Phytochemistry, Vol. 25, No. 8, pp. 1887–1894, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

THE NATURAL DISTRIBUTION IN ANGIOSPERMS OF ANTHOCYANINS ACYLATED WITH ALIPHATIC DICARBOXYLIC ACIDS

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(*Received* 17 *December* 1985)

Key Word Index—Angiospermae; flower pigments; acylated anthocyanins; malonic acid; malic acid; succinic acid; chemotaxonomy.

Abstract—An electrophoretic survey of mainly floral tissues of 282 species from 68 families showed the presence of zwitterionic anthocyanins in 38% of the species and in 30% of the families. Families recognised for the first time as having such pigments include the Alliaceae, Gramineae, Orchidaceae and Ranunculaceae. Families where they are rare or absent include the Boraginaceae, Geraniaceae, Iridaceae, Onagraceae and Umbelliferae. Acylating acids so far identified are malic, malonic and succinic and of these three, malonic seems to be the most common. The significance of this acylation appears to be related to the stabilization of anthocyanins in the acidic environment of the cell sap.

INTRODUCTION

Until recently, anthocyanins containing acyl substituents were thought to be confined to no more than 20 plant families and the acyl group present was either an aromatic acid, such as caffeic or *p*-coumaric, or else the simple aliphatic acetic acid [1, 2]. However, it is now clear from both a limited survey of floral pigments [3] and from detailed investigations of individual anthocyanins [e.g. 4] that anthocyanins are also acylated in nature with aliphatic dicarboxylic acids, such as malonic [4], malic [5] or succinic [6]. Such acylation renders the anthocyanin zwitterionic which means that it is possible to detect these pigments very simply by paper electrophoresis in a buffer of pH 4.4 [3, 7].

Another important feature of this type of acylation is the instability of the acyl linkage *in vitro*, particularly when compared with other types of acylation. If such acylated anthocyanins are extracted by standard procedures using methanolic HCl, there is intermediate methyl ester formation, but the main reaction is loss of the acyl group within quite a short time. Successful extraction of zwitterionic anthocyanins depends on the substitution of HCl by weaker acids, acetic or formic, in the aqueous methanolic solvent. Because of this unusual lability, a variety of pigments, particularly several present in flowers of the Compositae, now known to be acylated were previously reported to be unacylated. In particular, the flowers of cornflower, *Centaurea cyanus*, the classic source of cyanidin 3,5-diglucoside, have now been shown to contain the 3-(6″-succinylglucoside)-5-glucoside using milder methods of extraction and purification [6, 8].

There is the possibility that other pigments characterized in early investigations as lacking acylation might in fact carry these labile substituents. It was therefore important to extend the earlier limited survey of some 81 species for zwitterionic anthocyanins [3] to a wider sampling of families. The results of this more extended survey of an additional 200 species are presented here.

RESULTS AND DISCUSSION

The results of surveying 282 spp. from 68 families are shown in Table 1. Also included are the results of the earlier survey [3] and those species in which anthocyanins acylated with dicarboxylic acids have been characterized [3–13]. This survey was based largely on petal pigmentation, but other tissues were also examined where appropriate. Of the 68 families surveyed, 24 were positive (i.e. contained zwitterionic pigments), i.e. some 35% of the sample (Table 2). New families not previously recognised as having these acylated pigments include: in the dicotyledons, Euphorbiaceae, Malvaceae, Thymeliaceae and Verbenaceae; and in the monocotyledons, Alliaceae, Gramineae, Liliaceae and Orchidaceae. At the species level, the frequency of zwitterionic anthocyanins is about 38%. This probably over estimates the actual frequency among the angiosperms generally, since it is biased in terms of sampling towards those families in which zwitterionic pigments were first found. Nevertheless, it suggests that the actual frequency at the species level may be as high as 25%.

In those species with anionic pigments at pH 4.4, all the pigment moved from the origin in nearly all cases, indicating that acylation normally extends to each individual anthocyanin that may be present. This has generally been confirmed in those plants which have been the subject of detailed analysis. The possibility of obtaining false results, due to the complexing of anthocyanin with other constituents in crude plant extracts seems unlikely, in view of the relatively acid pH used for electrophoresis. This was tested for with flavone glucuronides which also move anionically at pH 4.4. However, it was found that non-zwitterionic anthocyanins were unaffected when electrophoresed in admixture with apigenin 7-glucuronide.

The positive families listed in Table 2 vary from those where acylation is universal (Labiatae, Alliaceae) or nearly so (Compositae, Ranunculaceae, Scrophulariaceae) to those with only occasional occurrence (Iridaceae,

Table 1. Electrophoretic survey of angiosperm flowers for zwitterionic anthocyanins

Plant family	Genus and species	Organ surveyed	Mobile pigment present
DICOTYLEDONEAE			
Acanthaceae	*Acanthus spinosus* L.	Se	–
Anacardiaceae	*Rhus cotinus* L.	L	–
Apocynaceae	*Rhazya orientalis* A. DC.	P	–
	Vinca major L.	P	–
Begoniaceae	*Begonia* cv. Black Jack	L	–
Berberidaceae	*Berberis vulgaris* L.	L, F	–
Bignoniaceae	*Catalpa bignonioides* Walter	P	–
Boraginaceae	*Borago officinalis* L.	P	–
	Cynoglossum officinale L.	P	–
	Echium rubrum Jacq.	P	–
	E. vulgare L.	P	–
	Echium cv. dwarf hybrid	P	–
	Lithospermum purpuro caeruleum L.	P	–
	Myosotis sylvatica Hoffm.	P	–
	Paracaryum heliocarpum Kern.	P	–
	Pulmonaria officinalis L.	P	–
	P. rubra Schott	P	–
	P. saccharata Mill.	P	–
	Symphytum officinale L.	P	–
Buddleiaceae	*Buddleia davidii* Franch.	P	–
Campanulaceae	*Campanula portenschlagiana* Roem. & Schult.	P	–
	C. cervicaria L.	P	–
	Codonopsis cardiophylla Diels ex Kom.	P	–
	Trachelium caeruleum L.	P	–
Cannaceae	*Canna edulis* Ker-Gawl.	P	–
Caprifoliaceae	*Wegelia* sp.	P	–
Caryophyllaceae	*Agrostemma githago* L.	P	–
	Dianthus carthusianorum L.	P	–
	D. caryophyllus L. pink forms	P	+
	deep red forms	P	–
	D. deltoides L.	P	+
	D. nitidus Waldst. & Kit.	P	–
	D. sylvestris Wulf.	P	+
Caryophyllaceae	*Lychnis chalcedonica* L.	P	–
	L. flos-cuculli L.	P	+
	L. flos-juvis Desv.	P	+
	Moehringia pentandra J. Gay	L	–
	Saponaria officinalis L.	P	–
	Silene dioica (L.) Clairville	P	–
Cistaceae	*Cistus roseus* Jacq.	P	–
	Helianthemum cv. Henfield Brilliant	P	–
Compositae	*Ageratum* cv. Ocean	P	+
	Arctotis sp.	P	+
	Aster cv.	P	+
	Bellis perennis L.	P	+
	Bidens sp.	P	–
	Callistephus chinensis Cass.	P	+
	Centaurea cyanus L.*	P	+
	C. grinensis Reuter*	P	+
	C. jacea L.*	P	+
	C. macroptilon Borb.*	P	+
	C. micranthes Gmel.*	P	+
	C. montana L.	P	+
	C. nigra L.	P	+
	C. pannonica (Heuff.) Simk*	P	+
	C. spinulosa Rochel*	P	+
	Cicerbita plumieri (L.) Kirschleger	P	+
	Cichorium endivia L.	P	+
	C. intybus L.*	L, P	+

Table 1. (*Continued*)

Plant family	Genus and species	Organ surveyed	Mobile pigment present
	Cirsium arvense (L.) Scop	P	+
	C. dissectum (L.) Hill	P	+
	Coleostephus myconis (L.) Reichenb. f.	St	+
	Cosmos atrosanguineus Hook.	P	−
	Cynara scolymus L.	P	+
	Dahlia variabilis L. cvs	P	+
	Erigeron tillingii Voroch	P	+
	Gaillardia grandiflora Hort.	P	+
	Gerbera jamesonii Bolus*	P	+
	Helenium cv. Bruno	P	+
	Helianthus annuus L. cv.	P	+
	Helichrysum cv. Swiss Giant	P	+
	Helipterum roseum Benth.	P	+
	Liatris spicata Willd	P	+
	Ligularia przewalski cv. The Rocket	St	+
	Osteospermum jucundum (Phillips) Norlindh	P	−
	Senecio coccinaeflorus Rowl.	P	−
	S. cruentus DC.*	P	+
	Stokesia laevis Hill	P	+
	Tanacetum coccineum Willd.	P	+
	Zinnia cv. Persian Carpet	P	+
Convolvulaceae	*Convolvulus arvensis* L.	P	−
	Convolvulus cv.	P	+
	Calystegia sepium (L.) R. Br.	P	−
Crassulaceae	*Crassula schmidtii* Regel	P	−
	Sedum spectabile Boreau	P	−
Cruciferae	*Aubretia deltoidea* DC.	P	+
	Brassica oleracea L.*	L	+
	Cheiranthus cheiri L.	P	−
	Lunaria annua L.	P	+
	Moricandia arvensis DC.	P	+
	Raphanus sativus L. cv.	root	+
Dipsacaceae	*Knautia arvensis* (L.) Coult	P	−
	K. macedonica Griseb.	P	−
	Scabiosa atropurpurea L.	P	−
Ericaceae	*Daboecia cantabrica* (Hudson) C. Koch	P	−
	Rhododendron ponticum L.	P	−
	Rhododendron cv.	P	−
Euphorbiaceae	*Ricinus communis* L.	P/L	+
Fagaceae	*Fagus sylvatica* L.	L	−
Fumariaceae	*Dicentra eximia* Torr.	P	−
Geraniaceae	*Erodium malacoides* Willd.	P	−
	Geranium endressii Gay 'Wargrave Pink'	P	−
	G. himalayense Klotzsch.	P	−
	G. maderense Yeo	P	−
	G. macrorrhizum L.	P	−
	G. pratense L.	P	−
	G. robertianum L.	P	−
	Pelargonium hortense cv. Red White stem	P	−
	cv. Red Minicascade	P	−
Gesneriaceae	*Columnea* x *vedrariensis* Hort	L	−
	Rechsteineria leucotricha	P	−
	Saintpaulia ionanthe H. Wendl.	P	−
	Streptocarpus saxorum Engl.	P	−
	Streptocarpus hybrida cv. Constant Nymph	P	+
Globulariaceae	*Globularia nudicaulis* L.	P	−
Haloragidaceae	*Gunnera manicata* Linden	stamen	−
Labiatae	*Coleus sylvatica*	P	+
	Hyssopus officinalis L.	P	+
	Lamium maculatum L.	P	+

Table 1. (*Continued*)

Plant family	Genus and species	Organ surveyed	Mobile pigment present
	Lavandula stoechas L.	P	+
	Monarda didyma L.	P	+
	Plectranthus argentatus L.	P/L	+
	P. luteus	P	+
	Rosmarinus officinalis L.	P	+
	Salvia nemorosa L.	P	+
	S. verticillata L.	P	+
	S. virgata L.	P	+
	S. viridis L.	P	+
	Stachys sp.	P	+
	S. officinalis (L.) Trevisan	P	+
	S. sylvatica L.	P	+
	Thymus praecox Opiz subs. *arcticus*	P	+
	T. pulegoides L.	P	+
Lardizabalaceae	*Akebia quinita* Decne	P	−
Leguminosae	*Anthyllis montana* L.	P	−
	Baptisia australia R. Br.	P	−
	Cercis siliquastrum L.	P	−
	C. chinensis Bunge	P	−
	Clitoria ternatea L.*	P	+
	Cytisus scoparius (L.) Link	P	−
Leguminosae	*Lathyrus latifolius* L.	P	−
	L. odoratus L. Sutton's hybrids	P	−
	Lotus berthelotii Masf.	P	−
	Lupinus cv. Russell hybrid	P	+
	Parochetus communis Buch. -Ham ex D. Don	P	−
	Sophora viciifolia Hance	P	−
	Trifolium repens L.	P	−
	Vicia sativa L. subsp. *sativa*	P	+
Lobeliaceae	*Lobelia* cv. Mrs. Clibran	P	+
	cv. String of Pearls	P	+
Lythraceae	*Lythrum salicaria* L.	P	−
Magnoliaceae	*Magnolia* x *soulangeana* Hort.	P	−
	Schizandra rubriflora Rehd. & Wils.	P	−
Malvaceae	*Abutilon hybridum* Hort.	P	−
	Althaea officinalis L.	P	+
	Hibiscus rosa-sinensis L.	P	−
	Malva sylvestris L.	P	+
Myrtaceae	*Psidium cattleianum* Sabine	F	−
Nymphaeaceae	*Nymphaea marliacea* cv. Attractum	P	−
Oleaceae	*Forsythia suspensa* Vah	stem	−
	Syringa vulgaris L.	P	−
Onagraceae	*Chamaenerion angustifolium* (L.) Scop.	P	−
	Epilobium montanum L.	P	−
	Fuchsia fulgens DC.	P/S	−
	Fuchsia cv. Gartenmuster Bonstadt	P/S	−
	Lopezia racemosa Cav.	P	−
Papaveraceae	*Eschsholtzia* cv.	P	−
	Papaver nudicaule L.*	P	+
	P. orientale L.	P	−
	P. rhoeas L.	P	−
Peoniaceae	*Paeonia officinalis* Auct.	P	−
	Paeonia cv.	P	−
Polemoniaceae	*Phlox drummondii* L.	P	+
	P. paniculata L.	P	+
	Polemonium caeruleum L.	P	−
Plumbaginaceae	*Armeria maritima* L.	P	−
	Ceratostigma willmottianum Stapf.	P	−
	Limonium cvs.	P	−
Polygonaceae	*Polygonum affine* D. Don	P	−
Primulaceae	*Cyclamen hederifolium* Aiton	P	−

Table 1. (*Continued*)

Plant family	Genus and species	Organ surveyed	Mobile pigment present
	Primula bulleyana Forrest	P	−
	P. yargonensis Petitm	P	−
Punicaceae	*Punica granatum* L.	P	−
Ranunculaceae	*Aconitum napellus* L.	P	+
	Actaea sp.	F	+
	Aquilegia vulgaris L.	P	+
	Aquilegia cv.	P	+
	Clematis alpina (L.) Miller	P	+
	C. × *jackmannii* Hort.	P	+
	C. viticella L.	P	+
	Consolida ambigua (L.) R. W. Ball & Heywood	P	−
	Pulsatilla montana Reichb.	P	+
	Pulsatilla vulgaris Miller	P	+
Rosaceae	*Chaenomeles japonica* Lindl.	P	−
	Crateagus monogyna Jacq.	P	−
	Malus domestica Borkh.	F	−
	Potentilla atrosanguinea Loddiges ex D. Don	P	−
	P. nepalensis Hook.	P	−
	Prunus cerasus L.	P	−
	Rosa multiflora cv. Crimson Rambler	P	−
	Spiraea salicifolia L.	P	−
Saxifragaceae	*Astilbe japonica* L.	P	−
	Bergenia cv. Dark Pink	P	−
	Escallonia cv.	P	−
	Saxifraga × *urbium* D. A. Webb	P	−
Solanaceae	*Atropa belladonna* L.	P/F	−
	Browallia demissa L.	P	+
	Hyoscyamus niger L.	P	−
	Nicandra physalodes (L.) Gaertn.	P	−
	Petunia hybrida cv. Colour Parade	P	−
	Solanum dulcamara L.	P	−
	S. crispum Ruiz. & Pav.	P	−
Scrophulariaceae	*Antirrhinum majus* L.	P	−
	Cymbalaria muralis	P	−
	Diascia rigescens	P	−
	Digitalis purpurea L.	P	−
	Hebe cv. Karl Teschet	P	+
	Melampyrum cristatum L.	P/S	+
	Mimulus guttatus DC.	P	+
	*Mimulus luteus**	P	+
	Parahebe catarractae (Forst. f.) Oliver	P	+
	P. perfoliata (R. Br.) Briggs et Ehrend.	P	+
	Penstemon cv. Skyline	P	+
	Veronica chamaedrys L.	P	+
	V. filiformis Sm.	P	+
	V. longifolia L.	P	+
Thymeliaceae	*Daphne retusa*	P	+
	D. cv.	P	+
Theaceae	*Camellia sansaqua* Thunb.	P	−
Umbelliferae	*Daucus carota* L.	P	−
	Anthriscus sylvestris (L.) Hoffm.	L	−
	Heracleum mantegazzianum Sommier & Levier	Stem	−
Valerianaceae	*Centranthus ruber* (L.) DC.	P	−
Verbenaceae	*Verbena* × *hybrida* Voss	P	+
Violaceae	*Viola riviniana* Reichenb.	P	−
	V. × *wittrockiana* Gams	P	−
MONOCOTYLEDONEAE			
Alliaceae	*Allium angulosum* L.	P	+
	A. cristophii Trautv.	P	+
	A. ochroleucum Waldst Kit.	P	+

Table 1. (*Continued*)

Plant family	Genus and species	Organ surveyed	Mobile pigment present
Alliaceae	*A. schoenoprasum* L.	P	+
Araceae	*Xanthosma violaceum*	stem/L	–
Amaryllidaceae	*Haemanthus katherinae* Baker	P	–
	Vallota speciosa Durand & Schniz	P	–
Bromeliaceae	*Billbergia decora* Poepp. & End.	P	–
Commelinaceae	*Commelina communis* L.*	P	+
	Rhoeo discolor Hance	L	–
	Setcreasea purpurea Boem	L	–
	Tradescantia navicularis Ortgies	P	–
	T. cv. Leonora	P	–
	Zebrina pendula Schnizl	L	–
Gramineae	*Phragmities australis* (Cav.) Trin. ex Steudel	P	+
	Zea mays L.	F	+
Iridaceae	*Anomatheca laxa* (Thunb.) Goldblatt	P	–
	A. verrucosa (Vogel) Goldblatt	P	–
	Crocus speciosus M. Bieb.	P	–
	Dietes vegetata (L.) N.E. Br.	P	–
	Gladiolus illyricus Koch	P	–
	G. papilio Hooker f.	P	–
	Iris cristata Solander	P	–
	I. douglasiana Herbert	P	–
	I. ensata Thunb.	P	–
	I. lutescens Lam.	P	–
	I. macrosiphon Torrey	P	–
	I. setosa Pall ex Link	P	–
	Lapeirousia corymbosa (Lam.) Goldbl. subsp. *fastigiata*	P	+
	Schizostylis coccinea Backh. & Harv.	P	–
	Sisyrinchium graminifolium Lindl.	P	–
	S. montanum Greene	P	–
	S. striatum Sm.	P	–
Lemnaceae	*Spirodela polyrhiza* L.*	L	+
Liliaceae	*Colchicum agrippinum* Baker	P	–
	C. byzantinum Ker. Gawl.	P	–
	C. cv. The Giant	P	–
	Endymion non-scriptus (L.) Garcke	P	+
	Hemerocallis cv. Pink Prelude	P	–
	Lilium leichtlinii Hook f.	P	–
	L. martagon L.	P	–
	Liriope spicata Lour.	P	–
	Scilla pensylvanica	P	+
	S. peruviana L.	P	+
	Tulipa gesnerana L. cv. Queen of the Night	P	–
Musaceae	*Musa sapientum* L.	S	–
Orchidaceae	*Bletilla striata* (Thunb.) Rech. f.	P	+
	Dactylorhiza elata (Poiret) Soo	P	+
	Epidendrum ibaguense HBK	P	+
Zingiberaceae	*Kaempferia galanga* L.	P	–

Key: P, petal; F, fruit; L, leaf; Se, sepal; St, stem.
*These species have been shown previously to contain zwitterionic anthocyanins, from detailed investigation [7–13].

Leguminosae, Papaveraceae, Solanaceae). It will be interesting to see what organic acids are present in these various sources, but from the limited number of pigments that have been fully characterized (Table 3), it would appear that malonic acid is the most common. Malonated anthocyanins have been characterized (Table 3) or detected [cf. ref. 9] in at least seven families.

The only source of an anthocyanin acylated with succinic acid is the genus *Centaurea* (Compositae). However, it does not appear to be generally present as an acyl substituent in this family, since all other genera examined so far have yielded malonated pigments [4]. The third acid, malic, has only been detected in *Dianthus* (Caryophyllaceae) [5]. However, at least one other genus,

Table 2. Families with zwitterionic anthocyanins

Family	Species frequency
DICOTYLEDONS	
Caryophyllaceae	5/12
Compositae	35/39
Convolvulaceae	1/3
Cruciferae	5/6
Euphorbiaceae	1/1
Gesneriaceae	1/5
Labiatae	17/17
Leguminosae	3/14
Lobeliaceae	1/1
Malvaceae	2/4
Papaveraceae	1/4
Polemoniaceae	2/3
Ranunculaceae	9/10
Scrophulariaceae	10/14
Solanaceae	1/7
Thymeliaceae	2/2
Verbenaceae	1/1
MONOCOTYLEDONS	
Alliaceae	4/4
Commelinaceae	1/6
Gramineae	2/2
Iridaceae	1/17
Lemnaceae	1/1
Liliaceae	3/11
Orchidaceae	3/3

Lychnis, in this family has zwitterionic pigments (Table 1) so that it is possible that it may be found to have a wider distribution. Other dicarboxylic acids, especially those which are already known to occur in conjugation with hydroxycinnamic acids such as tartaric [14], may well be found during detailed investigations of these novel anthocyanins.

Several dimalonated pigments have been described (Table 3) and multiple acylation is clearly possible, considering the number of free sugar hydroxyl groups present in anthocyanidin glycosides. Such derivatives would be immediately apparent from their increased electrophoretic mobility. So far, however, the indications of this survey are that the great majority of zwitterionic anthocyanins have only one acid substitution. The position of this substitution would appear to the 6-hydroxyl of glucose in those pigments that have been analysed, but again variation in position of substitution is clearly possible.

The significance of the negative results (i.e. lack of anionic movement on electrophoresis) at the family level (Table 1) is difficult to evaluate, due to the rather limited sampling. However, it would seem that some families either lack species with these anthocyanins or have them very rarely. This is probably true of the Boraginaceae (12 species in 8 genera all negative), Geraniaceae (8 spp., 3 genera), Plumbaginaceae (3 spp., 3 genera), Rosaceae (8 spp., 7 genera) and Umbelliferae (3 spp., 3 genera). In the case of the latter family, it was possible to examine pigmentation in the intact plant and in tissue culture of *Daucus carota* and in both cases, zwitterionic anthocyanins were absent.

From the chemotaxonomic viewpoint, the occurrence of this type of anthocyanin acylation would seem to be

Table 3. Known anthocyanins with aliphatic dicarboxylic acids as acylating groups

Pigment	Source	Reference
PELARGONIDIN GLYCOSIDES		
3-(6″-Malonylglucoside)	*Callistephus chinensis*	[4]
3-Malylglucoside	*Dianthus caryophyllus*	[5]
3-Malonylsophoroside	*Papaver nudicaule*	[7]
3-(6″-Malonylglucoside)-5-glucoside	*Dahlia variabilis*	[4]
3,5-Di(malonylglucoside)	*Dahlia variabilis*	[4]
CYANIDIN GLYCOSIDES		
3-(6″-Malonylglucoside)	*Cichorium intybus*	[9]
3-Malylglucoside	*Dianthus deltoides*	[5]
3-Dimalonylglucoside	*Dendranthema myconis*	[4]
3-Malonylglucuronosylglucoside	*Helenium* cv. Bruno	[4]
3-(6″-Malonylglucoside)-5-glucoside	*Dahlia variabilis*	[4]
3,5-Di(malonylglucoside)	*Dahlia variabilis*	[4]
3-(6″-Succinylglucoside)-5-glucoside	*Centaurea cyanus*	[6, 8]
3-(*p*-Coumarylglucoside)-5-malonylglucoside	*Stachys* sp.	[10]
Rubrocinerarin*	*Senecio cruentus*	[11, 12]
PEONIDIN GLYCOSIDE		
3,5-(Malonyl-*p*-coumaryl diglucoside)*	*Plectranthus argenteus*	[3]
DELPHINIDIN GLYCOSIDES		
3,5-Di(malonylglucoside)	*Cichorium intybus*	[4]
3-(*p*-Coumarylglucoside)-5-malonylglucoside	*Commelina communis*	[13]
Cinerarin*	*Senecio cruentus*	[11, 12]
Ternatins A–F*	*Clitoria ternatea*	[11]

*Complex pigments including both malonic acid and aromatic acid substituents.

haphazard. The families so far recorded as containing them (Table 2) are generally unrelated to each other. Even the five families in which they are commonly present—Compositae, Cruciferae, Labiatae, Ranunculaceae, Scrophulariaceae—are widely separated in any system of plant classification. This contrasts with the natural distribution of anthocyanins acylated with hydroxycinnamic acids, since the majority of family occurrences are in relatively advanced families [1] and acylation with hydroxycinnamic and is thus an advanced character.

Both types of acylation can be present in the same pigment (cf. Table 3) and pigments linked to both aromatic and aliphatic acids apparently occur widely in the Labiatae and also probably in several other families. The functions of the two types of acyl substituent would therefore appear to differ. The presence of an aliphatic dicarboxylic acid attached through sugar would appear to be important in stabilizing the anthocyanin in the acidic environment of the cell vacuole. *In vitro* experiments have indicated that malonated anthocyanins are more stable to the effects of light than other anthocyanins [11] so that a role for such acylation in flower colour production would seem likely. Further studies on the function and natural occurrence of these zwitterionic anthocyanins are in progress.

EXPERIMENTAL

Plant material. Plant tissues were freshly collected from the University of Reading Botanic Gardens or from wild and cultivated plants collected locally. Most of the wild species in the Botanic Gardens were grown from seed of spontaneous origin and identifications were verified by the curator, Mr. R. Rutherford. Other identifications were provided by the taxonomy staff of the Department.

Electrophoretic survey. Pigments were extracted for 8–24 hr in MeOH–HOAc–H_2O (19:2:19) and the extracts, after filtration, were concd at room temp. in an air draft. Electrophoresis was conducted on Whatman No. 3 paper in acetate buffer pH 4.4 for 2 hr at 40 V cm^{-1}. The papers were removed, dipped briefly in 1% aq. HCl and then dried. Cationic anthocyanins remained close to the origin, moving very slightly towards the cathode, whereas zwitterionic anthocyanins were clearly distinguished by their movement (3–4 cm) towards the anode. Rarely, diacylated pigments were present and these moved about 6–7 cm. Many of the crude extracts were also chromatographed on Whatman No. 1 paper in the standard anthocyanin solvents and pigments were classified into pelargonidin, cyanidin or delphinidin types on the basis of their colours in daylight and in the UV. Detailed separations and analysis of zwitterionic anthocyanins in about 20 species of those surveyed have been reported elsewhere [3–5, 10].

Acknowledgements—The author is grateful to departmental colleagues for plant identifications and Mr. N. Hind for provision of plant materials.

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