between the Liparieae and Podalyrieae but also agree with other evidence that *Hypocalyptus* is only superficially similar to the genera of these two tribes.

Introduction

The chemical basis for flower colour in the family Fabaceae is poorly known (Kay, 1987; Brouillard, 1988; Harborne, 1988) but may provide useful new character information for taxonomic and phylogenetic interpretations. For example, we have recently shown that the anthocyanins of the tribe Podalyrieae are unexpectedly conservative, and that they occur as acetyl and coumaroyl esters in the fresh flowers (van Wyk and Winter, 1994). This interesting result prompted us to expand our chemotaxonomic survey to the tribe Liparieae, which is generally considered to be closely related to the Podalyrieae (Polhill, 1981). Our main interest was a comparison between the anomalous *Hypocalyptus* and other genera of the two tribes.

Materials and Methods

Plant materials. The species studied, authorities for names and vouchers specimens are listed in Table 1.

Procedures. Fresh petals were extracted in 1% methanolic HCl or MeOH:acetic acid:H_2O (8:1:1) and the extracts studied by analytical HPLC with a diode array detector and the same gradient solvent system as described in detail elsewhere (van Wyk and Winter, 1994). The use of a different column (Phenomenex IB-SIL C18 reverse phase, 5 μm particle size, 250 mm × 4.6 mm i.d.) resulted in higher retention times than those reported in van Wyk and Winter (1994). Samples were subjected to partial hydrolysis (usually 0, 15, 30, 45 and 60 min in 2N HCl at 100°C), precleaned by filtration and concentrated by C_{18} solid phase extraction. Anthocyanins were identified by their spectral characteristics and by comparison and co-HPLC with known standards (Harborne, 1967). Further confirmation was obtained by a study of hydrolysis products (Strack *et al.*, 1980). The following standards were used: cy-3-sophoroside from *Hibiscus rosa-sinensis*; pg-3-sophoroside from *Tropaeolum majus*; cy-3-glucoside from *Acer* (leaves); pn-3-glucoside from *Fuchsia* (partially hydrolysed petals); mv-3-glucoside from *Fuchsia* and from *Tibouchina semidecandra*; cy-3-(acetylglucoside) from *Virgilia* and cy-3-coumaroylglucoside from *Hyacinthus orientalis* (bulb scales) and also *Podalyria* petals (see van Wyk and Winter, 1994).

Results

Table 1 shows that cyanidin-3-glucoside is the most common anthocyanin in the Liparieae, occurring in all the genera except *Hypocalyptus*, where malvidin-3-glucoside is the only compound. Esters of cyanidin-3-glucoside are found only in *Amphithalea*

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TABLE 1. DISTRIBUTION OF ANTHOCYANINS IN EXTRACTS FROM *AMPHITHALEA, COELIDIUM, HYPOCALYPTUS* AND *LIPARIA*. Anthocyanin distributions are given as percentages of total pigments, as estimated from HPLC results, using a diode array detector (see Materials and Methods)

	Major Anthocyanins*							
	glycosides					esters		
	1	2	3	4	5	6	7	8
Genus, species and sample	R_t†: 18.5	21.8	22.0	27.9	29.5	37.5	43.2	47.0
Amphithalea ericifolia Eckl. & Zeyh.								
Van Wyk 3240a	—	—	100	—	—	—	—	—
Van Wyk 3240b	—	—	63	—	—	37	—	—
Van Wyk 3240c	—	—	83	—	—	17	—	—
A rostrata Schutte & Van WYk								
Van Wyk 3231b	—	—	100	—	—	tr	—	—
Coelidium parvifolium (Thunb.) Druce								
Vlok & Schutte 123	—	—	6	—	—	25	7	62
C. villosum (Schltr.) Granby								
Vlok & Schutte 117	—	—	12	—	—	20	5	63
Hypocalyptus coluteoides (Lam.) Dahlg.								
Schutte 730a	—	—	—	—	100	—	—	—
Schutte 730b	—	—	—	—	100	—	—	—
Schutte 730c	—	—	—	—	100	—	—	—
Van Wyk et al. 3406	—	—	—	—	100	—	—	—
H. oxalidifolius (Sims) Baill.								
Schutte 698	—	—	—	—	100	—	—	—
H. sophoroides (Berg.) Baill.								
Van Wyk et al. 3448	—	—	—	—	100	—	—	—
Liparia parva J. R. T. Vogel ex Walp.								
Van Wyk 3243a (bracts)	—	—	55	45	—	—	—	—
L. splendens (Burm.f.) J. J. Bos & De Wit susp. *splendens*								
Winter 91 (petals)	91	9	—	—	—	—	—	—
subsp. *comantha* (Eckl. & Zeyh.) J. J. Bos								
Schutte 696 (bracts)	—	—	76	24	—	—	—	—

*Anthocyanins: **1** = cyanidin-3-sophoroside; **2** = pelargonidin-3-sophoroside; **3** = cyanidin-3-glucoside; **4** = peonidin-3-glucoside; **5** = malvidin-3-glucoside; **6** = cyanidin-3-(acetylglucoside); **7** = unidentified unstable minor compound); **8** = cyanidin-3-(*p*-coumaroylglucoside).

†Retention time in min (for HPLC system see van Wyk and Winter, 1994).

and *Coelidium*, in the former esterified with acetic acid, in the latter with coumaric acid. The red flower colour in *Liparia* is due to the 3-sophorosides of cyanidin and pelargonidin in the petals of *L. splendens*, and the 3-glucosides of cyanidin and peonidin in the bracts of both species.

Discussion

The results in Table 1 show that anthocyanins are taxonomically significant and that each of the genera has a unique and diagnostically different combination of flower anthocyanins. The almost universal occurrence of cyanidin-3-glucoside in pink- and purple-flowered species supports the idea of a close affinity between the Liparieae and Podalyrieae. Furthermore, the major flower anthocyanins of *Amphithalea* and *Coelidium* are esterified in the fresh flowers, exactly as in the Podalyrieae (van Wyk and Winter, 1994). The apparent difference between *Amphithalea* and *Coelidium* may be a result of the small number of samples, but the close similarity of both genera with the Podalyrieae is obvious. All the major anthocyanins are the same in the two tribes, except peonidin-3-glucoside and its acetic acid ester, which occur in *Virgilia* but not in *Amphithalea* and *Coelidium*. Differences in flower colour are also, as in the

Podalyrieae, not reflected in the anthocyanins. The two species of *Amphithalea*, for example, have very different flower colours but the same (single) anthocyanin. In *A. rostrata* the flowers are dark maroon–purple, while they are pale pink in *A. ericifolia.*

The inflorescences of the two *Liparia* species are orange–red in appearance, mainly as a result of the pigmentation of the large bracts which surround the flowers (for colour illustrations see van Wyk *et al.*, 1991). The petals of *L. parva* are lemon yellow and devoid of anthocyanins, but anthocyanins contribute to the orange colour of the petals in *L. splendens.* The expected lack of homology, not only between purple and red flowers, but also between bracts and petals, is clearly reflected in Table 1. The bracts of both species have the 3-glucosides of cyanidin and peonidin, while the petals of *L. splendens* have the 3-sophorosides of cyanidin and pelargonidin.

Hypocalyptus is clearly separated from all other genera by the unique occurrence of malvidin-3-glucoside as the only anthocyanin of all three species. Methylation of anthocyanins is considered to be a derived character (Heller and Forkmann, 1988) and this degree of hydroxylation and especially methylation does not occur in any of the other genera. The only convincing evidence to support an affinity between *Hypocalyptus* and the Liparieae–Podalyrieae alliance is the intrusive base of the calyx. This similarity may be superficial, because *Hypocalyptus* is very different in its general morphology, chromosome number and chemistry (Dahlgren, 1972; van Wyk and Schutte, in press). Amongst the genera of the two tribes, *Hypocalyptus* is the only known source of canavanine (Bell *et al.*, 1978), the only genus with proanthocyanins in the leaves (Harborne, 1969) and, with the exception of *Cyclopia*, the only genus which does not accumulate quinolizidine alkaloids (Van Wyk and Greinwald, unpublished). Anthocyanins can now be added to a growing list of characters, all indicating that *Hypocalyptus* is not closely related to the Liparieae and Podalyrieae.

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