Pergamon

PII: S0305-1978(96)00095-6

Biochemical Systematics and Ecology, Vol. 25, No. 1, pp. 39–42, 1997

0305–1978/97 $17.00+0.00

The Major Flower Anthocyanins of *Lobostemon* (Boraginaceae)

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Key Word Index—Boraginaceae; *Lobostemon*; anthocyanins; chemotaxonomy; flower colour.

Abstract—The chemical basis of flower pigmentation in the genus *Lobostemon* is presented. Six major anthocyanins were found in the species investigated. Delphinidin-3,5-diglucoside is the most common pigment and tends to be a major component of violet-blue flowers. When the level of delphinidin-3,5-diglucoside is low, then delphinidin-3-glucoside tends to be the major component, together with small amounts of delphinidin-3-rutinoside. Red flowers possess cyanidin-3-rutinoside as a major component. Cyanidin-3,5-diglucoside and cyanidin-3-glucoside occur sporadically in some species, particularly in red or pinkish flowers. The anthocyanins were found to be more or less related to flower colour and only of minor interspecific taxonomic value.

Introduction

A taxonomic revision of *Lobostemon* Lehm. is currently underway. As part of a multidisciplinary approach, a study of the anthocyanins was undertaken. Flower colour is a favourite taxonomic character, as is the case in existing *Lobostemon* keys (Levyns, 1934). Anthocyanin composition has proven to be of taxonomic value in certain taxa (Saito and Harborne, 1992). However, as mentioned by Baum (1976) and Van Wyk and Winter (1995), flower colour should be used with caution in that the homology of the pigments involved is rarely known. To date no data are available on the anthocyanin composition of flowers in *Lobostemon*. This paper identifies the anthocyanin components of *Lobostemon* and assesses the chemotaxonomic potential thereof.

Materials and Methods

Plant materials. Voucher specimens and locality data of the material used for extraction are listed in Table 1. The sample included 19 species, representing the full range of variation found in the genus, which has about 30 described species. The formal taxonomy of *Lobostemon* has not yet been finalised and we therefore prefer not to give authorities for names.

Procedures. Petals were extracted with 1% HCl (MeOH) and chromatographed by analytical HPLC using a diode array detector (Beckman ULTRASPHERE ODS column, 5 μm particle size, 250 mm × 4.6 mm i.d.; flow rate 1 ml min^{-1}; 20 μl sample loop). The solvent system was the same as described by Akavia and Strack (1980) and Strack *et al.* (1980) and comprised a 20–100% linear gradient of A in B. A: 1.5% H_3PO_4, 20% acetic acid and 25% MeCN in H_2O; B: 1.5% H_3PO_4 in H_2O. All samples were purified by ODS solid phase extraction prior to analysis. R_t values, UV-VIS spectral data and R_f values were used for comparisons with known standards (Harborne, 1967). Some extracts were co-chromatographed (HPLC and PC) with known standards (Harborne, 1967) to confirm equivalent R_t and R_f values. Samples were also subjected to partial hydrolysis (usually 10, 20, 40 and 60 min with 2N HCl @ B.P.) to determine the type of glycoside (Strack *et al.*, 1980). To check for the possible presence of unstable malonyl esters, some samples were extracted in MeOH: acetic acid:H_2O (8:1:1) but these extracts were practically devoid of anthocyanins, even after thorough

(*Received* 9 *June* 1996; *accepted* 9 *September* 1996)

TABLE 1. DISTRIBUTION OF ANTHOCYANINS IN *LOBOSTEMON* PETALS. Anthocyanin distributions are given as percentages of total pigments, as estimated from HPLC results, using a diode array detector (see Materials and Methods)

Anthocyanins*:			A	B	C	D	E	F
Retention times (mins):			12.6	17.4	18.7	15.5	20.4	22.0
Species	Voucher specimen	Petal colour						
Lobostemon sect. ***echioides***								
L. capitatus	MHB 450	cream	23	45	tr	10	22	—
L. echioides	MHB 512	blue-violet	84	13	tr	—	—	—
L. gracilis	MHB 443	light blue	71	13	—	4	tr	—
L. cf. *horridus*	MHB 484	blue-violet	84	12	—	4	tr	—
Lobostemon sect. ***trichotomi***								
L. cf. *glaucophyllus*	MHB 510	blue-violet	76	11	—	7	tr	—
L. cf. *glaucophyllus*	BVW 3488	blue-violet	63	14	tr	14	8	—
L. cf. *laevigatus*	MHB 401	blue-violet	69	27	tr	4	tr	—
L. cf. *laevigatus*	MHB 404	blue-violet	39	52	—	5	3	—
L. cf. *laevigatus*	MHB 511	blue-violet	75	25	—	tr	—	—
L. cf. *laevigatus*	MHB 519	blue-violet	71	25	—	4	—	—
L. glaber	MHB 397	white	67	33	tr	tr	tr	—
L. hottentoticus	MHB 378	pink-white	—	95	tr	tr	5	—
L. cf. *pearsonii*	MHB 514	blue-violet	73	24	tr	3	—	—
L. trichotomus	MHB 518	violet-blue	64	36	—	—	—	—
L. trichotomus	MHB 110	white	tr	84	5	tr	7	—
L. trichotomus	BVW 3520a	bright blue	77	14	—	5	tr	—
L. trichotomus	BVW 3520b	bright blue	89	7	—	tr	tr	—
L. trichotomus	MHB 513	violet-blue	90	10	tr	tr	—	—
Lobostemon sect. ***argentei***								
L. argenteus	MHB 436	violet-blue	71	15	tr	10	4	—
Lobostemon sect. ***fruticosi***								
L. curvifolius	MHB 389	violet-blue	94	6	tr	tr	—	—
L. decorus	MHB 488	violet-blue	75	7	—	4	6	—
L. fruticosus	BVW 3487	pink	15	2	tr	29	47	7
L. marlothii	MHB 470a	violet-blue	54	6	—	14	7	6
L. marlothii	MHB 470b	violet-blue	75	10	—	4	tr	tr
L. oederiaefolius	MHB 396	violet-blue	58	28	3	4	7	tr
L. strigosus	MHB 455	violet-blue	74	14	tr	12	tr	tr
Lobostemon sect. ***grandiflori***								
L. regulareflorus	MHB 439	red	—	tr	—	—	24	76
L. montanus	MHB 381	blue-violet	78	9	tr	13	tr	—
Other Boraginaceous taxa								
Echium violaceum	ex hort.	blue-violet	85	12	tr	—	—	tr

*Anthocyanins: A = Dp-3,5-diglucoside; B = Dp-3-glucoside; C = Dp-3-rutinoside; D = Cy-3,5-diglucoside; E = Cy-3-glucoside; F = Cy-3-rutinoside.

grinding of the flower petals. It appeared that the stronger acid was necessary for the release of anthocyanins from the petal tissue. Since malonylated anthocyanins occur in the related Labiatae (Saito and Harborne, 1992), it would be interesting to investigate the exact form in which the pigments occur in *Lobostemon* petals. This aspect, however, was beyond the scope of our investigation.

The anthocyanins were analyzed by PC in four standard solvent systems as described by Harborne (1967). All the compounds had relative R_t- and R_f-values closely equivalent to literature data. Co-HPLC with cyanidin-3,5-diglucoside and cyanidin-3-glucoside from *Kalanchoe blossfeldiana* petals, cyanidin-3-rutinoside from *Euphorbia pulcherrima* bracts and delphinidin-3-rutinoside from *Strelitzia reginae* petals confirmed preliminary identifications.

Results

The distribution of the six major anthocyanins in 28 samples from 19 *Lobostemon* species is given in Table 1. *Lobostemon* flowers predominately contain cyanidin and delphinidin glycosides. Delphinidin-3,5-diglucoside would appear to be most prevalent in *Lobostemon*, being undetectable in only two species sampled, i.e. *L. hottentoticus* and *L. regulareflorus*. Delphinidin-3-glucoside tends to be the major component in instances where delphinidin-3,5-diglucoside is present in low quantities. Delphinidin-3-rutinoside occurs only in minor quantities in the species studied. Red flowers sampled have cyanidin-3-rutinoside as the major component. Cyanidin-3-glucoside and cyanidin-3,5-diglucoside rarely occur in more than trace quantities.

Discussion

Lobostemon appears to be a conservative genus in terms of anthocyanin composition. Although the anthocyanins differ quantitively, all the species investigated had various combinations of the six major anthocyanins, which could all be positively identified.

The correlation between flower colour and anthocyanin type varies among taxa. Van Wyk and Winter (1994), for example, found no association in genera of the Podalyrieae (Fabaceae), whereas Harborne and Smith (1978) did in the Polemoniaceae. Violet flowers are generally associated with the presence of delphinidin, although exceptions are to be found (Brouillard, 1988). *Lobostemon* shows a tendency for delphinidin-3,5-diglucoside to be associated with violet-blue flowers. According to Saito and Harborne (1992), there is an evolutionary advantage in basing blue flower colour on delphinidin rather than cyanidin derivatives in that less flavone copigment is needed to shift the original purple colouration towards the blue region.

Saito and Harborne (1992) opine that in the Labiatae at least, pelargonidin is the preferred basis of a red colour attractive to birds. This is not the case in *Lobostemon* and in certain Crassulaceous taxa (Van Wyk and Winter, 1995), where cyanidin seems to be the basis of red pigmentation. Scogin (1991) could not confirm earlier results which suggested that cyanidin was frequently present in passerine-visited flowers, while pelargonidin was often present when hummingbirds were the pollinating vectors. The red flowers of *L. regulareflorus*, which is pollinated by sunbirds, possesses almost exclusively cyanidin-3-rutinoside.

Accepting that cyanidin derivatives contribute to the red colour, it seems surprising to observe cyanidin-3,5-diglucoside in quantity in non-red flowers. Closer examination of non-red flowers, however, reveals various sources of red pigmentation. Many species possess red stamens. *Lobostemon capitatus* has red markings on its cream petals and *L. strigosus*, for example, has in addition to red petals, a conspicuous red ring surrounding the staminal scales at the base of the petals. The *L. fruticosus* sample is unique in that it has cyanidin-3-glucoside as its major component. The elevated levels of cyanidin-3-glucoside is possibly indicative of degradation through partial hydrolysis due to storage (Withey *et al.*, 1993; Boyles and Wrolstad, 1993), but the presence of relatively large amounts of cyanidin-3,5-diglucoside more likely points to a biosynthetic shift from blue to red in this pink-flowered species.

The anthocyanins of *Lobostemon* species with similar flower colours differ largely quantitatively. The overall pattern is that flower colour is more or less directly related to pigment types, with bluish flowers tending to have delphinidin glycosides, and pink or red flowers generally with cyanidin glycosides. The anthocyanins are not sufficiently

conservative to be used as taxonomic markers, except perhaps for the red-flowered species. It is nevertheless useful to know that flower colour in *Lobostemon* can be judged visually and that similar colours are likely to be chemically homologous. A cursory look at other Boraginaceous taxa indicated that *Echium* L. has a composition of anthocyanins similar to that of *Lobostemon.* A more detailed comparison between *Lobostemon* and other Boraginaceous genera (especially *Echium*) is bound to be enlightening.

Acknowledgements—The Foundation for Research Development is thanked for financial support.

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